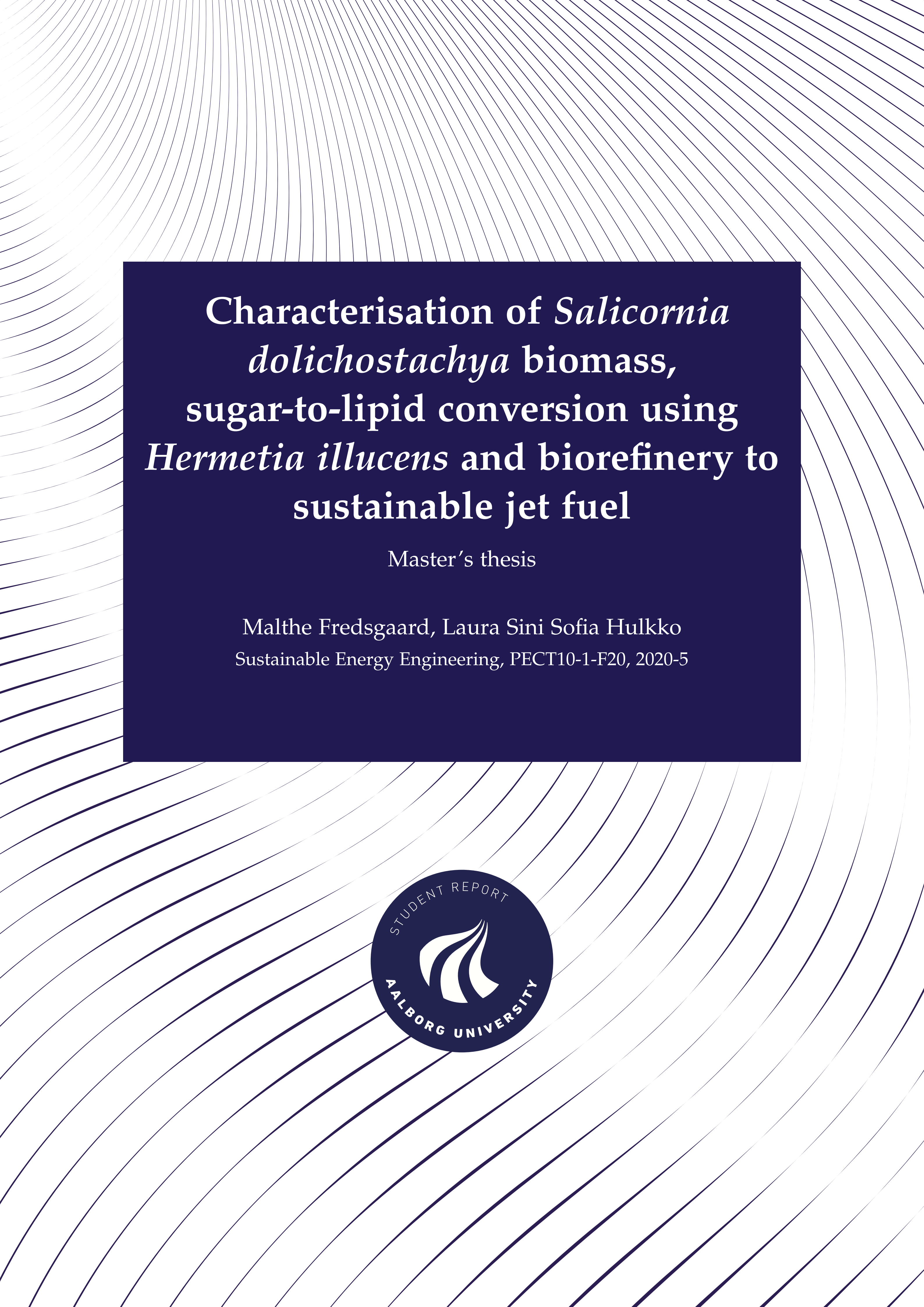

Characterisation of *Salicornia dolichostachya* biomass, sugar-to-lipid conversion using *Hermetia illucens* and biorefinery to sustainable jet fuel

Master's thesis

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Sustainable Energy Engineering, PECT10-1-F20, 2020-5

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Abstract:

Salt-tolerant succulent halophyte *S. dolichostachya* is characterised to evaluate its suitability to biorefinery in different stages of plant growth, as the soil salinisation has seen as a major agricultural issue and the worlds biofuel demand is increasing. The green fractionation approach is chosen, where the liquid and solid fractions of the biomass are considered separately. Soxhlet extractions are used to separate different biochemical groups from the pulp, and the lignocellulosic residue is fed to black soldier fly larvae (BSFL) for sugars-to-lipids conversion. The ASTM approved route of hydroprocessed esters and fatty acids is applied in *in silico* study of processing BSFL lipids to sustainable jet fuel.

The fractionation shows biomass consisting 47-69 w% green juice and 28-48 w% pulp. Up to 62 w% of the pulp dry matter (DM) is lignocellulose, and the ash content is also relatively high, due to accumulation of salts to halophyte tissues. Results show, that 12-14 w% of total DM is covered by protein. The total lipid content is low, but increases over time when plant produces seeds. Feed trials show, that up to 40 w% of the traditional BSFL feed can be replaced with *S. dolichostachya* biomass with only small effects in the growth, yielding 38 w% lipid content of BSFL.

Simulation and techno-economic analysis show, that with the applied process routes, inputs and production rates, the jet fuel production will be profitable only with large fresh biomass input and applied pretreatment. Anotherwise, the process will not be profitable due to high operational costs. Therefore, further research and optimisation studies are needed to evaluate the suitability of *S. dolichostachya* feedstock and sugars-to-lipids conversion method to biorefinery and liquid fuel production more accurately and in an aim to develop a robust biorefinery process.

Nomenclature

Symbol	**Description**	**Unit**
Latin symbols		
A	absorbance	
A	area	m^2, ha
a	conversion rate	%
C	Celsius	
C	concentration	mol/L, ppm
g	gram	
h	hour	
ha	hectar	
Hz	Hertz	
i	iso	
K	extinction coefficient	min^{-1}
K	Kelvin	
k	conversion factor	
k	reaction rate constant	$kmol/m^3s$
L	liter	
MT, t	metric ton	
M	million	
M	molar	
m	mass	g, t
max	maximum	
min	minimum	
min	minute	
mol	mole	
n	amount of substance	mol
n	normal	
n/a	not available	
P	pressure	Pa
Pa	Pascal	
ppm	parts per million	

Symbol	Description	Unit
R	recovery factor	
r	rate of reaction	
rpm	revolutions per minute	
s	second	
T	temperature	°C, K
t	time	s, min, h, yr
V	volume	m^3, L
v%	volume percentage	
W	Watt	
w%	weight percentage	
x	molar fraction	
yr	year	
Greek symbols		
ρ	density	g/L
Σ	sum	
σ	standard deviation	
Φ	fugacity coefficient	
Chemical formulas		
Al_2O_3	aluminium oxide	
$Ba(OH)_2$	barium hydroxide	
C	carbon	
Ca	calsium	
C_3H_8	propane	
$C_6H_{10}O_5$	cellulose	
C_2H_5OH	ethanol	
CO	carbon-monoxide	
CO_2	carbon-dioxide	
COOH	carboxyl group	
H	hydrogen	
H_2SO_4	suphuric acid	
K	potassium	
Mo	molybdeum	
N	nitrogen	
Na	sodium	
NaCl	sodium chloride	
NaOH	sodium hydroxide	
Ni	nickel	
NO_x	nitric-oxide	
O	oxygen	
OH	hydroxyl group	

Symbol	**Description**	**Unit**
Pd	palladium	
S	sulphur	
Si	silicon	
Ti	titanium	
Acronyms		
ABE	Acid Activated Adsorbent	
ASTM	ASTM International	
BSFL	Black Soldier Fly Larvae	
CAPEX	Capital Expenditures	\$, USD
CF	Chicken-Feed	
DM	Dry Matter	
DW	Dry Weight	
EFB	Empty Fruit Bunch	
EU	European Union	
FA	Fatty Acid	
FFA	Free Fatty Acid	
FP	Freezing Point	°C, K
FPU	Filter Paper Unit	2 mg mL^{-1}
FW	Fresh Weight	
GHG	Green House Gas	
HCA	Hydroxycinnamic Acid	
HDO	Hydrodeoxygenation	
HEFA	Hydroprocesses Esters and Fatty Acids	
HI	Hydrocracked and Isomerised	
HPLC	High Performance Liquid Chromatography	
HVO	Hydrotreated Vegetable Oil	
LC	Labour Cost	\$, USD
LHSP	Liquid Hourly Space Velociry	h^{-1}
MW	Molar Weight	g/mol
NBE	Neutral Absorbent	
NPV	Net Present Value	
OPEX	Operational Costs	\$, USD
PA	Phenolic Acid	
PFR	Plug Flor Reactor	
PBR	Packed Bed Reactor	
PUFA	Polyunsaturated Fatty Acid	
ROI	Return On Investment	%
SF	Severity Factor	
SFA	Saturated Fatty Acids	
SPD	SuperPro Designer	

Symbol	**Description**	**Unit**
SPK	Synthetic Paraffinic Kerosene	
UFA	Unsaturated Fatty Acid	
UV	Ultraviolet	
WGS	Water Gas Shift	
SI-Prefix		
nano	10^{-9}	n
micro	10^{-6}	µ
milli	10^{-3}	m
centi	10^{-2}	c
kilo	10^{3}	k
mega	10^{6}	M

Contents

Preface

This master's thesis is made at Aalborg University Esbjerg. The report and additive documents have been produced in the period from September 2019 to May 2020.

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Chapter 1

Introduction

1.1 Purpose and relevance of study

The global awareness of the effects of greenhouse gas (GHG) emissions, usage of fossil fuels and the limited oil reservoirs have increased the pressure towards new technologies and sustainable energy sources. International goals and political agreements are set to decrease these emissions and increase the exploitation of carbon dioxide (CO_2) reduced fuels [1, 2]. In line with The Paris Agreement [1], which obligates countries to reduce CO_2 emissions in an aim to limit the increase of the global temperature to 1.5 °C, in 2020 The European Parliament has set an ambitious goal called "European Green Deal" to make Europe carbon-neutral by 2050 [2] and the agreement involves all sectors of society and economy, including energy policies, transportation and agriculture. This a continuum for the 2019 resolution, which sets and objective of achieving net-zero GHG emissions by 2050 [3]. In 2020, The European Commission proposed the first European Climate Law [4], which legally binds the member countries of The European Union (EU) to work towards 2050 goals by cutting GHG emissions, protecting the natural environment and investing in sustainable technologies.

Figure 1.1: Sustainable Development Goals for 2030 set by the United Nations [5]

In 2015, United Nations defined their Sustainable Development Goals (Fig. 1.1) [5], to target the challenges the world's ecosystems and civilization are currently facing, and this project focuses on number 7, 12 and 13 with corresponding statements being *Affordable and Clean Energy*, *Responsible Consumption and Production* and *Climate Action*, respectively.

1.1.1 Transportation emissions

The largest amount of CO_2 emissions are caused by the usage of fossil fuels, either in energy production or transportation, and agriculture [6]. According to EU statistics [6], the whole transportation section, including international aviation, covers more than 25 w% of the total emission in EU in 2017. In the same year, 3 w% of the total GHG emissions is sourced from aviation (2 w% in global) [6]. Some improvements are done in the light transportation sector, as the number of electric and electric hybrid cars is increasing [7]. The challenge lays in the heavy transportation sector, as European Environment Agency shows in their statistics [8], out of all transportation emission, 13.3 w% is sourced from aviation 13.9 w% from maritime and approximately 19.2 w% from heavy-duty vehicles. Therefore, almost half of the total emissions from transportation comes from heavy fuel, such as kerosene, heavy fuel oil and diesel. The emissions from aviation are especially problematic, as the water vapour (contrails) and nitric-oxide (NO_x) emissions released in upper atmosphere increase the global warming effect [9]. Therefore, airlines and other transportation companies all around the world have to take actions to decrease the CO_2 emissions of their operations. The report of The International Air Transport Association [10] states, that the aviation industry answers the challenge by carbon offsetting, updating fleets and investing in new technologies.

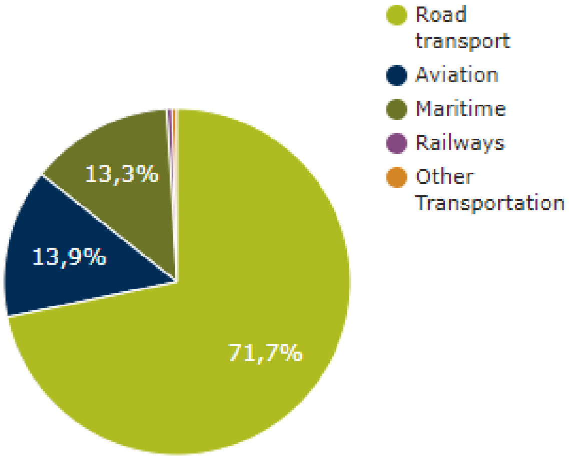


Figure 1.2: EU (Convention) - Share of transport greenhouse gas emissions 2017 [8]

1.1.2 Biofuels and biorefinery

One key implementation towards the climate goals is to increase the share of biofuels in transportation and energy production [2, 3]. This is a challenge, as in 2018, only 8.1 w% of the fuels used in transportation in EU was covered by biofuels, the leading countries being Sweden (32.1 w%), Finland (18.8 w%) and Austria (9.7 w%) [8]. Regulations and support systems, such as tax reduction for biofuels, are created in EU to increase the usage of sustainable energy sources and reduce the environmental impact of transportation sector [3]. In The United States, the share of biofuels in transportation sector is 7.1 w%, but a national goal was set in 2007 to substitute 20 w% of the fossil transportation fuels with sustainable alternatives by 2022 [11, 12]. The major liquid biofuels in the market are bioethanol and biodiesel, covering up to 90 w% of the total production [13]. In aviation, the global share of sustainable aviation fuels is less than 1 w% [14], hence International Energy Agency [15] has set a sustainable development goal to increase the share to 10 w% until 2030 and 20 w% until 2040. To reach the goals, the production capacity of sustainable aviation fuels has to be increased all over the world, as the current production is mainly centered to EU, and the capacity covers only 4 w% of the fossil kerosene demand in EU [14].

In biorefinery, a biomass can be processed in a sustainable way to produce bio-based fuel, chemicals and other valuable products, such as food ingredients and pharmaceuticals [16]. Improving biorefinery and integrating it as a part of circular economies are some of the main objectives of the bioenergy department of International Energy Agency [16]. Producing only fuel is rarely profitable, hence value-added products increase the process feasibility, and these multi-product systems are seen to be the most sustainable and robust options for the future [17]. The biofuels from the biorefineries can be classified to four groups, depending on the initial feedstock used. Even if the production of biofuels is still expensive, the costs are expected to come down though advanced technology [18].

Biomass used for 1st generation biofuels are conventional food crops high in starch or sugar, such as corn or sugarcane, or commonly used oil crops, such as rapeseed [19, 20]. Utilising these edible plants for fuel purposes has not seen as a sustainable option, as the strong competition with food resources creates an ethic problem (food vs. fuel) [19]. Cultivation of conventional crops also require fresh water irrigation, arable land area and farming investments [19, 20]. Nevertheless, the majority of biofuel productions worldwide is from 1st generation feedstocks, and the total annual production is more than 50 billion liters [18, 21]. To lower the amount of food-based biofuels, EU has also set limitations for using 1st biofuels and has committed to gradually decrease the limit until zero [22].

The group of 2nd generation biofuels are produced from municipality waste, waste on inedible fats or lignocellulosic biomass, such as straw, grasses, agricultural residue and wood [18]. Lignocellulosic biomass is cheap and abundant, and consist of three main parts: cellulose (35-50 w%), hemicellulose (20-35 w%) and lignin (10-25 w%) [23, 24]. The exact composition depends on the raw material. Breaking the strong structure of lignocellulose requires more processing than starch [23]. Therefore, the production of biofuel from lignocellulose has not shown to be cost effective yet, due to high operation costs and technical barriers [21]. However, some feedstocks have shown a potential for biorefinery and liquid fuel production [20]. Nowadays, most of the 2nd generation biofuels available in the market are usually produced from animal fats and used cooking oil [17].

The feedstock for 3rd generation biofuels is aquatic algae biomass, but the large-scale production of biofuels from aquatic biomass has still many challenges and limitations to overcome [20]. The 4th generation biofuels are still in the stage of early developments, as their production require advanced technologies like genetic modification of micro-organisms [20].

1.1.3 Soil salinisation

A great global issue is the increasing area of salt-affected soils around the world, which can be caused by natural processes, but also human activity, such as agriculture, problems with water management systems and deforestation [25]. Soil salinisation has reported to be major reason for agriculture land degradation [26].

Figure 1.3: Salt affected soils in Iran (left), China and Thailand [27]

Food and Agriculture Organization of the United Nations states in 2015 report, that 1 billion hectares (ha) of the world's surface is sodic or salt affected (salt concentration greater than 500 mg/L) soils [28]. The area increases continuously due to unsustainable agricultural actions and land use [29], as over-irrigation with fresh water increases soil salinity and irrigation water is also taken from limited and vitally important fresh water reservoirs [30]. According to The United States Department of Agriculture estimation, 10 million ha of land suitable for farming, is lost every year worldwide [30]. Only in Europe, approximately 6.7 million ha of soils are affected by salinisation and 72 million ha are considered sodic [27]. Most of the common crops are salt-sensitive and cannot tolerate the soil salinity, therefore these marginal land areas can no longer be utilised for traditional farming [25]. This causes economical losses and tightens the situation to meet the demand of food, water and energy to world's increasing population. In this situation, it is necessary to create new ways to produce food and energy.

1.1.4 Underutilised halophytes

Halophytes are plants growing in marsh lands, seashores and saline deserts, and the domestication of these naturally salt-tolerant plants is one of the main attempts to overcome previously mentioned issues [30]. Some plants can tolerate salinity enough to be fully irrigated with sea water [31]. *Salicornia* species and other halophytes have been used by humans for centuries: previously plants were gathered from marshes and deserts for medicinal herbs, but nowadays they are sold with high price in market for a gourmet vegetable and nutrient supplement [30, 32]. For many succulent halophytes, the harvesting period for food is short due to lignification, as shown in Fig. 1.4 [26]. *Salicornia herbacea* and *Salicornia bigelovii* have also shown to be suitable for animal feed for sheep, cattle and goats in small portions, depending on the salt content of the plant [30, 33]. Halophyte plants have high nutrient value, as they content a high concentrations of antioxidants and other health beneficial compounds [33]. Belal et al. [34] studied the replacement of fish meal with halophyte-based meal in feeds of Nile tilapia (*Oreochromis niloticus*), and show that up 40 w% of the traditional fish meal can be replaced with *Salicornia bigelovii* without affecting the growth or composition of fish.

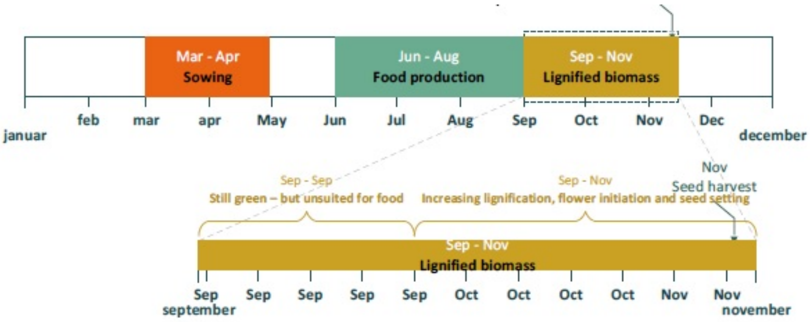


Figure 1.4: Cultivation phases of annual halophyte *Salicornia dolichostachya* [26]

Besides salt, some halophyte plants can tolerate other toxic compounds, for example, heavy metals, which make them ideal for bioremedation of metal polluted soils [35, 36]. Halophytes can also be used for biofiltering as a part of aquaculture systems [26, 30, 37] and the study of Shpigel et al. [37] shows, that *Salicornia persica* can be used as a biofilter for the effluents from fish ponds, removing up to 61.4 w% of total dissolved nitrogen, when the plantation is run with low nitrogen load. Also, the market price of *S. persica* improves the process profitability [37]. Besides cultivation for food, halophytes have shown potential for production of valuable chemicals, and the review article by Cybulska et al. [38] shows, that various compounds used in pharmaceutics, cosmetics and food industry can be found from halophytes native to the Arabian peninsula. Some research has also been done on the biofuel potential of halophytes [13, 39, 40, 41]. For these reasons, these plants can be seen as a great feedstock for biorefinery [26]. Despite their potential, halophyte plants are still very underutilised, even if they could play the key role in the upcoming change.

1.2 Problem statement

This study focuses on the biochemical characterisation and the biorefinery simulation of *Salicornia dolichostachya*, which is an annual halophyte native to seasidea and marsh land areas in Northern Europe. The characterisation is done based on laboratory experiments and analysis. The process simulation includes green fractionation, the production of value-added compounds, biochemical surgars-to-lipid converion, production of sustainable aviation fuel and techno-economical assessment. The problem statement can be written as:

Is Salicornia dolichostachya a suitable feedstock for biorefinery and biofuel production?

Chapter 2

Literature review

2.1 Properties of halophytes and *Salicornia* species

Only approximately 1 % of known plant species belong to halophytes [25]. As a result of evolutionary adaptation to the environment, these annual or perennial plants can survive and grow under the salinity conditions, which would be toxic for most of the plants [42]. Because of the salt-tolerant properties, halophyte plants have increased the interest as a source of food and biomass, but also as a possible platform for saline water agricultural systems and biofuel production [25, 13, 40]. The *Salicornia* species are flowering green succulent halophytes, that can be found all over the world (Tab. 2.1), excluding Australia [43]. Some of the species can be found only in relatively small regions, but some are widely spread in larger areas [44, 45].

Species	Origin	Cite
S. europaea	Eurasia	[45]
S. bigelovii	North America, Caribbean	[46]
S. neei	South America	[47]
S. ramosissima	France, Iberian Peninsula	[32]
S. sinus-persica	Arabian peninsula	[48]
S. herbacea	East Asia, North Africa	[49]
S. persica	Iran, East Mediterranean	[49]
S. virginica	The United States, Canada	[50]
S. brachiata	India, Bangladesh, Sri Lanka	[40]
S. dolichostachya	Northern Europe	[44]

Table 2.1: *Salicornia* species around the world

2.1.1 Salt-tolerance

Halophytes are naturally found in the seashores, marsh areas and salted deserts around the world [30]. *Salicornia* spp. and other very salt tolerant plant species are osmoconformers, meaning that plants can maintain a high osmotic pressure in their shoots, and they also accumulate salts to their tissues [49, 50]. According to the study of Lv et al. [51] the optimal soil salt concentration of 200-400 nM sodium chloride (NaCl) enhances the growth and photosynthesis activity of *S. europaea*, but the plant can also tolerate more than 1000 mM NaCl, which makes it one of the most salt tolerant plant species in the world. Similarly, Souza et al. [47] cultivate *S. neei* under different salinities and show, that the plant can be cultivated in high salinity conditions (513-769 mM NaCl) without affecting the growth and succulence. Katschnig et al. [43] studied the salt tolerance of *S. dolichostachya* and shows, that salt is actually required for optimal growth, and the ideal environment has the salinity of 300 mM NaCl, based on the amount of succulent shoots and stem diameter. Huiskes et al. [44] studied the growth of *S. dolichostachya* under seawater cultivation, an show that the species is fully adapted to flooding conditions and the regular flood with 250 mM NaCl water enhances the growth, especially when cultivating in clay soil. The research by Glenn et al. [49] states, that *S. bigelovii*, alongside some other halophyte plants, can yield as much biomass as as conventional crops (mean 18 t/ha) even under a full seawater irrigation, and soil salinity in cultivation can be up to 1.3 M NaCl. Yields are based on ecological studies, as there is relatively few field trials of agricultural activity in salt-affected land areas [49].

Figure 2.1: Left: The phenotype of *S. dolichostachya* differs significantly depending on the environment, all shrubs harvested from Mandø, Denmark 26th Sep. 2019. Right: *S. brachiata* shoot cross section [52]. P: palisade, SM: spongy mesophyll, V: vascular tissue.

2.1.2 *Salicornia* spp. as a source of food and biorefinery feedstock

Fresh and salty green shoots of *Salicornia* spp. succulent can be used as a gourmet vegetable and a nutrient supply, due to the high concentration of nutrients, such as minerals, healthy fatty acids and amino acids [31, 53, 26]. Food production of *Salicornia* is a highly seasonal [53] and as the plant matures, it starts to produce seeds and get lignified, making shrubs more woody and unsuitable for food [26].

The lignocellulosic fibre fraction of *Salicornia* is often seen as a waste, as it cannot be used for direct combustion due to high salt content, and it is shown to be suitable for animal feed only in small portions [26, 30, 34]. Salt content can be seen as a problem especially in the case of dicotyledonous halophytes, including *S. dolichostachya*, which accumulate more salt in shoot tissues, compared to monocotyledon halophytes, especially grasses [49]. However, the fibre fraction has a potential to be valorized to biofuels and several value-added products [26]. The composition of lignocellulosic fraction of halophyte plats differs from typical lignocellulosic biomass, being 26-37 % cellulose, 24-38 % hemicellulose and only 2–10 % lignin [41, 46]. Lignin content of halophytes is relatively low compared to most agricultural residues, such as wheat straw (19.8 %), sugarcane bagasse (28.4 %) and rice straw (20.2 %) [54], which makes the processing of halophytes easier compared to other 2nd generation feedstocks. Therefore, *S. dolichostachya* and other *Salicornia* species are an unique kind of raw material for biorefinery.

Figure 2.2: Different stages of lignification: the fresh shrub (left) is bright green and the most mature shrub has already produced seeds

The total lipid content of halophyte plants is low, and for succulent halophytes, the lipid content can be only 1.0-1.8 % [13]. Essaide et al. [55] determined the lipid content of *S. herbacea* being 1.7 g/100 gDM. However, some *Salicornia* species, such as *S. brachiata* and *S. bigelovii* are producing seeds containing up to 22.5 % of lipids, hence those plants can also be seen as an oil crops and a potential feedstock for liquid biofuels [40, 31, 13]. Using *Salicornia* spp. oil for biofuel purposes does not compete with food resources, as the high content of saponins makes the oil unsuitable for direct food purposes [40]. In *S. bigelovii* biomass, oil seeds can cover up to 11 % of the total weight of the shrub [56] and according to Glenn et al. [49], the seeds contain 28 % oil and 31 % protein, which is similar to the seed quality of soybean. The seeds of *Salicornia* plants are small, as the diameter can be less than 1.4 mm [45], which makes the seed separation a challenging process. However, the separation of seeds and lipids may be profitable, if the extracted oil consist of high fractions of desirable fatty acids or lipids soluble carotenoids. Lu et al. [53] show, that *S. bigelovii* oil has a high amount of healthy polyunsaturated fatty acids. Patel et al. [13] show, that the oil from *S. brachiata* contains 29.1 % of ω-3 fatty acids and 25.5 % ω-6 fatty acids. Maciel et al. [57] show even higher share of these fatty acids in the total lipids of *S. ramosissima*, being 40.4 % and 20.4 % of ω-3 and ω-6 fatty acids, respectively. Fatty acids in ω-3 family can also be found from *S. sinus-persica* [26].

Species	Type	Lipid [%]	Protein [%]	Fibres [%]	Ash [%]	Ref.
S. sinus-persica	Fresh pulp	n/a	13.0	37.4*	13.2	[48]
S. sinus-persica	Juice	4.6	7.7	11.0*	61.1	[48]
S. herbacea	Whole plant	1.7	22.1	36.2	8.1	[55]
S. brachiata	Stems and tips	n/a	10.0	55.0	12.2	[58]
S. bigelovii	Fresh	3.2	13.3	46.0	37.7	[53]
S. bigelovii	Fresh, seedless	n/a	n/a	63.5	5.0	[56]
S. bigelovii	Dry, seedless	n/a	n/a	36.9	43.1	[39]
S. bigelovii	Washed, seedless	n/a	n/a	66.3	10.0	[39]
S. bigelovii	Pods, seedless	n/a	n/a	~32	~31	[46]
S. bigelovii	Stems, seedless	n/a	n/a	~61	~24	[46]
S. ramosissima	Stems and tips	0.4	5.2	n/a	n/a	[32, 59]

Table 2.2: Content of lipids, protein, lignocellulosic fibres and ash in different types of *Salicornia* biomass dry matter. *) total sugar content excluding lignin.

2.1.3 Phytochemicals in *Salicornia* spp.

Salicornia spp. are used as a medicinal herb and folk medicine for centuries due to the various health benefits, which is now known to include antioxidant, anti-inflammatory, diabetes preventing and even cytotoxic effects [32, 60]. Halophytes are producing these bioactive compounds and secondary metabolites potentially due to their adaptation to harsh environmental conditions [38]. Phytochemicals are increasingly gaining interest in the field of nutritional supplements, pharmaceuticals and medicine, where extracted plant compounds, such as hydroxycinnamic acids (HCA), phenolic acids (PA), flavonoids and their respective derivatives show beneficial effects in *in vivo* and *in vitro* studies [61, 62, 63, 64, 65]. An article by Salomone et al. [66] shows high phenolic acid intake with lower prevalence of insulin resistance and non-alcoholic fatty liver disease. The beneficial hepatoprotective properties are backed up by study of Kumar and Goel [67] showing the many health properties of the plant phenolics, such as anti-inflammatory, anticancer, antimicrobial, antiallergic, antiviral, antithrombotic and hepatoprotective properties.

In a recent *in vivo* study by Karthivashan et al. [61] the ethanolic extracted, isolated non-toxic phytochemical acanthoside B of *S. europaea* shows anti-neuroinflammatory properties in mice, with properties for further developed into a potential drug for treating neurodegenerative conditions such as Alzheimer's disease. Similarly, the research by Kim et al. [68] studies the anti-neuroinflammatory effect of *S. europaea* targeting the changes typical to Parkinson's disease, and both *in vitro* and *in vivo* studies demonstrate, that compounds find in botanic extract could be potentially used as a therapeutic agent in the future. A study by Ferreira et al. [60] states, that the ethanol extract of *S. ramosissima* has considerable effects to mice metabolism, liver and kidneys. According to the review by Cybulska et al. [38] about phytochemicals find in halophytes, *S. europeae* contains phenolic compounds shown to work as anti-obesity agents, preventing diabetes in rats and having an anticancer effect in mice. *S. europaea* also contains flavonoids with anti-inflammatory effects, which are suspected also to cause the antimicrobial effect of botanical extract [38]. Kim et al. [69] studies the seasonal variation in the concentration of flavonols and phenylpropanoic acids in *S. herbacea* and show, that the contents are relatively higher when plant in the mature stage and harvested during August and September in Northern Hemispehere.

Shoots of *Salicornia* spp. are shown to be rich in bioactive compounds, and the study by Souza et al. [47] compares the concentrations of secondary metabolites and antioxidant activity of *S. neei* cultivated under different salinities. The study shows biomass being rich in various pigments that have shown being beneficial for health: chlorophyll (described in section 2.2.3), β-carotene and xantophylls, such as lutein, zeaxanthin, neoxanthin and violaxanthin [47]. Also, concentration of phenolic compounds and flavonoids are reported, but the types of compounds are not specified. The content of phenolic compounds are not affected by salinity, increasing salinity has a positive effect to flavonoid content, and content of syringic acid and vanillin are negatively affected by increasing salinity [47].

The phytochemicals found in *Salicornia* spp. are divided into different chemical classes in Tab. 2.3. These classes depend on the structure of the molecule [70, 26]. The research of phytochemicals in *Salicornia* spp. is inadequate, but a large variety of HCAs and phenolic acids have been shown to exist in these halophtye plants, and some *Salicornia* species might share a common composition of HCAs and phenolic acids.

Compounds	S.eur [71]	S.ram [32]	S.s-p [26]	S.her [55]	S.big [26]	S.nee [47]
PA						
Benzoic acid			X			
Gallic acid	X		X			X
M-salicylic acid				X	X	
Protocatechuic acid	X		X		X	X
Quinic acid	X		X		X	
HCA						
Caffeic acid	X		X	X		X
Cinnamic acid				X		
Dihydrocaffeic acid			X			
Ferulic acid			X	X	X	X
p-Coumaric acid	X		X			
Sinapic acid				X		
Esters						
Bornyl acetate			X		X	
Chlorogenic acid	X		X	X		X
Rosmarinic acid	X					
Flavonoids						
Acacetin				X		
Galangin				X		
Hesperetin	X			X		
Isorhamnetin				X		
Kaempferol				X		
Myricetin				X		
Rhamnetin	X			X		
Rutin	X					
Quercetin				X		X
Other						
Anthraquinone			X		X	
Azelaic acid		X	X			
Carvonic acid			X			
Eucalyptol			X			
Humulene			X		X	
Malic acid	X	X				
Neoxanthin					X	X
O-cymene			X			
Syringic acid			X	X		X
Thymol			X		X	
Vanillic acid					X	
Vanillin		X	X			X

Table 2.3: Detected phytochemicals of relevance. Chemical classes are made as described by Morales-Gonzalez [70]. PA: phenolic acids, HCA: hydrozycinnamic acids. S.eur: *S. europaea*, S.ram: *S. ramosissima*, S.s-p:*S. sinus-persica*, S.her: *S. herbacea*, S.big: *S. bigelovii*, S.nee: *S. neei*.

2.2 Fractionation and characterisation methods

In green fractionation, the biomass is divided to liquid (juice) and solid (pulp) fractions. After fractionation, both fractions are considered separately to create a detailed characterisation of the biomass and its fractions, as shown in Fig. 2.3. The aim of characterisation is to get a detailed information about the used feedstock and its biochemical composition. The contents of ash, carbohydrates, lignin, lipids and protein can be determined by laboratory analysis methods.

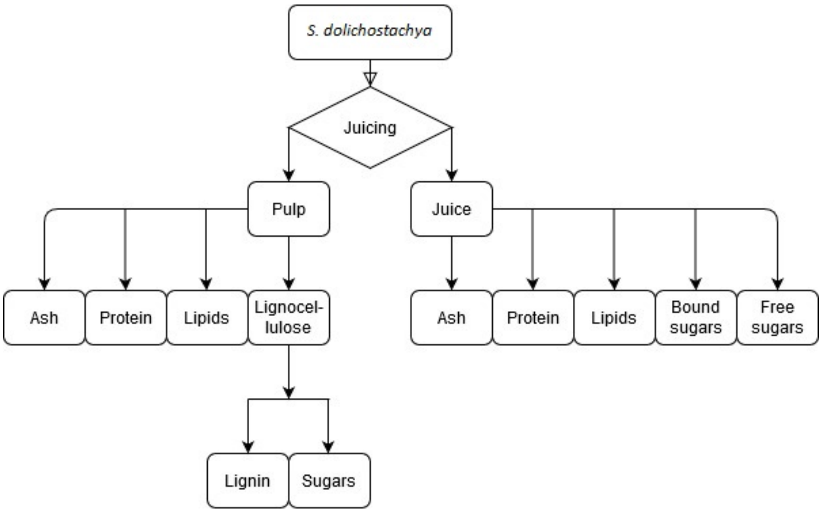


Figure 2.3: Overall fractions of *S. dolichostachya*

Research by Alassali et al. [48] uses green fractionation method in *S. sinus-persica* biomass characterisation, and the green fractionation is done with single auger juicer, resulting 67.8 % liquid from total initial biomass. Damborg et al. [72] use are twin auger screw press in wet fractionation of different green forages, resulting 57.4 - 70.6 % juice and 21.1 - 32.8 % pulp, depending on plant species. In both studies, the biomass fractionation is done directly without previous chopping or milling.

He et al. [54] introduce a different fractionation method for waste grains from breweries, where the biomass is milled first and then mixed with de-ionised water to make a suspension, which is incubated at 60 °C for four hours in the present of different reagents. This kind of method is not desired in the case of halophytes, as it requires addition of water and one of the main benefits of halophyte agriculture is to decrease the amount of used fresh water and therefore, improve the water management [30].

Figure 2.4: Juice (left) and pulp fractions of 26th Sep. 2019 picked *S. dolichostachya* biomass after green fractionation using single auger juicer

2.2.1 Lipid extraction from pulp

The lipid compounds of biomass are commonly extracted by Soxhlet extraction using n-hexane. Other known solvents used in lipid extraction with a Soxhlet apparatus are chloroform and the mixtures of chloroform/methanol, chloroform/ethanol, n-hexane/methanol and n-hexane/ethanol. Some of these mixtures have shown higher lipid extraction rates from microalgae biomass [73, 74]. Besides Soxhlet extraction, oils can be also obtained by using a solvent bath combined with pretreatment methods, such as ultrasonication. For example, Paisal et al. [75] extracted algal oil from *Chlorella* sp. by using 300 W ultrasonic bath at 42 kHz with 1:2 V/V mixture of methanol and n-hexane.

The argument for using pure non-polar compounds as n-hexane is, that pure n-hexane will only extract the non-polar compounds, such as lipids and some pigments, while more polar compounds extracted by methanol or ethanol are immiscible in n-hexane, hence two solvents in the mixture are needed to be separated, requiring post-processing of the extract. This separation could be done by evaporating the solvents, wash the products with n-hexane, and then separate the polar and non-polar phases.

Soxhlet extraction

The Soxhlet apparatus is a piece of laboratory glassware often used for biomass sample preparation. This is done by extraction of targeted molecules into the solvent, after which the solvent in the solution can be evaporated off, to yield concentration of the extracted molecules. The general Soxhlet extraction is split into a sequence of actions, as described by Luque de Castro and Priego-Capote [76]:

1. A sample is placed in a cellulose thimble. The thimble is closed with cotton, and put inside the extractor.
2. A distillation flask of at least two times the volume of the extractor, commonly a round bottom flask, is filled with solvent 1.5 times the volume of the extractor and a few boiling stones

3. The extractor, condenser and distillation flask are put together, and the extraction starts when heat is applied to the distillation flask to boil the solvent. Cold water is circulating in the condenser.
4. The evaporated solvent condenses in the condenser, and drips into the extractor and the thimble.
5. When the level of the solution containing solvent and extracted molecules reach the overflow limit, the entire amount of solution in the extractor siphons into the distillation flask. Cycle is completed and starts over.

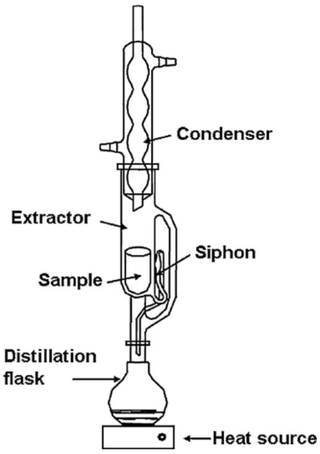


Figure 2.5: Schematic of conventional Soxhlet apparatus [76].

The sample to be extracted is constantly brought into contact with concentrated solvent, which enables the operator of the extraction to extract a large amount of the molecules soluble in the solvent, by only using a small amount of solvent, compared to a solvent bath extraction. This is due to the Soxhlet extractions ability to shift the transfer equilibrium [76]. To determine the amount of lipids and other extracted compounds after Soxhlet extraction, the mass of the dried thimble, including the dried biomass, is weighed before and after extraction, and the mass difference will be the amount of extracted compounds.

2.2.2 Liquid-liquid lipid extraction

In a study by Kech et al. [77] the process of liquid-liquid lipid extraction from wet urban sewage sludge is investigated. The method used for the extraction is the Bligh-Dyer method [78], using a mix of chloroform, methanol and water. When mixed in the right ratio, a monophasic solution is formed to improve the contact between the lipids and the organic solvent. Adding more water will then split the liquid into a biphasic solution, with chloroform containing the lipids. As seen on Fig. 2.6, the water content is required to relatively low, as a high water content will result in a biphasic solution, not mixing the lipids with chloroform.

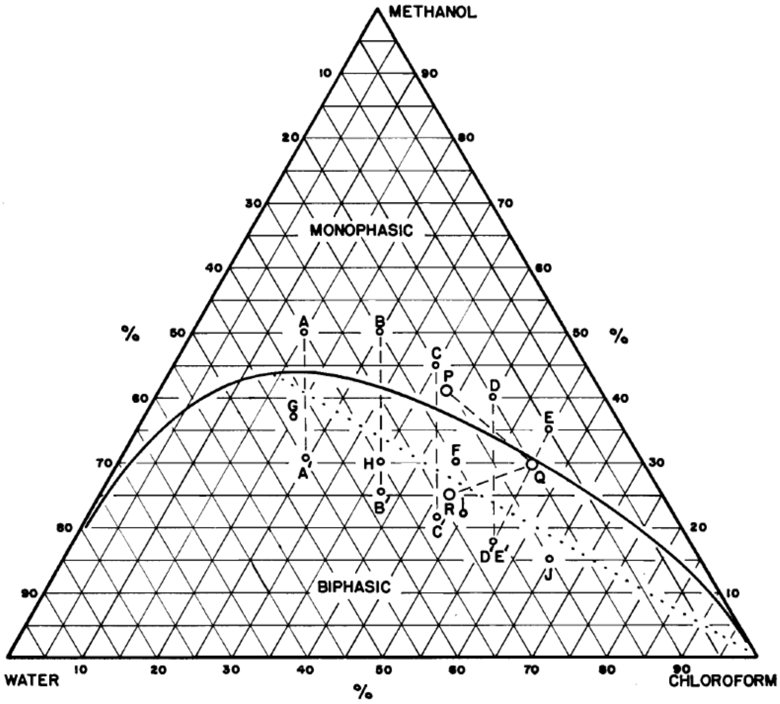


Figure 2.6: Points indicate measurements done by Bligh and Dyer, to determine the mixing ratio of chemicals to yield monophasic and biphasic conditions [78].

A study by Smedes [79] investigates alternative chemicals to replace chloroform, due to its toxicity and safety issues. A mixture of isopropanol, cyclohexane and water was proposed by Smedes, and is also shown to be more effective by Kech et al. [77].

As described by Kech et al. the lipid content of the lipid-containing liquid has to be lower than 5 %, as high lipid concentrations in the liquid may affect the nature of the organic solvents used [77]. The sludge used for the liquid-liquid extraction by Kech et al, using the Smedes mixture, had a mean water content of 92.7 % and 95.5 %, and not as high a salt content as *S. dolichostachya* juice [77, 78].

2.2.3 Chlorophyll concentration

Chlorophylls are the pigments of photosynthesis and responsible of the green colour of plants. Two types of chlorophyll are present in the green biomass: chlorophyll *a* and chlorophyll *b*. [80] These pigments are used as a natural colorant (E140) in food industry, cosmetic products, inks, resins and soaps [81]. Tavanandi et al. [82] states, that chlorophylls can also be used as a photo-dynamic agents in a cancer therapy. Therefore, chlorophyll can be seen as of the potential value-added products in biorefinery. Lu et al. [53]

studies the nutritional value of *S. bigelovii* and show the total amount of chlorophyll in biomass to be 569 mg/kgFW (fresh weight). In the research by Kaviani et al. [36], the effect of heavy metal concentration of soil to the pigment content of subspecies of *S. persica* is assessed, and the control samples show the chlorophyll concentration in the acetone extract being approximately 0.5 mg/mL. Souza et al. [47] studies the pigment content of *S. neei* under different cultivation conditions, and show the total chlorophyll content up to 293 µg/gDM and the chloprohyll concentration being negatively affected by increasing salinity, but the ratio of chlorophyll *a* and *b* remains constant.

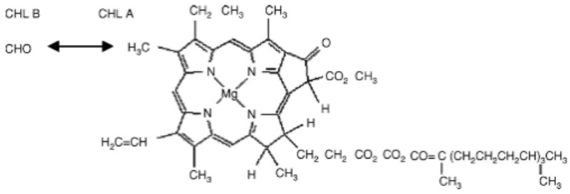


Figure 2.7: Chemical structure of chlorophyll *a* and *b* [81]

Chlorophyll can be separated from the biomass by extraction. Studies agree, that the best yield can be achieved by using ethanol, when compared to another solvents, such as acetone, methanol, dimethyl sulphoxide or NN dimethyl formamide [81, 82, 83]. Chlorophyll can be isolated by using chromatography methods, but the process is challenging, as the chlorophyll molecules are very sensitive for different kind of chemical transformations [80]. Hence, in this project, the main focus in on quantification the amount of chlorophyll in the extract and *S. dolichostachya* pulp.

The determination of the chlorophyll concentration can be done by using spectroscopy, where the absorbance is measured by using different wavelengths. Porra et al. [84] determined specific wavelengths and calculation coefficients for different solvents, and their work is widely cited in related studies [80, 82]. Tavanandi et al. [82] studied the chlorophyll isoaltion from microalgae, and uses Eq. 2.1 for chlorophyll concentration in ethanol solution, where A is absorbance in specific wavelength, *Chla* is chlorophyll *a* and *Chlb* is chlorophyll *b*.

$$C\ [\mu g/mL] = Chla + Chlb = (13.36 \cdot A_{664} - 5.19 \cdot A_{649}) + (27.43 \cdot A_{649} - 8.12 \cdot A_{664}) \quad (2.1)$$

2.2.4 Protein content and total nitrogen determination

Protein is considered one of the main value-added compounds in the biorefinery, as it has a good selling price in food and feed industry, which helps to create robust process and reduce the production costs of medium value products, like biofuels [54, 85]. Therefore, the accurate determination of the protein content is important for reliable biomass balance calculations. Mar Contreras et al. [86] review an extraction methods of proteins from lignocellulosic biomass and show that some of the alcohol soluble plant storage proteins can be extracted with organic solvents, such as ethanol and isopropanol, yielding 8-20 % of protein from rice bran and 11 % from soybean feedstock. However, alkaline extraction is more common method for protein extraction, as it usually has a higher yield compared to organic solvents and it is more environmentally sustainable compared to organic solvents [86]. According to He et al. [54], alkaline extraction followed by acid precipitation is the traditional method for protein isolation, but it has not been applied in industrial scale due to high amount of required alkaline and low cost efficiency. From the green juice, protein can be separated by lactic acid fermentation, where *Lacrobacillus* converts the free sugars to lactic acid, and decreasing pH causes protein precipitation [87].

Analytical methods for direct determination for total protein content are developed, and these methods can be applied, when it is desired to know the feedstock suitability for feed purposes, and the protein does not need to be extracted nor isolated. Spectrophotometric methods can give some relative results, but the process has come uncertainties regarding the solubility of the components and finding the standard protein sample similar to the proteins present in the biomass [85]. Another direct method is hydrochlorid acid hydrolysis and amino acid analysis using high performance liquid chromatography, but the method is expensive and very time consuming, as the hydrolysis time can be up to 48 hours [85]. However, the HPLC gives a detailed information about the different types of proteins present in the biomass.

Widely accepted method is to determine the total amount of nitrogen present in the biomass, and then approximate the nitrogen-to-protein conversion using Eq. 2.2 [88]. These methods are simple and cost-effective compared to spectroscopy or HPLC methods, but they lack the selectivity for specific kind of proteins [85, 88].

$$Protein\ [\%] = k \cdot \%N \tag{2.2}$$

The commonly used k factor 6.25 is based on the average nitrogen content of protein (16 %) and to the assumption that all the nitrogen is bound to protein, but this factor tends to overestimate the amount of protein, as nitrogen can also be found from other compounds, such as chlorophyll [85, 88]. Therefore, finding a right conversion factor may be challenging, especially for new generation feedstocks.

There is two mainly used ways to total nitrogen determination: Dumas combustion method and Kjeldahl wet digestion method. Both of them are more than a century old and still used in the industry, but Kjeldahl is considered more reliable [88], even if the they have shown similar repeatability and reproducibility with optimised systems [89, 90]. Some studies show, that Dumas method gives higher results for total nitrogen content compared to Kjeldahl method [89, 91].

There has been only a small amount of research considering the protein content of halophytes. Barreira et al. [59] studied the nutritional value of different succulent halophytes and show the protein content of *S. ramosissima*, *Sarcocornia perennis* and *Sarcocornia alpini* to be 5.2 g/100 gDM, 6.9 g/100 gDM and 8.1 g/100 gDM, respectively, when using Kjeldahl method and 6.25 conversion factor. The protein content is similar to corn (5-10 %)

which is already commonly used in a feed industry [89]. Alassali et al. [48] determined the protein content of *S. sinus-persica* to be 13.0 g/100 gDM by using Kjeldahl method and 6.25 conversion factor. Lu et al. [53] show similar protein content of 13.3 % for untreated *S. bigelovii* biomass, also determined by Kejldahl method and 6.25 conversion factor.

Kjeldahl method

The principle of Kjeldahl digestion is to transform the organic nitrogen to ammonium nitrogen by boiling in the sulfuric acid [90]. The process has three phases [89]:

1. Digestion: the sample is kept in boiling sulphuric acid (420 °C) for two and a half hours using mercury oxide and potassium sulfate as a reaction catalyst
2. Distillation: the result solution is collected, cooled, diluted with water and sodium hydroxide is added before distillation
3. Titration: gained ammonia solution is titrated against standard hydro-chloric acid, and the endpoint detects the concentration of collected ammonia

It has to be noted, that performing Kjeldahl digestion requires the use of heavy metals and handling of dangerous reagents in high temperatures [89]. It has seen as a reliable way to determine the total nitrogen content, but issues regarding the longer analysis time, safety and disposal of high amounts of hazardous chemical waste are decreased the popularity of the method in industrial applications [89, 90, 91].

Dumas combustion method

Marcó et al. [89] explains Dumas total nitrogen determination consisting of four parts:

1. Combustion in high temperature (800-900 °C) in the present of oxidation catalyst
2. Reduction in the copper column at 700 °C
3. Water and CO_2 traps
4. Separation with gas chromatography and thermal conductivity detection

The sample size < 1 g is weighed to a small tin capsule with the possible catalyst, and the capsule is fed to the system [89]. There is a continuous flow of helium through the process, as it is used as a carrier gas. Oxygen is fed directly to the combustion reactor only in the first phase of the analysis. The first two steps of the method are requiring high temperatures, but the traps and separation are working in less severe environment. The pros of Dumas combustion are short analysis time (only a few minutes), small sample size and environmental sustainability, as the method does not involve the use of dangerous chemicals and the amount of waste is practically insignificant, hence it has replaced the

Kjeldahl method in the industrial use in past years [89, 90, 91]. Also, modern Dumas combustion based technologies define also the concentration of other elements, such as hydrogen, sulphur and carbon.

2.2.5 Carbohydrates and lignin content

Lignocellulosic biomass consist of cellulose ($C_6H_{10}O_5$), hemicellulose and lignin, and it is a feedstock for 2nd generation biofuels. Besides biorefinery to biofuels, lignocellulose is used in forest industry and as a raw material for biomaterials and bio-based chemicals [92]. Cellulose and hemicellulose are polymerised from sugar monomers. Cellulose consists of 6-carbon glucose fibrils, which are bond with strong crystal bonds. Hemicellulose consist from various sugar monomers, mainly from 5-carbon sugars (pentoses) xylose and arabinose, but also 6-carbon glucose, mannose and galactose. Lignin is an organic aromatic compound, which can be processed to phenolics in biorefinery. [92] The analysis of total carbohydrates gives the information about the types of sugars monomers present in the biomass, amount of lignin and the size of the whole lignocellulose fraction of biomass. As most of the lignin is insoluble to acid [93], the total lignin content of the biomass can be approximated by studying this insoluble fraction, also known as Klason lignin.

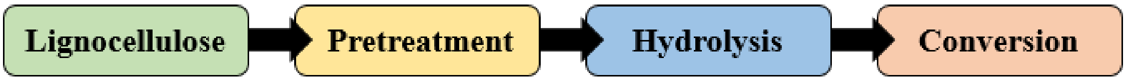


Figure 2.8: Traditional processing chain of lignocellulosic biomass

Hydrolysis

Prior sugar analysis, the sugar monomers need to be released from carbohydrates with saccharification, which can be done with enzymatic or acid hydrolysis [92]. Hydrolysis is also required, if biomass is going to be fermented or fed to micro-organisms [94], as the fibres cannot necessarily be digested with natural enzymes of the organism. Therefore, cellulase together with other type enzymes can be used prior the conversion to hydrolyse the cellulose and hemicellulose [95]. Cellulolytic enzymes are naturally produced by countless fungi and bacteria, and enzymes can be categorized based on the action mechanism, for example [96]:

- Exoglucanase breaks the crystalline cellulose
- Endoglucanase targets the amorphous and soluble forms of cellulose
- β-glucosidase acts on non-reducing ends of sugar molecules and and produces glucose by hydrolysis of cellodextrins and cellobiose. It does not work for crystalline or amorphous cellulose.

Used cellulases are mixtures of different types of cellulolytic enzymes. Lignocellulose is known to be a recalcitrant material, and pretreatments can be used to break the cell wall structure and enhance the sugar yield in hydrolysis [56]. The high enzyme cost and low enzyme activity without pretreatment have been the main obstacles for practical large-scale use of enzymatic hydrolysis [94]. Cellulases are promising to be utilised for industrial applications, but they are not commercialised yet. However, lots of research is going on and prices of enzymes are expected to down as the demand increases. It is approximated, that yearly cellulace market could be worth 400 million USD in thr future. [96]

Acid hydrolysis can be done by using either strong or diluted acid to break the biomass, depending on the type of feedstock. The dilute acid percolation, typically with 1 % sulphuric acid (H_2SO_4), is one of the simplest methods to convert lignocellulose to sugar monomers and up to 70 % glucose yield can be achieved [95]. Pentose sugars present in the hemicellulose fraction are easier to hydrolyse than crystalline cellulose, and the sugar degradation in too high hydrolysis temperatures decreases the sugar yield. Therefore, the dilute acid hydrolysis can be done in two phases, where the first phase operates in milder conditions to hydrolyse pentose sugars, and the conditions of next phase are optimised to separate lignin from recalcitrant cellulose [95]. In strong acid hydrolysis, the hydrogen bonds inside the plant cell walls are broken, and cellulose and hemicellulose are dissolved in concentrated, 72 % H_2SO_4. Then, the following dilution with water in mild temperatures causes rapid hydrolysis with low sugar degradation. [95]

High Performance Liquid Chromatography

High performance liquid chromatography (HPLC) analysis with organic acid or lead column gives the detailed information about the types of sugar monomers present in the solution. After hydrolysis, the this method can be applied to detect the types of sugars present in the biomass. It is one of the most powerful tools in analytical chemistry to separate, qualify and quantify the compounds present in the liquid solution. The samples are pumped with high pressure to the column, which is filled with different particles (stationary phase). The separation is based on the chemical properties of sample compounds and the physical interactions with these particles. Different compounds pass the column with different velocity: compounds with weak interactions pass through faster than the ones with strong interactions. Liquid mobile phase is used as a carrier to transfer the sample compounds through the column to the detector, which identifies the compounds by refractive index, absorbance or mass spectrometry. [97]

Sugars and Klason lignin in halophytes

Zaier et al. [98] studied the nutritional composition of three different *Amaranthaceae* family species native to Mediterranean coast and show, that the amount of dietary fibres increases, as the plant matures and turn from green to red-violet in colour. The biggest increase is in the amount of dietary fibres insoluble in enzymatic hydrolysis, being from 6.9 to 7.6 g/100 gFW for *Arthrocnemum indicum*, 5.9 to 8.0 g/100 gFW for *Halocnemum strobilaceum* and 6.8 to 7.6 g/100 gFW for *Suaeda fruticosa* [98]. Lu et al. [53] study for *S. bigelovii* shows the total carbohydrate content of untreated biomass to be 4.5 g/100 gFW.

A few studies have done considering the types of sugars in *Salicornia* biomass. Cybulska et al. [39] show, that dried *S. bigelovii* biomass grown in United Arab Emirates consist of 9.1 g/100 gDM glucan, 7.7 g/100 gDM xylan, 5.5 g/100 gDM arabinan and 6.8 g/100 gDM Klason lignin. After washing with fresh water, the glucan (26 g/100 gDM) and xylan (22 g/100 gDM) content of washed biomass are relatively high compared to traditional crops [39]. Bañuelo et al. [56] studies the fresh, seedless shoots of *S. bigelovii* grown in Mexico and show a very low Klason lignin content of 1.98 % of DM, cellulose and hemicellulose (holocellulose) covering total of 61.15 % of the washed raw material. Both studies use the acid hydrolysis method to release the sugar monomers from extracive free fibres. Alassali et al. [48] studies the green fractionated *S. sinus-persica* pulp an shows that the fresh, untreated pulp contains 15.6 g/100 gDM glucose, 11.1 g/100 gDM arabinose, and 10.7 g/100 gDM xylose and fructose combined. However, not all the sugars are present in the solid lignocellulose, hence some free sugar monomers can already be detected from the liquid fraction of the biomass, and Alassali et al. [48] show the free sugar content of green juice DM to be 1.0-1.5 %.

2.3 Treatment methods for sugar yield enhancement

After extractions, the remained material is nearly salt-free lignocellulosic fibre fraction of *S. dolichostachya*. The branch structure of hemicellulose breaks easily in hydrolysis, but the crystal structure of cellulose is more recalcitrant, hence pretreatment can be performed to loose the structure and enhance the hydrolysis and make biomass more easily digestible form for microbiological organisms [92].

Studies show, that hydrothermal pretreatment is an efficient way to increase the sugar conversion in and digestibility of cellulose [39, 48, 99, 100, 101, 102, 103]. The study of Cybulska et al. [39] show, that hydrothermal pretreatment can increase the glucan-to-glucose enzymatic convertability of extractive-free lignocellulosic *S. bigelovii* biomass from 26 % to 87–92 % and xylan-to-xylose convertibility from 6 % to 62–100 %. Alassali et al. [48] study the wet fractionation of *S. sinus-persica* and show, that a high cellulose recovery (94.6 %) of pulp can be achieved with a 60 minutes pretreatment at 120 °C and promising results are also gained with a 10 minute pretreatment at 170 °C, which lead high enzymatic convertibility and the ethanol yield up to 76.91 %.

The severity of hydrothermal pretreatment is described with a severity factor (SF), which can be calculated with following Eq. 2.3.

$$SF = log_{10}\left(t \cdot e^{\frac{T-100}{14.75}}\right) \tag{2.3}$$

where t is the reaction time [min] and T is the pretreatment temperature [°C]. The concept of severity factor is first introduced by Overend and Chornet in 1987 [104]. Many studies agree, that digestibility and sugar conversion increases with more severe treatment conditions [100, 103]. However, when high conversion is achieved, the effect of severity decreases [39]. Too severe conditions are also unfavourable, as higher temperatures enable the degradation of pentoses to undesirable products, such as furfural and other toxic compounds, which can inhibit the micro-organisms and therefore disturb the whole biochemical process [39, 100]. Cybulska et al. [39] reported high hemicellulose losses during the pretreatment process, which would advocate for milder pretreatment conditions for *Salicornia* biomass.

Hydrothermal pretreatment is tested for different types of biomass throughout the years, and sugar recoveries achieved for various treated lignocellulosic biomasses are collected to the Tab. 2.4. Saha et al. [99] study corn stover biomass and show, that 72 % total sugar yield can be achieved with a 5 minute pretreatment at 200 °C. Asuraf et al. [100] research the effect of hydrothermal pretreatment to the digestibility of green and woody lignocellulosic biomass and show, that with 10 minute pretreatment at 170 °C, digestibility of 75 % and 65 % can be achieved for Bermuda grass and jasmine, respectively, but < 20 % for woody date palm fronds. However, when the treatment temperature is increased to 200 °C, the digestibility increases to 65 % for date palm fronds and to approximately 80 % for green biomasses.

Biomass	Treatment conditions	SF	Glucose [%]	Xylose [%]	Ref.
S. bigelovii	10 min, 210 °C	4.24	87	30	[39]
S. bigelovii	10 min, 190 °C	3.65	79	52	[39]
S. sinus-persica	60 min, 120 °C	2.37	94.6	~100	[48]
S. sinus-persica	10 min, 150 °C	2.47	77.49	~90	[48]
Date palm leaflets	10 min, 180-190 °C	3.36-3.65	>90	>75	[101]
Date palm rachis	10 min, 180-190 °C	3.36-3.65	>90	>79	[101]
Pumpkin	30 min, 190 °C	4.13	86.3	74.2	[103]
Wheat straw	9 min, 195 °C	3.75	94*	70*	[102]
Wheat straw	6 min, 185 °C	3.28	>90*	90*	[102]

Table 2.4: Maximum achieved total sugar recovery for different types of biomass after hydrothetmal pretreatment. SF: severity factor. (* Recovery of cellulose/hemicellulose)

Another method to increase the amount of available sugars is an organosolv method. The basic principle is to fraction lignocellulosic biomass by using organic solvents to remove some parts of the biomass and leaving other parts as solid fraction [105]. In the case of *S. dolichostachya*, it would be convenient to remove lignin as a liquid and leave cellulose and hemicellulose as a solid fraction, as the solid material is easy to feed for biological organisms. Organosolv treatment has shown a considerable improve to the sugar conversion for hardwood feedstock, and according to the study of Romanì et al. [106], 74–93 % glucan recovery from *Eucalyptus nitens* bark biomass can be achieved. For softwood biomass, up to 90 % sugar yield has been achieved in enzymatic hydrolysis after using ethanol-water mixture solvent for fractionation [105]. One of the advantages of organosolv method is to isolate high-quality lignin without carbohydrate contamination or sulphur [106]. Lignin in an organic complex compound present in lignocellulosic biomass and interest in its valorisation to fine aromatic chemicals, such as phenols, has increased in recent years [24]. However, since organosolv method is relatively expensive and halophyte plants have significantly lower lignin content compared to other 2$^{\text{nd}}$ generation biofuel feedstocks, using this process path might be economically challenging.

2.4 Biochemical sugars-to-lipids conversion

Lignocellulose is an abundant type of biomass in the world, but the highest increase in biofuel demand considers lipid-based heavy transportation fuels, such as jet fuel and biodiesel [8]. Therefore, it is desired to study the conversion of lignocellulose to lipids, as the technology would allow the fuel production from more low-cost feedstocks, such as agricultural residues [107]. The field had increased a lot of interest in past years, and despite its great potential, some obstacles has to be overcome, such as the microbial inhibition caused by furfural, acidic phenolics or other lignocellulose degradation products [108, 109]. Considering halophytes, the possible high salt content of the biomass might also set challenges, if the micro-organisms cannot tolerate or digest the feedstock.

2.4.1 Oleaginous micro-organisms

Fermentation using oleaginous micro-organisms has shown potential to convert sugars in starch and polysaccharide hydrolysates, sourced from food processing and agricultural waste, to single cell oils [110]. Oleaginous micro-organisms are single cell yeasts, fungi, bacteria or microalgae, that can accumulate lipids to their tissues to cover more than 20 % of their total cellular dry matter [109, 110]. The lipid accumulation is enhanced, if micro-organism gets under a nutrient stress, such as nitrogen deprivation [107]. As lignocellulose can not be directly digested by micro-organisms, hydrolysis pretreatment is required to convert the complex cellulose and hemicellulose to sugars monomers [94, 109]. To achieve high lipid yields, hydrolysate has to be often detoxified, as it otherwise has too high concentration of furfural and other toxins, which inhibit the micro-organisms [111, 112]. However, some species, such as *Rhodotorula glutinis* has shown high tolerance to inhibitors, hence the hydrolysate can be directly used for microbial cultivation [113]. Carvalho et al. [114] show the simplified process of fuel production from lignocellulosic sugarcane bagasse through microbial sugars-to-lipids conversion, including the value-added streams of polyunsaturated fatty-acids (PUFA) and carotenoids (Fig. 2.9).

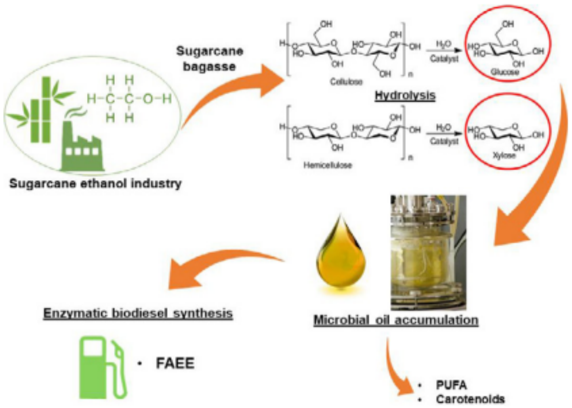


Figure 2.9: Heavy transportation fuel production from hydrolysed sugarcane bagasse feedstock using oleaginous fungi for conversion [114]

Slininger et al. [108] studies the lipid production from corn stover and switchgrass hydrolysates using different yeasts, and finds *Lipimyces tetrasporus* and *Lipomyces kononenkoae* consuming almost all of the available sugars in the hydrolysate and accumulating more than 50 % cell biomass as lipids, which makes these yeasts potential for heavy transportation fuel production. *Saitoella coloradoensis* has also shown to be potential strain for lipid production, not because of high lipid yield, but the high concentration of valuable β-carotene [108]. The same study estimates, that by optimizing the process, approximately 180 and 720 liters of single cell oil could be produced per acre of corn stover and switchgrass, respectively [108]. Mast et al. [115] studies the lipid production using *Rhodotorula glutinis* and shows that within 48 hours, yeast can consume 80 % of sugars from wheat straw hydrolysate and gain a lipid content of 12.5 %, major fatty acids being palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:2). Ahmad et al. [111] uses three different fungi strains to produce lipids from hydrolysates of oil pal empty fruit bunches (EFB), and show that the yielded oil from *Mucor plumbeus* is rich in unsaturaterd and polyunsaturated fatty acids, and microbial conversion and biodiesel production from EFB could increase the production of palm oi industry up to 25 % with cheaper raw material costs [111].

Strain	Type	Feedstock	Lipid content [%]	Comment	Ref.
L. tetrasporus	Yeast	Switchgrass	53.0		[108]
L. kononenkoae	Yeast	Switchgrass	59.0		[108]
T. cutaneum	Yeast	Corn cob	32.1	Fermented	[116]
R. glutinis	Yeast	Corn cob	47.2	Non-detoxified	[113]
R. glutinis	Yeast	Wheat straw	12.5	Non-detoxified	[115]
T. fermentans	Yeast	Rise straw	40.1		[112]
T. fermentans	Yeast	Sugarcane bagasse	39.9		[117]
M. circinelloides	Fungi	Sugarcane bagasse	25.0		[114]
M. plumbeus	Fungi	EFB	37.0	Enzymatic hyd.	[111]
M. plumbeus	Fungi	EFB	12.5	Acid hyd.	[111]

Table 2.5: Cell lipid content of different fungi after microbial lipid production from various lignocellulosic biomass hydrolysates

2.4.2 Black soldier fly larvae

Living organisms have developed digestion systems suitable for various kinds of substrates, and only in recent year, an interest of using insect digestion for lignocellulosic biomass conversion has increased [118]. Chatellard et al. [118] studies the degradation of wheat straw using microbiome of insects from several orders, and show that insects can efficiently digest cellulose and even hemicellulose, depending on the species used. Highest conversions are achieved when using guts of *Ergates faber* and *Locusta migratoria* [118].

Biowaste treatment using black soldier fly larvae (BSFL), *Hermetia illucens*, is an emerging technology for biochemical biowaste-to-feed conversion [119]. The types of biowaste converted using BSFL are described in a review paper by Gold et al. [119], and reviewed biowastes are human and farm animal manure, fruit and vegetable waste, municipality and brewery waste. As a control feed, poultry feed is used, as this is a frequently used feedstock across the field of research on BSFL. Based on 23 different research papers reviewed by Gold et al. [119], done on BSFL feeding experiments, the following protein and lipid composition was found as showed in Tab. 2.6.

Biowaste		Protein [w% DM]	Lipid [w% DM]	Comment
Manure	Cow	34-35	n/a	May include bedding material
	Poultry	34-35	n/a	May include bedding material
	Pig	32-43	33	May include bedding material
Fruit waste		35-58	15-38	Discarded fruits e.g. apples, pears & oranges
Vegetable waste		44	n/a	Discarded vegetables e.g. lettuce, beans & cabbage
Poultry feed		33-39	34	Feed used for poultry

Table 2.6: Types of biowastes fed for BSFL reweived by Gold et al. [119]

BSFL is used as a biowaste treatment to convert organic waste into high protein/lipid animal feed, which can be fed directly to chicken, and this is already done in some tropical areas [120, 121]. Trends also show, that insect-based animal feeds can be produced in more sustainable way than conventional feed crops [119]. If it is wanted to use larvae for feed purposes, legal limitations are set to define the type of biowaste allowed as larvae feed. Initial feed can not be harmful to the final user, hence the use of manure is restricted.

BSFL can be seen as an easy, cheap and scalable route from lignocellulosic biomass to lipids and valuable compounds, such as protein and chitin. In Tab. 2.11, the lipid composition of BSFL is compared to *S. brachiata* oil, single cell oil from *R. glutinis* and a few common 1st generation feedstocks used in fuel purposes . One main advantage of using BSFL for biochemical conversion is the easy rearing of insects compared to microorganisms, which often rely on constant cultivation conditions without contamination and the presence of cultivation media. Whereas, BSFL can be reared directly in the feedstock itself. Also, less biowaste pretreatment is needed in order to get the BSFL to feed efficiently from it, for example, hydrolysis of the feedstock is not required.

2.5 Lipids to jet fuel

Multiple feedstocks are available for oil to jet fuel conversion, as oils and paraffins are similar in molecular structure as alkanes in the jet fuel range. As jet fuel comprises of alkanes in the range of 8 to 16 carbon atoms per molecule, free fatty acids (FFA) from depropanation of oils and paraffins of the same molecule length are ideal feedstocks [125]. Lipids, such as soybean oil, canola oil, algal oil and waste cooking oil, are shown to be suitable and potential feedstocks for transesterification into biodiesel or to be processed into jet fuel. Soybean oil has been used extensively in the United States as a feedstock for biodiesel production using 30.0 % of the total soybean production of 2018 [126].

Jet fuel needs to be a very reliable with very specific properties, as the consequences of jet fuel with a high freezing point, low energy density or varying viscosity could be catastrophic. Therefore, the suitable jet fuels and allowed processes are highly standardised

Biomass	C10:0	C12:0	C14:0	C16:0	C16:1	C18:0	C18:1	C18:2	C18:3	Other	∑SFA	∑USFA	∑PUSFA	Ref.
BSFL	3.0	35.5	7.5	15.0	4.0	3.5	23.5	6.0	n/a	2.0	66.0	33.5	6.0	[122]
S. brachiata	n/a	n/a	n/a	26.0	0.5	7.0	6.0	26.0	29.0	5.5	33.0	61.5	55.0	[40]
Canola	n/a	n/a	n/a	4.0	n/a	1.5	62.0	20.5	8.5	3.5	5.5	91.0	29.0	[123]
Oil palm	n/a	n/a	1.0	42.5	n/a	4.0	41.0	10.0	0.5	1.0	47.5	51.5	10.5	[123]
Soybean	n/a	n/a	n/a	11.5	n/a	4.0	23.5	53.3	6.5	0.5	15.5	83.5	60.0	[123]
Coconut	6.0	48.0	18.5	8.5	n/a	3.0	7.5	2.0	n/a	6.5	90.0	9.5	2.0	[124]
R. glutinis	n/a	n/a	n/a	16.8	0.8	3.7	45.8	17.9	4.3	10.7	20.5	46.6	19.2	[107]

Figure 2.10: Approximate fatty acid profiles of oils from different feedstocks. Results presented as a mass percentage of total fatty acids. SFA: Saturated fatty acids, USFA: Unsaturated fatty acids, PUFA: Polyunsaturated fatty acids

and restricted.

Parameter	Jet A-1	Jet A	Jet B	Unit
Density @ 15 °C	775-840	803.3	821.0	kg/m^3
Max. sulfur content	3,000	697	5	wt ppm
Max. total aromatics*	26.5	18.9	20.4	vol%
Max. distillation Initial Point**		147.6	152.1	°C
Max. T10	max 205	167.0	170.2	°C
Max. T20		175.5	176.6	°C
Max. T50		199.0	201.5	°C
Max. T90		245.3	240.5	°C
Max. final point	max 300	270.6	258.9	°C
Max. T90-T10	min 22	78.3	70.0	°C
Max. freezing point	-47.0	-49.6	-64.9	°C
Max. viscosity @ -20 °C	8.0	4.04	4.19	mm^2/s
Flash point	38.0	40.5	42.5	°C

Table 2.7: Requirements for different types of jet fuel. Values from T10 to T90 are maximum temperature where corresponding mass fraction of products should be distilled. *: Determined by HPLC. **ASTM D86: Standard Test Method for Distillation of Petroleum Products at Atmospheric Pressure [127]

2.5.1 Pretreatment of lipids

After extraction, lipids from the biomass contain significant amounts of impurities that might be harmful to the catalysts, lower the final value and properties of the end product, or decrease shelf-life. These impurities can be unwanted organic compounds, soaps, metals, Na, K, Ca and large amounts of phosphorus. To ensure longer catalyst life, higher transesterification rates, and avoid the formation of gum when combusted, it is important to pretreat the lipids [75, 128]. The metals in vegetable oil are generally present with phospholipids, which makes the removal of metals and metal ions easy [129].

Degumming

Degumming can be done by both water degumming and acid degumming, in order to remove phospholipids. A phospholipid molecule has both hydrophilic end and a hydrophobic end, referred to as a hydrophilic head and a hydrophobic tail. This means that the molecule can form reverse micelles in a non-polar solution as hexane and oil [130]. In a study done by Paisan et al. [75], water degumming and acid degumming is performed to microalgal oil from *Chlorella* sp., which was reported to have a very high phospholipid content. The extracted algal oil was mixed with palm oil, which is reported to have a very low phospholipid content, to lower the viscosity. Water degumming done by Paisan et al. [75] was performed by adding deionised water to the *Chlorella* oil, heating and stirring the mixture, and separating the impurities from the oil by centrifuging. With this method, maximum phospholipid removal is 19.4 % after sensitivity analysis bu changing temperature, reaction time and water content of the mixture [75].

Acid degumming is similar to water degumming, but instead of mixing the oil with water, it is mixed with aqueous phosphoric acid, and washed with near-boiling deionised water after heating and stirring. With this method, Paisan et al. [75] reached a maximum phospholipid removal of 82.5 %. higher phospholipid removal by acid degumming indicates, that majority of phospholipids in *Chlorella* sp. cannot be hydrated.

Phospholipids form reverse micelles in a non-polar solution, ad according to Lin et al. [130],the size of a micella has a molecular weight of >20,000 g/mol. As free phospholipids and triglycerides have molecular weights of approximately 900 g/mol, it is also possible to filter the phospholipids from a hexane-oil solution [130]. These formed reverse micelles will, in a hexane-oil solution, also contain some hexane, oil, FFAs, the majority of pigments and other compounds [130]. Lin et al. [130], achieve a phospholipid rejection rate of 99.6 % for cottonseed oil, with an intial 100 µm filtration and DS-7 membrane filtration (Desalination Systems, Inc., Escondido, CA) with a molecular weight cut-off of 1000.

Bleaching

After degumming, further pretreatment of oil can be done to reach better quality. Bleaching is done to remove carotenoids, other pigments (chlorophyll), metals, soaps and oxidation products from the vegetable oil [131, 132].

Almeida et al. [133] studied the thermodynamic kinetics, properties and equilibria of bleaching clay in an aim to remove carotene pigments from palm oil. The study used two commercial bleaching clays widely used in the vegetable oil industry: acid activated adsorbent (ABE) and neutral adsorbent (NBE). ABE shows fast and efficient adsorption of carotenes present in palm oil, and the variation in the reaction temperatures between 90 °C and 120 °C shows only a small increase in adsorption, whereas carotene adsorption by NBE is very sensitive to reaction temperature. ABE exhibits equilibrium behaviour after 40 minutes of reaction regardless of reaction temperature, whereas NBE does not reach equilibrium after 120 minutes. The bleaching clay containing the carotene pigments can be removed by vacuum filtration.

2.5.2 Hydroprocessed esters and fatty acid

Hydroprocessed Esters and Fatty Acids Synthetic Paraffinic Kerosene (HEFA-SPK or HEFA) is an ASTM International (ASTM) approved biomass-based jet fuel and suitable for up to a 50 % blend-in with conventional fossil jet fuels [127]. In HEFA process, many process steps are happening simultaneously in one reactor:

1. Saturation unsaturated triglycerides
2. Removal of glycerin-group from the free fatty acids (FFA)
3. Saturation of glycerin to propane

4. Deoxygenation, decarboxylation and decarnoxylation of saturated FFA into saturated alkanes (n-alkanes)

The feedstock for HEFA is desired to be high in solid lipids, and is therefore a good process route for animal fats, algal lipids, used cooking oils and grease [134]. Overall, HEFA is a well described process route for saturating triglycerides and free fatty acids, and further processing of these into n-alkanes.

The jet fuel range n-alkanes with carbon numbers C8-C15 have freezing points between -57 °C and 10 °C, which in blended is higher than maximum limitation for Jet A-1. Therefore, n-alkanes with high freezing points has to be isomerised into i-alkanes and hydrocracked, to yield lower freezing points to meet the ASTM requirements. Isomerisation and hydrocracking are also happening simultaneously in one reactor, and the process yields a product with low freezing point, which meets the Jet A-1 requirements (freezing point $\leq$-47 °C) [135, 127, 136]. In the end, fuel products with different carbon ranges and properties can be separated from each other by distillation.

Due to the similarities in fatty acid compositions of BSFL lipids to palm oil and coconut oil, as seen in Tab. 2.11, similar process specifications as operation temperature, operation pressure, reactor catalysts are chosen as described by Martinez-Hernandez et al. [137], as no literature of HEFA from BSFL has been published. As the alkanes from biofuel production of the HEFA process will be in a limited range, it is important to make sure that the lipid profile corresponds with the wanted length of molecules, and can be manipulated by hydrocracking and isomerisation, as described in section 2.5.3. The readtion product also needs to correspond a fuel with jet fuel range carbon numbers and properties complying with the ASTM standards.

Hydrogenation of triglycerides

Hydrogenation of triglycerides is the reaction of saturating a double-bonded triglyceride to have only single bonds between carbon atoms, and use hydrogen to bond with the formed free electrons. As the triglyceride profile of terrestrial animals are often higher in saturated triglycerides than oil producing crops, the saturation of the unsaturated fatty acids from animal fat is less hydrogen intensive, meaning higher conversion to alkanes instead of alkenes, which have one double bond between carbon atoms [123, 122]. The reason to hydrogenate the unsaturated triglycerides is to make sure that the triglycerides will not be processed into n-alkenes, but n-alkanes in the HEFA process, as this class of hydrocarbons have better combustion properties [138].

An example of saturation of cis-unsaturated triglyceride is the saturation of monounsaturated triglyceride triolein (tri-C18:1) into the saturated triglyceride, tristrearin (tri-C18:0). This process can be done at temperature of 175 °C and pressure of 0.55 MPa with Ni as a catalyst [139]. When the saturation of cis-unsaturated triglyceride occurs, the formation of trans-unsaturated triglyceride will also occur. However, if the process is optimised to the right conditions, the trans-unsaturated triglyceride will get saturated into saturated triglyceride [139]. Polyunsaturated tryglycerides trilinolein (tri-C18:2) and trilinolenin (tri-C18:3) can also be saturated to tri-C18:0 [134, 140]. Swicklik et al. [139] describes the linear kinetic rate of reaction for tri-C18:1, without taking the reaction intermediate trans-unsaturated triglycerides into account, as the reaction will run until full conversion. This is shown in Eq. 2.4 and in reaction equation, where n is the amount of carbon atoms in the triglyceride, and m is the amount of double bonds in each FFA branches of the triglyceride.

$$-r_{tri-C_{18:1}} = k \cdot C_{tri-C_{18:1}} \tag{2.4}$$

$$\mathrm{C_nH_{(2n-1-6\cdot m)}(COOH)_3 + (3\cdot m)H_2 \xrightarrow{Ni} C_nH_{(2n-1)}(COOH)_3}$$

The reaction rate constant for saturation of tri-C18:1 can be found by applying the equations by Swicklik et al. [139]. The values used in the calculation can be found in Tab. 2.8. Considering a conversion rate 96.3 % of the 200 g tri-C18:1 in the reactor, molar weight of tri-C18:1 being 885.4 g/mol (Eq. 2.5) and a conversion of 0.218 mol per 40 min in a reactor with a volume of 181.56 cm^3 (Eq. 2.6):

$$n = \frac{a \cdot m}{MW} \tag{2.5}$$

$$k = \frac{n}{t \cdot V} \tag{2.6}$$

This gives the following kinetic reaction rate shown in Eq. 2.7.

$$-r_{tri-C_{18:1}} = 4.99 \cdot 10^{-4} \frac{kmol}{m^3 \cdot s} \cdot C_{tri-C_{18:1}} \tag{2.7}$$

Parameter	Symbol	Value	Unit
Extinction coefficient*	K	$3.7 \cdot 10^{-2}$	min^{-1}
Conversion rate*	a	96.3	%
Mass*	m	200	g
Molar weight	MW	885.4	g mol^{-1}
Amount of substance	n	0.218	mol
Time*	t	40	min
Volume*	V	181.56	cm $^{-3}$
Reaction rate constant	k	$4.99 \cdot 10^{-4}$	$\frac{kmol}{m^3 \cdot s}$

Table 2.8: Values used in the calculation of reaction rate constant. *) As provided in the research article by Swicklik et al. [139]

Depropanation

Triglycerides comprise of a glycerin molecule and three FFA molecules. These can be separated by a catalytic reaction using hydrogen as co-reactant, yielding propane and FFAs [129]. This process is called depropanation. The produced propane can be sold or used internally to heat the processing streams by combustion. In the reaction equation of the depropanation of saturated triglycerides, *n* is amount of carbon molecules in the FFAs. The used catalyst, nickel witj molybdenum promoted aluminium oxide (Ni-Mo/Al_2O_3), is described by Sinha et al. [129] in the reveiw of HEFA processing.

$$C_nH_{(2n-1)}(COOH)_3 + 3\,H_2 \xrightarrow{Ni-Mo/Al_2O_3} 3\,C_nH_{2n+1}COOH + C_3H_8$$

Reaction rate
$-r_{tri-\mathrm{C18:0}} = k_{tri-\mathrm{C18:0}}C_{tri-\mathrm{C18:0}}$

Table 2.9: Kinetic reaction expressions deduced from a study by Martinez-Hernandez et al. [137]

Hydrodeoxygenation

In order to produce n-alkanes from the saturated FFAs, the carboxyl groups of FFA molecules need to be removed, and replaced with hydrogen. This reaction is called hydrodeoxygenation (HDO). It is not a straight route, as some reaction intermediates will be produced. In a study by Arora et al. [141] investigating the synthesis of octadecane (C18) from stearic acid (C18:0) using the catalyst Ni-Mo/Al_2O_3, reaction intermediates octadecanal (C18=0) and octadecanol (C18-OH) were found, with heptadecane (C17) being a by-product from direct decaroxylation of C18:0 and decarbonylation of C18=O. These aldehyde and alcohol reaction intermediates, will further react with hydrogen to form octadecane. The entire reaction can be seen in Fig. 2.11, and similar reactions will happen when HDO of other saturated FFAs is considered.

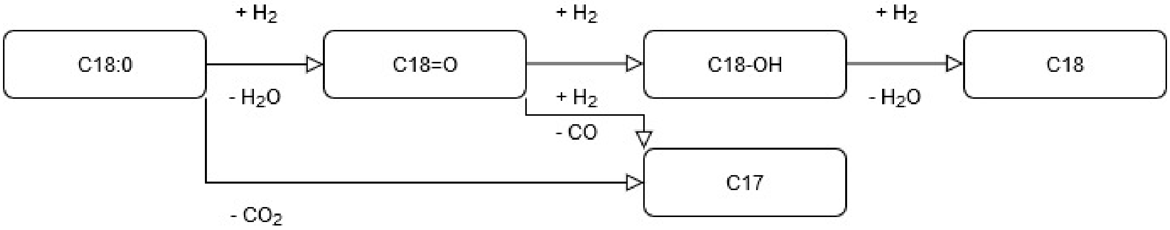


Figure 2.11: Reaction diagram for considered FFA to alkane reactions, and their reactions intermediates [141].

$$\text{Reaction 1: } C_{18:0} + H_2 \xrightarrow{Ni-Mo/Al_2O_3} C_{18}{=}O + H_2O$$

$$\text{Reaction 2: } C_{18:0} \xrightarrow{Ni-Mo/Al_2O_3} C_{17} + CO_2$$

$$\text{Reaction 3: } C_{18}{=}O + H_2 \xrightarrow{Ni-Mo/Al_2O_3} C_{17} + CO$$
$$\text{Reaction 4: } C_{18}{=}O + H_2 \xrightarrow{Ni-Mo/Al_2O_3} C_{18}-OH$$
$$\text{Reaction 5: } C_{18}-OH + H_2 \xrightarrow{Ni-Mo/Al_2O_3} C_{18} + H_2O$$

Reaction 1 is the reduction of the carboxyl group in C18:0 to form C18=O and H_2O in the presence of hydrogen. Reaction 2 is the removal of CO_2 from the C18:0 via decarboxylation, and reaction 3 removes carbon-monoxide (CO) from the C18=O via decarbonylation in the presence of hydrogen. Reaction 4 is the hydrodeoxygenation of C18=O to form C18-OH in the presence of hydrogen, and reaction 5 is the hydrodeoxygenation of C18-OH to form C18 and hot water in the presence of hydrogen [141].

Reaction rate
$r_1 = \frac{k_1 C_{C_{18:0}} C_{H_2}}{(1+K_{C_{18:0}} C_{C_{18:0}})}$
$r_2 = \frac{k_2 C_{C_{18:0}}}{(1+K_{C_{18:0}} C_{C_{18:0}})}$
$r_3 = \frac{k_3 C_{C_{18=O}} C_{H_2}}{(1+K_{C_{18:0}} C_{C_{18:0}})}(1 - \frac{aC_{18-OH}}{a_{C_{18=O}} a_{H_2} K_{eq}})$
$r_4 = \frac{k_4 C_{C_{18=O}}}{(1+K_{C_{18:0}} C_{C_{18:0}})}$
$r_5 = \frac{k_5 C_{C_{18-OH}}}{(1+K_{C_{18:0}} C_{C_{18:0}})}$

Table 2.10: Kinetic reaction expressions given by Arora et al. [141]. k_i is the rate constant, C_i is the concentration, K_i is the inhibition term

In Tab. 2.10, the kinetic reaction expression of decarbonylation (reaction 3) takes into account the activity a and equilibirum constant K_{eq}. The activity for component i is the product of the liquid phase fugacity coefficient of component i (Φ_i^l), the molar fraction of component i (x_i) and the total pressure. The equilibirum constant comprises of standard state thermochemical properties of the component (denoted with A, B, C etc.) and the reaction temperature.

$$a_i = \Phi_i^l x_i P \tag{2.8}$$

$$K_{eq} = exp(\frac{A}{T} + B \cdot ln(T) + CT + DT^2 + ET^3 + F) \tag{2.9}$$

As products from the described reactions are CO, CO_2 and H_2O, with the precence of hydrogen, a water gas shift (WGS) and methanation will happen as the operation temperature and pressure are high. However, as described by Kharaji et al. [142], the rate of WGS and methanation are highly temperature dependent, with the catalyst being Ni-Mo/Al_2O_3, and they start to occur around 300 °C.

The reactions of hydrodeoxygenation will happen as catalysed reactions inside a packed bed reactor (PBR). A schematic of the hydrodeoxygenation PBR with intermediate products shown in Fig. 2.12.

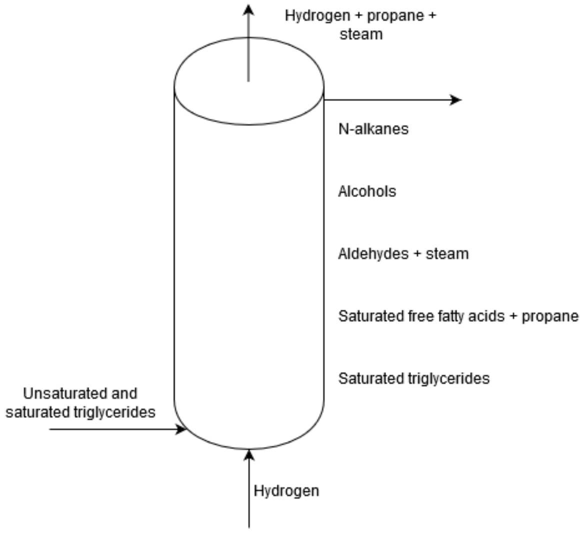


Figure 2.12: Schematic of hydrodeoxygenation PBR with start chemical, intermediate and final products (carboxylation and carbonylation left out). The molecule structures of the chemicals involved in the reaction can be seen in App. B, on a 18 carbon basis.

2.5.3 Hydrocracking and isomerisation

To make the fuel products meet the ASTM standards set for HEFA, the n-alkanes are hydrocracked and isomerised (HI). This will result a lower freezing point of the fuel, while maintaining high energy released from combustion. The two reaction types, hycrocracking and isomerisation, will happen simultaneously in a PBR, with the presence of free excess hydrogen. Hydrocracking is the reaction where long-chained alkanes are split into two smaller alkanes, and saturated with hydrogen to prevent the formation of alkenes. For the alkanes within the range from n-C10 to n-C26, but mainly in the range from n-C12 to n-C20, H-G hydrocracking process is suggested by Speight and Özüm [143]. The same method is used in the petrochemical industry for hydrocracking the lighter fractions of crude oil, and it is done in the presence of a catalyst and simultaneously with isomerisation. Isomerisation is the reaction of rearranging the atoms in alkanes, for example, from n-decane to i-decane. Some research has been done considering HI of vegetable oils (Tab. 2.11), but HI is typically performed to fossil crude hydrocarbons, in order to satisfy the demand for lighter hydrocarbons [137].

Lipid type & Ref.	Conditions	Catalyst	Gasoline [%]	Kerosene [%]	Diesel [%]
Canola oil [144]	350 °C, 9.0 MPa 3.0 h^{-1} LHSV	Mo–Ni/Al_2O_3	n/a	n/a	78
Palm oil [131]	477 °C, 5.6 MPa 1.5 h^{-1} LHSV	Pd/Al_2O_3	43	48	6
Palm oil [145]	350 °C, 4.0 MPa 8.0 h^{-1} LHSV	Mo-Ni/SiO_2-Al_2O_3	n/a	n/a	82
Soybean oil [146]	300 °C, 1.5 MPa 6.0 h^{-1} LHSV	Pd-based Zeolite	n/a	50	n/a
Coconut oil [135]	350 °C, 0.8 MPa 2.0 h^{-1} LHSV	Mo–Ni/Al_2O_3	n/a	71	n/a
Coconut oil [136]	350 °C, 4.0 MPa 1.0 h^{-1} LHSV	Mo–Ni/Al_2O_3	n/a	58	n/a

Table 2.11: Comparative yields from different feedstocks via catalytic HI. LHSV: Liquid Hourly Space Velocity in a PBR. Rapeseed oil was cracked in a batch reactor.

Studies of HI on n-alkanes in the range of C8-C18 are done by Flinn et al. [147] and Coonradt et al. [148], and backed up by Martinez-Hernandez et al. [137], giving a set of possible reactions of HI. It should be noted, that the range of carbon numbers are similar to the FFA chains in BSFL lipids. All hydrocracking reactions are splitting alkanes into range n-C8 to n-C15, which has molecules within the jet fuel range, described in section 2.5.4. These shorter alkanes will from thereon get isomerised. The reactions of HI will happen as catalysed reactions inside a packed bed reactor (PBR). A schematic of the HI PBR with intermediate products shown in Fig. 2.12.

Hydrocracking

Reaction 1: $C_nH_{2n+2} + H_2 \longrightarrow C_{n-3}H_{2n-4} + C_3H_8$ for n=13-18

Reaction 2: $C_nH_{2n+2} + H_2 \longrightarrow C_{n-8}H_{2n-14} + C_8H_{18}$ for n=17-18

Reaction 3: $C_nH_{2n+2} + H_2 \longrightarrow C_{n-7}H_{2n-12} + C_7H_{16}$ for n=17-18

Reaction 4: $C_nH_{2n+2} + H_2 \longrightarrow 2\,C_{n/2}H_{n+2}$ for n=16,18

Isomerisation

Reaction 5: $C_nH_{2n+2} \longrightarrow \text{iso}-C_nH_{2n+2}$ for n=6-18

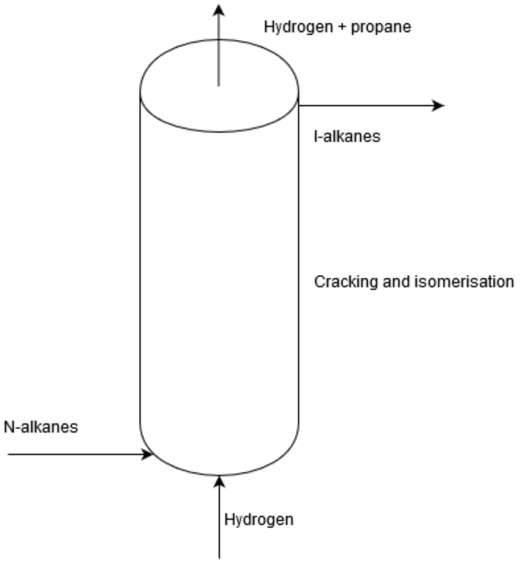


Figure 2.13: Schematic of HI PBR with start chemical, intermediate and final products.

2.5.4 Distillation

To separate the different fuels from each other, a distillation tower will heat and condense the fractions. The fractions from a crude oil distillation tower will be light gases, light naphtha, heavy naphtha, kerosene, light gas oil, heavy gas oil and residue. Each of these fractions can be fed back into the HI unit to match the demand set by consumers [149].

Jet A-1 fuel has a boiling point specification from 205 °C to 260 °C, but actually ranges from 175 °C to 315 °C, as some of the targeted molecules will end up in the heavier and lighter distillation fraction, but the distillation with larger temperature range will also blend in types of unwanted fuels in Jet A-1 [149, 150]. Boiling points from 205 °C to 260 °C roughly corresponds to alkane distillation products n-C12 to n-C17 and i-C14 to i-C21 [149]. The operation conditions of the HI process can therefore be optimised to yield a higher fraction of these alkanes, to yield a higher fraction of Jet A-1 in distillation.

As alkene yielding thermal cracking, which is a cracking process without hydrogen, occurs at temperatures >350 °C, it is not a concern when distilling jet fuel range alkanes in atmospheric pressure, as thermal cracking by distillation often happens at an approximate carbon length of the alkanes more than n-C25 and i-C30 [149].

Chapter 3

Project objectives

The project objectives to answer the initial problem stated in Section 1.2 are based on set international goals, the state-of-the-art literature and previous research results. The three main objectives of the projects are:

1. Broad characterisation of harvested *S. dolichostachya* biomass and study of the seasonal variation in the biomass composition
2. Experiments considering biological lignocellulose-to-lipids conversion using BSFL
3. Simulating the processing of lipids to sustainable aviation fuel through ASTM approved HEFA route

The biomass characterisation gives detailed information about the biochemical composition of *S. dolichostachya*. Biomass is harvested several times during the work period to observe the seasonal variations in the composition. This objective includes:

- Green fractionation of biomass to solid (pulp) and liquid (juice) fractions
- Dry matter and ash determination
- Determination of lipids: optimised Soxlhlet extraction for pulp and liquid-liquid lipid extraction for juice, both followed by gas chromatography and mass spectrometry analysis for fatty acid methyl esters.
- Approximation of protein content based on total nitrogen concentration
- Determination of carbohydrates and Klason lignin: weak acid hydrolysis for juice fraction and strong acid hydrolysis for pulp, both followed by HPLC analysis.

Lignocellulose-to-lipids conversion using black soldier fly larvae is an experimental study done in co-operation with ENORM ApS. This objective includes:

- 1st feeding trial using fresh *S. dolichostachya* pulp in different blending ratios with standard poultry-based feed
- 2nd feeding trial using extractive-free fibres from dry *S. dolichostachya* shrubs
- Observations of physical changes and analysis of the chemical composition of BSFL after the two-week trial period

Process simulation targets the issue of increasing demand for heavy transportation biofuels. The simulation covers the biorefinery process from harvested *S. dolichostachya* to ASTM approved aviation fuel. This objective includes:

- Building the biomass processing chart
- Biochemical sugars-to-lipids conversion
- Processing steps of HEFA route converting lipids to sustainable aviation fuel and other biofuel products
- Feasibility study ans scale-up scenarios of the process

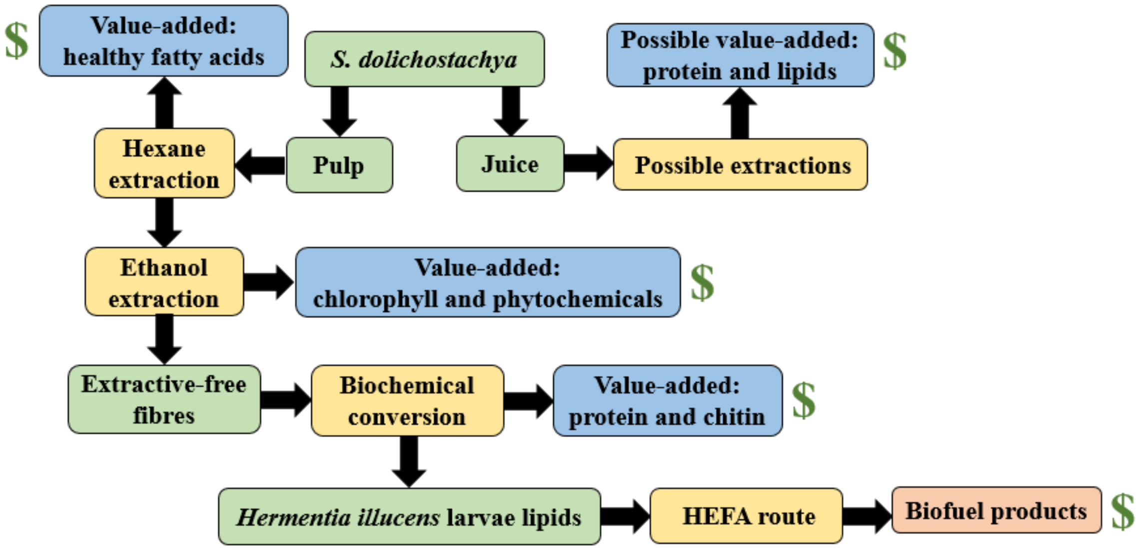


Figure 3.1: Simplified biorefinery process schematic

Chapter 4

Materials and methods

4.1 Raw material

Used *S. dolichostachya* biomass is harvested approximately once a month throughout the autumn in its different stages of lignification (Fig. 2.2) from the marsh land in Southern Denmark seashore (Fig. 4.1). The habitat undergoes strong tide changes up to 1.7 meters, and the local vegetation can be partly or entirely underwater during high tides. According to the World Weather and Climate Information [151], the annual average weather temperature varies between 5 °C and 11 °C, with the coldest and warmest months being January and August, respectively. The average annual precipitation is 728 mm. The measurements are from the nearest weather station in Esbjerg, Denmark. Harvested biomass is washed thoroughly with lukewarm tap water before processing in an aim to remove mud, sand, seaweed and other materials. The excessive water is drained and the clean shrubs are kept in the freezer before juicing. The processing in this chapter is done for each picked sample.

Figure 4.1: Marsh area in Mandø, Denmark 11th Nov. 2019

4.2 Green fractionation, dry matter and ash determination

Clean *S. dolichostachya* biomass is weighed and juiced with Omega Sana EUJ-707 domestic kitchen juicer with a single horizontal auger, to separate the liquid juice fraction from the woody pulp material. Gained juice and pulp are weighted and the mass fractions of the total initial biomass are calculated with Eq. 4.1. A small amount of the material is lost in the process.

$$Amount\ of\ juice\ or\ pulp\ [\%] = \frac{m_{juice\ or\ pulp}}{m_{initial}} \cdot 100\% \tag{4.1}$$

The dry matter (DM) and water content of the lignocellulosic pulp biomass is determined by weighing the mass of a plastic weighing boat, and the mass of the weighing boat with a pulp sample. The sample size is approximately 50 g and triplicates are taken for each batch. The weighing boats with the samples are placed in a drying oven at 37 °C for 24 hours, until the samples are completely dry. The dried samples are weighted and the dry matter content is calculated from the weight difference as shown in Eq.4.2.

$$DM_{pulp}\ [\%] = \frac{m_{pulp,dry}}{m_{pulp,wet}} \cdot 100\% \tag{4.2}$$

Dry biomass is milled with Retsch GM200 knife mill with rotating speed of 10,000 rpm for 45-90 seconds, and sieved through a 1 mm screen to ensure the fine particle size. This finely milled dry matter is used for the determination of ash, protein, lipids and sugars.

The dry matter content is also determined for the juice fraction of *S. dolichostachya* biomass and it is calculated as a mass fraction of dry solid matter, from the total mass of the juice. Approximately 10 g of juice is weighed to wide plastic weighing boats. Water is evaporated from the samples by keeping them in the oven at 50 °C for 24 hours. The dry residue is weighed and the mass is noted. Triplicates are taken for each batch of juice. It has to be noted, that salts and other water-soluble compounds are included in the definition of dry matter. To calculate the dry matter content of the juice, Eq. 4.3 is used.

$$DM_{juice}\ [\%] = \frac{m_{solids,dry}}{m_{juice}} \cdot 100\% \tag{4.3}$$

The ash content of the dry matter is determined by weighing approximately 5 g of pulp DM and 3 g of juice DM to cleaned, ashed and pre-weighed crucibles. Triplicates are taken for each sample. The samples are burnt in a muffle furnace at 550 °C for 3 hours. When the oven is cooled, crucibles with the ash are kept in the desiccator until room temperature. The crucibles and the residue are weighed, and the ash content is calculated from the weight difference.

4.3 Lipid extraction from *S. dolichostachya* pulp

The amount of lipid extracted from the biomass will vary depending the solvent or the solvent mixture, as described by Escorsim et al. [74], Sati et al. [73] and in section 2.2.1. The lipid content of *S. dolichostachya* pulp is determined with Soxhlet extraction by using n-hexane as a solvent, and the chose of solvent is based on the previous studies, where the n-hexane has shown to be the most commonly used. Three samples are taken for each batch of biomass.

Milled biomass is placed in the drying oven at 37 °C prior to the extraction to remove any possible excess moisture. Cellulose thimbles are dried prior to the extraction in the drying over at 37 °C for 24 hours and weighed. A sample size of 7-10 g is placed to the thimble and the total mass is noted.

The Soxhlet apparatus with an 100 mL extraction chambers is set up inside the fume hood, where 150 mL of n-hexane is added to the round bottom flask. A piece of hydrophilic cotton is used to block the opening of the thimble, which prevents the sample from being carried out of the thimble during the extraction. The samples are placed inside the extraction tubes and the extraction is run for approximately two hours, until the solvent in the extraction chamber is clear.

In the end of the extraction, the thimbles are taken out of the Soxhlet apparatus when the solution has stopped boiling. The cotton is removed and the samples are kept inside the fume hood for 24 hours to ensure that all solvents are evaporated. Then, samples are dried at 37 °C for another 24 hours. Thimble and the dry sample residue are weighed, and the lipid content is calculated as a mass percentage of the weight difference. It has to be noted, that halophtye biomass has a high salt content and salts are not soluble in n-hexane.

$$Lipids\ [\%] = 1 - \frac{m_{residue}}{m_{initial}} \cdot 100\% \tag{4.4}$$

After extraction, the solvent and extract solution is placed in the fume hood, to separate the solvent from the lipid. Solvent is separated by using rotary evaporator, and the lipid is transferred to a small sample tube and weighed. Lipids from the same harvest date are mixed, and impurities from the oil are removed by diluting samples with a small amount of n-hexane, and injecting the solution with a syringe to a glass veil through Q-max 0.45 µm filter. Samples are kept in the freezer (-20 °C) for further analysis.

Due to the spread of novel coronavirus COVID-19 in Denmark, the fatty acid profile determination by gas chromatography and mass spectormetry for fatty acid methyl esters was cancelled as there was no access to the laboratory facilities, and this further characterisation had to be left out from the project scope.

4.4 Liquid-liquid lipid extraction

As a big fraction of *S. dolichostachya* is liquid after green fractionation, it is desired to evaluate the lipid fraction of the juice. Lipids can be separated by drying the juice and performing the Soxhlet extraction as described in section 4.3, but as the liquid has a very low dry matter content, as seen in Tab. 5.1, evaporation of the water will require a great amount of energy in a large scale production.

To determine the lipid content of *S. dolichostachya* juice, the procedure of Bligh-Dyer [78] is used, with the solvent proposed by Smedes [79]. The ratio of solvents, i-propanol and cyclohexane, used for the liquid-liquid extraction from green juice, is investigated as seen in Fig. 4.2. The ratios of solvents and *S. dolichostachya* juice are seen in Tab. 4.1. After the monophasic solution has been mixed using solvents and the juice, the solution is vortex mixed for 1 minute. This is done to get a sufficient contact time between the lipids and the cyclohexane. Then added de-ionised water and cyclohexane are added to the solution. This was done to get a separation between the monophasic and biphasic solutions. The biphasic solution will consist of a polar and a non-polar phase.

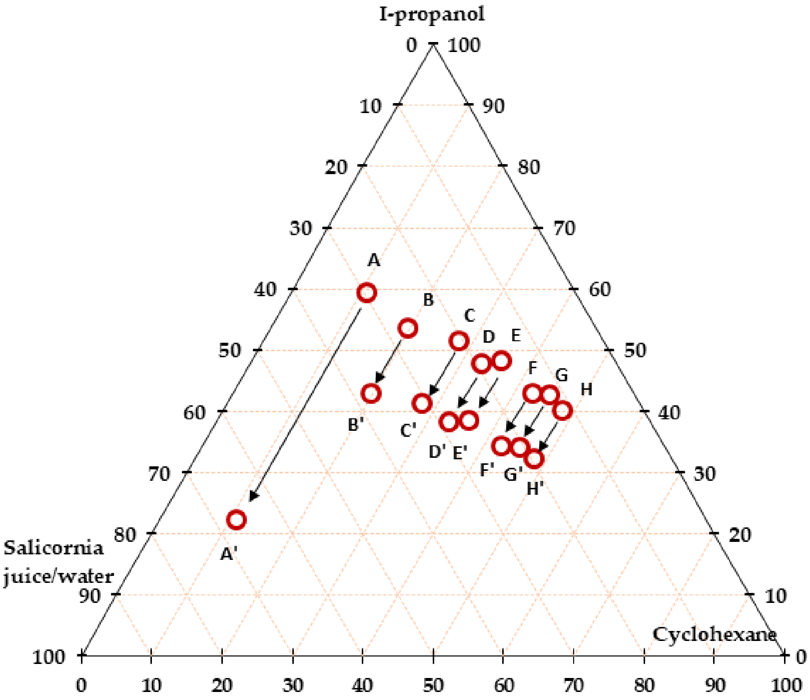


Figure 4.2: Points indicate measurements done in this study, to determine the mixing ratio of chemicals to yield monophasic and biphasic conditions. Done on *S. dolichostachya* juice sampled 26th of September. Point A to A' was initial test.

Sample	Cyclohexane [%]	i-propanol [%]	*S. dolichostachya* juice/water [%]
Monophasic			
26th Sep.			
1	14.3	66.7	19.0
2	25.7	62.9	11.4
3	32.1	60.4	7.5
15th Oct.			
1	14.3	66.7	19.0
2	29.0	58.1	12.9
3	37.8	63.3	8.9
11th Nov.			
1	15.8	63.2	21.0
2	29.0	58.1	12.9
3	41.4	48.8	9.8
Biphasic			
26th Sep.			
1	14.3	53.3	32.4
2	25.7	50.3	24.0
3	32.1	48.3	19.6
15th Oct.			
1	14.3	53.3	32.4
2	29.0	46.5	24.5
3	37.8	42.7	19.6
11th Nov.			
1	15.8	50.5	33.7
2	29.0	46.5	24.5
3	41.4	39.0	19.5

Table 4.1: Initial ratios of chemicals for monophasic conditions and ratios of chemicals for transitioning into biphasic conditions. All experiments are on basis of 4 mL of *S. dolichostachya* juice for monophasic conditions.

The non-polar phase, containing the lipids, is separated by using Finnpipette variable-volume pipettes with range of 10-200 µL. Remaining cyclohexane is evaporated in a fume hood, and the lipids are prepared for further lipid profile analysis as described in section 4.3. The analysis for the optimal processing point with the least amount of chemicals used per gained amount of lipids, can be found by performing triplicates for all three operation points for each sample.

Due to the spread of novel coronavirus COVID-19 in Denmark, the fatty acid profile determination were cancelled as there was no access to the laboratory facilities, and this characterisation had to be left out from the project scope.

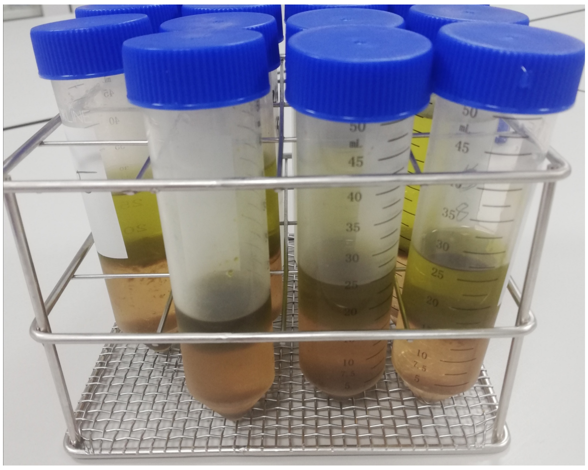


Figure 4.3: Biphasic solutions of liquid-liquid lipid extraction. Lipids are soluble in cyclohexane, which is evaporated off after the separation of the non-polar and polar phases.

4.5 Protein content of *S. dolichostachya*

To determine the total amount of nitrogen, an elemental analysis is performed for the pulp DM and juice DM samples. The modern application of Dumas combustion method is used for all the samples, as it is a fast, safe and cost-effective way to determine the amounts of nitrogen and several other elements. Kjeldahl wet digestion is performed for the juice and pulp samples from a batch harvested in 15th Oct. 2019. This is done to validate the results of the nitrogen content in the biomass, as the Kjeldahl method is considered more reliable. It has to be noted, that the Kjeldahl method gives a result for the whole green juice sample, not just the juice dry matter.

The pulp is dried prior to the analysis in the oven at 37 °C for 24 hours, to ensure that biomass is completely dry. Four samples are taken for each harvested batch. The approximation of protein content is done based on the total nitrogen content of the raw material, and Eq. 2.2 with the k factor of 6.00 is used to calculate the conversion from nitrogen to protein. This is assumed to give more accurate results than using the traditional factor of 6.25, which tends to overestimate the protein content, and as the Dumas combustion has shown to measure higher nitrogen contents compared to Kjeldahl digestion.

4.6 Chlorophyll content of pulp

Chlorophyll can be extracted from biomass by using ethanol as a solvent, and the ethanol extraction is performed sequentially after Soxhlet lipid extraction. Therefore, the mass of the sample is lower than the initial mass, as the n-hexane soluble fraction is already extracted, which is noted in the mass balance calculations. Similarly to lipid extraction, the volume of solvent is 150 mL, and the extraction is run for 2 hours using 100 mL Soxhlet extraction apparatus. Relatively short extraction time is chosen to prevent the chlorophyll degradation. During the Soxhlet extraction, the round-bottom flask is shielded from light and the heating mantle is set to a low temperature, as chlorophyll is sensitive to UV light and heat. Prior to the spectrophotometry, the extract is sealed and kept in the refrigerator at 4 °C to prevent chlorophyll degradation, as chlorophyll is also sensitive to oxidative degradation [80, 152].

The concentration of chlorophyll in ethanol solution is determined by measuring the absorbance using Agilent Technologies Cary 60 UV-Vis spectrophotometer with the wavelengths of 649 nm and 664 nm, as described in section 2.2.3. The absorbance is measured five times for each wavelengths, and the mean values are used in the calculations.

The chlorophyll content was planned to be determined for all *S. dolichostachya* pulp batches. However, due to the spread of novel coronavirus COVID-19 in Denmark, the limited access to the laboratory facilities allowed the analysis only for 4th December 2019 picked shrubs.

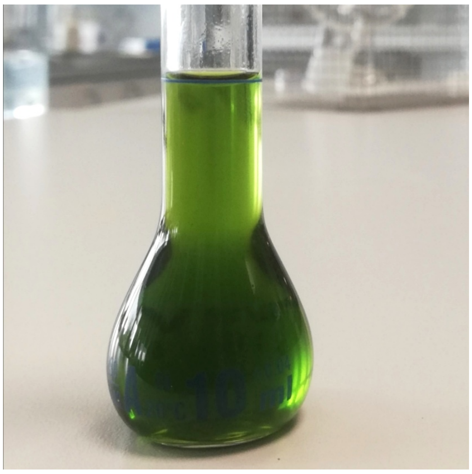


Figure 4.4: EtOH extract from 4th Dec. 2019 picked biomass

4.7 Sugar characterisation

In an aim to evaluate the total carbohydrate content of *S. dolichostachya* biomass bound in the lignocellulosic structure, HPLC analysis is performed for hydrolysates from both pulp and juice fractions. The sugar monomers are released from the juice and pulp with acid hydrolysis prior the HPLC. Dilute acid hydrolysis is applied for liquid fraction and strong acid hydrolysis for solid fraction. The sugar characterisation is performed by using the standard operation protocol which is modified according to ASTM standard E1758-01.

The standard has set the mobile phase used in the HPLC apparatus to be 0.005 molar H_2SO_4. The refractive index detector is used to identify the compounds exiting the column and the identification is done under a constant temperature of 5 °C. The analysis is performed using an organic acid column, as it is known to be suitable for straw and grass biomass and the method can determine the amounts of glucose, xylose and arabinose in the sugar solution. Those are also the types of sugars determined in the study by Cybulska et al. [46] for *S. bigelovii*. The oven with the column is set to be 63 °C for the sugar analysis and the flow rate inside the machine is 0.6 mL min^{-1}, which is the maximum allowed value for the used column. Standard samples and recovery standards are run at first before *S. dolichostachya* sugar samples.

4.7.1 Standard solution preparation

The baseline for analysis is set by running plain H_2SO_4 samples and recovery standard samples with known sugar concentration, also known as spiking samples. The spiking solution is prepared by weighting 3.00 g D-xylose and L-arabinose and 3.30 g D-glucose-monohydrate (dry) separately and transferring substances to a 100 mL measuring flask, which will be filled to mark with ultrapure water. Spiking samples are used to determine the sugar recovery factor, that accounts the sugar degradation and loss in hydrolysis process.

4.7.2 Weak acid hydrolysis of juice sample

Weak acid hydrolysis is used to hydrolyse the oligosaccharides present in the juice to sugar monomers. Weak acid hydrolysis is used for liquid phase materials, and reaction conditions are relatively mild, as only 8 w% H_2SO_4 is used as reagent.

Two recovery standards and two plain samples are run for each harvested batch of *S. dolichostachya*. A sample size of 10 g of juice is weighed to 60 mL glass tubes. Then, 10 mL of 8 w% H_2SO_4 is added to each sample tube and two empty reference tubes. For reference tubes and samples selected to be spiked, 1 mL of spiking solution is added. Glass tubes are closed with screw caps and thoroughly mixed with inversion before autoclaving in 121 °C for 10 minutes. Samples are cooled to room temperature and kept in the fridge (4 °C) for further analysis.

4.7.3 Strong acid hydrolysis for the pulp biomass

Strong acid hydrolysis is performed to release the sugars from solid pulp biomass. Prior to the process, an 8 hour ethanol extraction is performed for finely milled pulp to ensure the removal of all non-structural compounds. Using only the extractive free fraction of *S. dolichostachya* makes the process easier, as some compounds, such as waxes and fats, are already extracted prior to the strong acid hydrolysis. Also, as most of the salts are expected to be present in the juice faction, the pulp is assumed to have a low salt content. The salts present in the pulp are expected to be extracted even if salts are only sparingly soluble in ethanol.

The extractive free sample material is dried at 45 °C to at least 85 w% DM. The used sample size is 0.16 g and four samples are run for each *S. dolichostachya* batch, in which two are recovery standard samples. The strong acid hydrolysis consist of two reactions, where the sample is first made soluble to strong acid in lower temperature (30 °C) and hydrolysed in dilute acid at higher temperature (121 °C) afterwards. Strong 12 M (72 w%) and dilute 0.42 M (2.5 w%) H_2SO_4 are used as a reagent. Reaction time for each phase is set to be 60 ± 1 minutes and time is controlled with a stopwatch.

Samples are weighed and transferred to 60 mL glass tubes. Then, 1.5 mL of 72 w% H_2SO_4 is added to all sample tubes and two empty reference tubes. Tubes are incubated in 30 °C water bath for an hour, and vortex mixed every 20 minutes to ensure the biomass is in contact with the acid and stirred evenly. When the reaction time has passed, the samples are transferred to an ice bath and 42 mL of ultrapure water is added to dilute the acid and stop the reaction. It has to be noted, that only 41 mL of ultrapure water is used for reference tubes and the two samples that are selected to be spiked for recovery standards. For the spiking samples, 1 mL of spiking solution is added. The samples are then mixed by inversion and autoclaved at 121 °C for an hour.

The cooled samples are filtered using vacuum filtration through weighed filter crucibles. The ceramic crucibles are prepared prior to the analysis by cleaning with pressurised air, rinsing with ultrapure water and heating in the oven for one hour at 550 °C. Clean filter crucibles are kept in the oven at 105 °C and cooled down to room temperature in desiccator before use. The liquid hydrolysate is collected and used for further HPLC analysis. Lignin content is determined from the insoluble residue.

4.7.4 Klason lignin

Klason lignin can be determined from the solid residue after strong acid hydrolysis. The remaining material is washed carefully with hot ultrapure water to remove the remaining acid. The filter crucibles with samples are dried in the oven and cooled down to room temperature in the desiccator. Then, samples are weighed and ashed in the muffle furnace at 550 °C for three hours. Ashed samples are cooled in desiccator and weighed again. The lignin content of the sample is calculated from the weight difference, of the initial biomass and the residue, and the results are collected to Tab. 5.7. If the filter crucible weighs the same before adding the solids and after combustion in the muffle furnace, the upstream extraction has efficiently removed all salts, minerals, and acid-insoluble material apart from Klason lignin.

4.7.5 Sample preparation for HPLC analysis

Initial juice samples for free sugar determination are prepared by centrifuge for 10 minutes at 4000 rpm. As the sugar monomers are water soluble, the supernatant is assumed to contain all sugars in the juice. The supernatant is filtered through a 0.22 µm syringe filters to vials and kept in the refrigerator prior the HPLC. This step should only be done with fresh juice, as the free sugar monomers in the green juice can easily ferment into several different organic acids, such as acetic acid, lactic acid and malic acid and other organic compounds.

Juice samples from weak acid hydrolysis are transferred to smaller test tubes and centrifuged 5 minutes at 4000 rpm. The pH level of supernatant is adjusted to be 2-3 with sodium hydroxide (NaOH) to be suitable for the HPLC analysis with organic column. Then, the pH adjusted hydrolysate is filtered through 0.22 µm syringe filters to vials and kept in the refrigerator.

Hydrolysed samples from pulp biomass are prepared by weighting 0.50 g of barium hydroxide ($Ba(OH)_2$), which is a strong base, to plastic tubes. Then, 5 mL of hydrolysate sample from the strong acid hydrolysis is measured to each tube. The tubes are carefully mixed by inverting and using a vortex mixer. Tubes are left standing for 5 minutes and then mixed and inverted again. As the solubility of $Ba(OH)_2$ is not very high in water at low temperature, the solid particles need to be separated prior the analysis. Tubes are centrifuged for 5 minutes with 4000 rpm. The supernatant is decanted and pH is adjusted to be 2-3. Then, 1 mL of supernatant is filtered through a 0.22 µm syringe filter to a vial and kept in the refrigerator, ready to be analysed.

4.7.6 Sugar recovery calculations

The amounts of different sugars are calculated based on the mass balances and HPLC results. To be able to calculate the total volume of the juice sample used in weak acid hydrolysis, the mean density of juice is determined by weighing ten samples with known volume, and calculating the densities. Knowing the total volume and DM content of the sample, the dry weight (DW) of the sample is calculated:

$$DW\ [g] = m_{sample} \cdot \frac{DM_{sample}\ [w\%]}{100} \tag{4.5}$$

Based on the dry weight, all the concentration results from HPLC are converted from [g/liter] to [g/100 gDM]. The recovery factor (R), which accounts for the lost sugars in the lignocellulose hydrolysis process, is calculated separately to each sugar by using Eq. 4.6.

$$R = \frac{C_{spiked\ sample}}{C_{added} + C_{sample}} \tag{4.6}$$

Where $C_{spiked\ sample}$ is the measured sugar concentration in spiked sample [g/100 gDM], C_{added} is the concentration of sugar in spike solution [g/100 gDM], and C_{sample} concentration of sugar in the corresponding sample without spiking [g/100 gDM]. Then, the corrected sugar concentrations are calculated:

$$C_{corrected}\ [g/100gDM] = \frac{C_{sample}}{R} \tag{4.7}$$

Concentrations of oligomeric sugars glucan, xylan and arabinan are determined by using the conversion factor for each corresponding sugar monomer. A conversion factor of 0.90 is used for 6-carbon sugars, and 0.88 for 5-carbon sugars.

$$C_{oligomeric}\ [g/100gDM] = C_{corrected} \cdot Conversion\ factor \tag{4.8}$$

4.8 Hydrothermal pretreatment

Based on previous research of lignocellulosic biomass, hydrothermal pretreatment is planned to be investigated for each picked batch of fresh *S. dolichostachya* pulp and extractive free fibres, to see the possible a seasonal variation in the effect of pretreatment. As the biomass is already processed several times with extractions, the remaining fibre fraction is assumed to be less recalcitrant than the fresh, unprocessed biomass. The planned pretreatment conditions were 6 w% dry matter loading, and 76 minutes in 140 °C, based on previous hydrothermal pretreatment results [48, 99, 101]. The severity factor, SF = 3.06, is calculated using Eq. 2.3, and same severity factor was also tested by Alassali et al. [48] and yielded the best ethanol recovery for *S. sinus-persica*. The lower temperature and longer residence time are selected, as the pretreatment is wanted to perform in an autoclave.

However, due to the spread of novel coronavirus COVID-19 in Denmark, the experiments were cancelled as there was no access to the laboratory facilities. However, the pretreatment is still taken into account in process simulation, by applying the pretreatment unit with planned conditions, and using the convertability of 76.9 w% based on the results by Alassali et al. [48].

4.9 Lignocellulose-to-lipids conversion

As the demand for heavy transportation fuel is higher than for bioethanol, and the lipid is a better feedstock for processing liquid biofuels than lignocellulose [153], it is desired to study the biochemical conversion from lignocellulose to lipids. This part of the project is done in a co-operation with ENORM ApS, a company specialised in production and processing of black soldier fly (*Hermetia illucens*) larvae. Two different experiments are done to see, how well BSFL can digest lignocellulosic biomass. In the first experiment, fresh and green screw-pressed *S. dolichostachya* pulp harvested 26th Sep. 2019 is fed to BSFL in different ratios: 100 w% of *S. dolichostachya*, and 40/60 w% blend of *S. dolichostachya* and fermented chicken-feed, respectively.

In the second experiment, BSFL are fed with 40/60 w% of the extractive-free fibre fraction of *S. dolichostachya* sampled 4th Dec. 2019 and chicken-feed, respectively. The dry matter of both feeds are mixed, and blended with water to achieve the total dry matter content of 30 w%. 4.35 w% of dairy product, A38 by Arla, containing *Lactobacillus acidofilus* is added to the mixture, and the feed is fermented at 29 °C for 24 hours. The total mass of feedstock is 1150 g, consisting of 50 g A38, 770 g tap water, 198 g chicken-feed and 132 g extractive-free fibre fraction of *S. dolichostachya*. This feed can be used for approximately 1730 larvae.

Figure 4.5: The ready mixed feed for the second trial before fermentation

The fermented chicken-feed is currently the used feed for BSFL at ENORM ApS, and it is also used as a control batch for both experiments, and as a base for comparison. After a two week growth period, BSFL are devitalised with steam, rinsed from external impurities and frozen for later analysis. The physical and biochemical properties of BSFL are compared to see the effect of nutrition to size and growth of BSFL. Comparison is done by visual observations and measuring. Length of the larvae is noted, and the frozen BSFL are homogenised with Retsch GM 200 knife mill with the rotating speed of 10,000 rpm for 45 seconds. The water content of BSFL is high, up to 80 w%, hence the BSFL are dried in 50 °C oven for 48 hours.

Lipid content of BSFL dry matter is determined by Soxhlet extraction, as described in section 4.3 using n-hexane as a solvent for approximately 2 hours. Also, the amount of ethanol and water extractive material in BSFL is determined parallel by 6 hour Soxhlet extraction. Approximately 3 g of BSFL is used for each extraction, and triplicates are taken from each sample.

4.10 Statistics

The amount of each biochemical compound X (ash, lipids, protein or carbohydrates) is calculated separately for juice and pulp fractions, excluding the 4th Dec. 2019 harvested biomass, which is considered as a whole shrub. Result values shown in this report are mean values of three samples, unless anything else is stated, and the sample standard deviations are calculated for all of the result values with three or more sample values.

However, it is useful to know the content of each compound in the total dry matter of the biomass. Therefore, the total values are calculated for each harvested batch by using Eq. 4.9.

$$X_{total} = \frac{X_{pulp} \cdot m_{DM,pulp} + X_{juice} \cdot m_{DM,juice}}{m_{DM,total}} \tag{4.9}$$

Where $m_{DM,pulp}$ and $m_{DM,juice}$ are the masses of obtained dry matter from pulp and juice fractions, and $m_{DM,total}$ being the sum of $m_{DM,pulp}$ and $m_{DM,juice}$.

The statistical total variance of multiple sample sets can be calculated as the sum of multiple variances, if the variances are from independent normally distributed random sample sets. This means that a total standard deviation can be calculated as the square root of the weighted total variance as shown in Eq. 4.10.

$$\sigma_{total} = \sqrt{\frac{\sigma^2_{X,pulp} \cdot m_{DM,pulp} + \sigma^2_{X,juice} \cdot m_{DM,juice}}{m_{DM,total}}} \tag{4.10}$$

Chapter 5

Results from biomass processing

5.1 Biomass characterisation

In this chapter, the results of the biomass characterization are shown, and the results are used as a base for the biorefinery simulation described in section 6.1.

5.1.1 Fractionation and dry matter

As other succulent plants, *S. dolichostachya* accumulates high amount of water to its tissues, and the juice fraction covers up to 69.1 w% of the total biomass. The shares of liquid and solid fractions are shown in Table 5.1. A small amount of biomass was lost in the juicing process. The green fractionation of *Salicornia* spp. has not widely studied, but the resulted fractions of 26th September 2019 harvested biomass are very similar to the study of Alassali et al. [48] (67.8 w% juice), which indicates that the succulent biomass is harvested nearly similar stage of growth.

Date picked	Juice [w%]	Pulp [w%]	Waste [w%]
26th Sep. 2019	69.1	28.0	2.9
15th Oct. 2019	61.6	35.7	3.7
11th Nov. 2019	47.5	48.3	4.2

Table 5.1: Green fractionation of harvested *S. dolichostachya* biomass. Waste is the amount of biomass lost in the juicing process.

It was expected, that the share of solid fraction and the dry matter content increases as the plant gets more woody and the shrub loses the succulent structure. This trend can bee seen in Tab. 5.2. It has to be noted, that *S. dolichostachya* biomass picked in 4th Dec. 2019 is treated as a whole shrub and not juiced, and the sample of 4th Dec. 2019 is considered to have some remaining moisture trapped in the biomass which can not be evaporated, as it has not been fractionated.

Date picked	Juice DM [w%]	Pulp DM [w%]	Total DM [w%]
26th Sep. 2019	5.3	23.4 ± 0.3	10.2
15th Oct. 2019	2.9	24.9 ± 2.3	10.7
11th Nov. 2019	3.0	27.0 ± 0.1	9.6
4th Dec. 2019	n/a	n/a	16.6 ± 0.14

Table 5.2: Dry matter content of *S. dolichostachya* pulp and juice

The dried and milled biomass from different batches are very similar by texture, and only small changes in colour can be observed in the dry matter of pulp and juice, which is shown in Fig. 5.1 and 5.2, respectively.

Figure 5.1: Dry matter of *S. dolichostachya* pulp. From left: 26th Sep. 2019, 15th Oct. 2019, 11th Nov. 2019 and 4th Dec. 2019

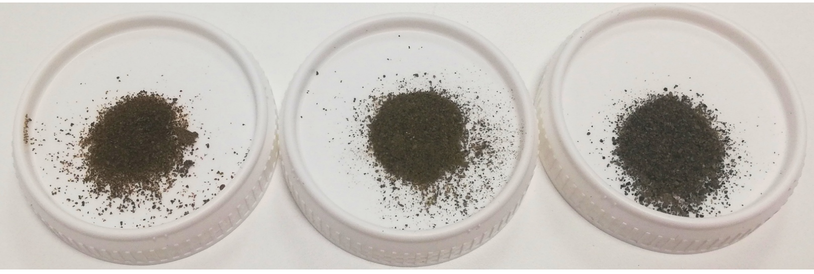


Figure 5.2: Dry matter of *S. dolichostachya* juice. From left: 26th Sep. 2019, 15th Oct. 2019 and 11th Nov. 2019

As the 4th December 2019 harvested shrub DM will be fed to BSFL in the second feed experiment, as described in section 4.9, the texture is further analysed. When DM is passed through 0.5 mm sieve, it is separated to small dusty particles, and long thin fibrous particles. The consistence of DM can be seen in Fig. 5.3. Fibres are covering 29 w% of the total mass of the DM. The dusty dusty part, including the seeds, covers 71 w% of the total mass of the DM. It is assumed, that most of the n-hexane and EtOH extractive material will be present in the dusty fraction.

Figure 5.3: Fibrous (left) and dusty dry matter of 4th Dec. 2019 picked *S. dolichostachya* shrubs

5.1.2 Ash

The results of ashing show, that the ash content of *S. dolichostachya* decreases as the plant matures. In juice, more than half of the dry matter is consisting of salts and minerals when the plant is still green succulent. Considering the DM content, the total amount of salts and minerals in juice is 7.4 - 35.1 g/L. When the plant matures, the fraction of ash in juice DM decreases, as the small seeds pass the juicer sieve and therefore, are present in the juice DM. For pulp fractions, the decrease in ash content is more moderate, but notable especially between the 26th Sep. 2019 and 15th Oct. harvests. Results are similar to the values determined by Alassali et al. [48] for green succulent *S. sinus-persica*, finding the ash content of juice and pulp dry matters to be 61.12 w% and 13.19 w%, respectively.

Date picked	Ash_{juice} [g/100 gDM]	Ash_{pulp} [g/100 gDM]	Ash_{total} [g/100 gDM]
26th Sep. 2019	66.2 ± 0.34	13.5 ± 0.04	32.3 ± 0.20
15th Oct. 2019	50.5 ± 0.23	6.4 ± 0.13	13.8 ± 0.13
11th Nov. 2019	35.7 ± 0.85	5.5 ± 0.03	8.5 ± 0.26
4th Dec. 2019	n/a	n/a	8.5 ± 0.10

Table 5.3: Ash content of *S. dolichostachya*

Visual observations from Fig. 5.4 and Fig. 5.5 also show the changes in the colour of ash. This indicates the changes in the elemental composition and properties of the ash. Changes are similar for both pulp and juice ash, where the grey colour turns more red when the plant get lignified, and the red colour can be sourced from oxidised iron present in ash. Different minerals present in the ash could be detected with atomic absorption spectrometry, but it is not included to project scope.

Figure 5.4: The ash of *S. dolichostachya* pulp. From left: 26th Sep. 2019, 15th Oct. 2019 and 11th Nov. 2019

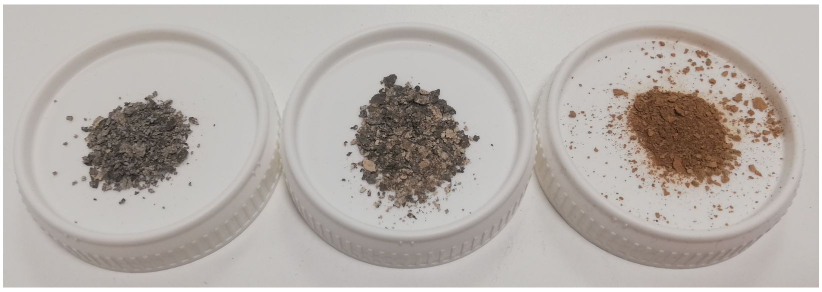


Figure 5.5: The ash of *S. dolichostachya* juice. From left: 26th Sep. 2019, 15th Oct. 2019 and 11th Nov. 2019

5.1.3 Content of extractive material

The extractions from pulp biomass are done in sequence. In the first extraction, n-hexane is used to separate lipids. The following ethanol extraction separates chlorophyll, most of the salts, possibly some of the protein and other structural compounds. As expected, the lipid content of the pulp increases over time, which is due to the seed production of plants. The corresponding lipid fractions of the fresh weight are between 1.2 - 1.8 w%, which is within the same range as results find by Patel et al. [13] for succulent halophytes. Similarly to the ash content, the amount of ethanol extractive material is decreasing over time, being almost negligible in 4th December harvested biomass.

Date picked	n-Hex. ext. [g/100 gDM]	EtOH ext. [g/100 gDM]	Fibres [g/100 gDM]
26th Sep. 2019	5.3 ± 0.2	10.9 ± 1.0	83.9 ± 1.0
15th Oct. 2019	6.1 ± 0.0	6.8 ± 0.0	87.1 ± 0.0
11th Nov. 2019	6.6 ± 0.0	3.3 ± 0.0	90.0 ± 0.0
4th Dec. 2019	8.3 ± 0.0	0.0	91.7

Table 5.4: Content of n-hexane extractives (lipids), EtOH extractives (chlorophyll + others) and lignocellulosic fibres in *S. dolichostachya* pulp after green fractionation. Not all extractions had enough data to be able to give a standard deviation.

The chlorophyll content of 4th December 2019 picked shrubs is determined to be 15.7

mg/kgFW, which is significantly lower than the chlorophyll content of *S. bigelovii* determined by Lu et al. [53] (569.1 mg/kgFW). The reason for the very low value is due to chlorophyll degradation, as the sample was kept in the laboratory room temperature of 22 °C and exposed to light from early December 2019 to the day of extraction, April 2020. In the study by Lu et al. [53], the total chlorophyll content is shown to decrease with 30 w% after eight days at the ambient temperature of 25 °C, which explains the great degradation in the longer time period. Thus, chlorophyll content should have been measured right after harvesting.

The amount of lipids present in the juice fraction is determined by liquid-liquid extraction using different initial mixtures described in section 4.4. In the method no. 1, the volumetric fractions of cyclohexane, juice and i-propanol are 14.3 v%, 19.0 v% and 66.7 v%, respectively. In the method no. 2, corresponding fractions are 29.0 v%, 12.9 v% and 58.1 v%, respectively. In the method no. 3, the corresponding fractions are 37.8 v%, 8.9 v% and 53.3 v%, respectively. Lipid yields are collected to Fig. 5.6, and it is possible to see the trend of the lipid content increasing over time. The lipid yield also increases, when the volume fraction of cyclohexane in the initial mixture increases. However, only the results of using the least amount of cyclohexane (method 1), fit into the undefined fraction shown in Fig. 5.10. The two other extractions show very high lipid content, up to 94 w% of juice DM. This conflicts with previous characterisation results. Possible reasons for unsuccessful experiments are the errors in measuring small masses, pipetting solid particles into the non-polar fraction, having some unevaporated leftover solvents, n-hexane or i-propanol, inside the vials.

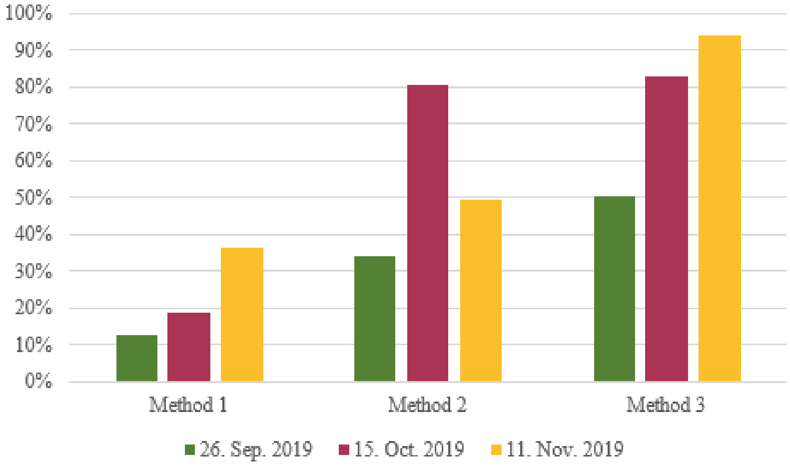


Figure 5.6: Lipid yield from *S. dolichostachya* juice based on the initial mixture of cyclohexane, juice and i-propanol [v%] of liquid-liquid extraction

5.1.4 Elemental composition and approximated protein

The total nitrogen content of samples from 15. Oct. 2019 are determined with both Kjeldahl and Dumas method, and rest on the samples are analysed only with Dumas method. For the juice samples, the mean nitrogen content determined with Kjeldahl method is very low, only 0.07 w%, corresponding to 0.41 w% protein content. For the pulp fraction, the mean nitrogen content of seven analysed pulp samples is 1.55 w%, corresponding to 9.3 w% protein. The total nitrogen content of pulp is similar to the results gained from Dumas combustion method, where the nitrogen content is only slightly higher. The results from Dumas method application can be seen from Tab. 5.5.

Date picked	N [w%]	C [w%]	H [w%]	S [w%]
Juice DM samples				
26th Sep. 2019	3.37 ±0.18	37.99 ±0.49	5.06 ±0.69	0.72 ±0.12
15th Oct. 2019	3.20 ±0.15	34.24 ±0.42	4.76 ±0.30	0.73 ±0.02
11th Nov. 2019	3.40 ±0.19	38.46 ±0.39	6.04 ±0.19	0.81 ±0.06
Pulp DM samples				
26th Sep. 2019	1.96 ±0.04	43.18 ±0.34	5.82 ±0.52	0.48 ±0.03
15th Oct. 2019	1.71 ±0.10	45.82 ±0.07	5.83 ±0.36	0.47 ±0.02
11th Nov. 2019	1.82 ±0.10	44.99 ±2.34	5.17 ±0.76	0.37 ±0.08

Table 5.5: Elemental composition of *S. dolichostachya* biomass. Machine accuracy ± 1 %.

The approximated protein content of *S. dolichostachya* is shown in Tab. 5.6. It has to be noted, that even if the protein content of juice DM seems relatively high, the dry matter content of the juice is low. Therefore, protein content of content of juice DM corresponds to 6.1 - 10.1 g/L of protein, content being highest in 26th Sep. 2019 and decreasing only slightly as the plant matures. Results for the pulp fraction and total dry matter are similar to protein content of other previously studied *Salicornia* species [48, 59].

Date picked	Protein [g/100 gDM]
Juice DM	
26th Sep. 2019	19.08±0.01
15th Oct. 2019	18.88±0.00
11th Nov. 2019	20.38±0.01
Pulp DM	
26th Sep. 2019	11.78±0.00
15th Oct. 2019	10.26±0.00
11th Nov. 2019	10.92±0.01
Total DM	
26th Sep. 2019	14.39
15th Oct. 2019	11.71
11th Nov. 2019	11.84

Table 5.6: Approximated amount of protein in *S. dolichostachya* pulp and juice

5.1.5 Klason lignin and acid-soluble material

Klason lignin content of the pulp was determined from the solid residue of extractive-free fibres after a strong acid hydrolysis. As shown in Tab. 5.7, the amount of lignin in the biomass increases over time. This is an expected results, since the plant lignifies and turns more woody as it matures. Compared to previous studies for matured *S. bigelovii* (7 %) [39] and 23 different annual and perennial halophytes (2-10 %) [41], the lignin content of *S. dolichostachya* is higher, up to 14 %.

Component(s)	Content [g/100 gDM]
26th Sep. 2019	
Acid-solubles	74.10 ± 0.08
Klason lignin	9.81 ± 0.29
15th Oct. 2019	
Acid-solubles	75.19 ± 0.35
Klason lignin	11.95 ± 0.35
11th Nov. 2019	
Acid-solubles	75.85 ± 0.90
Klason lignin	14.19 ± 0.75

Table 5.7: Klason lignin content of *S. dolichostachya* pulp. Acid-soluble material does not include the fractions of n-hexane and ethanol extractive material.

5.1.6 Sugars in *S. dolichostachya*

Amounts of different monosaccharides can be read as a percentage of area under peaks of chromatogram, and HPLC chromatograms are collected to Appendix A. After the calculations, the amount of different sugars in the pulp and juice are collected to Tab. 5.8 and Tab. 5.9. The content of different sugars in hydrolysed juice and pulp are calculated using equations described in section 4.7.6. The recovery factor for all sugars after strong acid hydrolysis, and arabinose after weak acid hydrolysis, varies between 0.69 and 0.98. The recovery factors of glucose and xylose from weak acid hydrolysis are lower, 0.41 - 0.54. Low recovery factor indicates, that some of the sugars have degraded and degradation products, such as furfural, could be found from the hydrolysate.

For the pulp fraction, the results show a steady increase in the amounts of glucan and xylan. Only a small decrease can be seen in the content of arabinan in 11. Nov. 2019 sample. Glucan is the most abundant type of sugar, covering approximately 50 w% of the sugars present in the pulp biomass, and it also covers 20-25 w% of the whole pulp DM, shown in Fig. 5.7. The contents of glucan and xylan in *S. dolichostachya* pulp are higher compared to previous studies, but Alassali et al. [48] shows almost the double amount of arabinan in the solid fraction of *S. sinus-persica*. Sugar content is also much higher compared to dried and untreated *S. bigelovii* studied by Cybulska et al. [39]. However, the same study shows the increase in the glucan content of washed biomass (approximately 20 g/100 gDM), which is in the same range as the amount of glucan in *S. dolichostachya*.

Date picked	Glucan [g/100 gDM]	Xylan [g/100 gDM]	Arabinan [g/100 gDM]
26th Sep. 2019	19.88±0.09	12.23±0.08	5.24±0.10
15th Oct. 2019	21.55±0.08	13.11±0.09	6.57±0.01
11th Nov. 2019	24.53±0.17	14.86±0.16	6.22±0.00

Table 5.8: Oligomeric sugars in *S. dolichostachya* pulp after green fractionation and strong acid hydrolysis

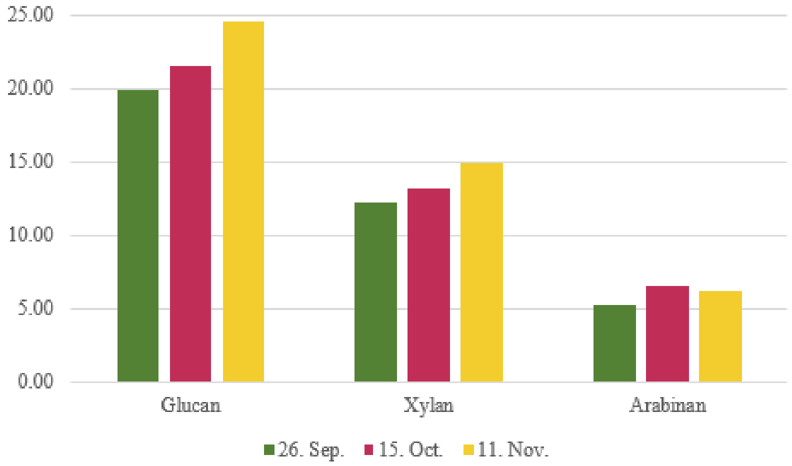


Figure 5.7: Sugar composition [g/100 gDM] of *S. dolichostachya* pulp DM

The amount of free sugars in the juice is similar to the results from Alassali et al. [48]

for *S. sinus-persica* (1.0 - 1.5 %). In 26th September 2019 harvested biomass, the content of free sugars in the highest (2.5 g/100 gDM), and the amount decreases below 1.0 g/100 gDM as the plant matures. The amount of oligomeric sugars are calculated to hydrolysed juice samples, and it can be seen, that sugar content increases drastically after 15th Oct. 2019. The results show a small decrease in glucan and xylan content between 26. Sep. 2019 and 15. Oct. 2019, and only the amount of arabinan is increasing. However, the big change is occurring after 15. Oct. 2019, as the amount of sugars in the juice DM from 11. Nov. 2019 harvested biomass is nearly four times higher than previously, glucan content being up to 4.6 g/100 gDM, as shown in Fig. 5.8.

Type	Glucan [g/L]	Xylan [g/L]	Arabinan [g/L]	Total [g/L]
26th Sep. 2019				
Free sugar	1.12±0.01	1.34±0.01	0.04±0.00	2.50
Hydrolysed	0.76±0.01	0.61±0.01	0.16±0.13	1.53
15th Oct. 2019				
Free sugar	0.50±0.01	0.56±0.01	0.13±0.00	1.19
Hydrolysed	0.36±0.01	0.22±0.01	0.18±0.01	0.76
11th Nov. 2019				
Free sugar	0.45±0.014	0.39±0.01	0.10±0.00	0.94
Hydrolysed	1.37±0.03	0.85±0.02	0.77±0.02	2.99

Table 5.9: Free and hydrolysed oligomeric sugars in *S. dolichostachya* juice. Note: free sugar concentrations are given to corresponding sugar monomers.

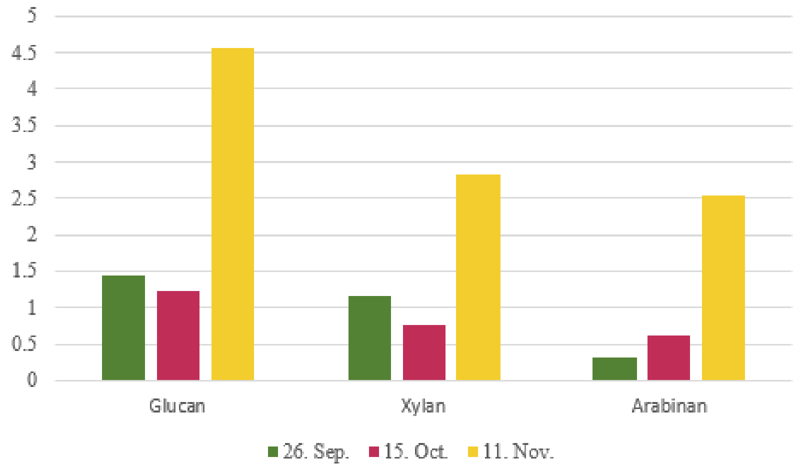


Figure 5.8: Sugars in *S. dolichostachya* juice DM [g/100 gDM]

5.1.7 Characterisation overview

The information gained from each characterisation method is collected to Fig. 5.9 and Fig. 5.10. Lignocellulose is the major biochemical fraction in *S. dolichostachya* pulp, covering in total 47.21 - 59.8 w% of DM. Protein content is similar to other *Salicornia* spp. and the content is relatively constant throughout the harvesting period. Lipid content is low, similarly to other succulent halophyte plants, but the amount increases when the plant start to produce seeds rich in oils. The ash content of the biomass decreases over time, and visual observations indicate the changes in the mineral composition. Approximately 11 - 16 w% of the pulp DM is left undefined, which is the fraction of dry matter that is left after mass balance calculations, when all the experimental results are considered.

Halophytes accumulate salts to their tissues, and as expected, ash content of *S. dolichostachya* juice DM is up to 66.2 w%, due to the presence of water soluble salts. However, as the plant matures, the ash content decreases almost to half, whereas the amount of sugars and lipids are increasing. The increasing in the lipid content can be indicated from liquid-liquid extraction results, and from increase of undefined fraction. The undefined fraction is the fraction of dry matter left after mass balance calculations, when all the experiment results, excluding lipid extraction, are considered. Similarly to the pulp fraction, the increase of lipid content in due to the seed production, as small seeds pass the juicer sieve and therefore are present in the juice DM. The amount of free sugars in the juice is similar to results from previous research. Protein content of juice DM covers up to 20.4 w% of the juice DM, and it stays constant over harvesting period. Therefore, protein precipitation from juice could be a potential value-added source in biorefinery.

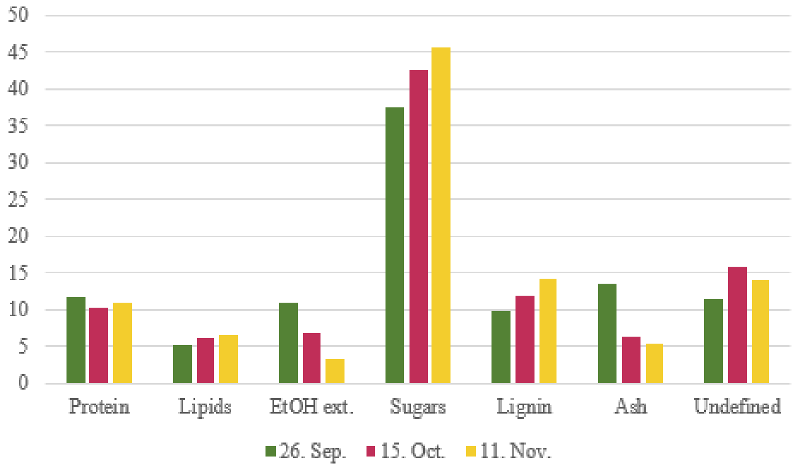


Figure 5.9: Seasonal variation of *S. dolichostachya* pulp dry matter composition. Content of different biochemical groups given as [g/100 gDM].

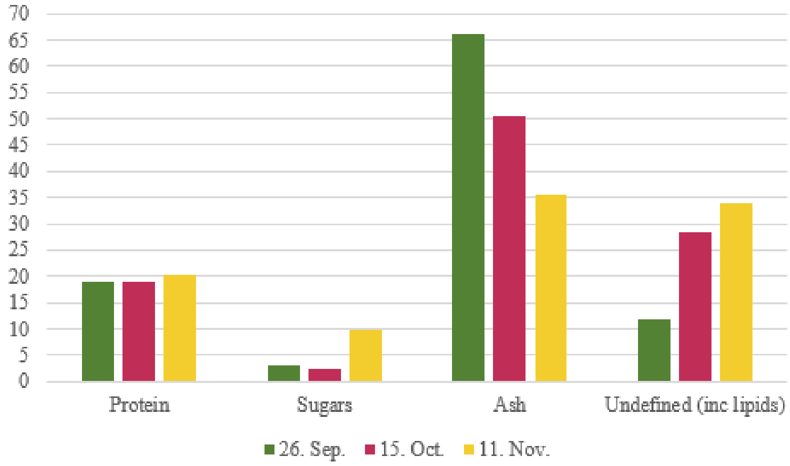


Figure 5.10: Seasonal variation of *S. dolichostachya* juice dry matter composition. Content of different biochemical groups given as [g/100 gDM].

From the experimental characterization work, the key results are collected and summarised to the list below:

- Juice fraction covers 45.5 - 69.1 w% of the total mass of a succulent plant. The juice fraction decreases, as the plant matures and lignifies.

- Ash covers 8.5 - 32.3 w% of the total dry matter, and the ash content is very high especially in the juice fraction due to water soluble salts. The ash content decreases drastically as the plant matures. The changes in the ash colour indicates the changes in the mineral composition.

- Lipid content of the succulent halophyte is low, only 1.2 - 1.8 w% of the total fresh biomass. The amount of lipids increase over time, when the plant produces oil-containing seeds.

- The largest fraction of the biomass is lignocellulose, covering up to 59.8 w% of the total dry matter. Glucose is the most abundant sugar monomer present in the dry matter. The total amount of sugars increases over time in both fractions, but the change is the most significant in the juice fraction between 15th October 2019 and 11th November 2019.

- As the plant lignifies, the Klason lignin content increases from 9.8 g/100 gDM to 14.2 g/100 gDM. The lignin content of *S. dolichostachya* is slightly higher compared to previous studies considering *Salicornia* spp.

- The amounts of protein in pulp and juice are approximately 10 g/100 gDM and 20 g/100 gDM, respectively, corresponding to total protein content of 12-14 g/100 gDM. The protein content stays nearly constant over time.

5.2 Analysis of black soldier fly larvae

Two feed experiments are carried out, and the 1st feed experiment uses fresh untreated *S. dolichostachya* pulp and chicken feed, with ratios described in section 4.9. The 2nd feed experiment used dried, milled, and n-hexane and ethanol Soxhlet extracted whole *S. dolichostachya* shrubs, also with ratios as described in section 4.9.

From Fig. 5.11 and Fig. 5.12 shows, how 100 w% *S. dolichostachya* fed BSFL are smaller and less matured compared to two other samples. However, the BSFL fed with the mixed feedstock, in both feed experiments, seem to be further in the life cycle compared to entirely *S. dolichostachya* fed. This might be due to the depletion of accessible feed at the time of the harvest.

Figure 5.11: BSFL from the 1st feed experiment. From left: 100 w% Sal., 40 w%/60 w% Sal./CF and 100 w% CF

Figure 5.12: BSFL from the 2nd feed experiment: 100 w% CF (left) and 40 w%/60 w% Sal./CF fed BSFL

The measured length of the BSFL is the mean value of ten samples, and shown in Tab. 5.10. It can be seen, that the size of larvae decreases significantly, when fed with 100 w% *S. dolichostachya*. However, when *S. dolichostachya* is blended with previously used chicken feed, the change in the size of larvae is smaller. The results from the second feed experiments shows, that the larvae fed with 40 w% extractive-free *S. dolichostachya* are smaller than ones fed with 40 w% fresh biomass. However, the dry matter content of larvae from the second feed trial is higher than from the first feed trial.

Feedstock	Length [mm]	DM [w%]
1st feed experiment		
100 w% CF	16.6 ± 1.0	31.9
60 w% CF 40 w% Sal.	15.4 ± 0.8	25.3
100 w% Sal.	11.4 ± 1.6	11.8
2nd feed experiment		
100 w% CF	17.3 ± 1.6	36.6 ± 1.4
60 w% CF 40 w% Sal.	13.6 ± 1.3	32.7 ± 0.4

Table 5.10: Physical characteristics and dry matter content of BSFL. CF: Chicken feed, Sal.: *S. dolichostachya*. *S. dolichostachya* used in 2nd feed experiment was assumed to be free from lipids, salts and phytochemicals.

The amounts of water, ethanol and n-hexane soluble materials in BSFL fed with different feedstock are reported in Tab. 5.11. Parallel extraction results show, that 100 w% CF fed BSFL has nearly same amount of ethanol and n-hexane extractive material. However, when BSFL is fed with 100 w% *S. dolichostachya*, the amount of ethanol extractive material is nearly double to the n-hexane extractive material, therefore, replacing part of the feed with *S. dolichostachya* might activate the production of some compounds, that are not present in the 100 w% CF fed BSFL oil and are not soluble in n-hexane. In Fig. 5.13, lipids from different feedstocks are shown, and it can be seen, that all the oils are in solid form in room temperature, which indicates a relatively high amount of saturated fatty acids. Visual observations also show a clear differences in colour and texture of BSFL lipids, when BSFL is fed entirely with CF or partly with extractive-free, 4th December 2019 harvested *S. dolichostachya*. Oil from partly *S. dolichostachya* fed BSFL is more green, and the texture is more smooth compared to lipids from entirely CF fed BSFL. These changes indicate the differences in fatty acid profiles.

Feedstock	Water [g/100 gDM]	EtOH [g/100 gDM]	n-hex. [g/100 gDM]
1st feed experiment			
100 w% CF	n/a	n/a	44.1 ± 0.7
60 w% CF 40 w% Sal.	n/a	n/a	35.8 ± 0.5
100 w% Sal.	33.9	20.6	12.0
2nd feed experiment			
100 w% CF	24.3	38.9	37.4 ± 0.6
60 w% CF 40 w% Sal.	20.8	24.8	22.5 ± 0.3

Table 5.11: Water, ethanol and n-hexane soluble material of BSFL. Extractions performed in parallel. Not all extractions had enough data to be able to give a standard deviation. CF: Chicken feed, Sal.: *S. dolichostachya*.

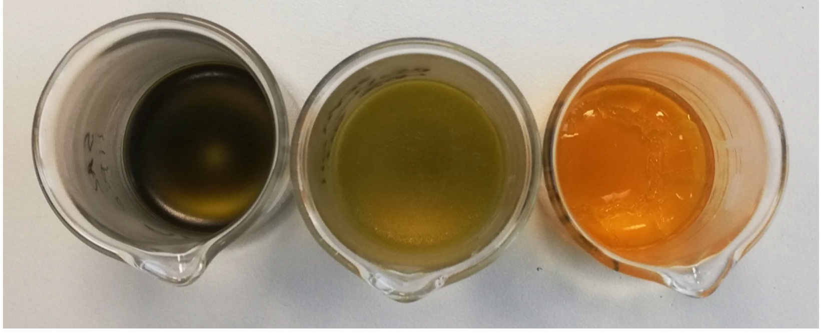


Figure 5.13: Lipids and other n-hexane soluble material from different feedstocks: 4th December 2019 harvested *S. dolichostachya* DM (left), 40 w% extractive-free Sal. fed BSFL and 100 w% CF fed BSFL

After the second feedstock experiment, the frass from the cultivation tray is isolated from the BSFL. The frass residue consists of undigested feedstocks, excrement and larvae exoskeletons. This is done to determine the amount of undigested matter, which is assumed to be *S. dolichostachya* lignocellulose. As seen in Fig. 5.14, the frass from 100 w% chicken-feed fed larvae has high amount of exoskeletons, which indicates that the larvae has grown to more mature stage. It is also possible to observe undigested fibres in the frass from 40 w% *S. dolichostachya* fed larvae.

Figure 5.14: Frass from 2nd feed experiment: 100 w% CF (left) and 40%/60% Sal./CF fed BSFL

Assuming all of the chicken-feed is consumed and the frass from the 100 w% CF fed batch of BSFL does not contain any digestible feed or BSFL exoskeletons, and consist entirely of excrement, the amount of excrement from chicken-feed can be calculated in the frass of the 60% and 40% chicken-feed and *S. dolichostachya*, respectively, fed batch by using Eq. 5.1.

$$w_{Sal.\ eaten} = \frac{m_{DM\ Sal.\ in\ mix} - m_{frass\ of\ mix} - m_{100w\%\ CF\ frass} \cdot 0.60}{m_{DM\ Sal.\ in\ mix}} \tag{5.1}$$

	100 w% CF	60%/40% CF/Sal.
Sal. in feedstock [g]	0.0	138.0
<0.5 mm particles in feed [w%]	n/a	71.0
Dry frass [g]	52.4	152.9
Frass from CF [g]	52.4	31.4
Sal. in frass [g]	0.0	121.4
Sal. eaten [w%]	0.0	12.0

Table 5.12: Frass analysis from 2nd feed experiment of BSFL

As shown in Tab. 5.12, only a small amount of extractive-free *S. dolichostachya* fibres were consumed by BSFL. Therefore, it does not seem to be an ideal feedstock for the BSFL farming. A reason for poor accessibility can be, that the extractive-free fibres were not soaked in water long enough to provide suitable consistency and texture for feedstock, as the provided extractive-free *S. dolichostachya* fibres consist of small dusty particles and longer, thin fibrous particles of up to 4 mm. As most the extractive material is assumed to be present in the dusty form, the fraction of fibres in the larvae feed will be higher than described in section 5.1.1. Another reason for poor accessibility can be, that the lignocellulosic structure of the extractive-free *S. dolichostachya* has not been broken down sufficiently during the fermentation for the BSFL to access, thus a hydrothermal pretreatment possibly followed by an enzymatic hydrolysis could improve the digestibility of biomass.

The key results from both BSFL feed experiments are collected and summarised:

- *S. dolichostachya* can be seen as a potential feed for BSFL farming when blended with currently used feedstock
- Too high amount of *S. dolichostachya* in feed has a negative effect to the growth, as BSFL seems to be more dependent on protein than sugars.
- Replacing part of the feedstock with halophyte based feed has an effect to the colour and texture of the BSFL lipid. This indicates the changes in the fatty acid profile.
- Fresh *S. dolichostachya* seems to be more suitable feedstock than extractive-free fibres. Pretreatment is needed to make the sugars from fibres more accessible to BSFL.
- As the amount of EtOH extractive material in 100 w% *S. dolichostachya* fed biomass is higher to the n-hexane extractive material, it would be worth investigating, if some of the phytochemicals are accumulating in the food chain.
- Farming residue, as known as frass, can be valorized and sold as a natural fertilizer

Chapter 6

Process simulation

The process simulation includes biomass processing and the HEFA process route to the production of sustainable aviation fuel. The biomass simulated is set to have the same properties and extraction characteristics as the *S. dolichostachya* sampled 11$^{\text{th}}$ November 2019, and the biofuel processing from BSFL lipids is based on the data from feeding trials and existing literature of the HEFA process. Two simulations are made separately, to ensure stable simulations that will reach convergence.

After each simulation has been run, the two simulations are merged in a single simulation, and different parameters will be investigated. These parameters are three different labour costs depending on the location of the biorefinery, biomass input mass flow rate, and biomass pretreatment before feedstock fermentation as a dummy variable. Each simulation will be presented as schematics in Fig. 6.2 and Fig. 6.9.

All heating and cooling units will be placed separate from reactors, evaporators, distillation towers, etc. as this will allow for inter-stream energy recovery heat exchangers.

6.1 Processing of *S. dolichostachya* and black soldier fly larvae

By an *in silico* analysis approach, using Intelligen SuperPro Designer (SPD) as process design tool, a simulation of a biomass processing plant using *S. dolichostachya* raw material is carried out. This simulation will refine the biomass into value-added streams as determined in the laboratory experiments, with the end product being lipids from BSFL fed partly with *S. dolichostachya* fibres. This end product will later in the HEFA process simulation react to become biofuels. Using the data from chapter 5, information from ENORM ApS and data from literature studies, it is possible to simulate the extraction yields, amount of extracts, operational costs (OPEX) and capital expenditures (CAPEX) and sizing of equipment and efficiency.

To ease building the process, the user can use the unit procedure *Continuous 1x1 reaction generic box*, see Fig. 6.1. This unit procedure allows the user to make a reaction that does not affect the techno-economic analysis, nor the energy and mass balances. This allows the formation of non-existing intermediate products, for example splitting one specified component into its sub-components to simulate an extraction.

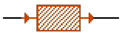


Figure 6.1: Continuous 1x1 reaction generic box

Operational data for the used equipment in the process simulation, that cannot be found in literature nor experimental data, such as total suspended solids in the cake after screw-pressing *S. dolichostachya*, are approximated using the default values of SPD.

When the SPD simulation is set up and the iterative loops have reached convergence, techno-economic analysis is done to find the optimal processing conditions. The schematic of biomass processing is shown in Fig. 6.2.

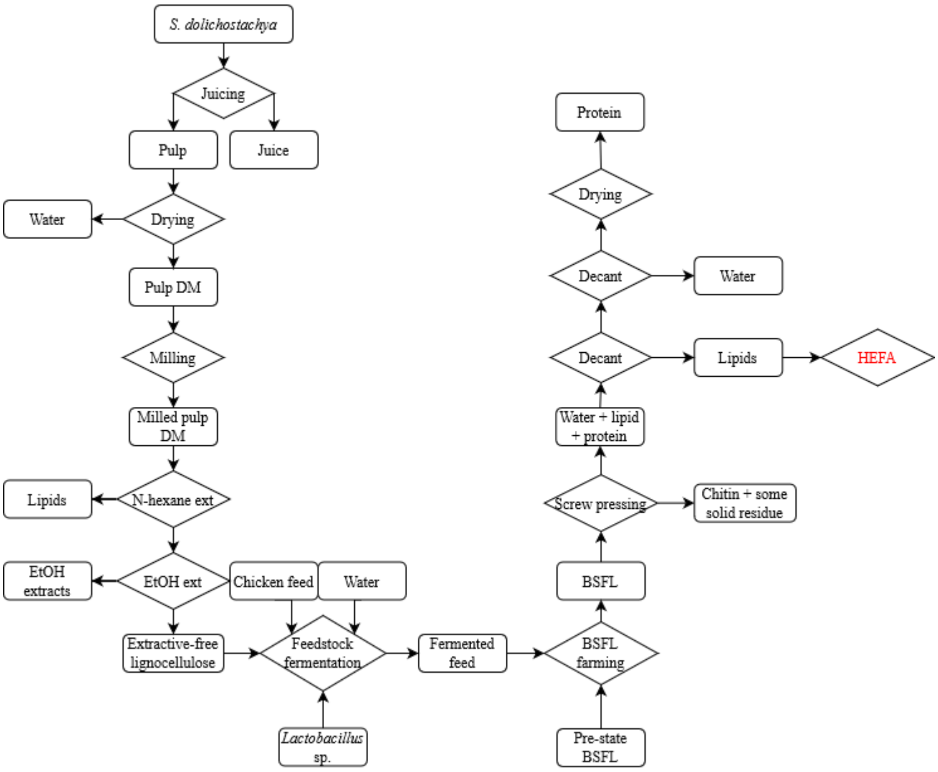


Figure 6.2: Process schematic of *S. dolichostachya* processing for production of BSFL, without hydrothermal pretreatment and enzymatic hydrolysis of the biomass. Rectangle shaped boxes are products, diamond shaped boxes are processes. Final process is marked red.

Tables will be shown on the basis of 10,768 kg h^{-1} fresh *S. dolichostachya* into the first simulation process unit.

6.1.1 Biomass preparation

The first step of biorefinery is to separate the juice from the *S. dolichostachya* biomass by green fractionation. This is done by using a screw press, and drying the pulp fraction by using drum dryer. The experimental data from Tab. 5.1 is used to define the pulp and juice fractions in the simualtion.

Parameter	Value	Unit
Biomass input	10,768	kg h^{-1}
Biomass DM output	1,472	kg h^{-1}
Outlet temperature	70	°C
Pressure	101	kPa
Labour	0.66	$h_{labour}\ h_{operation}^{-1}$

Table 6.1: Operation conditions in fractionation and drying units

Milling of the dried *S. dolichostachya* is simulated by using a bead milling unit. In this process step, the dried pulp will be simulated to be fractionated into different biochemical groups: ash, lipids, lignin, proteins, EtOH soluble material, cellulose and hemicellulose. Used fractions are based on the biomass characterisation, and from now on, the fractions are considered separately.

Parameter	Value	Unit
Outlet temperature	50	°C
Pressure	101	kPa
Labour	0.33	$h_{labour}\ h_{operation}^{-1}$

Table 6.2: Operation conditions in the bead milling unit

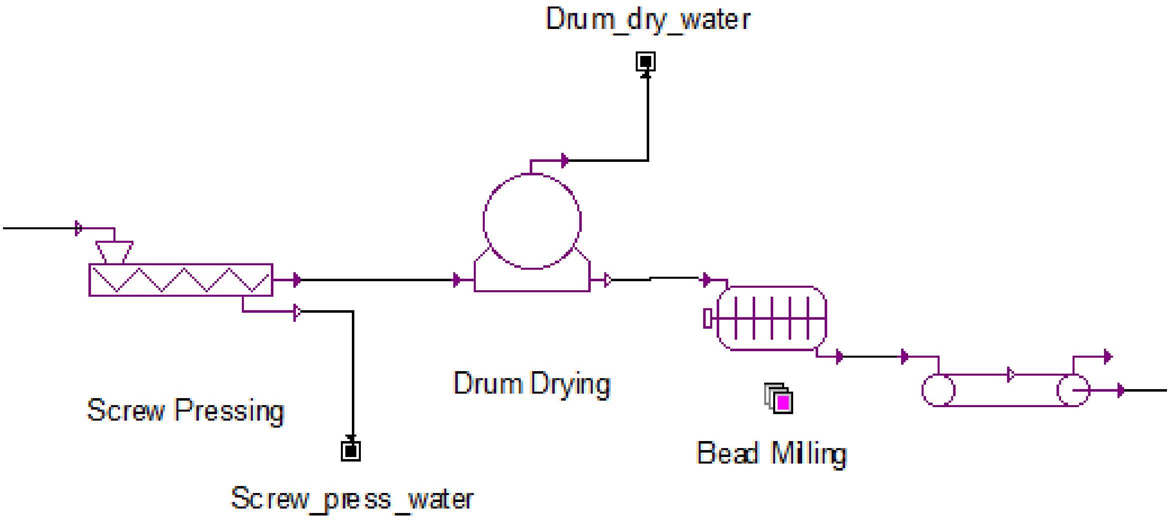


Figure 6.3: Pre-processing of *S. dolichostachya*

6.1.2 Extractions from *S. dolichostachya* biomass

As the feedstock for BSFL is a mixture of fermented chicken feed and pure lignocellulosic fibres from *S. dolichostachya*, the lipids and EtOH soluble material, including salts, are to be extracted. The extractions are be done with n-hexane and EtOH, using a semi-closed loop solvent recycle system, where make-up solvent is introduced to recycled solvent, replacing the amount of solvent lost in the extraction process. It should be noted that other solvents could be used for these extractions, but as the laboratory extractions are performed using n-hexane and EtOH, the same solvents are also used in the simulation. If the extract would be sold for human feed or additive to skin care products, the solvents should be non-toxic and safe for consumption. The lipids extracted could be used as biofuel input for HEFA production, but as the lipids are assumed to be high in healthy ω-3 and ω-6 fatty acids, the lipids are extracted and sold as value added products, to increase the possibility for feasibility.

As there is no Soxhlet extractor unit specified in SPD, a modified Soxhlet cycle is build by using other accessible units. The cycle is semi-continuous reaction, as new solvent will continuously enter the extractor unit, but the solid will be suspended in the recycling with a retention time of 1 hour for n-hexane extraction and 6 hours for EtOH extraction. The cycle consists of a continuous storage unit (Soxhlet extraction chamber), evaporation unit, using mechanical vapour recompression (Soxhlet siphon), custom mixing make-up valve (continuously added solvent) and solvent buffer tank (round bottom flask). The stream will get condensed after the evaporation unit to ensure a liquid phase, and then compressed to 101 kPa, so there will be no phase change inside the rotational machinery that might cause equipment damage. The evaporator unit will recycle 99 w% of the solvent, which is the default value provided by SPD. 99 w% of the solvent and solvent solubles will get decanted off and stored in a storage unit, which is again default values given by SPD. To provide a pure concentrate of the extracted compounds, the solvents will get fully vented off in the storage unit, and recycled back into the Soxhlet extraction cycle. The overall recycle of the solvent is therefore 99.99 %, and the remaining solvent will evaporate off during biomass transportation.

It has to be noted that an industrial scale Soxhlet extractor contains of fewer parts, and the purchase cost of this Soxhlet cycle will therefore be unrealistically high. The Soxhlet cycle is modelled as shown in Fig. 6.5 in order to get the right energy and mass balances, so the OPEX of the cycle will be a close estimation of an industrial scale Soxhlet extractor.

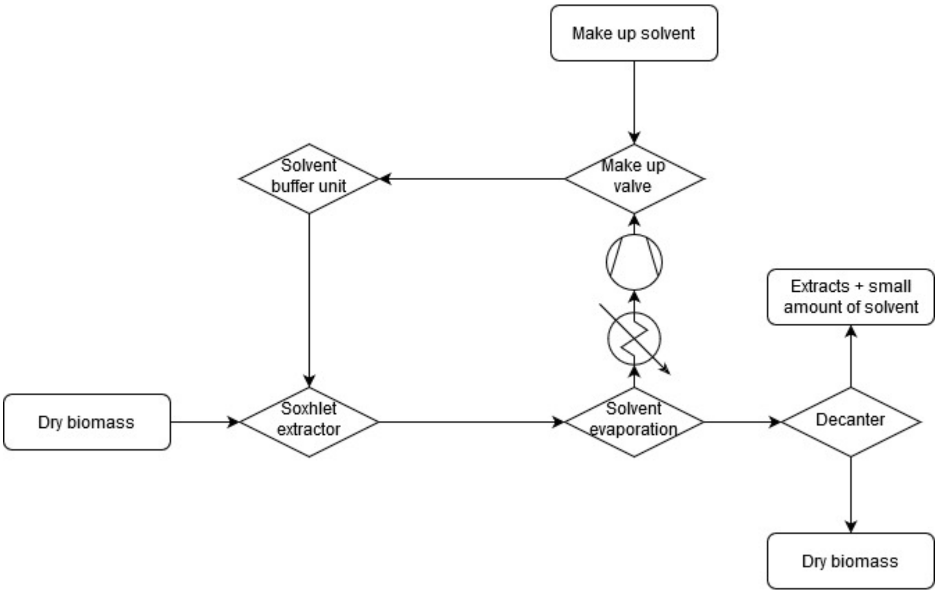


Figure 6.4: Process schematic of simulated Soxhlet extraction in SPD. Rectangle shaped boxes are products, diamond shaped boxes are processes. Solvent evaporation is followed by a cooling unit and a pump unit.

Parameter	Value	Unit
Biomass input ($\dot{m}_1$)	1,472	kg h^{-1}
Continuous storage unit		
Thermal mode	Adiabatic	-
Pressure	101	kPa
Residence time	1	h
Labour	0.33	$h_{labour}\ h^{-1}_{operation}$
Evaporation unit		
Evaporation temperature	40	°C
Pressure	37	kPa
Solvent recycle	99	w%
Labour	0.33	$h_{labour}\ h^{-1}_{operation}$
Make-up valve		
Output flow	$\dot{m}_1 \cdot 9.825$	kg h^{-1}
Pressure	101	kPa
Labour	0	$h_{labour}\ h^{-1}_{operation}$
Solvent buffer tank		
Thermal mode	Adiabatic	-
Pressure	101	kPa
Labour	0.33	$h_{labour}\ h^{-1}_{operation}$
Lipid storage		
Thermal mode	Adiabatic	-
Pressure	101	kPa
Solvent recycle	100	w%
Labour	0	$h_{labour}\ h^{-1}_{operation}$

Table 6.3: Operation conditions of the lipid extraction unit, where 15 liters of solvent is used per 1 kg of biomass, as determined by laboratory work

Parameter	Value	Unit
Biomass input ($\dot{m}_2$)	1,376	kg h^{-1}
Continuous storage unit		
Thermal mode	Adiabatic	-
Pressure	101	kPa
Residence time	6	h
Labour	0.33	$\text{h}_{labour}\ \text{h}^{-1}_{operation}$
Evaporation unit		
Evaporation temperature	40	°C
Pressure	18	kPa
Solvent recycle	99	w%
Labour	0.33	$\text{h}_{labour}\ \text{h}^{-1}_{operation}$
Make-up valve		
Output flow	$\dot{m}_2 \cdot 11.835$	kg h^{-1}
Pressure	101	kPa
Labour	0	$\text{h}_{labour}\ \text{h}^{-1}_{operation}$
Solvent buffer tank		
Thermal mode	Adiabatic	-
Pressure	101	kPa
Labour	0.33	$\text{h}_{labour}\ \text{h}^{-1}_{operation}$
EtOH extract storage		
Thermal mode	Adiabatic	-
Pressure	101	kPa
Solvent recycle	100	w%
Labour	0	$\text{h}_{labour}\ \text{h}^{-1}_{operation}$

Table 6.4: Operation conditions of the EtOH solubles extraction unit, where 15 liters of solvent is used per 1 kg of biomass, as determined by laboratory work

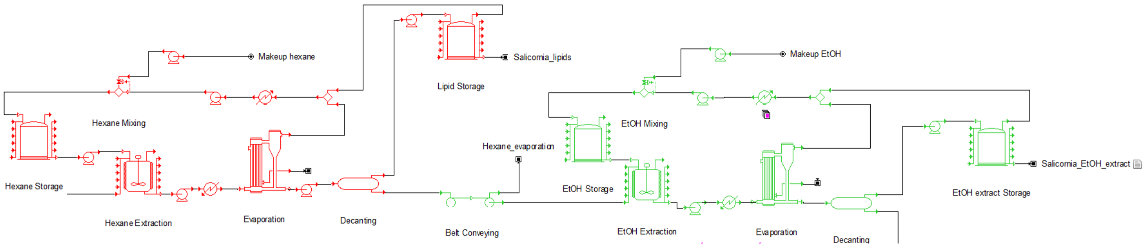


Figure 6.5: Soxhlet extractions of *S. dolichostachya* DM

6.1.3 Hydrothermal pretreatment and enzymatic hydrolysis

As described in section 4.8, the simulation of hydrothermal pretreatment and enzymatic hydrolysis is carried out based on the literature study, as experimental data for *S. dolichostachya* pretreatment is not available. The hydrothermal pretreatment and enzymatic hydrolysis are only simulated for half of the scenarios, as the effect of this step will be investigated. The pretreatment is done in a batch reactor, with the user-specified SPD operation sequence: transfer in, pretreatment reaction and transfer out. Before entering the reactor tank, the biomass fibres are mixed with water to achieve the required DM loading of 6 w%. After mixing, the slurry is heated to the pretreatment temperature of 140 °C, and inputted to the batch reactor for 76 minutes. Properties of the pretreatment unit are describes in Tab. 6.5, and they are corresponding to the planned pretreatment laboratory experiments described in section 4.8. However, maintaining the high pretreatment temperature for longer period of time increases the amount of energy needed for heating, thus, in the actual industrial application, it would be more feasible to increase the temperature and shorter the residence time while maintaining the same severity factor. Therefore, applying the conditions described by Alassali et al. [48] with the SF of 3.06 could be considered to save in utility costs.

Parameter	Value	Unit
Inputs		
S. dolichostachya	1,238	kg h^{-1}
Water	19,352	kg h^{-1}
Operation conditions		
Temperature	140	°C
Total residence time	76	min
Labour		
Transfer in	2	$h_{labour}\ h_{operation}^{-1}$
Operation time	15	min
Reaction	0.25	$h_{labour}\ h_{operation}^{-1}$
Operation time	46	min
Transfer out	2	$h_{labour}\ h_{operation}^{-1}$
Operation time	15	min

Table 6.5: Inputs and operation conditions of the pretreatment unit

After the pretreatment, the pretreated slurry is stored in a tank before fermentation in a batch fermentor. As the enzymatic hydrolysis takes place in the tank, cellulase enzyme is added. The used cellulase is an enzyme mixture of endoglucanase, exoglucanase and β-glucosidase. Residence time being 24 hours, the hydrolysis conditions are similar as in the study by Alassali et al. [48] for fractionated *S. sinus-persica*. As there is no available data about how well BSFL can digest the pretreated and hydrolysed halophyte biomass, some assumption are made considering the conversion rate. Alassali et al. [48] reported the ethanol yield of 76.9 %, and this conversion rate is also used in the simulation, assuming that 76.9 % of the hydrolysed glucose can be fermented into edible food for the BSFL, reaching similar biochemical conversion yields as the CF fed BSFL. The hemicellulose, protein, cellulase and *Lactobacillus* is considered to be converted 100 % into edible feedstock [118].

The enzymatic hydrolysis is carried out by using the data from the research article by Alassali et al. [48], as this study describes enzymatic hydrolysis of *S. sinus-persica* using 15 filter paper units (FPU) gDM^{-1} . The activity of cellulase, also known as filter paper activity measured by FPU, varies from each enzyme batch supplied, and is measured in 2.0 mg glucose mL^{-1}. A typical filter paper activity is in the range of 70-80 FPU, meaning that 1 mL of the enzyme will release 140-160 mg of glucose, if let to hydrolyse pure cellulose, but it is not uncommon to each even higher concentrations above 115 FPU [101, 100]. The activity can decrease over time if the batch of enzymes is not properly stored, as enzymes can easily denature.

Measurement of the cellulase activity is calculated from laboratory experiments, and derived by Eq. 6.1, where the numerator, 0.37, is the amount of glucose in mmoles equivalent to 2.0 mg of glucose from the volume of the assay tested (0.5 mL). The denominator, [enzyme], is the amount of enzymes in the tested solution in mmoles, that will release 2.0 mg glucose [154].

$$FPU = \frac{0.37}{[enzyme]\ releasing\ 2.0\ mg\ glucose} \tag{6.1}$$

Using data from literature [48, 101, 100, 155, 154] and the value for glucan in Tab. 5.8, the amount of cellulase added to the hydrothermal pretreated slurry is calculated as described in Eq. 6.2, where $\dot{m}_{cellulase}$ is the mean mass flow rate of cellulase provided to the batch enzymatic hydrolysis reactor, $\dot{m}_{glucan}$ is the mean mass flow rate of glucan of the slurry provided to the batch enzymatic hydrolysis reactor, $FPU_{loading}$ is the filter paper activity per unit mass of lignocellulosic DM, FPU_{batch} is the filter paper activity of the batch of enzymes per mL, $\rho_{cellulase}$ is the density of cellulase and 76.9 % is the conversion from the paper of Alassali et al. [48].

$$\dot{m}_{cellulase} = \frac{\dot{m}_{glucan} \cdot FPU_{loading} \cdot \rho_{cellulase}}{FPU_{batch} \cdot 76.9\ \%} \tag{6.2}$$

Parameter	Symbol	Value	Unit
Mass flow, glucan	$\dot{m}_{glucan}$	359.6	kg h^{-1}
Activity	$FPU_{loading}$	15	FPU g^{-1}
Activity	FPU_{batch}	75	FPU mL^{-1}
Density	$\rho_{cellulase}$	1.2	g mL^{-1}
Mass flow, enzyme	$\dot{m}_{cellulase}$	113.6	kg h^{-1}

Table 6.6: Enzyme calculation data

Parameter	Value	Unit
Inputs		
S. dolichostachya slurry	20,590	kg h^{-1}
S. dolichostachya glucan	359.6	kg h^{-1}
Cellulase	113.6	kg h^{-1}
Operation conditions		
Temperature	30	°C
Total residence time	24	h
Labour	0.33	$h_{labour}\ h^{-1}_{operation}$

Table 6.7: Inputs and operation conditions of enzymatic hydrolysis unit

The hydrolysed biomass slurry with 6 w% dry matter loading is mixed with chicken-feed used in the BSFL feed. As the mixture of chicken-feed and *S. dolichostachya* still has a DM content less than 30 w%, which is required for the BSFL feed fermentation, some of the water in the slurry has to be evaporated. This is done in a sludge drying unit.

Parameter	Value	Unit
Inputs		
Slurry	22,525	kg h^{-1}
DM w% in slurry	14.1	w%
Outputs		
Slurry	10,580	kg h^{-1}
DM w% in slurry	30.0	w%
Steam	11,945	kg h^{-1}
Operation conditions		
Temperature of steam	115	°C
Temperature of dried slurry	60	°C
Labour	0.2	$h_{labour}\ h^{-1}_{operation}$

Table 6.8: Inputs and operation conditions of sludge drying unit

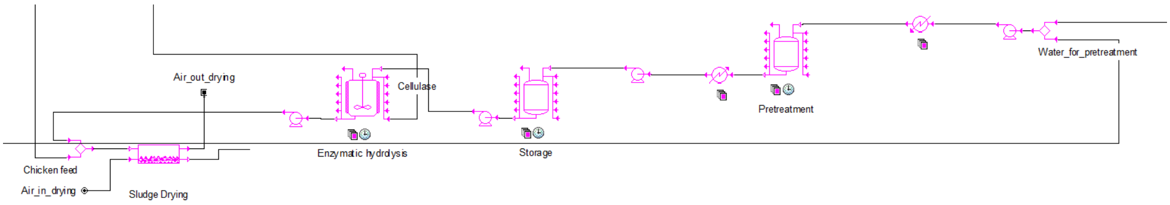


Figure 6.6: Hydrothermal pretreatment and enzymatic hydrolysis of extractive-free *S. dolichostachya* biomass

6.1.4 Feedstock fermentation

Information about the process types of the fermentation section, prices and yields are given by ENORM ApS. As ENORM ApS is fermenting their feedstock using a *Lactobacillus acidophilus* culture, this process step is also included to the simulation. *Lactobacillus acidophilus* used in the fermentation is from a dairy culture of A38 from the Danish dairy producer Arla. The mass flow rate and purchasing price of the added culture is modelled based on information from ENORM ApS. As the biomass pretreatment is a dummy variable, two different scenarios are considered:

1. When the pretreatment and hydrolysis are applied, the mixture of *S. dolichostachya* and CF is coming directly from the sludge drying unit with the right DM loading.
2. If there is no pretreatment, the extractive-free and dried *S. dolichostachya* is mixed with chicken-feed in a 40/60 w/w% ratio. Water is added to reach a slurry with demanded 30 w% DM loading.

A small amount of *Lactobacillus acidophilus* culture is added to the fermentation unit. This anaerobic fermentation is done in a batch fermentor.

Parameter	Value	Unit
Inputs		
Feedstock	10,580	kg h^{-1}
Lactobacillus acidophilus culture	480	kg h^{-1}
Operation conditions		
Temperature	29	°C
Total residence time	24	h
Pressure	101	kPa
Labour		
Transfer in	2	$h_{labour}\ h^{-1}_{operation}$
Operation time	1.25	h
Heating	0.5	$h_{labour}\ h^{-1}_{operation}$
Operation time	2.08	h
Fermentation	0.25	$h_{labour}\ h^{-1}_{operation}$
Operation time	24	h
Transfer out	2	$h_{labour}\ h^{-1}_{operation}$
Operation time	1.25	h

Table 6.9: Inputs and operation conditions in the feedstock fermentor. Fermentation conditions given by ENORM ApS [156].

6.1.5 BSFL cultivation

As BSFL are larvae hatched from fly eggs, an initial larvae culture needs to be made. ENORM ApS is doing this in simple fly-cages, where the flies lay eggs in between pieces of cardboard. This is simulated to happen continuously in a drum, with a small portion of the produced prepupae larvae as an input and fly eggs as output. Black soldier flies do not need food as they are mating, but only an insignificant amount of water. In SPD, the cultivation of BSFL is simulated as a silo with specific conditions and the residence time of 14 days. In reality, the cultivation is happening in separate parallel rooms with suitable ambient conditions, and the larvae is laying in trays placed in racks to make the transferring easier.

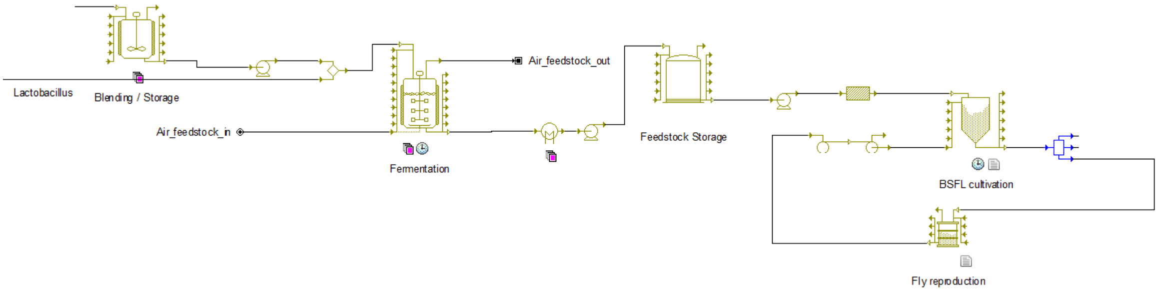


Figure 6.7: Feedstock fermentation and cultivation of BSFL

Parameter	Value	Unit
Inputs		
Fermented feedstock	4,542	kg h^{-1}
BSFL larvae eggs	5.29	kg h^{-1}
Output		
BSFL larvae	1317	kg h^{-1}
Frass	204	kg h^{-1}
Non-edible biomass	234	kg h^{-1}
Water	2381	kg h^{-1}
Emissions and undefined	411	kg h^{-1}
Operation conditions		
Cultivation time	14	days
Labour	0.16	$h_{labour}\ h^{-1}_{operation}$

Table 6.10: Inputs and operation conditions in the BSFL cultivation unit

6.1.6 Processing of BSFL

After cultivation of BSFL, the larvae and frass are separated by a coarse screen. The frass is stored in a big silo. A small amount, 1 w% of the total amount of cultivated BSFL, is transferred into the drum to allow for pupation and lay eggs to maintain the amount of input larvae. The remaining BSFL are devitalised using steam, and transported to a storage unit. The storage unit is needed as the cultivation of BSFL is a batch cultivation, and an intermediate storage will allow for a continuous output flow rate. From the storage unit, the BSFL are pressed using a hot screw press. Given by data from ENORM ApS, the screw press separates the liquid phase containing lipids, water and protein from the solid phase containing the BSFL exoskeleton and residual matter from the BSFL. The exosceleton is high in chitin, and can be sold as a value-added product. After screw pressing, the lipid and water phases are separated. Solid protein is separated from the water, dried and sold in the market as a value-added product.

Parameter	Value	Unit
Biomass input	876	kg h^{-1}
Outlet temperature	70	°C
Pressure	101	kPa
Labour	0.33	$h_{labour}\ h^{-1}_{operation}$

Table 6.11: Operation conditions of the BSFL screw press unit

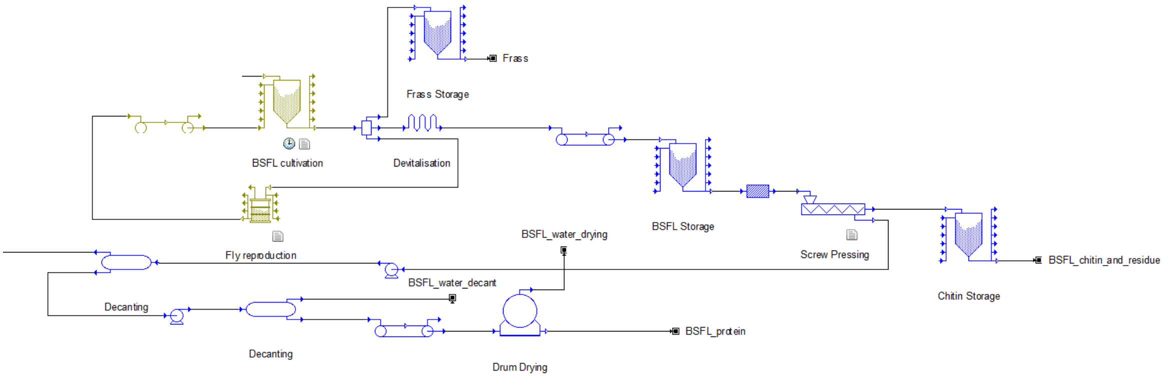


Figure 6.8: Processing of BSFL

6.2 HEFA processing

By an *in silico* analysis approach, HEFA process is evaluated using mainly SPD as process design tool. As SPD does not show the user all needed thermodynamic fluid properties, such as the freezing point, kinematic viscosity and density of the mixture of molecules, Aspen HYSYS is used for modelling the fractional distillation units. The end products of the HEFA process simulation are the biofuels light naphtha, HEFA-SPK and hydrotreated vegetable oils (HVO), in the range of gasoline, ASTM standardised Jet A-1 fuel and diesel, respectively. The simulation of the HEFA process will target the highest possible fraction of jet fuel, and it will be determined by the blend of distilled alkanes that will follow the ASTM standards for HEFA-SPK, as described by Starck et al. [127]. This will lower the amount of fuels produced in the range of naphtha and HVO, and possibly also lower their drop-in potential with conventional fossil fuels, as for example, the freezing point of HVO will be lower as the distillation temperature decreases. In this section the words naphtha, HEFA-SPK and HVO will be used interchangeable with gasoline, jet fuel and diesel, respectively.

Feedstock oil for the process is set to be 100 w% BSFL lipids. The fatty acid composition of BSFL lipids is simulated using data shown in Tab. 2.11, with the fatty acid composition reported by Li et al. [122].

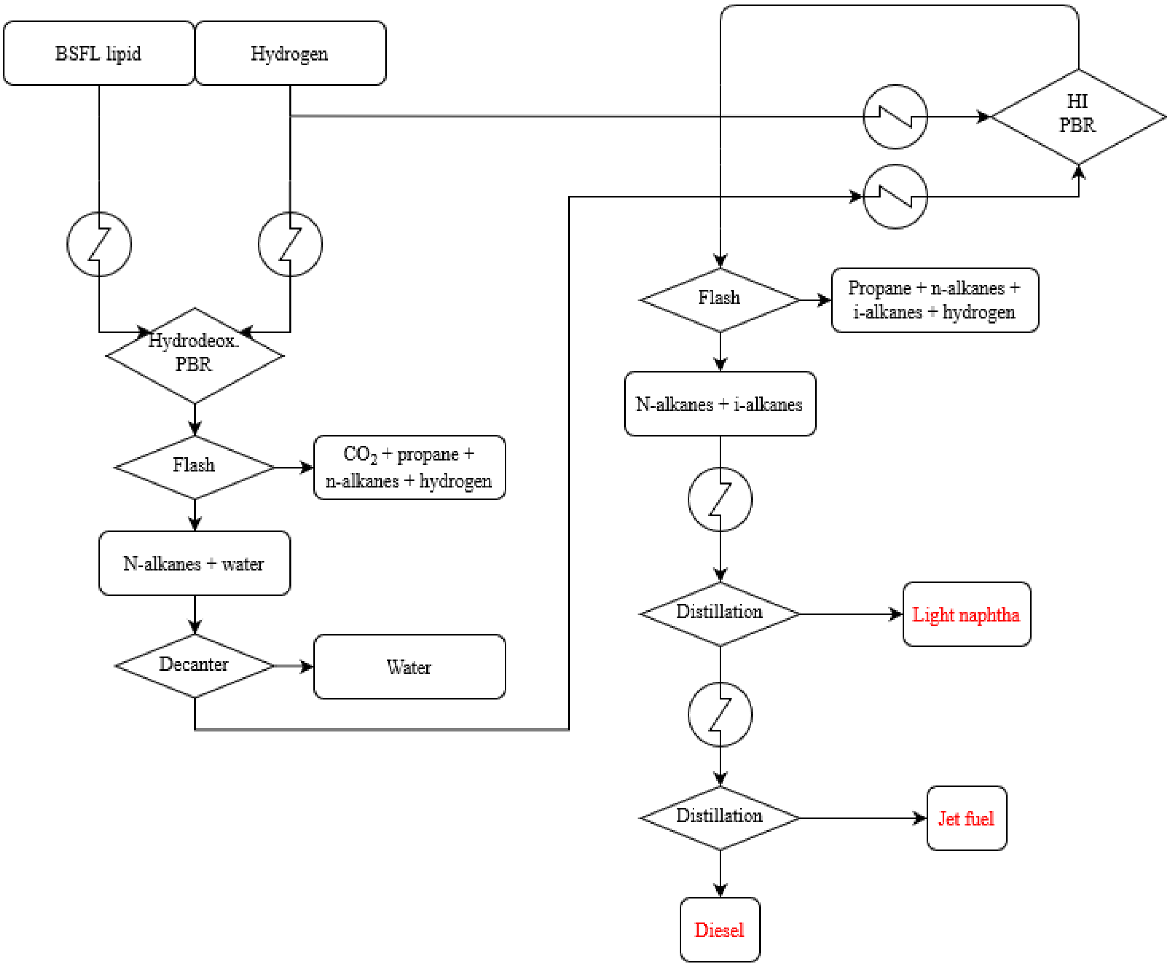


Figure 6.9: Process schematic of BSFL lipids for production of biofuels. Rectangle shaped boxes are products, diamond shaped boxes are processes, and circular symbols are heat exchangers. End products are marked with red colour.

6.2.1 Hydrodeoxygenation unit

In the first reactor, four different reactions are occurring simultaneously: saturation of unsaturated triglycerides, depropanation, hydrodeoxygenation and decarboxylation. These reactions are simulated to take place in a plug flow reactor (PFR), as there is no pack bed reactor unit in SPD.

Saturation of unsaturated triglycerides

As described in section 2.5.2, mono- and polyunsaturated triglycerides can be hydrogenated into saturated triglycerides. Kinetic rates for saturation of tri-C18:1 to tri-C18:0 are taken from experimental research done by Swicklik et al. [139], who found the kinetic rate of reaction to be linear, as shown in Eq. 2.4. As there is not sufficient experimental research done for saturation of tri-C18:2 to tri-C18:1, the kinetic rate of reaction is set to be to be the same as for the saturation of tri-C18:1 to tri-C18:0, as this is thought to be a reasonable assumption. Alkane tri-C18:0 is the only end product considered, as there are no other unsaturated triglycerides in the lipid feedstock. It is also assumed, that no other reactions will occur inside the hydrogenation PBR. The conversion is set to be 100 w%, as a simulation with the rate constant of 4.99 $\cdot 10^{-4} m^{-3} s^{-1}$ shows a complete reaction in less that the specified LHSV.

Parameter	Value	Unit
Temperature	175	°C
Catalyst (Ni)	0.9	w%
Pressure	0.55	MPa
LHSV	0.66	h^{-1}
k	$4.99 \cdot 10^{-4}$	$m^{-3}s^{-1}$
Conversion	100	w%

Table 6.12: Operation conditions of hydrogenation

Depropanation

In a study by Martinez-Hernandez et al. [137] the conversion of tri-C18:0 to C18:0 is considered to be rapid, and full conversion can be achieved by using the listed parameters in Tab. 6.13. The reaction rate constant is set to be the value that provides 100 w% reactant conversion, as only the conversion is given, and not the kinetics of the depropanation. This is possible to do within SPD tool. All lengths of triglycerides are assumed to react with hydrogen in the same rate, meaning identical reaction rate constants.

Parameter	Value	Unit
Temperature	310	°C
Catalyst TiO_2/NiMo	n/a*	w%
LHSV	1	h^{-1}
k	0.1	$m^{-3}s^{-1}$
Conversion	100	w%

Table 6.13: Operation conditions of depropanation [137]. *) No studies describe the amount of catalyst.

Hydrodeoxygenation

The amount of decarbonylated aldehydes are found to be very low (3.8 w%) at 310 °C and 4.0 MPa, as described by Martinez-Hernandez et al. [137]. Furthermore, decarbonylation cannot be simulated in SPD using conventional reaction rate equations, as it is a function of liquid phase fugacity, which is not a thermodynamic calculation that can be done by SPD. Therefore, decarbonylation is neglected in the simulation. In a study by Kharaji et al. [142], the effects of temperature and catalyst are investigated, to determine the potential WGS reactions of HEFA. Using the thermodynamic properties and catalyst described in Tab. 6.12, and considering the amount of produced CO described by Arora et al. [141], the WGS reactions are non-existing. This is both due to too relatively low temperature in the reactor, and negligible amount of CO produced from decarbonylation, as seen in subsection 2.5.2. The rate of reaction of WGS and CO will increase as the temperature over 300 °C increases. The reaction coefficients in Tab. 6.14 are corresponding the reactions shown in Tab. 2.10, and hydrodeoxygenation and decarboxylation conversions are calculated.

Parameter	Value	Unit
Temperature	300	°C
FFA/H_2 ratio	96.7	w%
Catalyst (Ni-Mo/Al_2O_3)	0.28	w%
Pressure	5	MPa
LHSV	3	h^{-1}
k_1	$5.52 \cdot 10^{-2}$	$\frac{m^6}{kmol\ kg\ s}$
k_2	$8.63 \cdot 10^{-3}$	$\frac{m^3}{kg\ s}$
k_4	$4.72 \cdot 10^{-2}$	$\frac{m^3}{kg\ s}$
k_5	$2.70 \cdot 10^{-2}$	$\frac{m^3}{kg\ s}$
$K_{C_{18:0}}$	51.4	$\frac{m^3}{kmol}$
Hydrodeoxygenation conversion	63.1	w%
Decarboxylation conversion	36.9	w%

Table 6.14: Operation conditions of hydrodeoxygenation using C18:0 as reagent [141, 137]

Hydrodeoxygenation unit summary

The operation conditions of the first PBR are set based on the highest required reaction temperature, pressure and lowest LHSV, being 310 °C, 5 MPa and 0.66 h^{-1} respectively. The conversion of saturation and depropanation is set to be 100 w%, and hydrodeoxygenation and decarboxylation conversions from SPD are 63.1 w% and 36.9 w%, respectively. Input streams and output streams are shown in Tab. 6.15, with the lipids being the BSFL lipids.

	Input [kg/h]	Output [kg/h]
H_2	8.47	1.13
H_2O	-	25.96
CO_2	-	18.54
C3	-	16.78
C11	-	30.29
tri-C12:0	111.85	-
C12	-	56.44
C13	-	6.09
tri-C14:0	21.58	-
C14	-	11.20
C15	-	15.38
tri-C16:0	52.80	-
C16	-	28.04
C17	-	29.37
tri-C18:0	14.20	-
tri-C18:1	67.00	-
tri-C18:2	16.47	-
C18	-	53.15

Table 6.15: Mass balance for hydrodeoxygenation PBR

After exiting the hydrodeoxygenation PBR, the stream is separated to vapour and liquid phases in a flash separator, as it contains a large amount of undesired propane, hydrogen, water and CO_2. Due to the azeotropic nature of alkane mixtures, a small amount of heavier alkanes will be in the vapour phase with the propane, and a small amount of the propane will also be in the liquid phase. This explains the lower amount of alkanes in Tab. 6.18 compared to Tab. 6.15. A large amount of water is still present in the stream, as the temperature of the hydrodeoxygenation PFR output stream is below its boiling point. This water is removed by decantation.

6.2.2 Hydrocracking and isomerisation

When designing the operation conditions for the HI unit, some constraints must be made. These constraints are set to meet the ASTM standards, and to make sure the end product is in the jet fuel carbon range, including alkanes n-C12 to n-C17 and isomers i-C14 to i-C21.

Parameter	Value	Unit
Temperature	250	°C
n-alkane/H_2 weight ratio	98.1	-
Catalyst IMP*	n/a	w%
Pressure	3	MPa
LHSV	1	h^{-1}

Table 6.16: Operation conditions in the HI PBR [137]. *) No studies describe the amount of catalyst.

As described by Martinez-Hernandez et al. [137], the conversion rate in the HI unit is given as percentage, shown in Tab. 6.17. All cracking reactions and isomerisation of remaining n-alkanes are describes in Fig. 6.10. It has to noted, that some of the cracking products, for example n-C15 from the cracking of n-C18, will crack forward in the same way as corresponding input alkanes, for example n-C15 to n-C12 and propane. All the cracked and non-cracked n-alkanes, besides heptane and propane, are isomerised afterwards, and all the reactions are happening parallel inside the PBR.

As SPD does not have an operation unit for PBR, and simulating a parallel processes in PFR sets challenges defining and iterating the reaction rate constant, the process is simulated with a generic box, where the output streams are calculated based on the conversion rates and mass balance. All meta-data, such as reactor prices and labour costs, are defined by user to match the costs of PFR with similar size and properties.

	Hydrocracked to C3	Hydrocracked to C8	Hydrocracked to C7	Hydrocracked to other	Conversion to isomers
C3	-	-	-	-	-
C7	-	-	-	-	-
C8	-	-	-	-	90
C9	-	-	-	-	88
C10	-	-	-	-	90
C11	-	-	-	-	90
C12	-	-	-	-	90
C13	5.8	-	-	-	90
C14	61.7	-	-	-	53.9
C15	51.3	-	-	-	27.2
C16	37.1	-	-	18.0	6.0
C17	50.4	17.3	2.0	-	12.6
C18	4.9	41.9	13.9	23.2	18.9

Table 6.17: HI reactions defined in mass percentage as suggested by Martinez-Hernandez et al. [137]

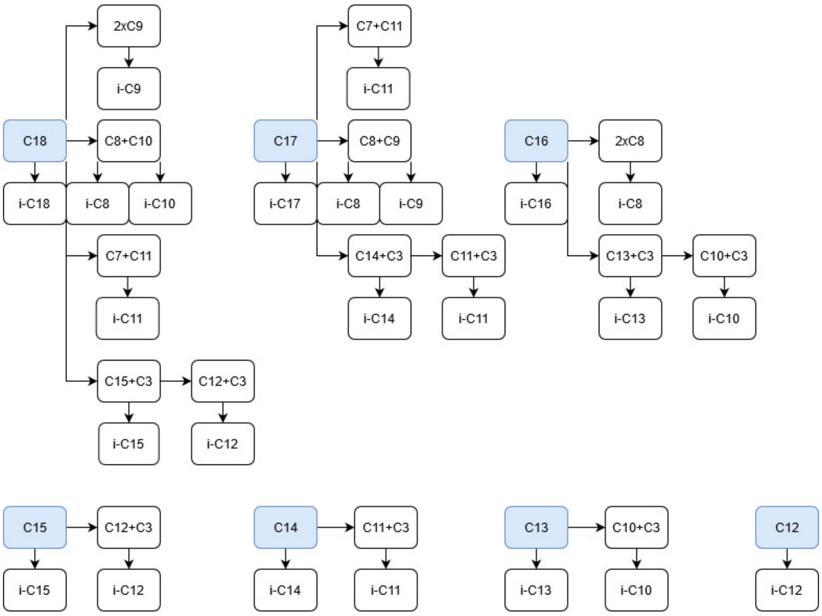


Figure 6.10: Possible HI reactions as suggested by Martinez-Hernandez et al. [137]. Hydrogen saturation of cracked compounds not shown.

Input streams, output streams, and mass balance of the HI unit are shown in Tab 6.18. A small amounts of unreacted n-alkanes can be detected from the output stream, as well as high amounts of leftover excess hydrogen and undesired propane. Vapour-liquid separation is performed in a flash separator after HI reactions, to separate propane and hydrogen, which is then recycled and reused. Due to the azeotropic nature of alkane mixtures, a significant amount of the lighter alkanes will also be separated, which explains the lower amount of distilled alkanes in Tab. 6.20 compared to Tab. 6.18.

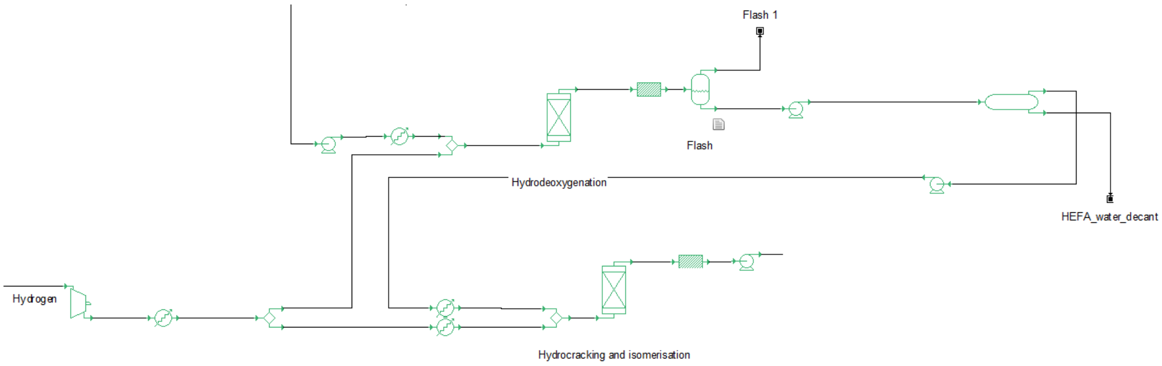


Figure 6.11: HEFA process of BSFL lipids

Alkane	Input [kg/h]	Output [kg/h]
H2	8.47	7.57
C3	1.61	12.08
C7	-	3.15
C8	-	1.75
i-C8	-	15.75
C9	-	1.82
i-C9	-	13.32
C10	-	1.35
i-C10	-	12.10
C11	30.24	4.62
i-C11	-	41.54
C12	56.41	6.36
i-C12	-	57.27
C13	6.09	1.37
i-C13	-	12.34
C14	11.21	4.13
i-C14	-	4.84
C15	15.38	6.22
i-C15	-	2.33
C16	28.04	11.83
i-C16	-	0.76
C17	29.37	7.78
i-C17	-	1.12
C18	53.38	6.94
i-C18	-	1.62
Σ	239.94	239.94

Table 6.18: Mass balances of simulated HI reactions

6.2.3 Distillation

Distillation is simulated with Aspen HYSYS process design tool, as SPD does not calculate some thermodynamic properties of fluids, which are necessary to know to comply with ASTM standards shown in Tab. 2.7. Distillation takes place in two separate columns, where the first column separates the HI alkanes in gasoline range, and the second column separates the jet fuel range from diesel range.

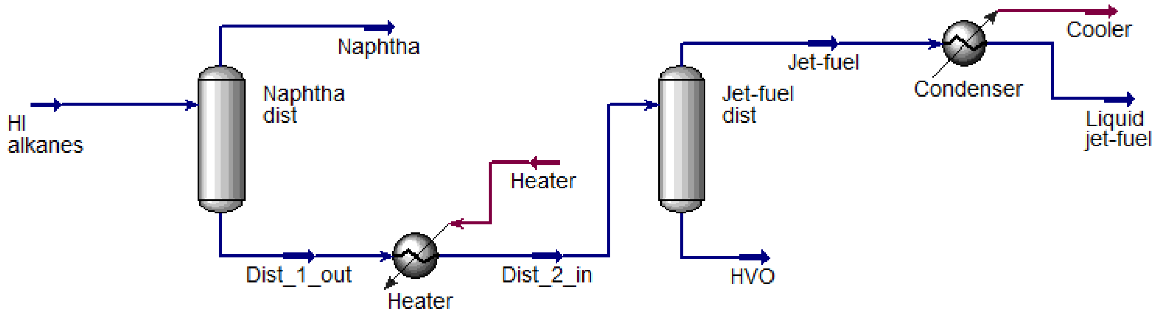


Figure 6.12: Naphtha and jet fuel distillation units simulated in Aspen HYSYS

For naphtha distillation, the heavy key distillation temperature, where the distilled molecule with the highest boiling point will boil, is 190 °C, which is the mean value of 175 °C and 205 °C. This temperature is considered to be a reasonable light key distillation temperature, where the distilled molecule with the lowest boiling point will boil, of Jet A-1, as the maximum light key distillation temperature is 205 °C, as described in Tab. 2.7. The mean temperature of 190 °C is chosen, as Jet A-1 fuel range molecules can be distilled in the temperatures of 175-315 °C, but temperatures of 175-205 °C and 300-315 °C can yield unwanted physical properties for the jet fuel product, as described in section 2.5.4.

The freezing point of Jet A-1 is set as one of the primary constraints in the simulation in Aspen HYSYS. As the freezing point is not determined directly in Aspen HYSYS, it is calculated using Eq. 6.3 based on the data of freezing points of n-alkanes, estimated freezing points of i-alkanes from Martinez-Hernandez et al. [137], and the molar fractions of alkanes of the distilled jet fuel stream.

$$FP_{Jet\ A-1} = \sum_{n=1}^{i} FP_n y_n \tag{6.3}$$

Therefore, the heavy key distillation temperature of Jet A-1 is set to be 228 °C, as it enables the separation of heavier hydrocarbons and yields the jet fuel product matching the ASTM standards. Distillation at higher temperatures results the product including too many heavy alkanes, increasing the freezing point above the standardised limit of -47 °C, when HI as described in section 6.2.2 and the feedstock for the HEFA process is BSFL lipids with the lipid profile described in Tab. 2.11.

Parameter	Naphtha [157]	HEFA-SPK [127]	HVO [158, 157, 159]
Limits/common values			
Freezing point [°C]	-	<-47.0	<-10.0
Density at 15 °C [kg m^{-3}]	-	775 - 840	780 - 890
Kinematic viscosity [mm^2 s^{-1}]	-	<8.0*	1.9 - 6.0**
Distillation temp. [°C]	70-180	>205 - <300	240-340
Distillation products			
Freezing point [°C]	-66.0	-47.3	-19.2
Density at 15 °C [kg m^{-3}]	773.5	786.4	781.1
Kinematic viscosity [mm^2 s^{-1}]	2.6*	4.1*	2.0**
Distillation temp. [°C]	<190	190-228	>228

Table 6.19: Properties of distilled fuels products compared to ASTM standard limitations and distillation temperature specifications. *: Kinematic viscosity at -20 °C, **: Kinematic viscosity at 40 °C.

The resulted properties of HVO meets the corresponding ASTM D6751 standard values, but the minimum distillation temperature is slightly lower than the common value. This is due to the high amount of relatively light alkanes in the HVO range molecules distilled off [158]. The kinematic viscosity at 40 °C is within the accepted limits (2.6 mm^2 s^{-1}), and the density at 15 °C is just above the accepted minimum value of 780 kg m^{-3}. Therefore, the produced HVO can be used as a ASTM certified fuel. The freezing point of HVO is relatively low, which again indicates a high concentration of relatively light alkanes in the HVO fuel mix [159]. The produced naphtha can be blended to petroleum fuels, and it has shown to be suitable for gasoline combustion engines [160].

The mass balance of distillation units, and the alkane composition is shown in Tab. 6.20. The SPD and Aspen HYSYS simulations show, that with the input flow of 100 kg h^{-1} BSFL lipid and 8 kg h^{-1} hydrogen, it is possible to achieve the fuel productions of 20.00 kg h^{-1}, 22.30 kg h^{-1} and 33.90 kg h^{-1} of naphtha, HVO and ASTM approved jet fuel, respectively. Tab. 6.20 shows the alkane profiles of naphtha, HEFA-SPK and HVO on a 100 kg h^{-1} BSFL lipid basis.

Alkane	Input [kg h^{-1}]	Naphtha [kg h^{-1}]	HEFA-SPK [kg h^{-1}]	HVO [kg h^{-1}]
C3	0.01	0.01	0.00	0.00
C7	0.79	0.59	0.19	0.01
C8	0.55	0.35	0.18	0.02
i-C8	4.91	3.24	1.55	0.12
C9	0.61	0.31	0.26	0.04
i-C9	4.5	2.43	1.84	0.23
C10	0.47	0.18	0.24	0.05
i-C10	4.2	1.77	2.04	0.4
C11	1.62	0.44	0.88	0.3
i-C11	14.57	4.45	7.77	2.34
C12	2.24	0.42	1.19	0.62
i-C12	20.16	4.4	10.84	4.92
C13	0.48	0.06	0.23	0.19
i-C13	4.34	0.65	2.19	1.51
C14	1.46	0.11	0.57	0.77
i-C14	1.7	0.15	0.75	0.8
C15	2.19	0.11	0.71	1.37
i-C15	0.82	0.06	0.31	0.46
C16	4.17	0.14	1.04	2.99
i-C16	0.27	0.01	0.08	0.17
C17	2.74	0.06	0.5	2.18
i-C17	0.39	0.01	0.09	0.29
C18	2.44	0.03	0.32	2.09
i-C18	0.57	0.02	0.12	0.44
Σ	76.20	20.00	33.90	22.30

Table 6.20: Composition of distilled fuel products from BSFL lipid based HI alkanes

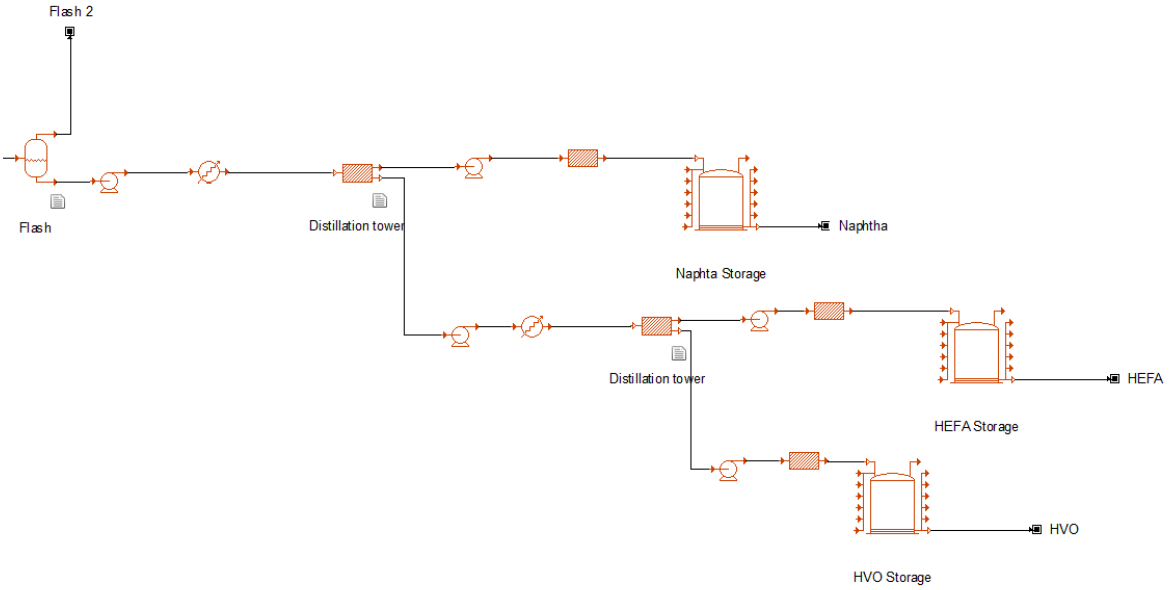


Figure 6.13: Distillation of alkanes and i-alkanes from the HEFA process.

6.3 Processing costs and economic evaluation

The total cost originates from different sources and in this process, the three largest costs are building and equipment costs (CAPEX), material costs (OPEX) and manpower (OPEX). Utility costs sourced from heating and cooling can be efficiently decreased by running energy recovery calculations.

The equipment cost is a function of equipment volume and volume flow rate. All pumps in the system has a standby pump modelled, so if one pump is to break down or shut down for maintenance, the standby pump will operate to ensure few bottlenecks and disturbances in the production. SPD tends to overestimate some of the equipment costs, and these are noted in the techno-economic analysis in section 7.1.

Labour rates are assigned for all processing units and for simplification, all equipment types have the same type of labour assigned. In SPD, the labour cost (LC) is determined from the basic salary x, and the model is run using the mean salary of operators in Denmark being 20.6 UDS/h [161]. On top of that, SPD takes account other labour related costs in the form of coefficients and required labour hours h. This is shown in Eq. 6.4.

$$LC = x_i \cdot (1 + a + b + o + s) \cdot h \tag{6.4}$$

where a is administrative costs (0.6), b is benefits (0.4), o is operating supplies (0.1) and s comes from supervision (0.2). The default coefficients from the SPD are used.

Materials and chemicals are having fixed prices per mass unit of *S. dolichostachya* input, and will therefore change as the input mass flow rate changes. Some assumptions have been made regarding the purchase prices of the consumable materials and chemicals, such as the purchase price of enzyme, as the cellulase bought for laboratory purposes are too expensive for industrial scale applications [162].

Parameter	Value [USD kg^{-1}]	Ref.	Note
Buying prices			
Hydrogen	2.00	[137]	
Water	0.0025	[163]	
Lactobacillus acidophilus culture	1.50	[156]	
Cellulase	0.50	[162]	
Chicken-feed	0.55	[156]	
Ethanol	6.75	[164]	USD L^{-1}
N-hexane	8.35	[164]	USD L^{-1}
Selling prices			
S. dolichostachya lipids	1.00		Assumption
S. dolichostachya EtOH extract	7.00	[26]	
Frass	2.00	[165]	
BSFL screw-pressed cake	0.72	[156]	
BSFL protein	2.17	[156]	
Hydrogen	2.00	[137]	
Propane	0.80	[137]	
Gasoline range	0.55	[137]	
Jet A-1 range	0.62	[137]	
Diesel range	1.00	[137]	

Table 6.21: Input data for material and chemical costs

Chapter 7

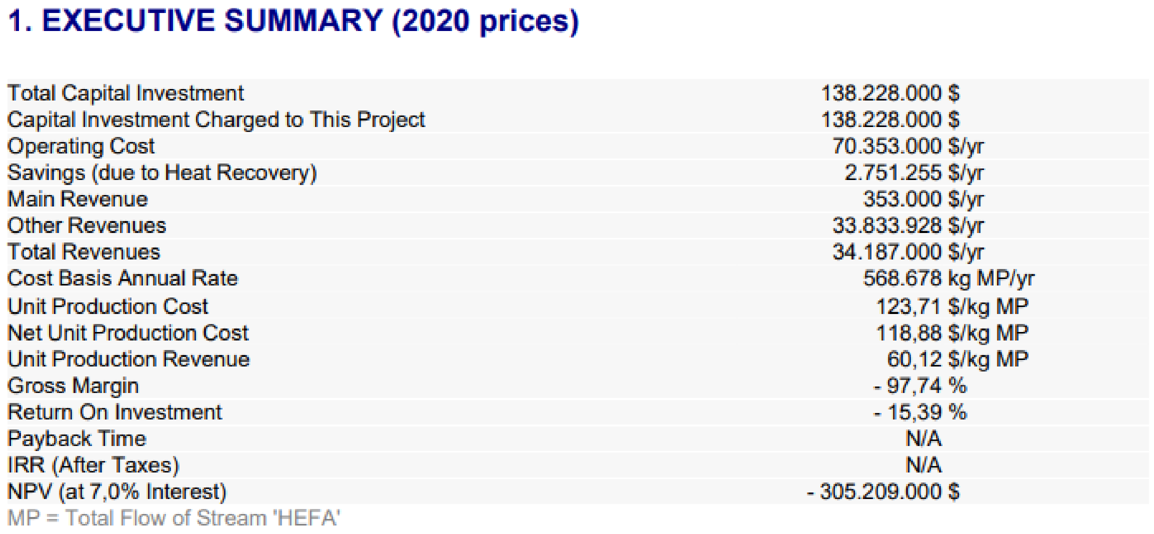
Techno-economic assessment

Techno-economic evaluation is a crucial step when planning the construction of a processing plant or inputting new process units and material streams to existing biorefineries. To analyse the costs and revenues correlated to production of sustainable jet fuel, the SuperPro Designer tool allows the user to examine the Economic Evaluation Report (EER) showing detailed information about costs and revenues from each production stream. CAPEX and OPEX are functions of the process plant size, and corresponding building costs, staff salaries, material and chemical costs, and utility used for heating and cooling.

7.1 Economic evaluation

In this section the Economic Evaluation Report (EER) of the biorefinery with *S. dolichostachya* input mass flow rate of 21,536 kg h^{-1} is discussed and no pretreatment. The pages 8 and 9 of the EER, considering various consumables cost and waste treatment/disposal cost, are left out from the section, as they are blank.

The first page of the EER shows a cash flow analysis and a summary of the process economics. As the operating costs in this process are much higher than the revenue, the cash balance is negative and the process does not reach profitability. Therefore, payback time cannot be determined for this biorefinery process.



1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	138.228.000 $
Capital Investment Charged to This Project	138.228.000 $
Operating Cost	70.353.000 $/yr
Savings (due to Heat Recovery)	2.751.255 $/yr
Main Revenue	353.000 $/yr
Other Revenues	33.833.928 $/yr
Total Revenues	34.187.000 $/yr
Cost Basis Annual Rate	568.678 kg MP/yr
Unit Production Cost	123,71 $/kg MP
Net Unit Production Cost	118,88 $/kg MP
Unit Production Revenue	60,12 $/kg MP
Gross Margin	- 97,74 %
Return On Investment	- 15,39 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7,0% Interest)	- 305.209.000 $

MP = Total Flow of Stream 'HEFA'

Figure 7.1: EER page 1, executive summary

All the equipment purchasing costs and specifications are described in the EER, and the total equipment cost is 19.7 M$. The most expensive components are the fermentor and feedstock blending tank, costs being nearly 4.3 M$ and 1.2 M$, respectively. SPD sets the prices for the reactors very high, hence in the reality, the cost could be lower than estimated in the report. As described in section 6.1.2, the cost of equipment used in the extraction is expected to be overestimated, due to the high amount of unit operations used in SPD. In the case of the fermentor, SPD also takes into account the expensive costs related to the equipment, such as sterilisation. Other expensive units are the large storage tanks for organic solvents, screw presser, pulp milling unit, large-sized BSFL protein decanter and heat exchangers. Even if the purchasing price of the implementing inter-stream heat exchangers is high, it is beneficial to apply these to decrease the amount of utility costs needed for heating and cooling, as seen in Fig. 7.8. The arrangement of the heat exchanger network can also be optimised by performing a pinch analysis.

The default length for a conveyor belt in SPD is 100 meters, which in reality will vary depending on the refinery layout, which can increase or decrease the length of a single conveyor. However, the total price is not expected to change a lot, as most of conveyor price comes from the motors, not the belt itself. A biorefinery process plant requires a high amount of pumps, where each are modelled with a standby unit, to ensure the undisturbed process even in the case of broken pump or maintenance. Therefore, the total amount of pump is the process is 50, and the purchase price in total is 677,000 $. The power used by the majority of the pumps is very low, as the pumps are only included to overcome any friction loss in the pipes and to ensure flow between process units.

It has to be noted, that 3.9 M$ of the equipment cost comes from undefined equipment, such as valves. All the equipment costs can be seen in Fig. 7.2, Fig. 7.3 and Fig. 7.4.

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor Vessel Volume = 624,89 m3	4.247.000	4.247.000
1 / 0 / 0	V-103	Flat Bottom Tank Vessel Volume = 4764,28 m3	1.237.000	1.237.000
1 / 0 / 0	V-111	Blending Tank Vessel Volume = 687,01 m3	1.186.000	1.186.000
1 / 0 / 0	V-104	Flat Bottom Tank Vessel Volume = 4466,75 m3	1.181.000	1.181.000
1 / 0 / 0	SP-101	Screw Press Throughput = 21536,12 kg/h	506.000	506.000
1 / 0 / 0	EC-102	Heat Exchanger Heat Exchange Area = 876,12 m2	463.000	463.000
1 / 0 / 0	ST-101	Heat Sterilizer Rated Throughput = 7028,30 L/h	456.000	456.000
1 / 0 / 0	V-115	Decanter Tank Vessel Volume = 65,79 m3	414.000	414.000
1 / 0 / 0	V-101	Blending Tank Vessel Volume = 1133566,97 L	397.000	397.000
1 / 0 / 0	BM-101	Bead Mill Bead Volume = 623,25 L	383.000	383.000
1 / 0 / 0	BC-104	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-103	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-105	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-101	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-106	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-102	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-107	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	EC-101	Heat Exchanger Heat Exchange Area = 358,52 m2	271.000	271.000
1 / 0 / 0	V-112	Flat Bottom Tank Vessel Volume = 534,26 m3	262.000	262.000
1 / 0 / 0	DDR-102	Drum Dryer Drum Area = 45,67 m2	246.000	246.000
1 / 0 / 0	EV-102	Multi-Effect Evaporator Mean Heat Transfer Area = 210,62 m2	221.000	221.000
1 / 0 / 0	SP-102	Screw Press Throughput = 7050,48 kg/h	207.000	207.000
1 / 0 / 0	SL-104	Silo Vessel Volume = 664174,17 L	204.000	204.000
1 / 0 / 0	V-102	Blending Tank	193.000	193.000

Figure 7.2: EER page 2, equipment cost 1/3

		Vessel Volume = 403537,17 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator Mean Heat Transfer Area = 68,25 m2	102.000	102.000
1 / 0 / 0	G-101	Centrifugal Compressor Compressor Power = 46,48 kW	75.000	75.000
1 / 0 / 0	SL-101	Silo Vessel Volume = 19963,23 L	73.000	73.000
1 / 0 / 0	SL-103	Silo Vessel Volume = 646,11 L	73.000	73.000
1 / 0 / 0	SL-102	Silo Vessel Volume = 10368,36 L	73.000	73.000
1 / 0 / 0	PFR-101	Plug Flow Reactor Vessel Volume = 285,98 L	71.000	71.000
1 / 0 / 0	PFR-102	Plug Flow Reactor Vessel Volume = 164,84 L	71.000	71.000
1 / 0 / 0	GBX-103	Generic Box Rated Throughput = 164,08 kg/h	58.000	58.000
1 / 0 / 0	GBX-104	Generic Box Rated Throughput = 120,54 kg/h	58.000	58.000
1 / 0 / 0	EH-102	Heat Exchanger Heat Exchange Area = 25,31 m2	55.000	55.000
1 / 0 / 0	V-105	Flat Bottom Tank Vessel Volume = 53484,30 L	51.000	51.000
1 / 0 / 0	DDR-101	Drum Dryer Drum Area = 0,74 m2	50.000	50.000
1 / 0 / 0	V-114	Flat Bottom Tank Vessel Volume = 49733,82 L	48.000	48.000
1 / 1 / 0	PM-101	Centrifugal Pump Pump Power = 7,09 kW	44.000	88.000
1 / 1 / 0	PM-107	Centrifugal Pump Pump Power = 6,65 kW	43.000	86.000
1 / 0 / 0	EH-101	Heat Exchanger Heat Exchange Area = 14,53 m2	40.000	40.000
1 / 0 / 0	V-107	Decanter Tank Vessel Volume = 30,38 L	34.000	34.000
1 / 0 / 0	V-108	Decanter Tank Vessel Volume = 68,15 L	34.000	34.000
1 / 0 / 0	V-110	Decanter Tank Vessel Volume = 0,95 L	34.000	34.000
1 / 0 / 0	V-113	Decanter Tank Vessel Volume = 693,49 L	34.000	34.000
1 / 1 / 0	PM-125	Centrifugal Pump Pump Power = 0,72 kW	17.000	34.000
1 / 1 / 0	PM-114	Centrifugal Pump Pump Power = 0,71 kW	16.000	32.000
1 / 1 / 0	PM-126	Centrifugal Pump Pump Power = 0,68 kW	16.000	32.000
1 / 1 / 0	PM-109	Centrifugal Pump Pump Power = 0,41 kW	13.000	26.000
1 / 1 / 0	PM-103	Centrifugal Pump Pump Power = 0,28 kW	11.000	22.000
1 / 1 / 0	PM-106	Centrifugal Pump Pump Power = 0,08 kW	10.000	20.000
1 / 1 / 0	PM-105	Centrifugal Pump	10.000	20.000

Figure 7.3: EER page 3 equipment cost 2/3

		Pump Power = 0,08 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,03 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-113	Centrifugal Pump	10.000	20.000
		Pump Power = 0,06 kW		
1 / 1 / 0	PM-102	Centrifugal Pump	10.000	20.000
		Pump Power = 0,12 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,03 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 26,39 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 1,48 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 11,37 kW		
1 / 0 / 0	V-117	Flat Bottom Tank	4.000	4.000
		Vessel Volume = 1506,33 L		
1 / 0 / 0	V-118	Flat Bottom Tank	4.000	4.000
		Vessel Volume = 1487,26 L		
1 / 0 / 0	V-116	Flat Bottom Tank	4.000	4.000
		Vessel Volume = 1328,64 L		
1 / 0 / 0	V-106	Flash Drum	2.000	2.000
		Vessel Volume = 41,80 L		
1 / 0 / 0	V-109	Flash Drum	1.000	1.000
		Vessel Volume = 12,16 L		
1 / 0 / 0	SD-101	Solids Drum	1.000	1.000
		Vessel Volume = 14,21 L		
		Unlisted Equipment		3.935.000
			TOTAL	19.674.000

Figure 7.4: EER page 4, equipment cost 3/3

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	19.674.000
2. Installation	12.771.000
3. Process Piping	6.886.000
4. Instrumentation	7.870.000
5. Insulation	590.000
6. Electrical	1.967.000
7. Buildings	8.853.000
8. Yard Improvement	2.951.000
9. Auxiliary Facilities	7.870.000
TPDC	69.432.000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	17.358.000
11. Construction	24.301.000
TPIC	41.659.000

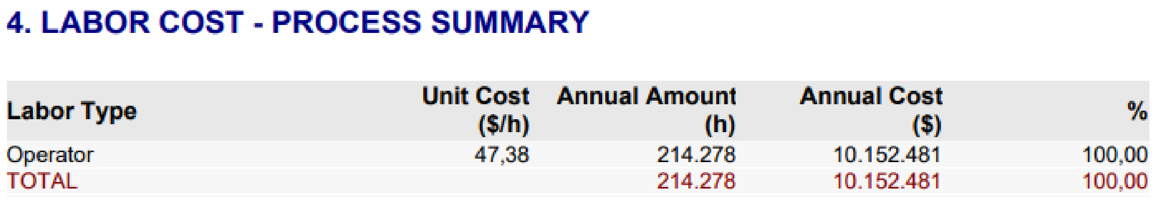
3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	111.091.000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	5.555.000
13. Contingency	11.109.000
CFC = 12+13	16.664.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	127.754.000

Figure 7.5: EER page 5, fixed capital expenditures

Fig. 7.5 shows the capital expenditures sourced from the land area, construction, equipment installation, piping and other costs with respect to building the biorefinery. These are the costs SPD tends to overestimate, as the costs like buildings, yard improvement and auxiliary facilities are different for each individual case. For example, it could possible to purchase an old existing factory, where some of the previously mentioned costs can be significantly lowered or nearly neglected. All fixed capital costs will also vary depending on the economical state of the country where the biorefinery is located. It has to be noted, SPD calculates costs like installation and instrumentation as a percentage of the purchase price of equipment, hence the fixed CAPEX is overestimated if the equipment price is overestimated. From the total biorefinery cost, 62.5 % comes from the direct costs, such as installation and instrumentation, and remaining part from indirect costs, such as engineering work.



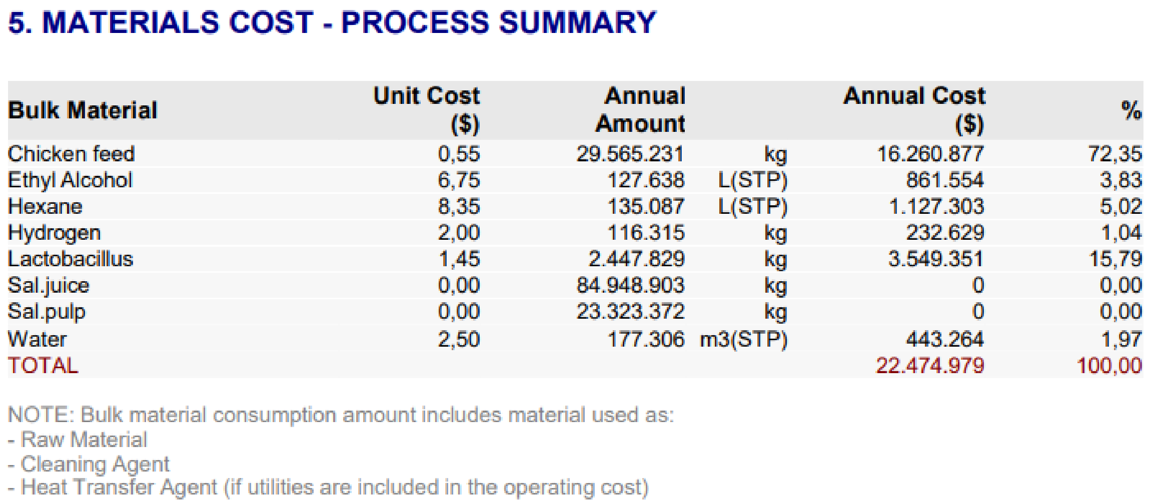
4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	47,38	214.278	10.152.481	100,00
TOTAL		214.278	10.152.481	100,00

Figure 7.6: EER page 6, labout costs

Labour costs shown in Fig. 7.6 are calculated as describes in section 6.3. Labour covers 14.43 % of the total operational costs of this process.

Large amount of operational costs are from materials and chemicals, covering 31.95 % of the total OPEX. The majority of these costs, 72.35 %, comes from purchasing the chicken-feed used as a BSFL feed. Therefore, it could be desirable to investigate an alternative feedstocks for BSFL cultivation, if the cost cannot be decreased. The second highest contributor to the material costs is *Lactobacillus acidophilus*, with a 15.79 % share of the total material costs. This price is overestimated, since the used *Lactobacillus* culture comes from the commercial dairy product A38 by Arla, which would not be the case in large scale industrial production. Purchasing the culture from the other vendor or possible *in situ* production could decrease the cost significantly. Also, the type of *Lactobacillus* strain used could be investigated to find the ideal option for this process. Purchasing the organic solvents covers 8.85 % of the total material costs, and the share could be decreased by optimising the amount of chemicals used in extractions and improving the solvent recycle even further. All the raw material and chemical costs are shown in Fig. 7.7.

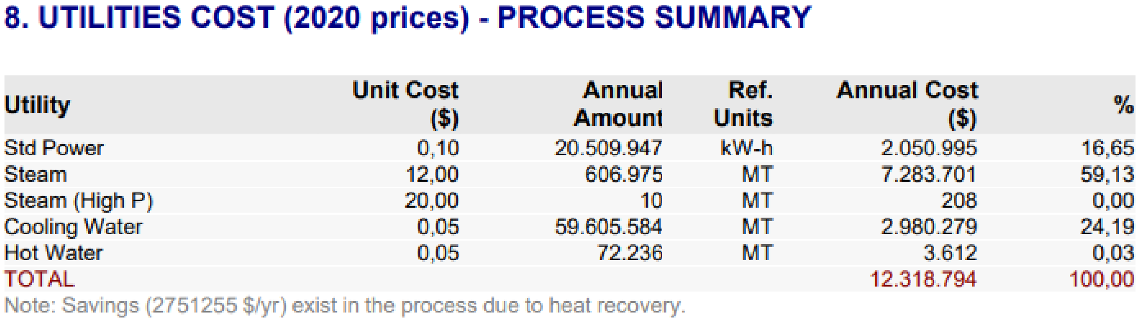


5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Chicken feed	0,55	29.565.231	kg	16.260.877	72,35
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	3,83
Hexane	8,35	135.087	L(STP)	1.127.303	5,02
Hydrogen	2,00	116.315	kg	232.629	1,04
Lactobacillus	1,45	2.447.829	kg	3.549.351	15,79
Sal.juice	0,00	84.948.903	kg	0	0,00
Sal.pulp	0,00	23.323.372	kg	0	0,00
Water	2,50	177.306	m3(STP)	443.264	1,97
TOTAL				22.474.979	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

Figure 7.7: EER page 7, raw material and chemical costs



8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	20.509.947	kW-h	2.050.995	16,65
Steam	12,00	606.975	MT	7.283.701	59,13
Steam (High P)	20,00	10	MT	208	0,00
Cooling Water	0,05	59.605.584	MT	2.980.279	24,19
Hot Water	0,05	72.236	MT	3.612	0,03
TOTAL				12.318.794	100,00

Note: Savings (2751255 $/yr) exist in the process due to heat recovery.

Figure 7.8: EER page 8, utility cost and savings due to heat recovery

Energy recovery analysis is performed manually in SPD to yield an utility cost reduction of 18.3 %. This cost could be further decreased by analysing using the pinch analysis optimisation operation. Pinch analysis will use inter stream heat exchangers to recover the most possible energy between multiple hot and cold streams. The energy recovery tool in SPD will only allow for one heat exchange interaction per stream, so not all energy can be recovered using this tool, unless there is an equal part of hot and cold streams.

Utility cost in Fig. 7.8 shows a significant part of the utility cost comes from the usage of steam and cooling water. The steam accounts for 59.13 % of the total utility cost. The price of 1 metric ton (MT) of cooling water at 25 °C is determined by SPD to be 0.05 $. The share of cost from cooling water to the total utility cost is 24.19 %. This could be significantly decreased by placing the process plant close to a large body of cold water, for example on a harbour. This will allow for easy access to free cooling water, as the only cost associated with this is the power required to pump the water into the process plants and through the heat exchangers. Using sea water for cooling might give other problems like a higher amount of maintenance for pumps and heat exchangers, as the salt in the water will lead to problems with oxidation.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

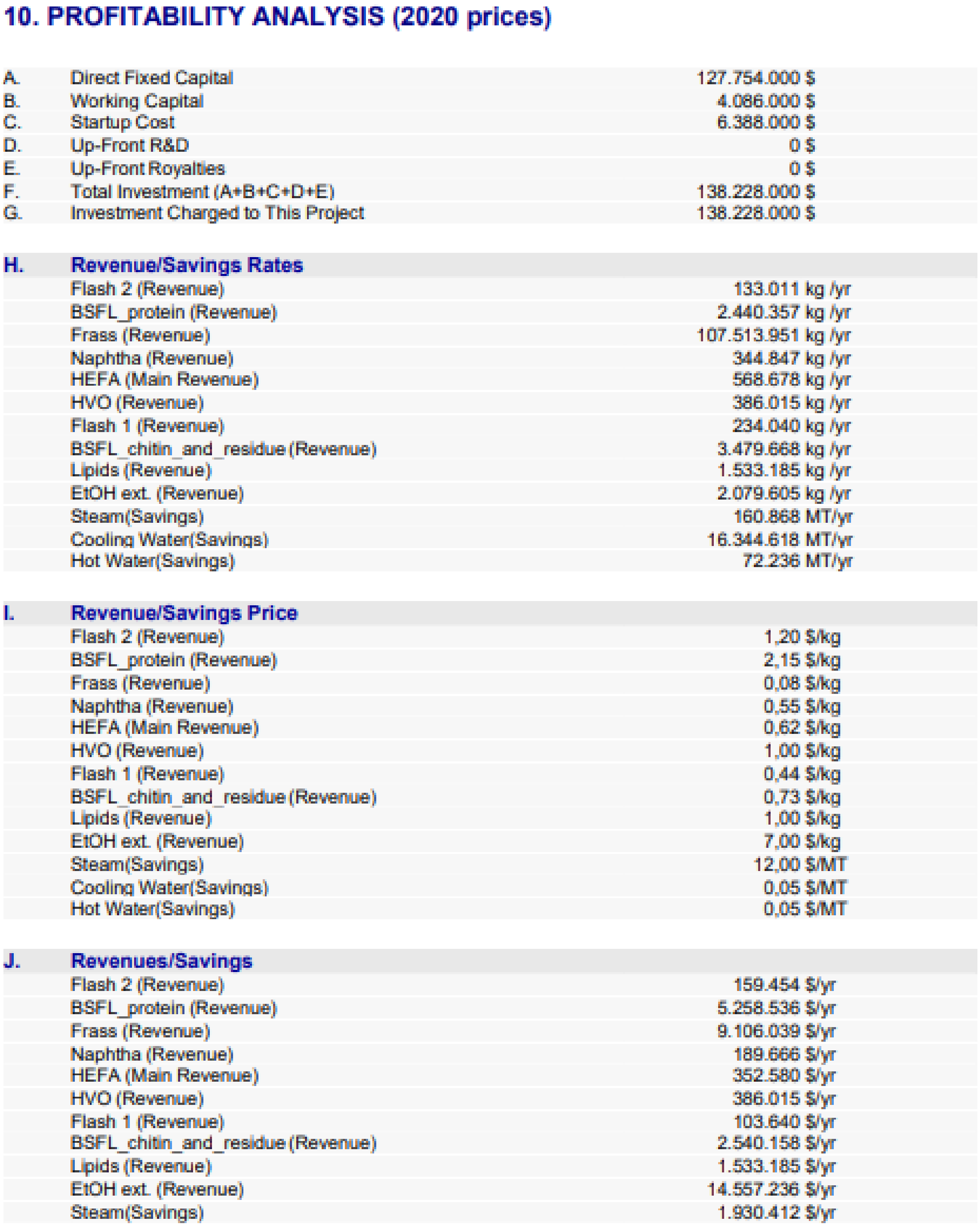
Cost Item	$	%
Raw Materials	22.475.000	31,95
Labor-Dependent	10.152.000	14,43
Facility-Dependent	23.884.000	33,95
Laboratory/QC/QA	1.523.000	2,16
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	12.319.000	17,51
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	70.353.000	100,00

Figure 7.9: EER page 9, operational costs

The summary of the total OPEX of the process is shown in Fig. 7.9, and the yearly costs are nearly 70.4 M$. The highest share of total costs (33.95 %) comes from facility dependent costs being 23.9 M$, which is assumed to be overestimated by SPD. As expected, the material costs are covering the second largest share of total OPEX, and the yearly costs could be decreased by optimising processes and material recycle. When Danish mean salary is applied, labour covers only 14.43 % of OPEX, and the share could be lower, if the process plant would be located in some other country, for example in Southern Europe.

Fig. 7.10 and 7.11 show the profitability analysis including revenues and savings. The end-product, HEFA, is only a small fraction of the overall revenues with 1.03 %, and it is set as the main revenue only as it is the end-product of the process. The another fuel product, HVO, has higher revenue compared to the sustainable jet fuel.

It can be seen that a large amount of value added products, 49.45 %, are derived from the production of BSFL. Especially the frass has a high sales value. This is due to the high price paid for organic fertilizers, and frass is an excellent fertilizer. Also the revenue from BSFL protein is relatively high. The single revenue stream with the highest value is the *S. dolichostachya* ethanol solubles, 42.58 %, and the price of this stream could be further increased by downstream processing to purify the phytochemicals, but these process steps are not included in the modelling. The revenue of the *S. dolichostavhya* lipids is low, but it has a potential to be increased, as the lipids are assumed to have a high amount of healthy fatty acids and the extraction method is modified so the oils can be sold as a nutrient supplement, like fish oil.



10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	127.754.000 $
B.	Working Capital	4.086.000 $
C.	Startup Cost	6.388.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	138.228.000 $
G.	Investment Charged to This Project	138.228.000 $

H.	**Revenue/Savings Rates**	
	Flash 2 (Revenue)	133.011 kg /yr
	BSFL_protein (Revenue)	2.440.357 kg /yr
	Frass (Revenue)	107.513.951 kg /yr
	Naphtha (Revenue)	344.847 kg /yr
	HEFA (Main Revenue)	568.678 kg /yr
	HVO (Revenue)	386.015 kg /yr
	Flash 1 (Revenue)	234.040 kg /yr
	BSFL_chitin_and_residue (Revenue)	3.479.668 kg /yr
	Lipids (Revenue)	1.533.185 kg /yr
	EtOH ext. (Revenue)	2.079.605 kg /yr
	Steam(Savings)	160.868 MT/yr
	Cooling Water(Savings)	16.344.618 MT/yr
	Hot Water(Savings)	72.236 MT/yr

I.	**Revenue/Savings Price**	
	Flash 2 (Revenue)	1,20 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,08 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,44 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	Lipids (Revenue)	1,00 $/kg
	EtOH ext. (Revenue)	7,00 $/kg
	Steam(Savings)	12,00 $/MT
	Cooling Water(Savings)	0,05 $/MT
	Hot Water(Savings)	0,05 $/MT

J.	**Revenues/Savings**	
	Flash 2 (Revenue)	159.454 $/yr
	BSFL_protein (Revenue)	5.258.536 $/yr
	Frass (Revenue)	9.106.039 $/yr
	Naphtha (Revenue)	189.666 $/yr
	HEFA (Main Revenue)	352.580 $/yr
	HVO (Revenue)	386.015 $/yr
	Flash 1 (Revenue)	103.640 $/yr
	BSFL_chitin_and_residue (Revenue)	2.540.158 $/yr
	Lipids (Revenue)	1.533.185 $/yr
	EtOH ext. (Revenue)	14.557.236 $/yr
	Steam(Savings)	1.930.412 $/yr

Figure 7.10: EER page 10, profitability 1/2

	Cooling Water(Savings)	817.231 $/yr
	Hot Water(Savings)	3.612 $/yr
1	Total Revenues	34.186.508 $/yr
2	Total Savings	2.751.255 $/yr
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	70.353.000 $/yr
2	Net AOC (K1-J2)	67.602.000 $/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	123,71 $/kg MP
	Net Unit Production Cost	118,88 $/kg MP
	Unit Production Revenue	60,12 $/kg MP
M.	Gross Profit (J-K)	- 33.416.000 $/yr
N.	Taxes (40%)	0 $/yr
O.	Net Profit (M-N + Depreciation)	- 21.279.000 $/yr
	Gross Margin	- 97,74 %
	Return On Investment	- 15,39 %
	Payback Time	N/A

MP = Total Flow of Stream 'HEFA'

Figure 7.11: EER page 11, profitability 2/2

7.2 Process scenarios

Different processing scenarios are modelled in an aim to see how different parameters affect the feasibility of the process. In this project, the investigated parameters are biomass input flow rate, dummy variable of hydrothermal pretreatment and the mean salary of the country of location for process with the lowest payback time. The economic evaluation reports from other process scenarios are collected to Appendix D.

EER	Page
Mass flow rate input sensitivity analysis	
No pretreatment, 10.77 t h^{-1}	159
No pretreatment, 21.54 t h^{-1}	174
No pretreatment, 43.07 t h^{-1}	188
No pretreatment, 86.14 t h^{-1}	202
Pretreatment, 10.77 t h^{-1}	216
Pretreatment, 21.54 t h^{-1}	231
Pretreatment, 43.07 t h^{-1}	246
Pretreatment, 86.14 t h^{-1}	261
Labour cost sensitivity analysis	
Pretreatment, 86.14 t h^{-1}, Danish	261
Pretreatment, 86.14 t h^{-1}, default	276
Pretreatment, 86.14 t h^{-1}, Portuguese	291

Table 7.1: Overview of economic evaluation reports in Appendix D

An important financial concept to examine when evaluating feasibility of the process is Return On Investment (ROI). ROI is the ratio between profits and CAPEX. This can be calculated as shown in Eq. 7.1.

$$ROI = \frac{profit}{CAPEX} \cdot 100\% \tag{7.1}$$

ROI can therefore be considered as the inverse of the payback time. If the process plant is considered to have a life time of 15 year without any big capital investments throughout the 15 years, the ROI has to be more than 6.66 % to reach profitability. A ROI higher than 6.66 % will therefore result in a profitable process plant in a reasonable time.

The key numbers from different scenarios are collected in Tab. 7.2, and it can be seen, that most of the simulations do not reach profitability in reasonable time or at all. The profitability of the process can be reached in reasonable time only when the biomass input stream is 86.1 tons of fresh *S. dolichostachya* per hour, pretreatment is applied and the annual operation time of 7920 hours. This is the equivalent of the yearly harvest from 23,200 ha or 23.2 km^2 per year as reported with a yearly yield of 4,000 kg DM ha^{-1} in Denmark by Thomsen [26]. Applying the hydrothermal pretreatment and enzymatic hydrolysis increases the performance and profitability of the process, which is due to the increase in the production of BSFL protein and frass, and the high revenue of these streams. However, as the simulated pretreatment is based on literature and assumptions, a pretreatment study and several additional feeding trials should be applied in an aim to make more accurate estimations of the biorefinery feasibility. Process optimisation prospects and considerable improvements are discussed in chapter 8.

Biomass input [t h^{-1}]	CAPEX [M$]	OPEX [M$ yr^{-1}]	Revenue [M$ yr^{-1}]	ROI [%]	Payback time [yr]
No pretreatment					
10.77	117.1	52.8	17.1	-21.7	n/a
21.54	138.2	67.6	34.2	-15.4	n/a
43.07	174.6	96.2	68.4	-7.2	n/a
86.14	238.4	151.8	136.8	2.4	42.5
Pretreatment applied					
10.77	125.5	62.0	27.8	-18.5	n/a
21.54	151.4	84.3	55.6	-10.2	n/a
43.07	194.5	127.8	111.2	0.1	775
86.14	269.2	213.2	222.4	10.6	9.5

Table 7.2: Total costs and revenue for different processing scenarios based on the biomass input and possible application of biomass pretreatment

As seen in Tab. 7.2, the ROI of the cases *No pretreatment, 86.14 t h*$^{-1}$ and *Pretreatment, 43.07 t h*$^{-1}$ is positive while the *revenue-OPEX* is negative. This is due to the depreciation value of the initially bought equipment and facility, and can therefore be seen as a net profit, while *Pretreatment, 86.14 t h*$^{-1}$ is the only simulated scenario giving a gross profit, where the *revenue-OPEX* is positive.

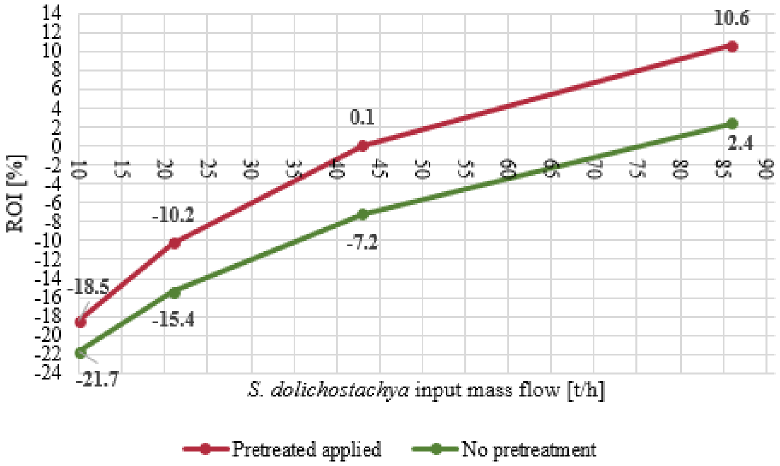


Figure 7.12: Return On Investment (ROI) as a fuction of *S. dolichostachya* biomass input for processes with pretreatment and without pretreatment

The labour cost varies a lot depending the economical state and salary level of the country where the biorefinery is located. The best scenario, the process with pretreatment and 86.14 t h^{-1} biomass input, is run by applying a mean Portuguese process operator salary 5.1 USD h^{-1} [166], and it is compared to the Danish salary level and high SPD default basic salary of 30 USD h^{-1}. The salary unit cost noted in Tab. 7.3 includes the additives describes in section 6.3. Analysis shows, that moving the production from Denmark to Portugal would decrease the share of labour costs from 5.0 % to 1.3 % of the total OPEX.

	Unit cost [$ h^{-1}]	OPEX [M$ yr^{-1}]	Total LC [M$ yr^{-1}]	LC from OPEX [%]	Payback time [yr]
Danish	20.6	213.3	10.73	5.0	9.5
Portuguese	5.1	204.0	2.67	1.3	7.9
Default	30.0	218.9	15.62	7.1	10.7

Table 7.3: The effect of labour cost (LC) to the overall economics of the process.

Chapter 8

Discussion

As the increasing soil salinisation degrades the agricultural land area, and the political agreements to fight the effects of greenhouse gas emission are increasing the demand for sustainable CO_2-reduced biofuels, characterisation and process simulation is done for *S. dolichostachya* halophyte biomass to evaluate its suitability for biorefinery purposes. The first project objective is the broad characterisation of green fractionated *S. dolichostachya* and studying the seasonal variation in the biochemical composition of the plant from September 2019 to December 2019. Based on the laboratory experiment results, *S. dolichostachya* has shown similar properties as other closely related species. However, since many of the literature studies consider fresh green biomass, harvesting the less mature succulent shrubs during summer months and performing the characterisation for that biomass would help to make more accurate comparisons between *Salicornia* species. Similarly, determination of sugars and protein for the 4th December 2019 harvested dry shrubs would have been desired, but not all analysis were able to carried out due to the spread of coronavirus COVID-19 in Denmark. Therefore, many properties of *S. dolichostachya* are yet to be researched and discovered.

Characterisation results show very low lipid content, which is typical for the succulent halophytes [13]. However, the extraction of the lipids can be beneficial, if the non-polar fraction includes high amounts of health beneficial fatty acids, such as ω-3 and ω-6 fatty acids, or lipid soluble pigments, such as β-carotene. Besides β-carotene, xanthophylls and other carotenoids have also shown antioxidant properties, and the extracts including these compounds have good market values, as they can be used for nutritional and pharmaceutical purposes [47]. The fatty acid profile could be defined by running gas chromatography and mass spectrometry for fatty acid methyl esters, and the concentration of pigments in botanical extract could be determined with spectrophotometric methods. Determining the fatty acid profile of the biomass gives important information, when deciding the possible target products of biorefinery, for example, knowing the ratio of ω-3 and ω-6 fatty acids is crucial, if the biomass is wanted to be used for aquaculture feed purposes [167] or as a nutritional supplement. This could also affect the decision of the extraction method. If the fractions of ω-3 and ω-6 of total fatty acids are low, extracted lipids could be inputted to biofuel processing together with BSFL lipids, as the sales values of bio fuels are lower than the sales values healthy fatty acids.

Quantifying the chlorophyll content in the *S. dolichostachya* can be considered unsuccessful, as the results are significantly lower than the reference values from the literature [53, 47], and most of the chlorophyll pigment in the biomass had degraded due to incorrect and unsuitable storage. Therefore, ethanol extraction and chrolophyll determination from the extract should be done right after next harvest, to validate the results or find the accurate chlorophyll content of *S. dolichostachya*. Regarding the extractive products, in this project, the type and amount of phytochemicals are not considered, because based in literature studies, phytochemicals are usually extracted from the whole dried shrub, not green fractionated biomass. However, analysing the concentrations of hydroxycinnamic acids, phenolic acids and flavonoids would open a possibility to add new value-added production streams.

Ashing of *S. dolichostachya* dry matter samples gives an interesting results considering the visual appearance of the ash, since the colour of the ash changed over time in both juice and pulp fractions. Further analysis using atomic absorption would determine the amount and type of different metals and minerals present in the ash, which is a relevant information, especially when ash covers a large fraction of the total dry matter, especially in less mature halophytes.

Protein separation could be one of the considered value-added processes for biorefinery, as protein can be valorized for feed production [54], and based on the experimental results, the protein content of *S. dolichostachya* remains nearly constant as the plant matures and lignifies. Extracting proteins from the juice fraction could give value to the juice fraction, which is not considered in this process simulation, and therefore increase the overall process feasibility. One potential way for the protein extraction from the green juice could be lactic acid fermentation, which is relatively simple but commonly used technology [87].

Pretreatment study for sugar yield enhancement is left out from the project scope due to the spread of coronavirus COVID-19 in Denmark. Previous research considering the whole shrubs [39] and green fractionated *Salicornia* sp. [48] are showing positive results considering the enzymatic conversion and sugar recovery. Literature-based hydrothermal pretreatment is simulated, and it is assumed, that pretreatment would increase the digestibility of extractive-free fibres and increase the sugars-to-lipids conversion rate. The economic evaluation report shows, that applying the pretreatment increases the overall performance of the process. Fractionated and extractive-free *S. dolichostachya* is assumed to be less recalcitrant as typical conventional lignocellulose, hence, relatively mild pretreatment conditions could have been adapted. It could be interesting to study, if lower temperatures can actually provide sufficient sugar recovery, without harmful by-products from pentose degradation. Also, modest operation temperature would save energy and lower the utility costs of the biorefinery. Even if furfural and toxic furan compounds derived from hemicellulose and lignin are not considered in this project, the content of these compounds in the pretreatment product should be examined and possible extraction of those chemicals should be added to the process schematic. The lignin content of *Salicornia* spp. are relatively low, but for the most mature shrubs, oragnosolv pretreatment could be considered to separating the lignin and preparing more pure feedstock to be used in sugars-to-lipids conversion. Lignin can be utilised in the production of aromatic chemicals [24].

The second project objective, sugars-to-lipids conversion using BSFL, included the feeding trials which were carried out successfully with a good co-operation with ENORM ApS. The post-processing of larvae included the extractions with different solvents, to find the amount of lipids and other extractive compounds in the larvae dry matter. Sugars-to-lipids conversion using BSFL is a novel technology with a lot of potential, but as the field is still under development and focused on feed production and waste treatment [119], further research and *in vivo* studies will be needed to evaluate the suitability of this method for green biomass-based fuel production. As the process simulation shows, 44-46 % of total material costs come from the purchasing the chicken-feed. Therefore, further feeding trials considering alternative, low-cost feedstocks could be considered.

Determining the fatty acid profile of the oil yielded from chicken-feed and partly *S. dolichostachya* fed larvae would give a knowledge considering the effects of feed to the BSFL lipids, as some changes could be expected based on the colour and texture of the yielded oils. Currently, the BSFL production is focused on protein and animal feed manufacturing, hence in the future studies, it would be important to analyse the effect of used feedstock to the protein content of larvae. It would also be desired to run detailed analysis for the extracts, as *Salicornia* spp. are previously shown to produce high amount of bioactive secondary metabolites [26], and it would be interesting to see, if some of these phytochemicals are accumulating in a food chain, which would open the door for food science and medical research.

Considering the liquid biofuel production, the conversion using oleaginous micro-organisms, such as yeasts and fungi, should also be investigated. Even if utilizing micro-organisms usually requires specific cultivation conditions and biomass pretreatment, studies have shown high yields of single cell oils, even when fed with non-detoxified lignocellulose hydrolysate [113]. The growth cycle of oleaginous micro-organisms are shorter compared to BSFL, only a few days instead of two weeks, and operational costs would be lower, as expensive additive feed would not be needed. Fungi would also provide protein and chitin as value-added products, similarly to BSFL. If the extractive-free fibre fraction of *S. dolichostachya* is less recalcitrant compared to conventional lignocellulosic biomasses as assumed, it could be seen as a potential feedstock for this type of process, due to milder pretreatment requirements.

Optimisation of extraction processes could be done to increase the profitability, for example, by selecting the right solvents, using minimum amount of solvent per mass unit of dry matter, and modifying the extraction time. This could be done by laboratory experiments, and implementing results to process simulation. Chemicals used in the extractions are evaporated off for recycle purposes, but as the evaporation unit is modelled to only partly recycle the solvent, some additive chemicals are required. This cost is up to 16.3 % of total material costs from the process with a low input mass flow, thus, the possible improvements of solvent recycling should be investigated. Also, it could be desired to run the simulation of biomass processing by using the data from each harvested batch of *S. dolichostachya* to find out, which biochemical composition gives the most profitable end result, and when would be the optimal time to harvest the biomass.

Considering the third project objective, process simulation, a large process schematic is made and all planned process scenarios are executed. The schematic includes the biomass and biofuel processing, and possible hydrothermal pretreatment. In the future, the process could be expanded to cover processing of value-added products also from the juice fraction of *S. dolichostachya*, and lipid pretreatment, as the BSFL lipids extracted with screw press will include high amounts of impurities. Including these process phases will give a more accurate estimations about the process profitability.

In techno-economic analysis, the overall feasibility of the process is evaluated, and the process does not show to be feasible for most of scenarios due to high operational costs. The process is profitable only when the fresh biomass input is 86.1 tons hr^{-1}, and the pretreatment is applied to the fibers before using them as a BSFL feed. The payback time would be 7.9 or 9.5 years, depending if the biorefinery is located in Portugal or Denmark, respectively. The capital expenditures estimated by SPD are high, but as described in 7.1, some of the CAPEX and facility dependent OPEX are overestimated, as the model is run only with the default settings considering the plant construction. In all the scenarios, a high share of OPEX is sourced from purchasing the raw materials and chemicals, and the share increases as the biomass input increases. Material costs cover 27.8 - 58.2 % or 22.1 - 54.0 % of total OPEX, depending if the pretreatment is applied or not, respectively. The share of labour cost from total OPEX is decreasing, when the biomass input increases, and as expected, it is lowest when the biorefinery is located in the county with lowest average salary level. Utility costs for heating and cooling covers 14.8 - 17.3 % or 12.2 - 20.2 % of total OPEX, depending if the pretreatment is applied or not, respectively. Large amount of these costs can be saved by using inter-stream heat exchangers, and utilizing the excess heat from the processing units is an environmentally sustainable option, as less power would be needed from outer energy sources.

In the case of process optimization, mathematical programming could be applied. It is a powerful tool for optimisation of industrial processes, for example, fuel produced or product extracted per USD over the lifetime of the process. Single process steps could be optimised by running laboratory experiments, but in order to do a full optimisation of the overall process, solving the optimisation problem is often considered. Mathematical programming solves problems by optimising objective functions, for example, to maximise fuel production per USD, through constraints, financial analysis, and experimentally defined mass balances and reaction rates. Constraints can be defined by available input material streams, labour hours or physical limitations, such as maximum amount of biomass inside a reactor per hour. Financial analysis would take into account CAPEX, OPEX and revenue of the process equipment and products. This would give information about each process step of the biorefinery: which ones are profitable, and which ones should be left out to maximise the profits.

In this project, the sustainable aviation fuel is chosen to be produced by ASTM approved HEFA route, which is a process using oils as a feedstock. It might be relevant to assess other technologies, such as kerosene production by Ficher-Tropsch synthesis, or hydrothermal liquefaction of fibres, which is seen as a promising future technology for the production of bio-oil. Fischer-Tropsch synthetic paraffinic kerosene is already ASTM approved in up to a 50 w% blend-in with conventional fossil fuels [127]. Also, even if sugar-based biofuels are not ASTM approved yet, according to National Renewable Energy Laboratory [125], continuous research is done in the field, successful trials are performed and airlines are trying to advocate for the approval of these fuels. Therefore, it may also be relevant to take a look at these processing routes in the future.

Overall, the projects shows that only 0.7 w% of fresh *S. dolichostachya* biomass and 4.8 w% of the dry matter ends up to the fuel products, and the fraction of jet fuel from all the fuel product is 43.8 w%. With the selected process routes and parameters, the simulation does show the biorefinery to be feasible within the reasonable time. This is true only when the fresh *S. dolichostachya* input is high and the pretreatment unit is applied. Therefore, further research and optimisation studies are needed to evaluate the suitability of *S. dolichostachya* to liquid fuel production purposes more accurately, in an aim to develop a robust biorefinery process.

Chapter 9

Conclusion

Based on the experimental results and literature studies, *S. dolichostachya* has shown similar properties as other closely related succulent *Salicornia* species. It has a low lipid content, and relatively large fractions of lignocellulose and ash. Characterisation of green fractionated biomass gives a lot of novel information about this one of the least studied *Salicornia* species, yet more research and optimisation studies needs to be carried out to determine the species full potential as a biorefinery feedstock.

Considering the sugars-to-lipids conversion, part of the conventional BSFL feed can be replaced with *S. dolichostachya* without effecting the growth and lipid content of larvae. However, visual observations of the colour and texture of the extracted oils indicated the changes in the fatty acid profile. Based on the results from feeding trials, fresh pulp seems to be more suitable feedstock compared to extractive-free fibres. However, the purchasing price of conventional chicken-feed covers up to 75 % of total material costs, depending on the biomass input.

S. dolichostachya biomass processing and biofuel production from BSFL lipids through ASTM approved HEFA route does show to be profitable within ten years, only if the input flow is 86.14 tons per hour and fibrous biomass is pretreated before conversion. Pretreatment of fibres increases the performance of the biorefinery, but the simulation is only based on literature studies and assumptions. Therefore, laboratory experiments, feeding trials and an optimisation study is needed to improve the process and evaluate the feasibility of each step. The biorefinery process is the most profitable, when the possible plant is located Portugal, as the local economical situation and the mean salary level is lower compared to Denmark. According the energy recovery analysis, up to 18.3 % of the utility cost can be saved yearly by using inter-stream heat exchangers, when the biomass input is 21.54 t h^{-1} and pretreatment is not applied.

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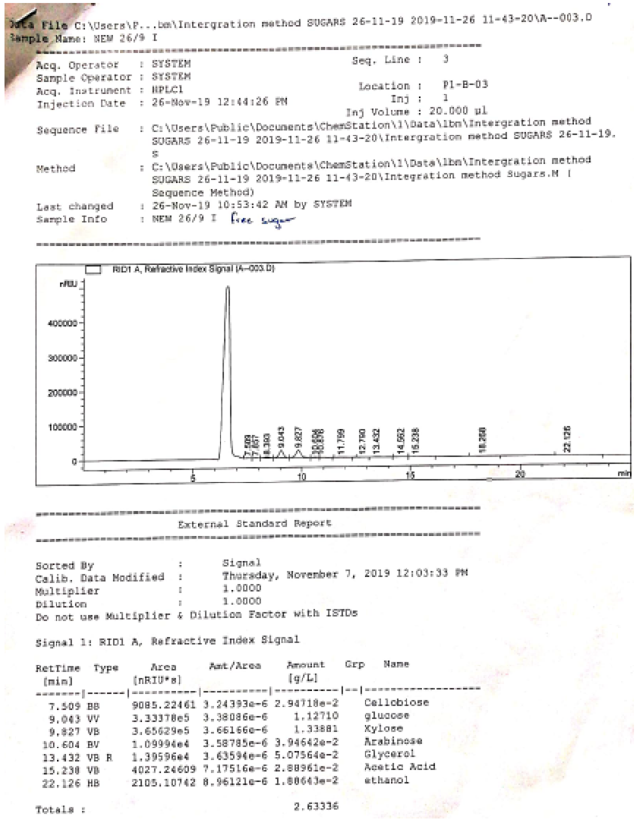
Appendices

Appendix A

HPLC chromatograms



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=====================================================================
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Sample Operator : SYSTEM
Acq. Instrument : HPLC1                            Location :   P1-B-03
Injection Date  : 26-Nov-19 12:44:26 PM                 Inj :   1
                                               Inj Volume : 20.000 µl
Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 26-11-19 2019-11-26 11-43-20\Intergration method SUGARS 26-11-19.
                  S
Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
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                  Sequence Method)
Last changed    : 26-Nov-19 10:53:42 AM by SYSTEM
Sample Info     : NEM 26/9 I   free sugar
=====================================================================
```

```
=====================================================================
                     External Standard Report
=====================================================================

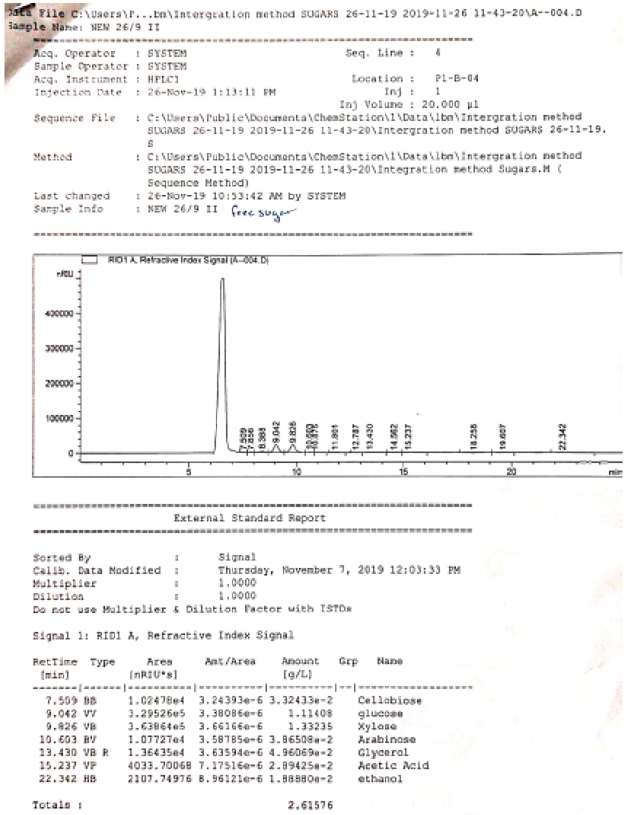
Sorted By             :      Signal
Calib. Data Modified  :      Thursday, November 7, 2019 12:03:33 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount   Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.509 BB     9085.22461 3.24393e-6 2.94718e-2    Cellobiose
  9.043 VV     3.33378e5  3.38086e-6    1.12710    glucose
  9.827 VB     3.65629e5  3.66166e-6    1.33881    Xylose
 10.604 BV     1.09994e4  3.58785e-6 3.94642e-2    Arabinose
 13.432 VB R   1.39596e4  3.63594e-6 5.07564e-2    Glycerol
 15.238 VB     4027.24609 7.17516e-6 2.88961e-2    Acetic Acid
 22.126 HB     2105.10742 8.96121e-6 1.88643e-2    ethanol

Totals :                                2.63336
```

Figure A.1: Free sugars, 26th Sep. 2019, sample 1



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=====================================================================
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Sample Operator : SYSTEM
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Injection Date  : 26-Nov-19 1:13:11 PM                 Inj :   1
                                               Inj Volume : 20.000 µl
Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 26-11-19 2019-11-26 11-43-20\Intergration method SUGARS 26-11-19.
                  S
Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 26-11-19 2019-11-26 11-43-20\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 26-Nov-19 10:53:42 AM by SYSTEM
Sample Info     : NEW 26/9 II   Free sugar

=====================================================================

=====================================================================
                     External Standard Report
=====================================================================

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Calib. Data Modified  :      Thursday, November 7, 2019 12:03:33 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type    Area      Amt/Area    Amount    Grp   Name
 [min]         [nRIU*s]                [g/L]
-------|------|----------|----------|----------|--|------------------
  7.509 BB     1.02478e4  3.24393e-6 3.32433e-2    Cellobiose
  9.042 VV     3.29526e5  3.38086e-6    1.11408    glucose
  9.826 VB     3.63864e5  3.66166e-6    1.33235    Xylose
 10.603 BV     1.07727e4  3.58785e-6 3.86508e-2    Arabinose
 13.430 VB R   1.36435e4  3.63594e-6 4.96069e-2    Glycerol
 15.237 VP     4033.70068 7.17516e-6 2.89425e-2    Acetic Acid
 22.342 HB     2107.74976 8.96121e-6 1.88880e-2    ethanol

Totals :                               2.61576
```
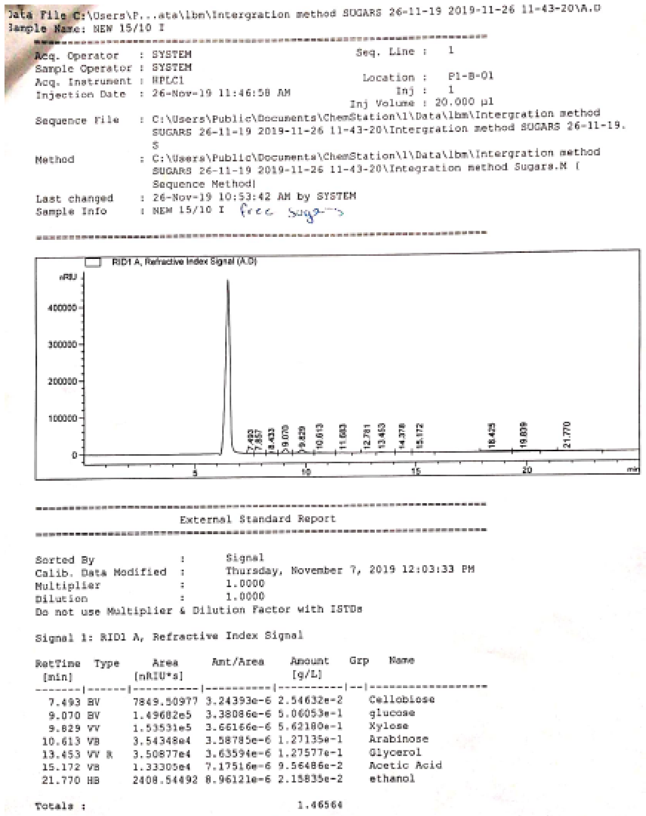

Figure A.2: Free sugars, 26th Sep. 2019, sample 2



```
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=====================================================================
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Sample Operator : SYSTEM
Acq. Instrument : HPLC1                          Location :   P1-B-01
Injection Date  : 26-Nov-19 11:46:58 AM                Inj :   1
                                               Inj Volume : 20.000 µl
Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 26-11-19 2019-11-26 11-43-20\Intergration method SUGARS 26-11-19.
                  S
Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 26-11-19 2019-11-26 11-43-20\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 26-Nov-19 10:53:42 AM by SYSTEM
Sample Info     : NEW 15/10 I   Free sugars

=====================================================================

=====================================================================
                     External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, November 7, 2019 12:03:33 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type    Area      Amt/Area    Amount    Grp   Name
 [min]         [nRIU*s]                [g/L]
-------|------|----------|----------|----------|--|------------------
  7.493 BV     7849.50977 3.24393e-6 2.54632e-2    Cellobiose
  9.070 BV     1.49682e5  3.38086e-6 5.06053e-1    glucose
  9.829 VV     1.53531e5  3.66166e-6 5.62180e-1    Xylose
 10.613 VB     3.54348e4  3.58785e-6 1.27135e-1    Arabinose
 13.453 VV R   3.50877e4  3.63594e-6 1.27577e-1    Glycerol
 15.172 VB     1.33305e4  7.17516e-6 9.56486e-2    Acetic Acid
 21.770 HB     2408.54492 8.96121e-6 2.15835e-2    ethanol

Totals :                               1.46564
```
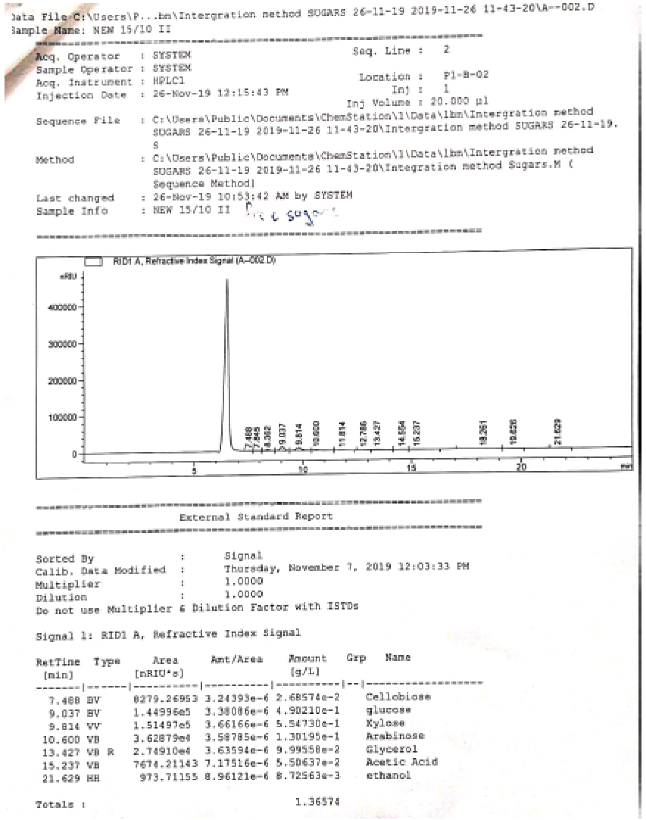

Figure A.3: Free sugars, 15th Oct. 2019, sample 1



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=====================================================================
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Sample Operator : SYSTEM
Acq. Instrument : HPLC1                       Location :   P1-B-02
Injection Date  : 26-Nov-19 12:15:43 PM            Inj :    1
                                          Inj Volume : 20.000 µl
Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 26-11-19 2019-11-26 11-43-20\Intergration method SUGARS 26-11-19.
                  S
Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 26-11-19 2019-11-26 11-43-20\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 26-Nov-19 10:53:42 AM by SYSTEM
Sample Info     : NEW 15/10 II  free sugars
=====================================================================

=====================================================================
                        External Standard Report
=====================================================================

Sorted By                 :      Signal
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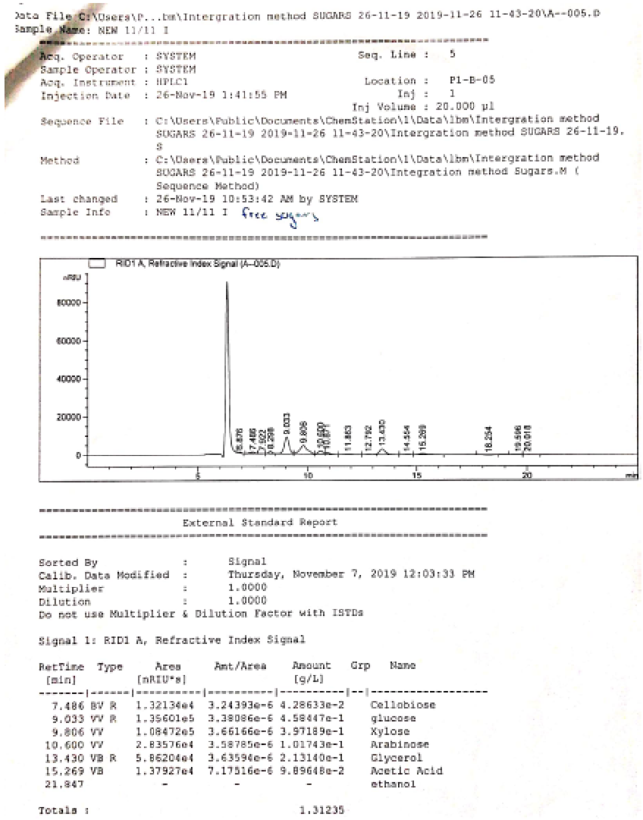
Dilution                  :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.488 BV     8279.26953 3.24393e-6 2.68574e-2    Cellobiose
  9.037 BV     1.44996e5  3.38086e-6 4.90210e-1    glucose
  9.814 VV     1.51497e5  3.66166e-6 5.54730e-1    Xylose
 10.600 VB     3.62879e4  3.58785e-6 1.30195e-1    Arabinose
 13.427 VB R   2.74910e4  3.63594e-6 9.99558e-2    Glycerol
 15.237 VB     7674.21143 7.17516e-6 5.50637e-2    Acetic Acid
 21.629 HH      973.71155 8.96121e-6 8.72563e-3    ethanol

Totals :                             1.36574
```

Figure A.4: Free sugars, 15th Oct. 2019, sample 2



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=====================================================================
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Injection Date  : 26-Nov-19 1:41:55 PM             Inj :    1
                                          Inj Volume : 20.000 µl
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                  S
Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
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Last changed    : 26-Nov-19 10:53:42 AM by SYSTEM
Sample Info     : NEW 11/11 I  free sugars
=====================================================================

=====================================================================
                        External Standard Report
=====================================================================

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Dilution                  :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.486 BV R   1.32134e4  3.24393e-6 4.28633e-2    Cellobiose
  9.033 VV R   1.35601e5  3.38086e-6 4.58447e-1    glucose
  9.806 VV     1.08472e5  3.66166e-6 3.97189e-1    Xylose
 10.600 VV     2.83576e4  3.58785e-6 1.01743e-1    Arabinose
 13.430 VB R   5.86204e4  3.63594e-6 2.13140e-1    Glycerol
 15.269 VB     1.37927e4  7.17516e-6 9.89648e-2    Acetic Acid
 21.847            -          -          -         ethanol

Totals :                             1.31235
```
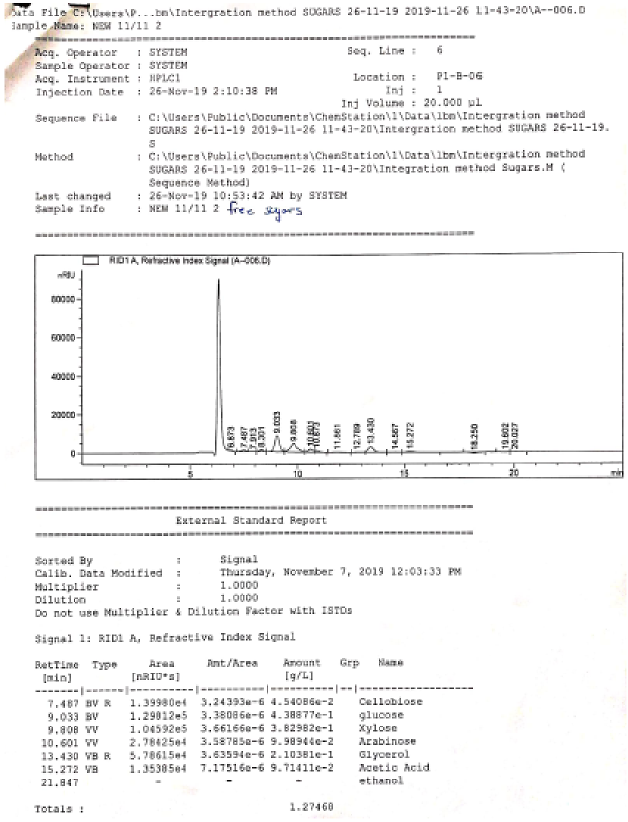
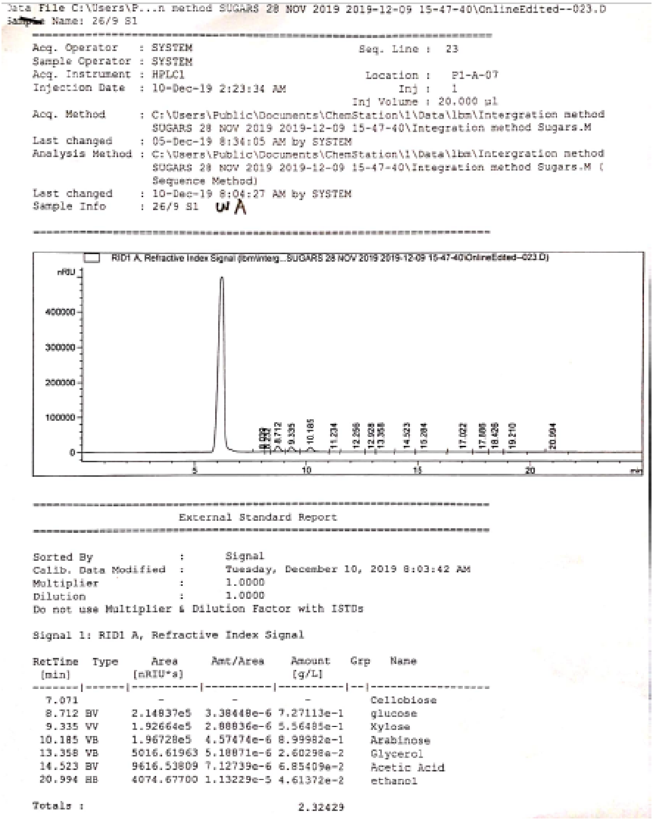

Figure A.5: Free sugars, 11th Nov. 2019, sample 1



```
Data File C:\Users\P...bm\Intergration method SUGARS 26-11-19 2019-11-26 11-43-20\A--006.D
Sample Name: NEW 11/11 2
=====================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   6
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                          Location :   P1-B-06
Injection Date  : 26-Nov-19 2:10:38 PM                Inj :   1
                                               Inj Volume : 20.000 µl
Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 26-11-19 2019-11-26 11-43-20\Intergration method SUGARS 26-11-19.
                  S
Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 26-11-19 2019-11-26 11-43-20\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 26-Nov-19 10:53:42 AM by SYSTEM
Sample Info     : NEW 11/11 2 free sugars
=====================================================================
```

```
=====================================================================
                    External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Thursday, November 7, 2019 12:03:33 PM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area     Amt/Area    Amount    Grp   Name
 [min]          [nRIU*s]               [g/L]
-------|------|----------|----------|----------|--|------------------
  7.487 BV R   1.39980e4  3.24393e-6 4.54086e-2     Cellobiose
  9.033 BV     1.29812e5  3.38086e-6 4.38877e-1     glucose
  9.808 VV     1.04592e5  3.66166e-6 3.82982e-1     Xylose
 10.601 VV     2.78425e4  3.58785e-6 9.98944e-2     Arabinose
 13.430 VB R   5.78615e4  3.63594e-6 2.10381e-1     Glycerol
 15.272 VB     1.35385e4  7.17516e-6 9.71411e-2     Acetic Acid
 21.847           -          -          -           ethanol

Totals :                              1.27460
```

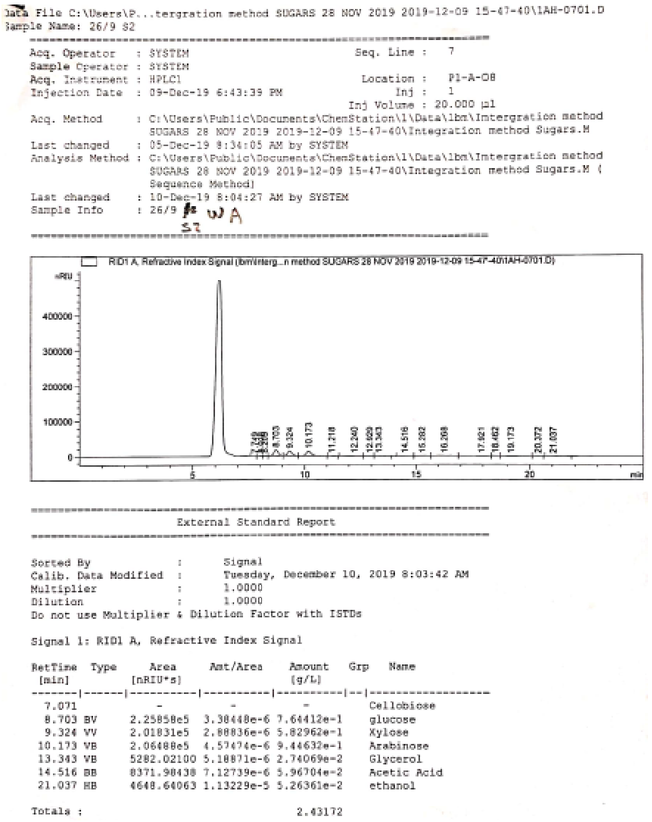
Figure A.6: Free sugars, 11th Nov. 2019, sample 2



```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--023.D
Sample Name: 26/9 S1
=====================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  23
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                          Location :   P1-A-07
Injection Date  : 10-Dec-19 2:23:34 AM                 Inj :   1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
Last changed    : 05-Dec-19 8:34:05 AM by SYSTEM
Analysis Method : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
Sample Info     : 26/9 S1   WA
=====================================================================
```

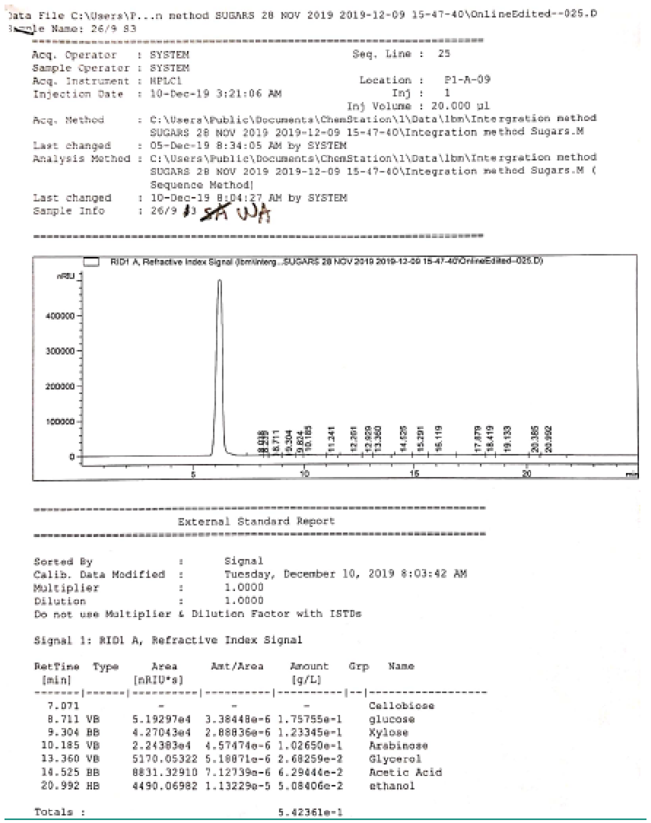
```
=====================================================================
                    External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area     Amt/Area    Amount    Grp   Name
 [min]          [nRIU*s]               [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071            -          -          -          Cellobiose
  8.712 BV     2.14837e5  3.38448e-6 7.27113e-1     glucose
  9.335 VV     1.92664e5  2.88836e-6 5.56485e-1     Xylose
 10.185 VB     1.96728e5  4.57474e-6 8.99982e-1     Arabinose
 13.358 VB     5016.61963 5.18871e-6 2.60298e-2     Glycerol
 14.523 BV     9616.53809 7.12739e-6 6.85409e-2     Acetic Acid
 20.994 BB     4074.67700 1.13229e-5 4.61372e-2     ethanol

Totals :                              2.32429
```

Figure A.7: Juice WAH, 26th Sep. 2019, sample 1, spiked



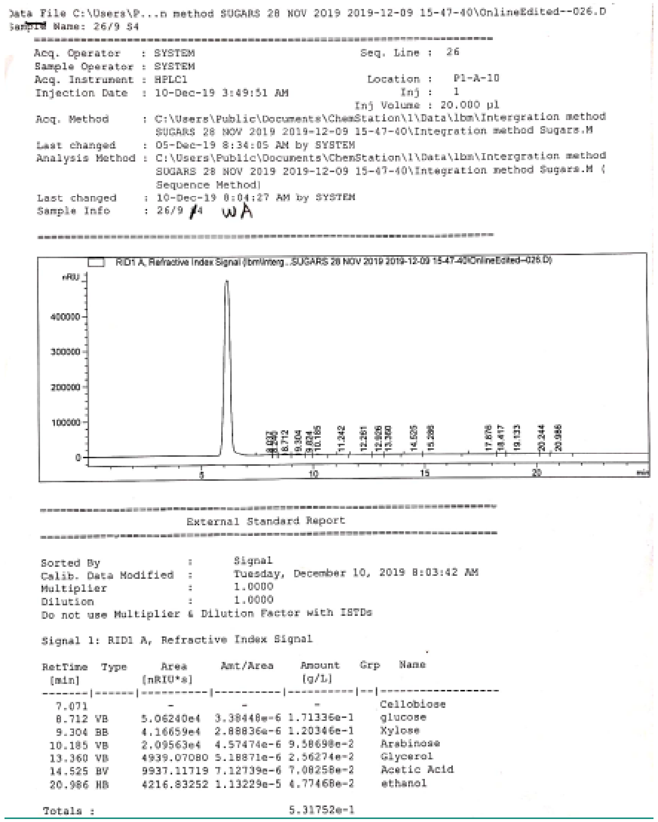
```
Data File C:\Users\P...tergration method SUGARS 28 NOV 2019 2019-12-09 15-47-40\1AH-0701.D
Sample Name: 26/9 S2
=====================================================================
Acq. Operator   : SYSTEM                        Seq. Line :   7
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                          Location :   P1-A-08
Injection Date  : 09-Dec-19 6:43:39 PM                Inj :   1
                                              Inj Volume : 20.000 µl
Acq. Method     : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
Last changed    : 05-Dec-19 8:34:05 AM by SYSTEM
Analysis Method : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
Sample Info     : 26/9 #S WA
                       S2
=====================================================================

=====================================================================
                    External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type    Area      Amt/Area    Amount    Grp   Name
 [min]         [nRIU*s]               [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071           -          -          -         Cellobiose
  8.703 BV    2.25858e5  3.30448e-6 7.64412e-1    glucose
  9.324 VV    2.01831e5  2.88836e-6 5.82962e-1    Xylose
 10.173 VB    2.06488e5  4.57474e-6 9.44632e-1    Arabinose
 13.343 VB    5282.02100 5.18871e-6 2.74069e-2    Glycerol
 14.516 BB    8371.98438 7.12739e-6 5.96704e-2    Acetic Acid
 21.037 HB    4648.64063 1.13229e-5 5.26361e-2    ethanol

Totals :                            2.43172
```

Figure A.8: Juice WAH, 26th Sep. 2019, sample 2, spiked



```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--025.D
Sample Name: 26/9 S3
=====================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  25
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                          Location :   P1-A-09
Injection Date  : 10-Dec-19 3:21:06 AM                Inj :   1
                                              Inj Volume : 20.000 µl
Acq. Method     : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
Last changed    : 05-Dec-19 8:34:05 AM by SYSTEM
Analysis Method : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
Sample Info     : 26/9 #3 ~~SA~~ WA
=====================================================================

=====================================================================
                    External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type    Area      Amt/Area    Amount    Grp   Name
 [min]         [nRIU*s]               [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071           -          -          -         Cellobiose
  8.711 VB    5.19297e4  3.38448e-6 1.75755e-1    glucose
  9.304 BB    4.27043e4  2.88836e-6 1.23345e-1    Xylose
 10.185 VB    2.24383e4  4.57474e-6 1.02650e-1    Arabinose
 13.360 VB    5170.05322 5.18871e-6 2.68259e-2    Glycerol
 14.525 BB    8831.32910 7.12739e-6 6.29444e-2    Acetic Acid
 20.992 HB    4490.06982 1.13229e-5 5.08406e-2    ethanol

Totals :                            5.42361e-1
```

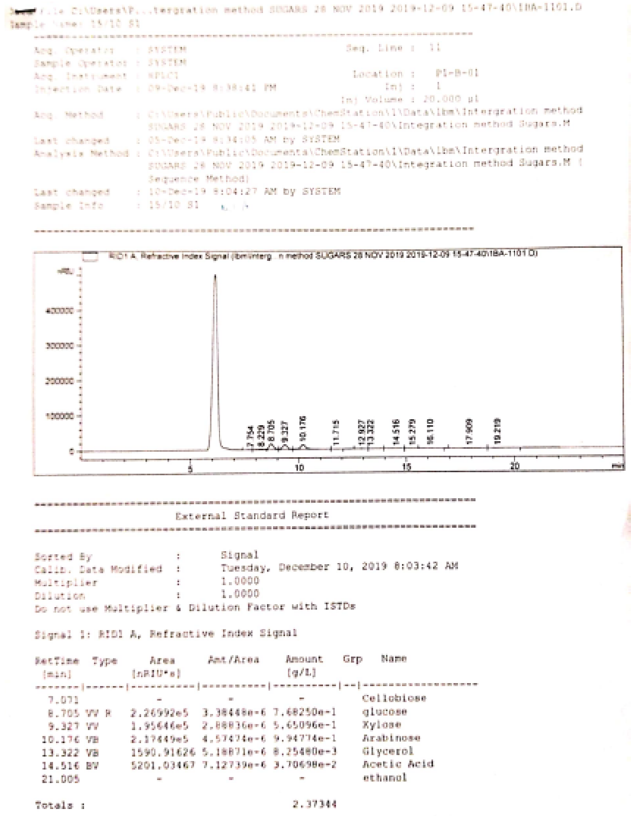
Figure A.9: Juice WAH, 26th Sep. 2019, sample 3



```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--026.D
Sample Name: 26/9 S4
=====================================================================
   Acq. Operator    : SYSTEM                          Seq. Line :  26
   Sample Operator  : SYSTEM
   Acq. Instrument  : HPLC1                            Location :   P1-A-10
   Injection Date   : 10-Dec-19 3:49:51 AM                  Inj :   1
                                                     Inj Volume : 20.000 µl
   Acq. Method      : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
   Last changed     : 05-Dec-19 8:34:05 AM by SYSTEM
   Analysis Method  : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                      Sequence Method)
   Last changed     : 10-Dec-19 8:04:27 AM by SYSTEM
   Sample Info      : 26/9 /4   WA
=====================================================================
```

```
=====================================================================
                      External Standard Report
=====================================================================

Sorted By              :      Signal
Calib. Data Modified   :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier             :      1.0000
Dilution               :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071             -          -          -          Cellobiose
  8.712 VB     5.06240e4  3.38448e-6 1.71336e-1      glucose
  9.304 BB     4.16659e4  2.88836e-6 1.20346e-1      Xylose
 10.185 VB     2.09563e4  4.57474e-6 9.58698e-2      Arabinose
 13.360 VB     4939.07080 5.18871e-6 2.56274e-2      Glycerol
 14.525 BV     9937.11719 7.12739e-6 7.08258e-2      Acetic Acid
 20.986 HB     4216.83252 1.13229e-5 4.77468e-2      ethanol

Totals :                             5.31752e-1
```

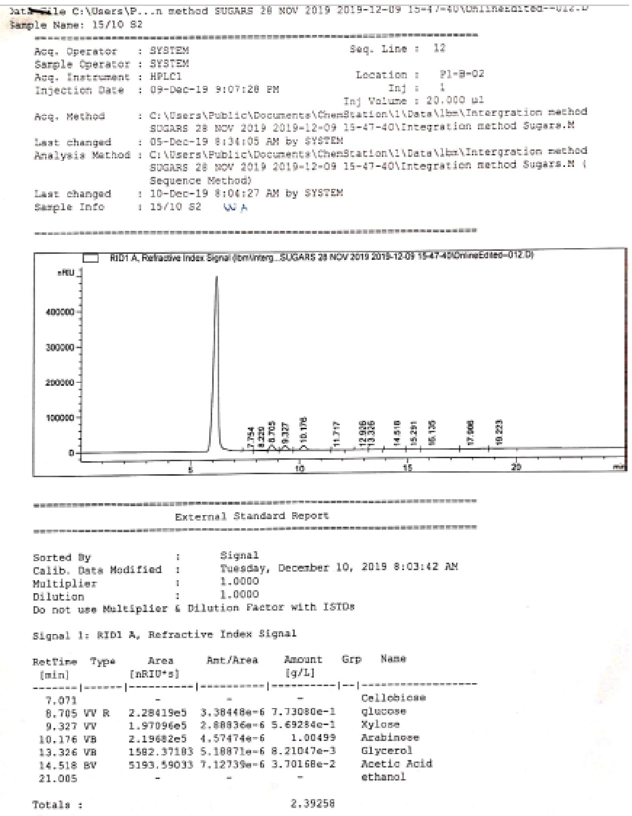
Figure A.10: Juice WAH, 26th Sep. 2019, sample 4



```
[illegible] File C:\Users\P...tergration method SUGARS 28 NOV 2019 2019-12-09 15-47-40\IBA-1101.D
Sample Name: 15/10 S1
=====================================================================
   Acq. Operator    : SYSTEM                          Seq. Line :  11
   Sample Operator  : SYSTEM
   Acq. Instrument  : HPLC1                            Location :   P1-B-01
   Injection Date   : 09-Dec-19 8:38:41 PM                  Inj :   1
                                                     Inj Volume : 20.000 µl
   Acq. Method      : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
   Last changed     : 05-Dec-19 8:34:05 AM by SYSTEM
   Analysis Method  : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                      Sequence Method)
   Last changed     : 10-Dec-19 8:04:27 AM by SYSTEM
   Sample Info      : 15/10 S1
=====================================================================
```

```
=====================================================================
                      External Standard Report
=====================================================================

Sorted By              :      Signal
Calib. Data Modified   :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier             :      1.0000
Dilution               :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071             -          -          -          Cellobiose
  8.705 VV R   2.26992e5  3.38448e-6 7.68250e-1      glucose
  9.327 VV     1.95646e5  2.88836e-6 5.65096e-1      Xylose
 10.176 VB     2.17449e5  4.57474e-6 9.94774e-1      Arabinose
 13.322 VB     1590.91626 5.18871e-6 8.25480e-3      Glycerol
 14.516 BV     5201.03467 7.12739e-6 3.70698e-2      Acetic Acid
 21.005             -          -          -          ethanol

Totals :                             2.37344
```

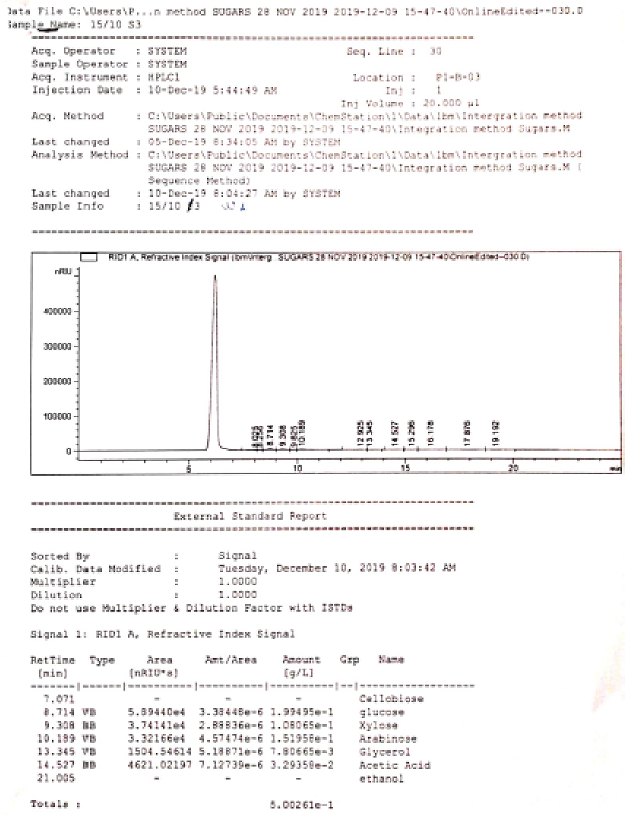
Figure A.11: Juice WAH, 15th Oct. 2019, sample 1, spiked



```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--012.D
Sample Name: 15/10 S2
=====================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  12
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                          Location :  P1-B-02
Injection Date  : 09-Dec-19 9:07:28 PM                Inj :  1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
Last changed    : 05-Dec-19 8:34:05 AM by SYSTEM
Analysis Method : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
Sample Info     : 15/10 S2    WA

=====================================================================
```

```
=====================================================================
                      External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type    Area      Amt/Area     Amount    Grp   Name
 [min]         [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071             -          -          -           Cellobiose
  8.705 VV R  2.28419e5  3.38448e-6 7.73080e-1      glucose
  9.327 VV    1.97096e5  2.88836e-6 5.69284e-1      Xylose
 10.176 VB    2.19682e5  4.57474e-6    1.00499      Arabinose
 13.326 VB    1582.37183 5.18871e-6 8.21047e-3      Glycerol
 14.518 BV    5193.59033 7.12739e-6 3.70168e-2      Acetic Acid
 21.005             -          -          -         ethanol

Totals :                               2.39258
```

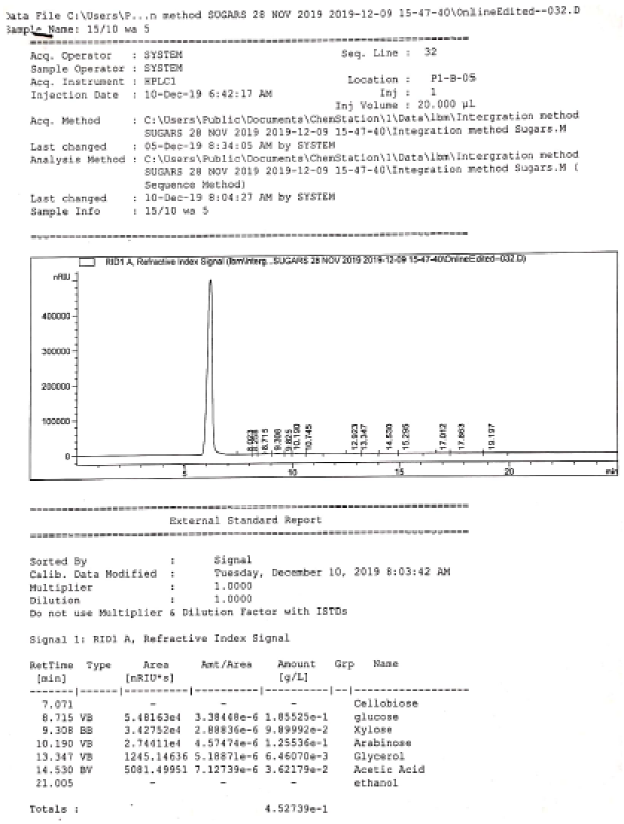
Figure A.12: Juice WAH, 15th Oct. 2019, sample 2, spiked



```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--030.D
Sample Name: 15/10 S3
=====================================================================
Acq. Operator   : SYSTEM                        Seq. Line :  30
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                          Location :  P1-B-03
Injection Date  : 10-Dec-19 5:44:49 AM                Inj :  1
                                               Inj Volume : 20.000 µl
Acq. Method     : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
Last changed    : 05-Dec-19 8:34:05 AM by SYSTEM
Analysis Method : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
Sample Info     : 15/10 S3

=====================================================================
```

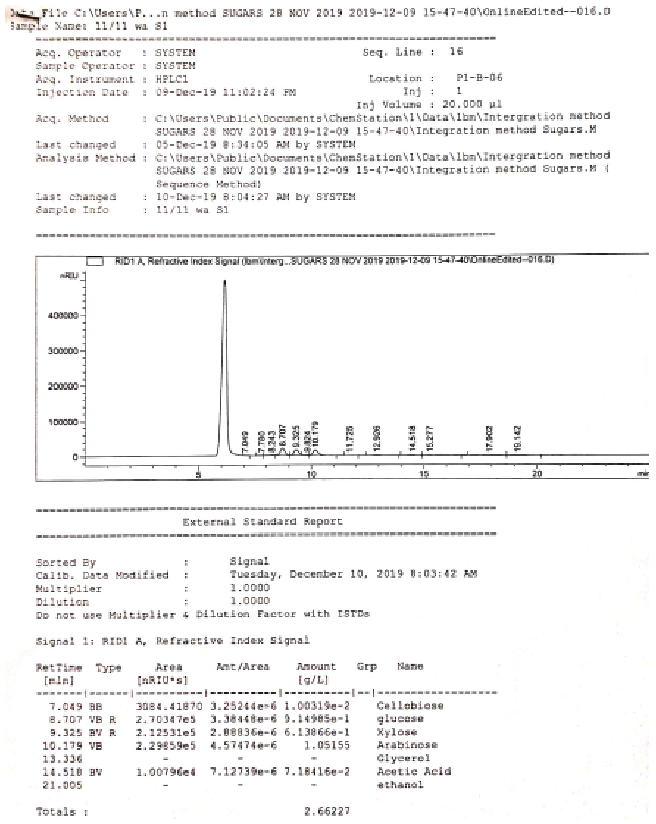
```
=====================================================================
                      External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type    Area      Amt/Area     Amount    Grp   Name
 [min]         [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071             -          -          -           Cellobiose
  8.714 VB    5.89440e4  3.38448e-6 1.99495e-1      glucose
  9.308 BB    3.74141e4  2.88836e-6 1.08065e-1      Xylose
 10.189 VB    3.32166e4  4.57474e-6 1.51958e-1      Arabinose
 13.345 VB    1504.54614 5.18871e-6 7.80665e-3      Glycerol
 14.527 BB    4621.02197 7.12739e-6 3.29358e-2      Acetic Acid
 21.005             -          -          -         ethanol

Totals :                             5.00261e-1
```

Figure A.13: Juice WAH, 15th Oct. 2019, sample 3



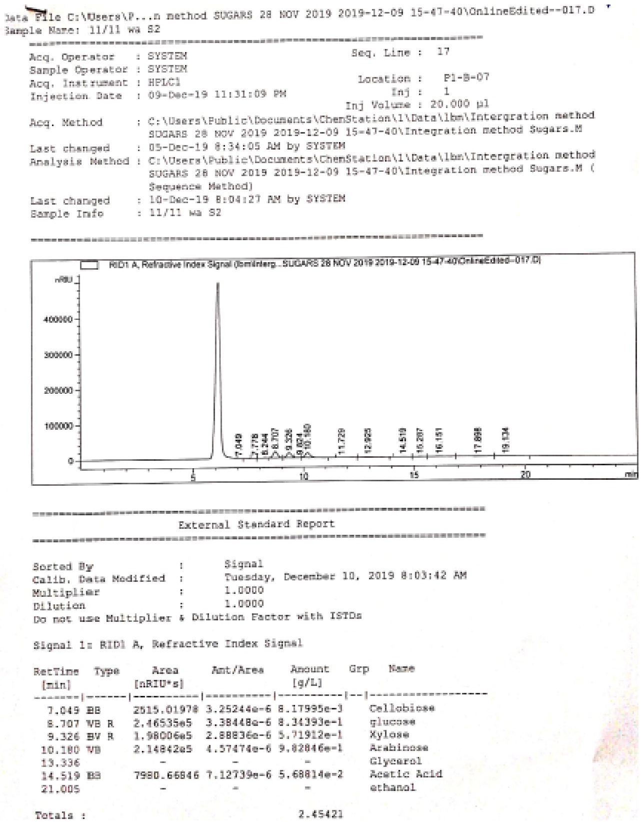
```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--032.D
Sample Name: 15/10 wa 5
=====================================================================
   Acq. Operator   : SYSTEM                           Seq. Line :  32
   Sample Operator : SYSTEM
   Acq. Instrument : HPLC1                             Location :   P1-B-05
   Injection Date  : 10-Dec-19 6:42:17 AM                   Inj :   1
                                                     Inj Volume : 20.000 µl
   Acq. Method     : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                     SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
   Last changed    : 05-Dec-19 8:34:05 AM by SYSTEM
   Analysis Method : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                     SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                     Sequence Method)
   Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
   Sample Info     : 15/10 wa 5

=====================================================================

=====================================================================
                         External Standard Report
=====================================================================

Sorted By                  :       Signal
Calib. Data Modified       :       Tuesday, December 10, 2019 8:03:42 AM
Multiplier                 :       1.0000
Dilution                   :       1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount   Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071             -          -          -         Cellobiose
  8.715 VB    5.48163e4  3.38448e-6 1.85525e-1    glucose
  9.308 BB    3.42752e4  2.88836e-6 9.89992e-2    Xylose
 10.190 VB    2.74411e4  4.57474e-6 1.25536e-1    Arabinose
 13.347 VB    1245.14636 5.18871e-6 6.46070e-3    Glycerol
 14.530 BV    5081.49951 7.12739e-6 3.62179e-2    Acetic Acid
 21.005             -          -          -         ethanol

Totals :                             4.52739e-1
```

Figure A.14: Juice WAH, 15th Oct. 2019, sample 4



```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--016.D
Sample Name: 11/11 wa S1
=====================================================================
   Acq. Operator   : SYSTEM                           Seq. Line :  16
   Sample Operator : SYSTEM
   Acq. Instrument : HPLC1                             Location :   P1-B-06
   Injection Date  : 09-Dec-19 11:02:24 PM                  Inj :   1
                                                     Inj Volume : 20.000 µl
   Acq. Method     : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                     SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
   Last changed    : 05-Dec-19 8:34:05 AM by SYSTEM
   Analysis Method : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                     SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                     Sequence Method)
   Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
   Sample Info     : 11/11 wa S1

=====================================================================

=====================================================================
                         External Standard Report
=====================================================================

Sorted By                  :       Signal
Calib. Data Modified       :       Tuesday, December 10, 2019 8:03:42 AM
Multiplier                 :       1.0000
Dilution                   :       1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount   Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.049 BB    3084.41870 3.25244e-6 1.00319e-2    Cellobiose
  8.707 VB R  2.70347e5  3.38448e-6 9.14985e-1    glucose
  9.325 BV R  2.12531e5  2.88836e-6 6.13866e-1    Xylose
 10.179 VB    2.29859e5  4.57474e-6    1.05155    Arabinose
 13.336             -          -          -         Glycerol
 14.518 BV    1.00796e4  7.12739e-6 7.18416e-2    Acetic Acid
 21.005             -          -          -         ethanol

Totals :                                2.66227
```

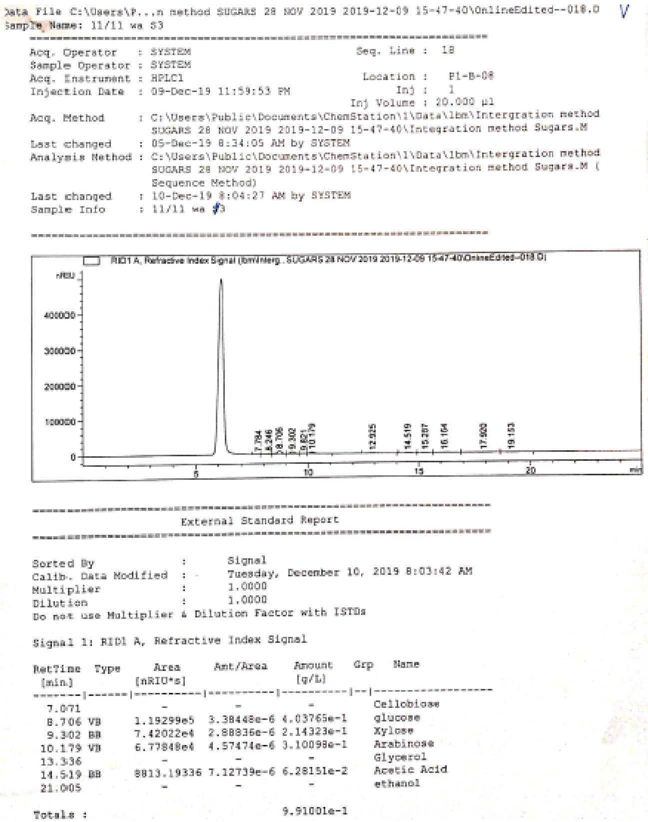
Figure A.15: Juice WAH, 11th Nov. 2019, sample 1, spiked



```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--017.D
Sample Name: 11/11 wa S2
=====================================================================
Acq. Operator   : SYSTEM                          Seq. Line :  17
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                            Location :   P1-B-07
Injection Date  : 09-Dec-19 11:31:09 PM                 Inj :   1
                                                 Inj Volume : 20.000 µl
Acq. Method     : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
Last changed    : 05-Dec-19 8:34:05 AM by SYSTEM
Analysis Method : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
Sample Info     : 11/11 wa S2

=====================================================================
```

```
=====================================================================
                     External Standard Report
=====================================================================

Sorted By             :       Signal
Calib. Data Modified  :       Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :       1.0000
Dilution              :       1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.049 BB    2515.01978 3.25244e-6 8.17995e-3    Cellobiose
  8.707 VB R  2.46535e5  3.38448e-6 8.34393e-1    glucose
  9.326 BV R  1.98006e5  2.88836e-6 5.71912e-1    Xylose
 10.180 VB    2.14842e5  4.57474e-6 9.82846e-1    Arabinose
 13.336             -          -          -       Glycerol
 14.519 BB    7980.66846 7.12739e-6 5.68814e-2    Acetic Acid
 21.005             -          -          -       ethanol

Totals :                              2.45421
```

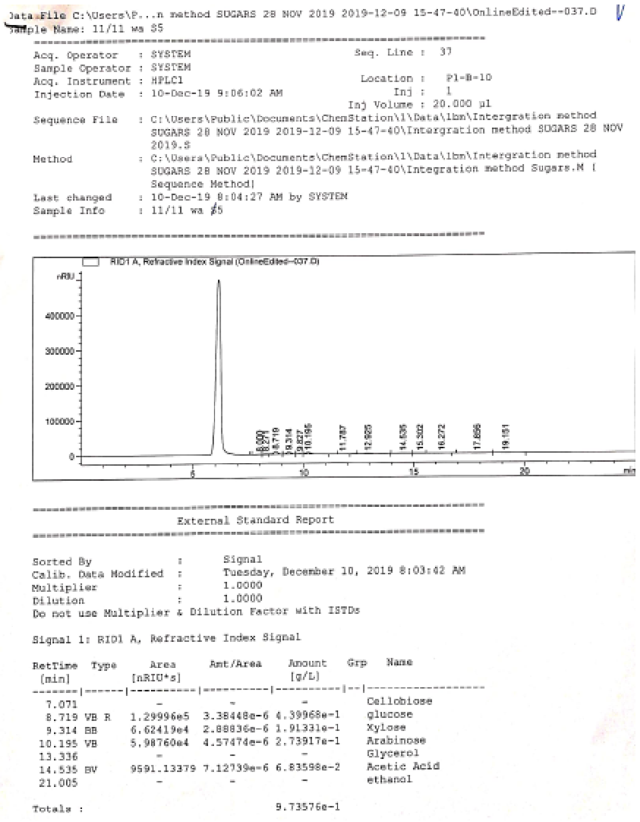
Figure A.16: Juice WAH, 11th Nov. 2019, sample 2, spiked



```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--018.D
Sample Name: 11/11 wa S3
=====================================================================
Acq. Operator   : SYSTEM                          Seq. Line :  18
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                            Location :   P1-B-08
Injection Date  : 09-Dec-19 11:59:53 PM                 Inj :   1
                                                 Inj Volume : 20.000 µl
Acq. Method     : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M
Last changed    : 05-Dec-19 8:34:05 AM by SYSTEM
Analysis Method : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
Sample Info     : 11/11 wa S3

=====================================================================
```

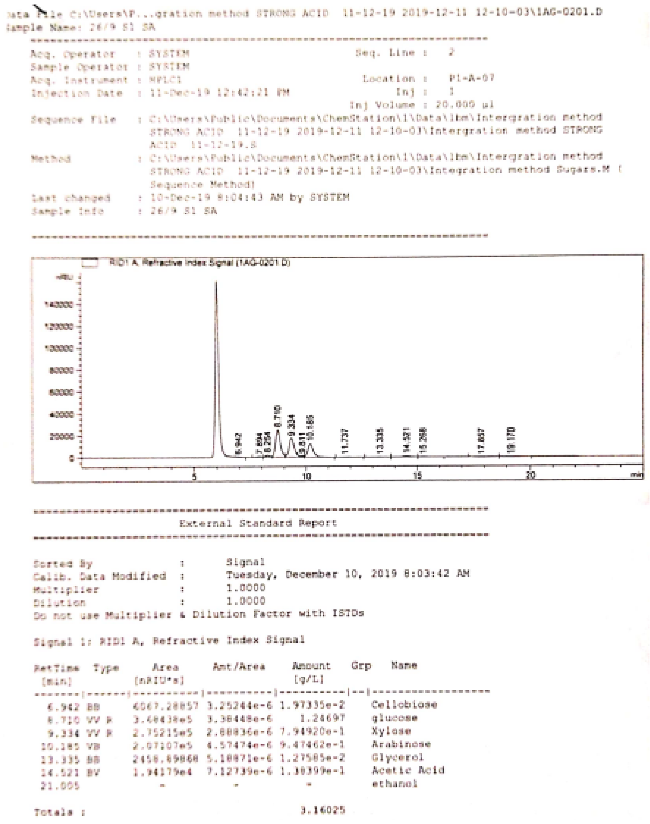
```
=====================================================================
                     External Standard Report
=====================================================================

Sorted By             :       Signal
Calib. Data Modified  :       Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :       1.0000
Dilution              :       1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071             -          -          -       Cellobiose
  8.706 VB    1.19299e5  3.38448e-6 4.03765e-1    glucose
  9.302 BB    7.42022e4  2.88836e-6 2.14323e-1    Xylose
 10.179 VB    6.77848e4  4.57474e-6 3.10098e-1    Arabinose
 13.336             -          -          -       Glycerol
 14.519 BB    8813.19336 7.12739e-6 6.28151e-2    Acetic Acid
 21.005             -          -          -       ethanol

Totals :                              9.91001e-1
```

Figure A.17: Juice WAH, 11th Nov. 2019, sample 3



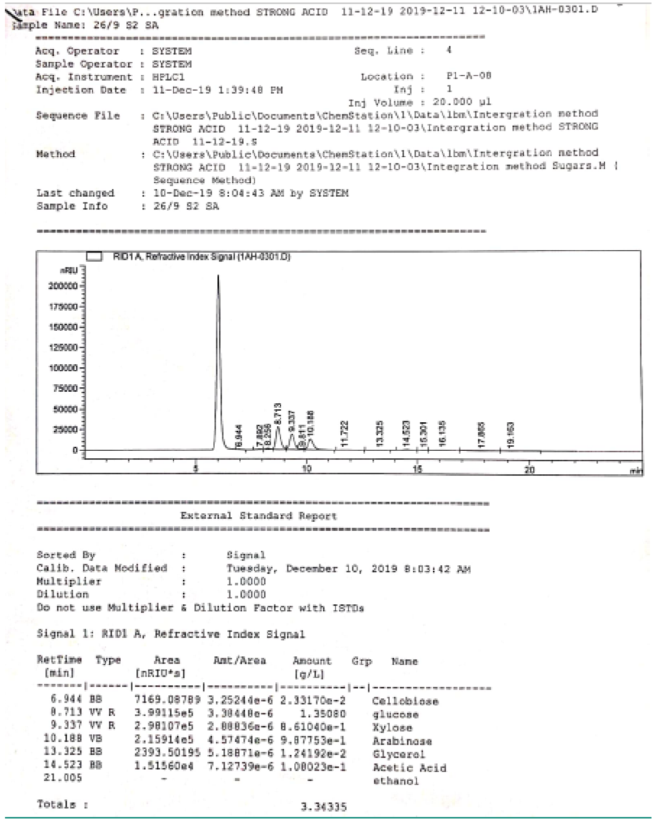
```
Data File C:\Users\P...n method SUGARS 28 NOV 2019 2019-12-09 15-47-40\OnlineEdited--037.D
Sample Name: 11/11 wa S5
=====================================================================
Acq. Operator   : SYSTEM                         Seq. Line :  37
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                           Location :   P1-B-10
Injection Date  : 10-Dec-19 9:06:02 AM                 Inj :   1
                                              Inj Volume : 20.000 µl
Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Intergration method SUGARS 28 NOV
                  2019.S
Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  SUGARS 28 NOV 2019 2019-12-09 15-47-40\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 10-Dec-19 8:04:27 AM by SYSTEM
Sample Info     : 11/11 wa S5

=====================================================================

=====================================================================
                     External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount   Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  7.071            -          -          -           Cellobiose
  8.719 VB R  1.29996e5  3.38448e-6 4.39968e-1       glucose
  9.314 BB    6.62419e4  2.88836e-6 1.91331e-1       Xylose
 10.195 VB    5.98760e4  4.57474e-6 2.73917e-1       Arabinose
 13.336            -          -          -           Glycerol
 14.535 BV    9591.13379 7.12739e-6 6.83598e-2       Acetic Acid
 21.005            -          -          -           ethanol

Totals :                            9.73576e-1
```

Figure A.18: Juice WAH, 11th Nov. 2019, sample 4



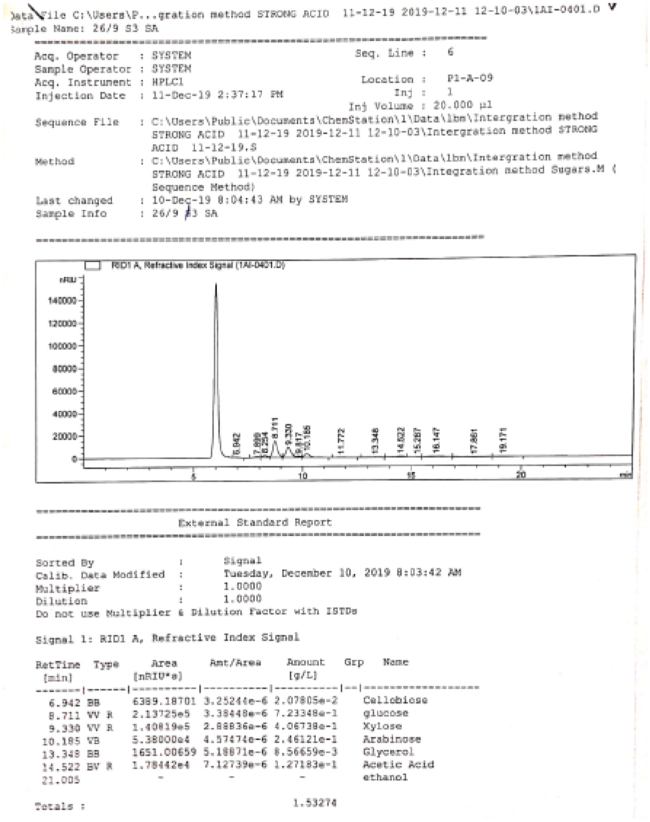
```
Data File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1AG-0201.D
Sample Name: 26/9 S1 SA
=====================================================================
Acq. Operator   : SYSTEM                         Seq. Line :   2
Sample Operator : SYSTEM
Acq. Instrument : HPLC1                           Location :   P1-A-07
Injection Date  : 11-Dec-19 12:42:21 PM                Inj :   1
                                              Inj Volume : 20.000 µl
Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                  ACID  11-12-19.S
Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                  STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                  Sequence Method)
Last changed    : 10-Dec-19 8:04:43 AM by SYSTEM
Sample Info     : 26/9 S1 SA

=====================================================================

=====================================================================
                     External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount   Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  6.942 BB    6067.28857 3.25244e-6 1.97335e-2       Cellobiose
  8.710 VV R  3.48438e5  3.38448e-6    1.24697       glucose
  9.334 VV R  2.75215e5  2.88836e-6 7.94920e-1       Xylose
 10.185 VB    2.07107e5  4.57474e-6 9.47462e-1       Arabinose
 13.335 BB    2458.89868 5.18871e-6 1.27585e-2       Glycerol
 14.521 BV    1.94179e4  7.12739e-6 1.38399e-1       Acetic Acid
 21.005            -          -          -           ethanol

Totals :                               3.16025
```

Figure A.19: Pulp SAH, 26th Sep. 2019, sample 1, spiked



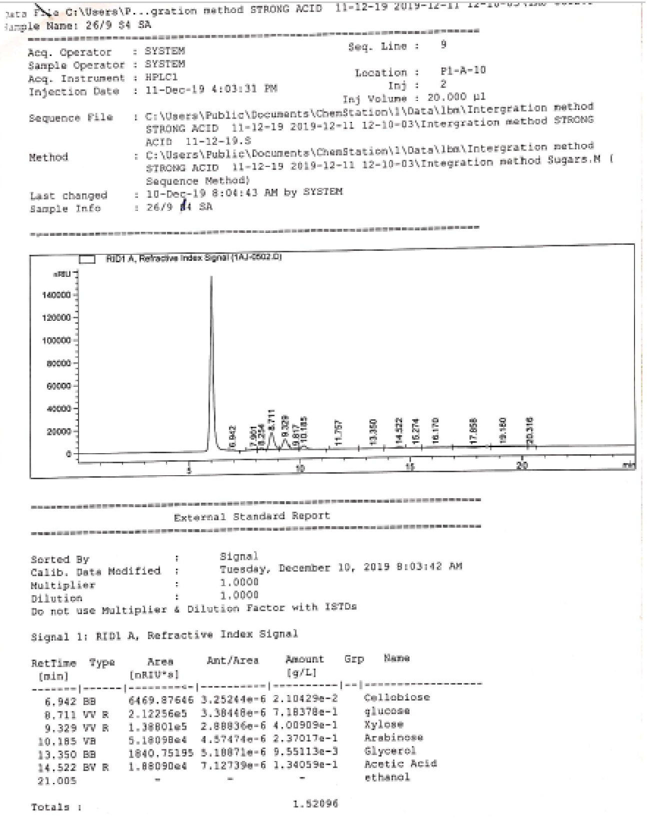
```
Data File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1AH-0301.D
Sample Name: 26/9 S2 SA
    =====================================================================
    Acq. Operator   : SYSTEM                          Seq. Line :    4
    Sample Operator : SYSTEM
    Acq. Instrument : HPLC1                            Location :   P1-A-08
    Injection Date  : 11-Dec-19 1:39:48 PM                  Inj :    1
                                                     Inj Volume : 20.000 µl
    Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                      ACID  11-12-19.S
    Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                      Sequence Method)
    Last changed    : 10-Dec-19 8:04:43 AM by SYSTEM
    Sample Info     : 26/9 S2 SA

    =====================================================================

    =====================================================================
                             External Standard Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Do not use Multiplier & Dilution Factor with ISTDs

    Signal 1: RID1 A, Refractive Index Signal

    RetTime  Type    Area      Amt/Area     Amount   Grp   Name
     [min]         [nRIU*s]                 [g/L]
    -------|------|----------|----------|----------|--|------------------
      6.944 BB    7169.08789 3.25244e-6 2.33170e-2    Cellobiose
      8.713 VV R  3.99115e5  3.38448e-6    1.35080    glucose
      9.337 VV R  2.98107e5  2.88836e-6 8.61040e-1    Xylose
     10.188 VB    2.15914e5  4.57474e-6 9.87753e-1    Arabinose
     13.325 BB    2393.50195 5.18871e-6 1.24192e-2    Glycerol
     14.523 BB    1.51560e4  7.12739e-6 1.08023e-1    Acetic Acid
     21.005            -          -          -        ethanol

    Totals :                             3.34335
```

Figure A.20: Pulp SAH, 26th Sep. 2019, sample 2, spiked



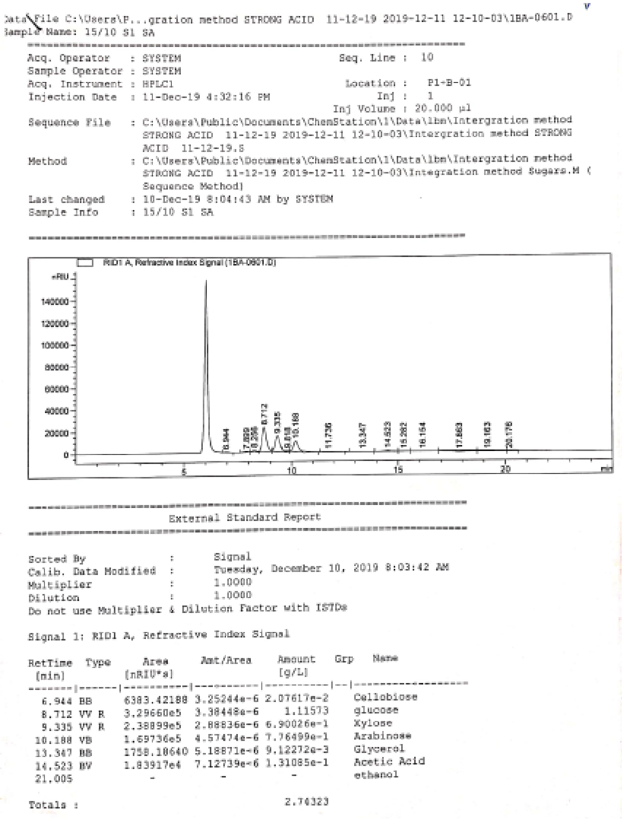
```
Data File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1AI-0401.D
Sample Name: 26/9 S3 SA
    =====================================================================
    Acq. Operator   : SYSTEM                          Seq. Line :    6
    Sample Operator : SYSTEM
    Acq. Instrument : HPLC1                            Location :   P1-A-09
    Injection Date  : 11-Dec-19 2:37:17 PM                  Inj :    1
                                                     Inj Volume : 20.000 µl
    Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                      ACID  11-12-19.S
    Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                      Sequence Method)
    Last changed    : 10-Dec-19 8:04:43 AM by SYSTEM
    Sample Info     : 26/9 S3 SA

    =====================================================================

    =====================================================================
                             External Standard Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Do not use Multiplier & Dilution Factor with ISTDs

    Signal 1: RID1 A, Refractive Index Signal

    RetTime  Type    Area      Amt/Area     Amount   Grp   Name
     [min]         [nRIU*s]                 [g/L]
    -------|------|----------|----------|----------|--|------------------
      6.942 BB    6389.18701 3.25244e-6 2.07805e-2    Cellobiose
      8.711 VV R  2.13725e5  3.38448e-6 7.23348e-1    glucose
      9.330 VV R  1.40819e5  2.88836e-6 4.06738e-1    Xylose
     10.185 VB    5.38000e4  4.57474e-6 2.46121e-1    Arabinose
     13.348 BB    1651.00659 5.18871e-6 8.56659e-3    Glycerol
     14.522 BV R  1.78442e4  7.12739e-6 1.27183e-1    Acetic Acid
     21.005            -          -          -        ethanol

    Totals :                             1.53274
```

Figure A.21: Pulp SAH, 26th Sep. 2019, sample 3



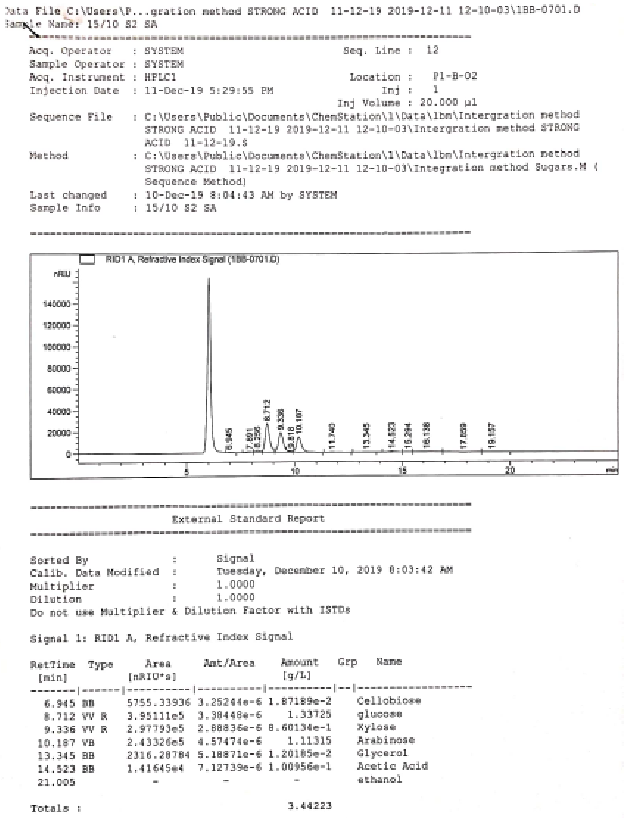
```
ata File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03
ample Name: 26/9 S4 SA
=====================================================================
Acq. Operator    : SYSTEM                        Seq. Line :   9
Sample Operator  : SYSTEM
Acq. Instrument  : HPLC1                          Location :   P1-A-10
Injection Date   : 11-Dec-19 4:03:31 PM                Inj :   2
                                              Inj Volume : 20.000 µl
Sequence File    : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                   ACID  11-12-19.S
Method           : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                   Sequence Method)
Last changed     : 10-Dec-19 8:04:43 AM by SYSTEM
Sample Info      : 26/9 S4 SA

=====================================================================

=====================================================================
                      External Standard Report
=====================================================================

Sorted By                :      Signal
Calib. Data Modified     :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier               :      1.0000
Dilution                 :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type    Area      Amt/Area    Amount    Grp   Name
 [min]         [nRIU*s]               [g/L]
-------|------|----------|----------|----------|--|------------------
  6.942 BB     6469.87646 3.25244e-6 2.10429e-2      Cellobiose
  8.711 VV R   2.12256e5  3.38448e-6 7.18378e-1      glucose
  9.329 VV R   1.38801e5  2.88836e-6 4.00909e-1      Xylose
 10.185 VB     5.18098e4  4.57474e-6 2.37017e-1      Arabinose
 13.350 BB     1840.75195 5.18871e-6 9.55113e-3      Glycerol
 14.522 BV R   1.88090e4  7.12739e-6 1.34059e-1      Acetic Acid
 21.005            -          -          -           ethanol

Totals :                             1.52096
```

Figure A.22: Pulp SAH, 26th Sep. 2019, sample 4



```
ata File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1BA-0601.D
ample Name: 15/10 S1 SA
=====================================================================
Acq. Operator    : SYSTEM                        Seq. Line :  10
Sample Operator  : SYSTEM
Acq. Instrument  : HPLC1                          Location :   P1-B-01
Injection Date   : 11-Dec-19 4:32:16 PM                Inj :   1
                                              Inj Volume : 20.000 µl
Sequence File    : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                   ACID  11-12-19.S
Method           : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                   Sequence Method)
Last changed     : 10-Dec-19 8:04:43 AM by SYSTEM
Sample Info      : 15/10 S1 SA

=====================================================================

=====================================================================
                      External Standard Report
=====================================================================

Sorted By                :      Signal
Calib. Data Modified     :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier               :      1.0000
Dilution                 :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type    Area      Amt/Area    Amount    Grp   Name
 [min]         [nRIU*s]               [g/L]
-------|------|----------|----------|----------|--|------------------
  6.944 BB     6383.42188 3.25244e-6 2.07617e-2      Cellobiose
  8.712 VV R   3.29660e5  3.38448e-6    1.11573      glucose
  9.335 VV R   2.38899e5  2.88836e-6 6.90026e-1      Xylose
 10.188 VB     1.69736e5  4.57474e-6 7.76499e-1      Arabinose
 13.347 BB     1758.18640 5.18871e-6 9.12272e-3      Glycerol
 14.523 BV     1.83917e4  7.12739e-6 1.31085e-1      Acetic Acid
 21.005            -          -          -           ethanol

Totals :                             2.74323
```

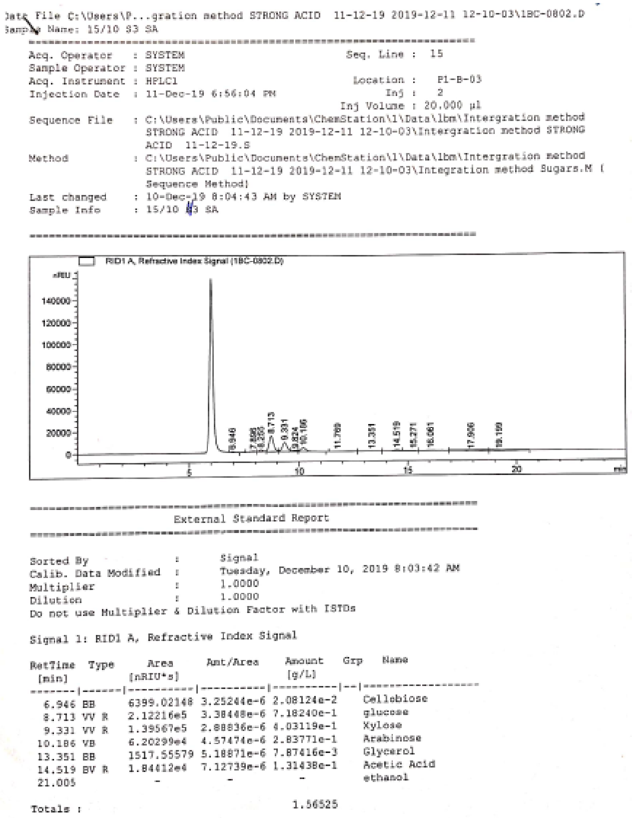
Figure A.23: Pulp SAH, 15th Oct. 2019, sample 1, spiked



```
)ata File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1BB-0701.D
Sample Name: 15/10 S2 SA
=====================================================================
 Acq. Operator   : SYSTEM                          Seq. Line :  12
 Sample Operator : SYSTEM
 Acq. Instrument : HPLC1                            Location :   P1-B-02
 Injection Date  : 11-Dec-19 5:29:55 PM                  Inj :   1
                                               Inj Volume : 20.000 µl
 Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                   ACID  11-12-19.S
 Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                   Sequence Method)
 Last changed    : 10-Dec-19 8:04:43 AM by SYSTEM
 Sample Info     : 15/10 S2 SA

=====================================================================
```

```
=====================================================================
                      External Standard Report
=====================================================================

 Sorted By              :       Signal
 Calib. Data Modified   :       Tuesday, December 10, 2019 8:03:42 AM
 Multiplier             :       1.0000
 Dilution               :       1.0000
 Do not use Multiplier & Dilution Factor with ISTDs

 Signal 1: RID1 A, Refractive Index Signal

 RetTime  Type     Area      Amt/Area     Amount   Grp   Name
  [min]          [nRIU*s]                 [g/L]
 -------|------|----------|----------|----------|--|------------------
   6.945 BB     5755.33936 3.25244e-6 1.87189e-2    Cellobiose
   8.712 VV R   3.95111e5  3.38448e-6    1.33725    glucose
   9.336 VV R   2.97793e5  2.88836e-6 8.60134e-1    Xylose
  10.187 VB     2.43326e5  4.57474e-6    1.11315    Arabinose
  13.345 BB     2316.28784 5.18871e-6 1.20185e-2    Glycerol
  14.523 BB     1.41645e4  7.12739e-6 1.00956e-1    Acetic Acid
  21.005             -          -          -        ethanol

 Totals :                                3.44223
```

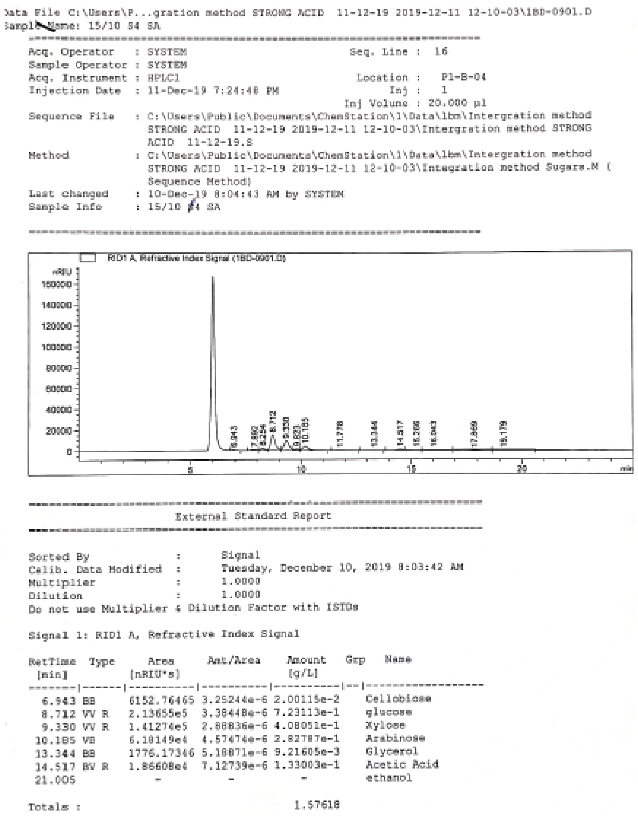
Figure A.24: Pulp SAH, 15th Oct. 2019, sample 2, spiked



```
)ata File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1BC-0802.D
Sample Name: 15/10 S3 SA
=====================================================================
 Acq. Operator   : SYSTEM                          Seq. Line :  15
 Sample Operator : SYSTEM
 Acq. Instrument : HPLC1                            Location :   P1-B-03
 Injection Date  : 11-Dec-19 6:56:04 PM                  Inj :   2
                                               Inj Volume : 20.000 µl
 Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                   ACID  11-12-19.S
 Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                   Sequence Method)
 Last changed    : 10-Dec-19 8:04:43 AM by SYSTEM
 Sample Info     : 15/10 S3 SA

=====================================================================
```

```
=====================================================================
                      External Standard Report
=====================================================================

 Sorted By              :       Signal
 Calib. Data Modified   :       Tuesday, December 10, 2019 8:03:42 AM
 Multiplier             :       1.0000
 Dilution               :       1.0000
 Do not use Multiplier & Dilution Factor with ISTDs

 Signal 1: RID1 A, Refractive Index Signal

 RetTime  Type     Area      Amt/Area     Amount   Grp   Name
  [min]          [nRIU*s]                 [g/L]
 -------|------|----------|----------|----------|--|------------------
   6.946 BB     6399.02148 3.25244e-6 2.08124e-2    Cellobiose
   8.713 VV R   2.12216e5  3.38448e-6 7.18240e-1    glucose
   9.331 VV R   1.39567e5  2.88836e-6 4.03119e-1    Xylose
  10.186 VB     6.20299e4  4.57474e-6 2.83771e-1    Arabinose
  13.351 BB     1517.55579 5.18871e-6 7.87416e-3    Glycerol
  14.519 BV R   1.84412e4  7.12739e-6 1.31438e-1    Acetic Acid
  21.005             -          -          -        ethanol

 Totals :                                1.56525
```

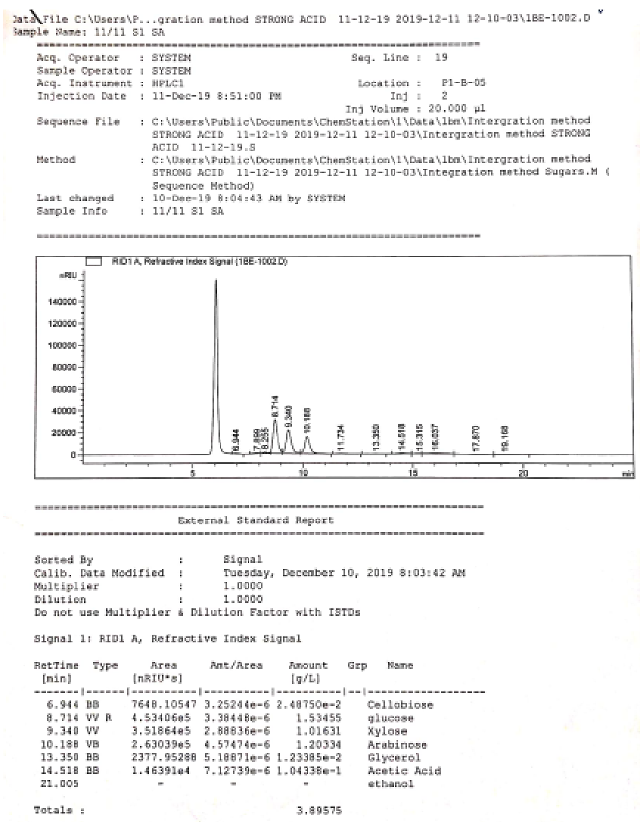
Figure A.25: Pulp SAH, 15th Oct. 2019, sample 3



```
Data File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1BD-0901.D
Sample Name: 15/10 S4 SA
    =====================================================================
    Acq. Operator   : SYSTEM                          Seq. Line :  16
    Sample Operator : SYSTEM
    Acq. Instrument : HPLC1                            Location :   P1-B-04
    Injection Date  : 11-Dec-19 7:24:48 PM                  Inj :   1
                                                     Inj Volume : 20.000 µl
    Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                      ACID  11-12-19.S
    Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                      Sequence Method)
    Last changed    : 10-Dec-19 8:04:43 AM by SYSTEM
    Sample Info     : 15/10 S4 SA

    =====================================================================
```

```
    =====================================================================
                         External Standard Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Do not use Multiplier & Dilution Factor with ISTDs

    Signal 1: RID1 A, Refractive Index Signal

    RetTime  Type     Area      Amt/Area    Amount    Grp   Name
     [min]          [nRIU*s]                [g/L]
    -------|------|----------|----------|----------|--|------------------
      6.943 BB      6152.76465 3.25244e-6 2.00115e-2    Cellobiose
      8.712 VV R    2.13655e5  3.38448e-6 7.23113e-1    glucose
      9.330 VV R    1.41274e5  2.88836e-6 4.08051e-1    Xylose
     10.185 VB      6.18149e4  4.57474e-6 2.82787e-1    Arabinose
     13.344 BB      1776.17346 5.18871e-6 9.21605e-3    Glycerol
     14.517 BV R    1.86608e4  7.12739e-6 1.33003e-1    Acetic Acid
     21.005            -          -          -          ethanol

    Totals :                              1.57618
```

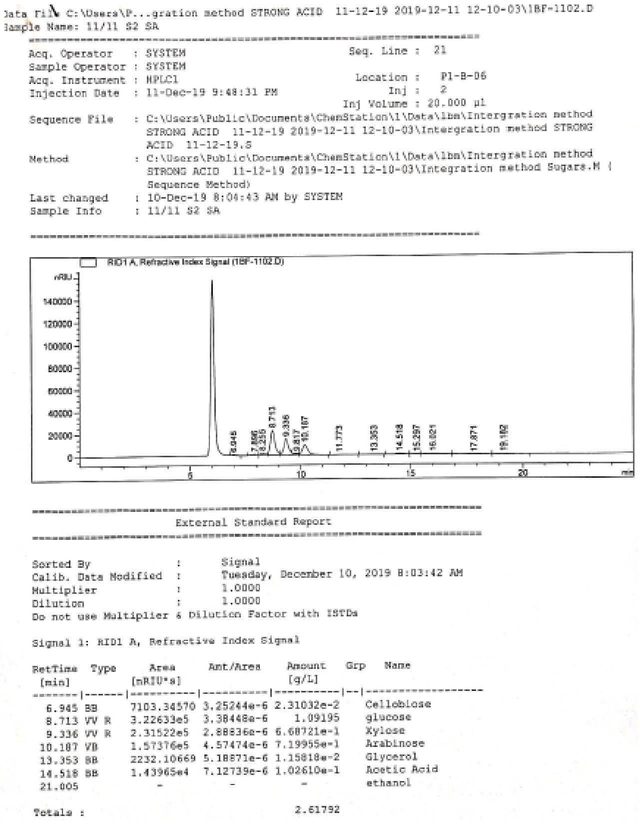
Figure A.26: Pulp SAH, 15th Oct. 2019, sample 4



```
Data File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1BE-1002.D
Sample Name: 11/11 S1 SA
    =====================================================================
    Acq. Operator   : SYSTEM                          Seq. Line :  19
    Sample Operator : SYSTEM
    Acq. Instrument : HPLC1                            Location :   P1-B-05
    Injection Date  : 11-Dec-19 8:51:00 PM                  Inj :   2
                                                     Inj Volume : 20.000 µl
    Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                      ACID  11-12-19.S
    Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                      STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                      Sequence Method)
    Last changed    : 10-Dec-19 8:04:43 AM by SYSTEM
    Sample Info     : 11/11 S1 SA

    =====================================================================
```

```
    =====================================================================
                         External Standard Report
    =====================================================================

    Sorted By             :      Signal
    Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
    Multiplier            :      1.0000
    Dilution              :      1.0000
    Do not use Multiplier & Dilution Factor with ISTDs

    Signal 1: RID1 A, Refractive Index Signal

    RetTime  Type     Area      Amt/Area    Amount    Grp   Name
     [min]          [nRIU*s]                [g/L]
    -------|------|----------|----------|----------|--|------------------
      6.944 BB      7648.10547 3.25244e-6 2.48750e-2    Cellobiose
      8.714 VV R    4.53406e5  3.38448e-6    1.53455    glucose
      9.340 VV      3.51864e5  2.88836e-6    1.01631    Xylose
     10.188 VB      2.63039e5  4.57474e-6    1.20334    Arabinose
     13.350 BB      2377.95288 5.18871e-6 1.23385e-2    Glycerol
     14.518 BB      1.46391e4  7.12739e-6 1.04338e-1    Acetic Acid
     21.005            -          -          -          ethanol

    Totals :                              3.89575
```

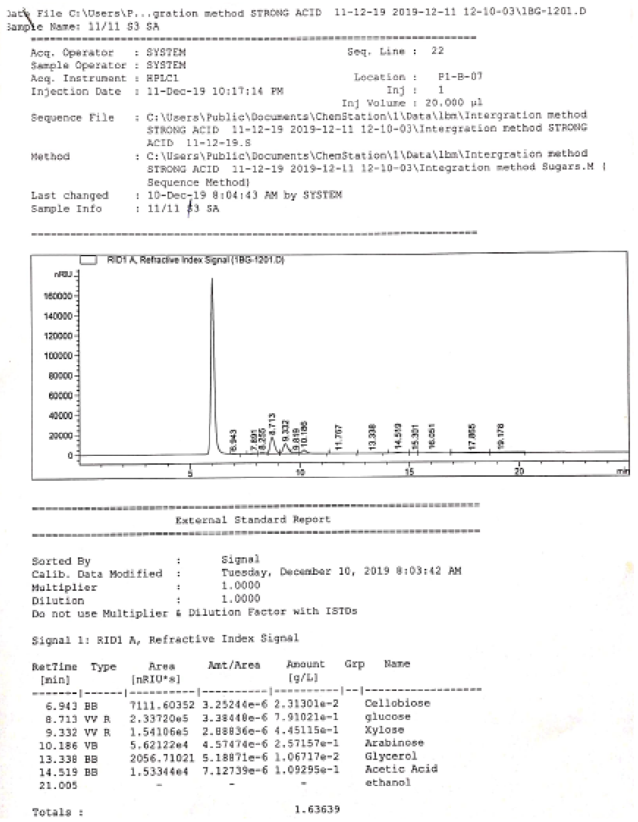
Figure A.27: Pulp SAH, 11th Nov. 2019, sample 1, spiked



```
Data File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1BF-1102.D
Sample Name: 11/11 S2 SA
=====================================================================
 Acq. Operator   : SYSTEM                       Seq. Line :  21
 Sample Operator : SYSTEM
 Acq. Instrument : HPLC1                         Location :   P1-B-06
 Injection Date  : 11-Dec-19 9:48:31 PM               Inj :   2
                                               Inj Volume : 20.000 µl
 Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                   ACID  11-12-19.S
 Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                   Sequence Method)
 Last changed    : 10-Dec-19 8:04:43 AM by SYSTEM
 Sample Info     : 11/11 S2 SA

=====================================================================
```

```
=====================================================================
                     External Standard Report
=====================================================================

Sorted By              :      Signal
Calib. Data Modified   :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier             :      1.0000
Dilution               :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  6.945 BB     7103.34570 3.25244e-6 2.31032e-2     Cellobiose
  8.713 VV R   3.22633e5  3.38448e-6    1.09195     glucose
  9.336 VV R   2.31522e5  2.88836e-6 6.68721e-1     Xylose
 10.187 VB     1.57376e5  4.57474e-6 7.19955e-1     Arabinose
 13.353 BB     2232.10669 5.18871e-6 1.15818e-2     Glycerol
 14.518 BB     1.43965e4  7.12739e-6 1.02610e-1     Acetic Acid
 21.005            -          -          -          ethanol

Totals :                                2.61792
```

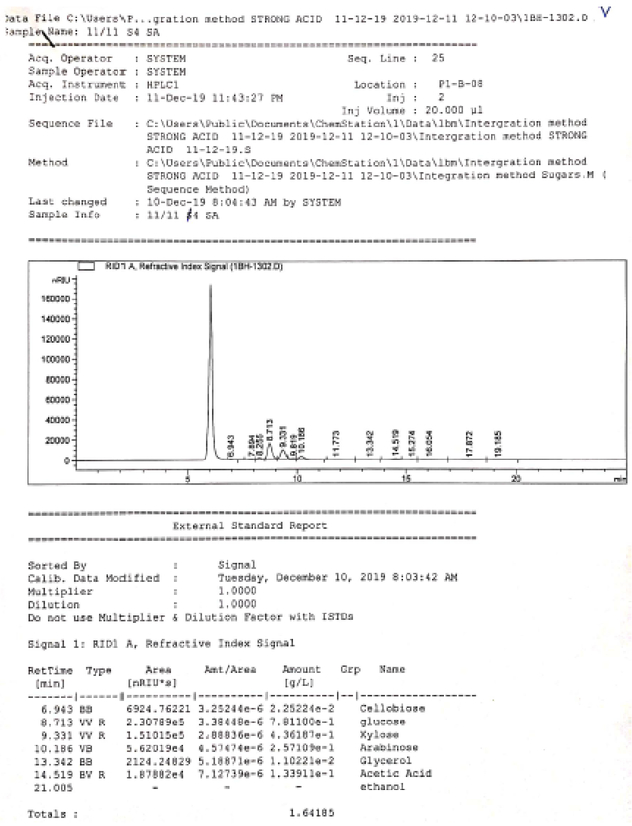
Figure A.28: Pulp SAH, 11th Nov. 2019, sample 2, spiked



```
Data File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1BG-1201.D
Sample Name: 11/11 S3 SA
=====================================================================
 Acq. Operator   : SYSTEM                       Seq. Line :  22
 Sample Operator : SYSTEM
 Acq. Instrument : HPLC1                         Location :   P1-B-07
 Injection Date  : 11-Dec-19 10:17:14 PM              Inj :   1
                                               Inj Volume : 20.000 µl
 Sequence File   : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                   ACID  11-12-19.S
 Method          : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                   Sequence Method)
 Last changed    : 10-Dec-19 8:04:43 AM by SYSTEM
 Sample Info     : 11/11 S3 SA

=====================================================================
```

```
=====================================================================
                     External Standard Report
=====================================================================

Sorted By              :      Signal
Calib. Data Modified   :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier             :      1.0000
Dilution               :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type     Area      Amt/Area     Amount    Grp   Name
 [min]          [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  6.943 BB     7111.60352 3.25244e-6 2.31301e-2     Cellobiose
  8.713 VV R   2.33720e5  3.38448e-6 7.91021e-1     glucose
  9.332 VV R   1.54106e5  2.88836e-6 4.45115e-1     Xylose
 10.186 VB     5.62122e4  4.57474e-6 2.57157e-1     Arabinose
 13.338 BB     2056.71021 5.18871e-6 1.06717e-2     Glycerol
 14.519 BB     1.53344e4  7.12739e-6 1.09295e-1     Acetic Acid
 21.005            -          -          -          ethanol

Totals :                                1.63639
```

Figure A.29: Pulp SAH, 11th Nov. 2019, sample 3



```
ata File C:\Users\P...gration method STRONG ACID  11-12-19 2019-12-11 12-10-03\1BH-1302.D
ample Name: 11/11 S4 SA
=====================================================================
Acq. Operator    : SYSTEM                        Seq. Line :  25
Sample Operator  : SYSTEM
Acq. Instrument  : HPLC1                          Location :   P1-B-08
Injection Date   : 11-Dec-19 11:43:27 PM               Inj :   2
                                               Inj Volume : 20.000 µl
Sequence File    : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Intergration method STRONG
                   ACID  11-12-19.S
Method           : C:\Users\Public\Documents\ChemStation\1\Data\lbm\Intergration method
                   STRONG ACID  11-12-19 2019-12-11 12-10-03\Integration method Sugars.M (
                   Sequence Method)
Last changed     : 10-Dec-19 8:04:43 AM by SYSTEM
Sample Info      : 11/11 S4 SA

=====================================================================
```

```
=====================================================================
                     External Standard Report
=====================================================================

Sorted By             :      Signal
Calib. Data Modified  :      Tuesday, December 10, 2019 8:03:42 AM
Multiplier            :      1.0000
Dilution              :      1.0000
Do not use Multiplier & Dilution Factor with ISTDs

Signal 1: RID1 A, Refractive Index Signal

RetTime  Type      Area      Amt/Area     Amount   Grp   Name
 [min]           [nRIU*s]                 [g/L]
-------|------|----------|----------|----------|--|------------------
  6.943 BB     6924.76221 3.25244e-6 2.25224e-2     Cellobiose
  8.713 VV R   2.30789e5  3.38448e-6 7.81100e-1     glucose
  9.331 VV R   1.51015e5  2.88836e-6 4.36187e-1     Xylose
 10.186 VB     5.62019e4  4.57474e-6 2.57109e-1     Arabinose
 13.342 BB     2124.24829 5.18871e-6 1.10221e-2     Glycerol
 14.519 BV R   1.87882e4  7.12739e-6 1.33911e-1     Acetic Acid
 21.005            -          -          -          ethanol

Totals :                             1.64185
```

Figure A.30: Pulp SAH, 11th Nov. 2019, sample 4

Appendix B

Input, intermediate products and final product of hydrodeoxygenation PFR

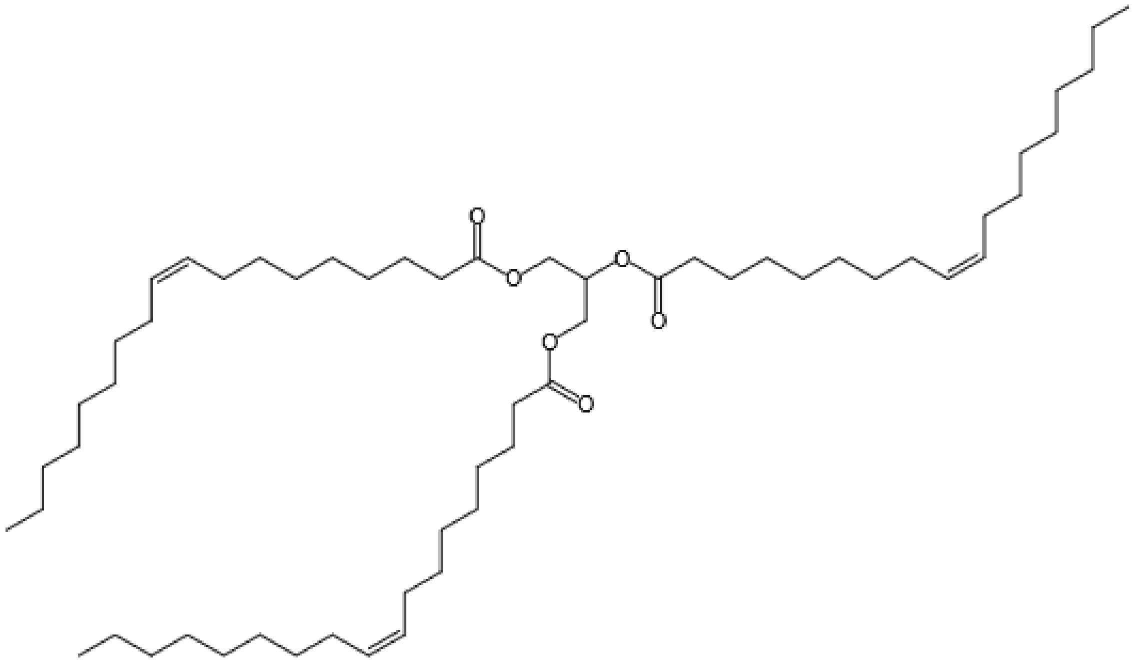


Figure B.1: Input chemical for hydrodeoxygenation, unsaturated triglyceride, triolein, (tri-C18:1)

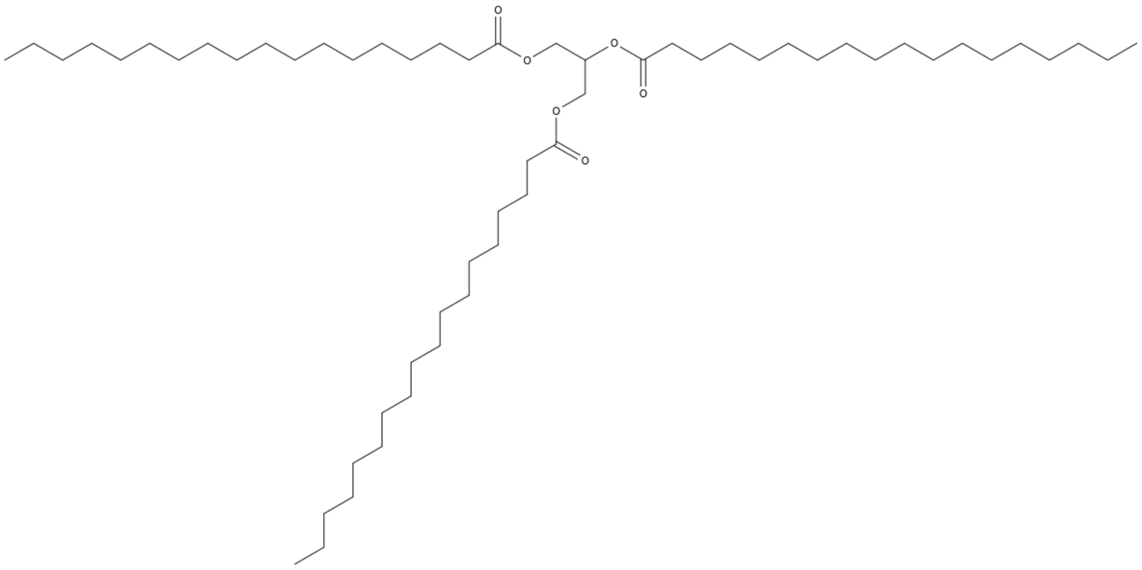


Figure B.2: Input and intermediate chemical for hydrodeoxygenation, saturated triglyceride, tristreatin, (tri-C18:0)

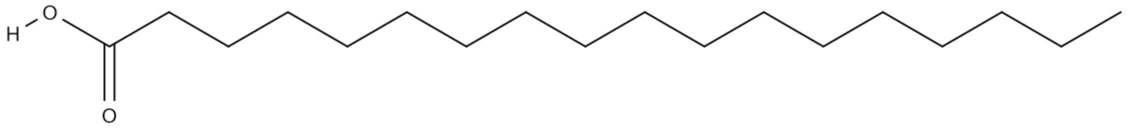


Figure B.3: Intermediate chemical for hydrodeoxygenation, saturated free fatty acid, stearic acid, (C18:0)

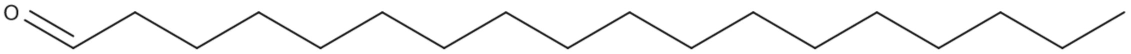


Figure B.4: Intermediate chemical for hydrodeoxygenation, aldehyde, octadecanal, (C18=O)

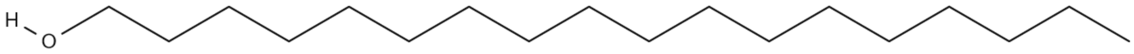


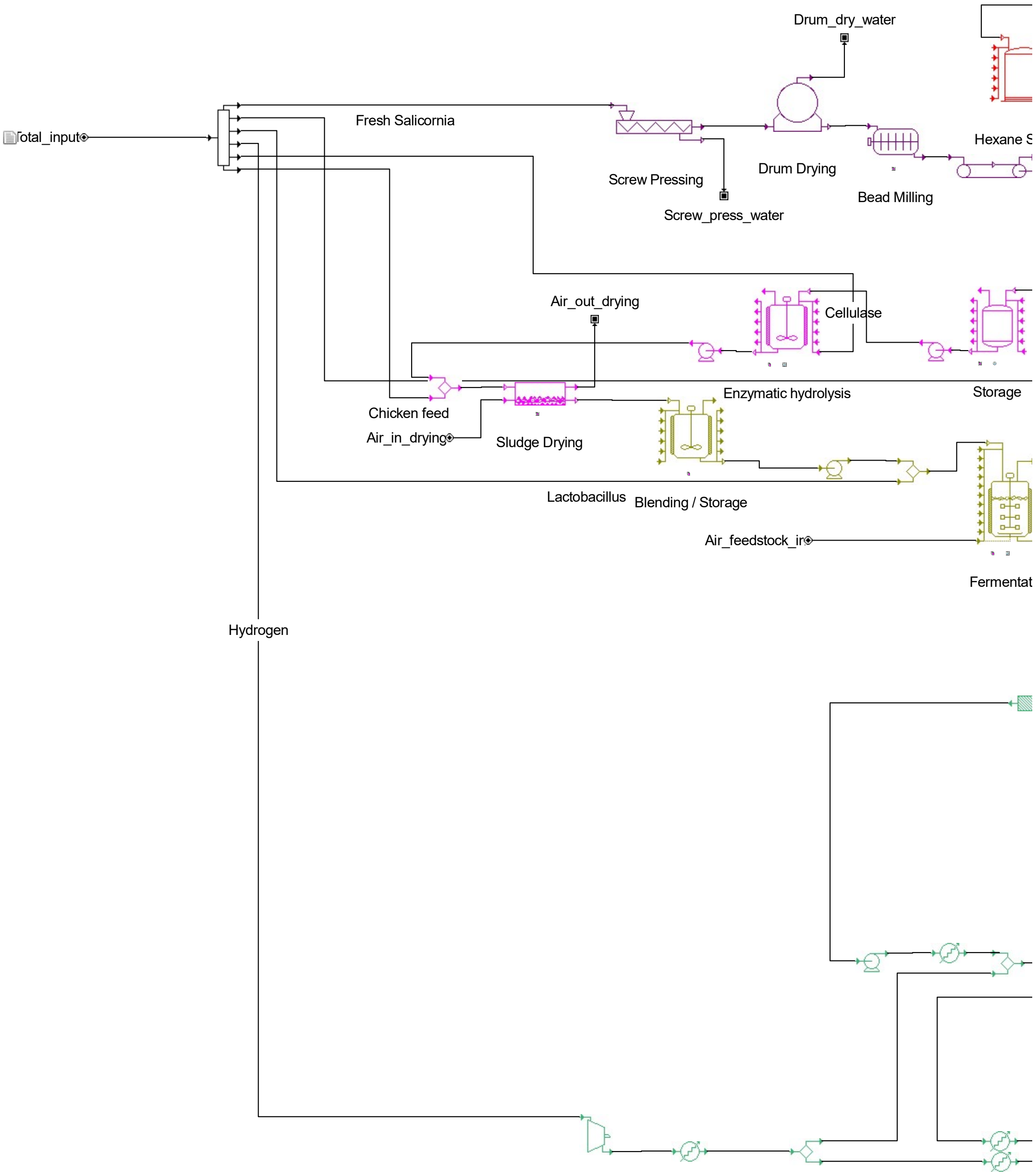
Figure B.5: Intermediate chemical for hydrodeoxygenation, alcohol, octadecanol, (C18-OH)

Figure B.6: Final chemical for hydrodeoxygenation, n-alkane, octadecane, (C18)

Appendix C

SuperPro Designer model

SuperPro Designer model overview.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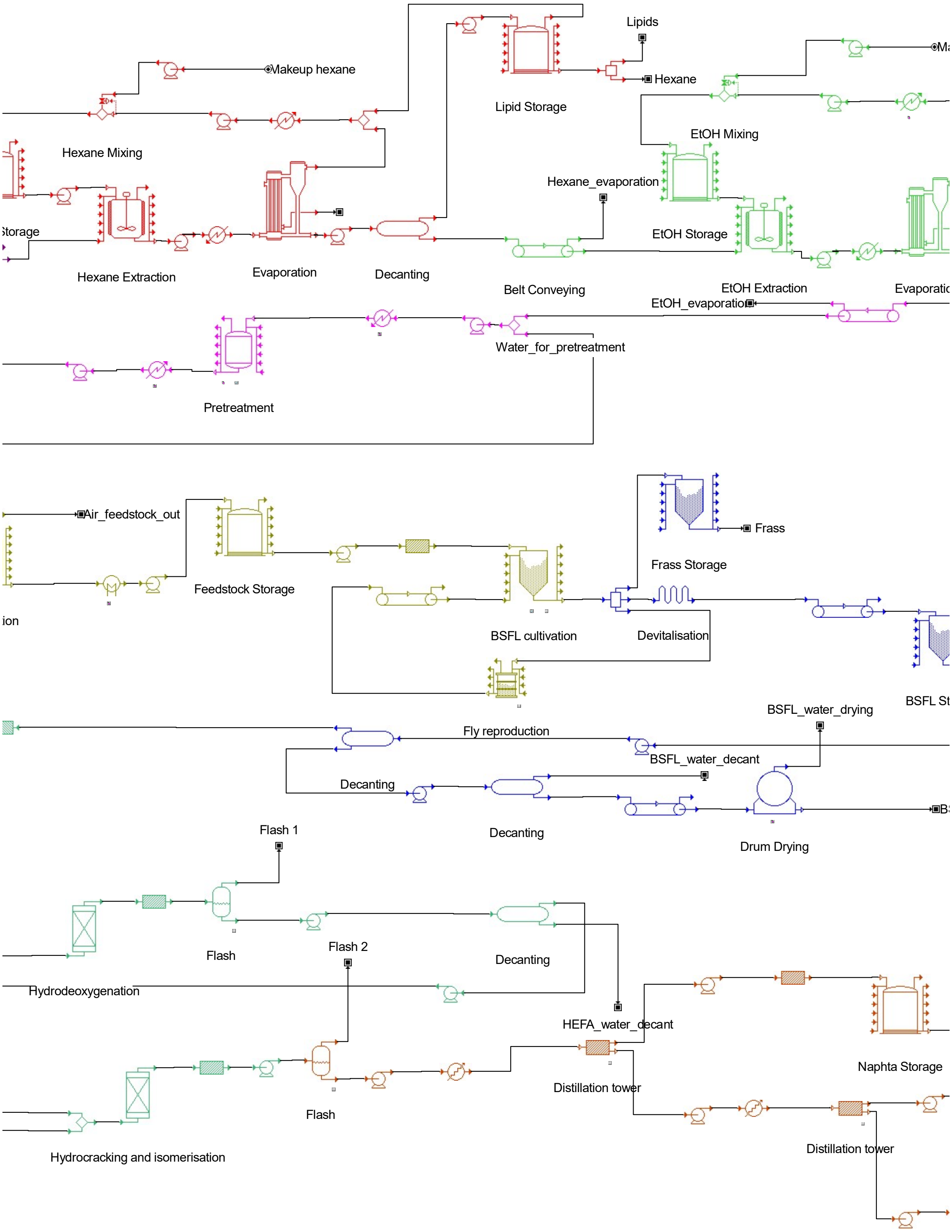
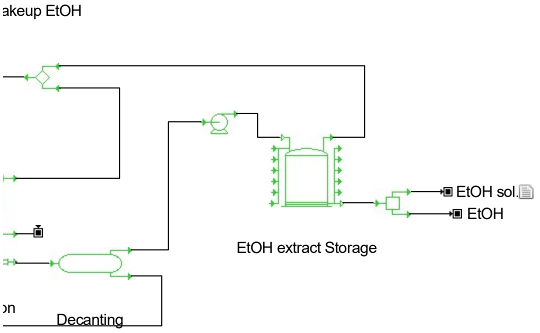
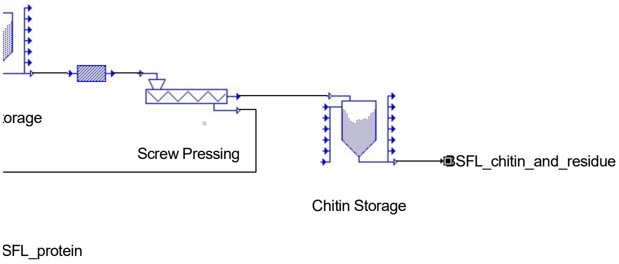
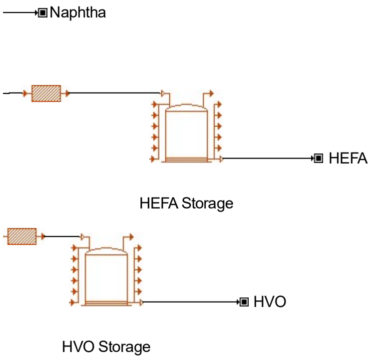
Fresh Salicornia
Total_input
Hexane S
Screw Pressing
Drum Drying
Bead Milling
Screw_press_water
Air_out_drying
Cellulase
Enzymatic hydrolysis
Storage
Chicken feed
Air_in_drying
Sludge Drying
Lactobacillus
Blending / Storage
Air_feedstock_in
Fermentat
Hydrogen


Lipids
Makeup hexane
Hexane
Lipid Storage
EtOH Mixing
Hexane Mixing
Hexane_evaporation
Storage
EtOH Storage
Hexane Extraction
Evaporation
Decanting
Belt Conveying
EtOH Extraction
Evaporatio
EtOH_evaporation
Water_for_pretreatment
Pretreatment
Air_feedstock_out
Frass
Frass Storage
Feedstock Storage
ion
BSFL cultivation
Devitalisation
BSFL_water_drying
BSFL St
Fly reproduction
BSFL_water_decant
Decanting
Decanting
Drum Drying
Flash 1
Flash
Flash 2
Decanting
Hydrodeoxygenation
HEFA_water_decant
Naphta Storage
Distillation tower
Flash
Distillation tower
Hydrocracking and isomerisation


akeup EtOH
EtOH sol.
EtOH
EtOH extract Storage
on
Decanting


orage
Screw Pressing
SFL_chitin_and_residue
Chitin Storage
SFL_protein


Naphtha
HEFA
HEFA Storage
HVO
HVO Storage

Appendix D

Economic evaluation reports

EER	Page
Mass flow rate input sensitivity analysis	
No pretreatment, 10.77 t h^{-1}	159
No pretreatment, 21.54 t h^{-1}	174
No pretreatment, 43.07 t h^{-1}	188
No pretreatment, 86.14 t h^{-1}	202
Pretreatment, 10.77 t h^{-1}	216
Pretreatment, 21.54 t h^{-1}	231
Pretreatment, 43.07 t h^{-1}	246
Pretreatment, 86.14 t h^{-1}	261
Labour cost sensitivity analysis	
Pretreatment, 86.14 t h^{-1}, Danish	261
Pretreatment, 86.14 t h^{-1}, default	276
Pretreatment, 86.14 t h^{-1}, Portuguese	291

Table D.1: Overview of EERs in Appendix D

Economic Evaluation Report

for Total_simulation_pretreatment_no_pre_new

maj 28, 2020

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	117.053.000 $
Capital Investment Charged to This Project	117.053.000 $
Operating Cost	55.393.000 $/yr
Savings (due to Heat Recovery)	2.600.580 $/yr
Main Revenue	176.000 $/yr
Other Revenues	16.916.964 $/yr
Total Revenues	17.093.000 $/yr
Cost Basis Annual Rate	284.339 kg MP/yr
Unit Production Cost	194,81 $/kg MP
Net Unit Production Cost	185,67 $/kg MP
Unit Production Revenue	60,12 $/kg MP
Gross Margin	- 208,85 %
Return On Investment	- 21,69 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7,0% Interest)	- 313.847.000 $

MP = Total Flow of Stream 'HEFA'

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor	2.908.000	2.908.000
		Vessel Volume = 312,45 m3		
1 / 0 / 0	V-103	Flat Bottom Tank	1.237.000	1.237.000
		Vessel Volume = 4766,18 m3		
1 / 0 / 0	V-104	Flat Bottom Tank	1.181.000	1.181.000
		Vessel Volume = 4468,09 m3		
1 / 0 / 0	V-111	Blending Tank	843.000	843.000
		Vessel Volume = 343,51 m3		
1 / 0 / 0	EC-102	Heat Exchanger	463.000	463.000
		Heat Exchange Area = 876,39 m2		
1 / 0 / 0	V-101	Blending Tank	395.000	395.000
		Vessel Volume = 1125515,81 L		
1 / 0 / 0	ST-101	Heat Sterilizer	368.000	368.000
		Rated Throughput = 3514,15 L/h		
1 / 0 / 0	SP-101	Screw Press	291.000	291.000
		Throughput = 10768,06 kg/h		
1 / 0 / 0	BC-101	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-104	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-102	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-103	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-107	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-106	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-105	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BM-101	Bead Mill	271.000	271.000
		Bead Volume = 311,63 L		
1 / 0 / 0	V-115	Decanter Tank	222.000	222.000
		Vessel Volume = 23,26 m3		
1 / 0 / 0	EV-102	Multi-Effect Evaporator	221.000	221.000
		Mean Heat Transfer Area = 211,00 m2		
1 / 0 / 0	V-102	Blending Tank	192.000	192.000
		Vessel Volume = 400461,06 L		
1 / 0 / 0	DDR-102	Drum Dryer	184.000	184.000
		Drum Area = 22,84 m2		
2 / 0 / 0	EC-101	Heat Exchanger	179.000	358.000
		Heat Exchange Area = 179,33 m2		
1 / 0 / 0	V-112	Flat Bottom Tank	160.000	160.000
		Vessel Volume = 267,13 m3		
1 / 0 / 0	SL-104	Silo	127.000	127.000
		Vessel Volume = 332087,08 L		
1 / 0 / 0	SP-102	Screw Press	119.000	119.000

		Throughput = 3525,24 kg/h		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	102.000	102.000
		Mean Heat Transfer Area = 68,51 m2		
1 / 0 / 0	G-101	Centrifugal Compressor	75.000	75.000
		Compressor Power = 23,24 kW		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 9981,61 L		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 5184,18 L		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 323,05 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 82,42 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 142,99 L		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 60,27 kg/h		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 82,04 kg/h		
1 / 0 / 0	EH-102	Heat Exchanger	55.000	55.000
		Heat Exchange Area = 24,91 m2		
1 / 0 / 0	DDR-101	Drum Dryer	50.000	50.000
		Drum Area = 0,37 m2		
1 / 0 / 0	V-105	Flat Bottom Tank	49.000	49.000
		Vessel Volume = 50510,89 L		
1 / 0 / 0	V-114	Flat Bottom Tank	47.000	47.000
		Vessel Volume = 47119,94 L		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,09 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,65 kW		
1 / 0 / 0	EH-101	Heat Exchanger	39.000	39.000
		Heat Exchange Area = 14,40 m2		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 52,19 L		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 0,33 L		
1 / 0 / 0	V-113	Decanter Tank	34.000	34.000
		Vessel Volume = 245,19 L		
1 / 0 / 0	V-108	Decanter Tank	34.000	34.000
		Vessel Volume = 103,54 L		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,72 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000
		Pump Power = 0,67 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-102	Centrifugal Pump	10.000	20.000
		Pump Power = 0,06 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-109	Centrifugal Pump	10.000	20.000

		Pump Power = 0,21 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	10.000	20.000
		Pump Power = 0,04 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	10.000	20.000
		Pump Power = 0,04 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-103	Centrifugal Pump	10.000	20.000
		Pump Power = 0,14 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	10.000	20.000
		Pump Power = 0,04 kW		
1 / 1 / 0	PM-113	Centrifugal Pump	10.000	20.000
		Pump Power = 0,03 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 5,68 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 0,74 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 13,20 kW		
1 / 0 / 0	V-117	Flat Bottom Tank	3.000	3.000
		Vessel Volume = 753,17 L		
1 / 0 / 0	V-118	Flat Bottom Tank	3.000	3.000
		Vessel Volume = 743,63 L		
1 / 0 / 0	V-116	Flat Bottom Tank	3.000	3.000
		Vessel Volume = 664,32 L		
1 / 0 / 0	V-109	Flash Drum	1.000	1.000
		Vessel Volume = 6,08 L		
1 / 0 / 0	V-106	Flash Drum	1.000	1.000
		Vessel Volume = 20,90 L		
1 / 0 / 0	SD-101	Solids Drum	1.000	1.000
		Vessel Volume = 7,11 L		
		Unlisted Equipment		3.297.000
			TOTAL	16.483.000

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	16.483.000
2. Installation	11.534.000
3. Process Piping	5.769.000
4. Instrumentation	6.593.000
5. Insulation	494.000
6. Electrical	1.648.000
7. Buildings	7.417.000
8. Yard Improvement	2.472.000
9. Auxiliary Facilities	6.593.000
TPDC	59.006.000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	14.751.000
11. Construction	20.652.000
TPIC	35.403.000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	94.409.000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	4.720.000
13. Contingency	9.441.000
CFC = 12+13	14.161.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	108.571.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	47,38	214.278	10.152.481	100,00
TOTAL		214.278	10.152.481	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Chicken feed	0,55	14.782.616	kg	8.130.439	66,47
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	7,04
Hexane	8,35	135.087	L(STP)	1.127.303	9,22
Hydrogen	2,00	58.157	kg	116.315	0,95
Lactobacillus	1,45	1.223.914	kg	1.774.676	14,51
Sal.juice	0,00	42.474.451	kg	0	0,00
Sal.pulp	0,00	11.661.686	kg	0	0,00
Water	2,50	88.653	m3(STP)	221.632	1,81
TOTAL				12.231.919	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	10.770.588	kW-h	1.077.059	9,61
Steam	12,00	596.563	MT	7.158.752	63,87
Steam (High P)	20,00	5	MT	104	0,00
Cooling Water	0,05	59.399.280	MT	2.969.964	26,50
Hot Water	0,05	36.118	MT	1.806	0,02
TOTAL				11.207.684	100,00

Note: Savings (2600580 $/yr) exist in the process due to heat recovery.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	$	%
Raw Materials	12.232.000	22,08
Labor-Dependent	10.152.000	18,33
Facility-Dependent	20.278.000	36,61
Laboratory/QC/QA	1.523.000	2,75
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	11.208.000	20,23
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	55.393.000	100,00

10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	108.571.000 $
B.	Working Capital	3.054.000 $
C.	Startup Cost	5.429.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	117.053.000 $
G.	Investment Charged to This Project	117.053.000 $

H.	**Revenue/Savings Rates**	
	Flash 2 (Revenue)	66.506 kg /yr
	BSFL_protein (Revenue)	1.220.179 kg /yr
	Frass (Revenue)	53.756.976 kg /yr
	Naphtha (Revenue)	172.424 kg /yr
	HEFA (Main Revenue)	284.339 kg /yr
	HVO (Revenue)	193.008 kg /yr
	Flash 1 (Revenue)	117.020 kg /yr
	BSFL_chitin_and_residue (Revenue)	1.739.834 kg /yr
	Lipids (Revenue)	766.593 kg /yr
	EtOH ext. (Revenue)	1.039.803 kg /yr
	Steam(Savings)	152.322 MT/yr
	Cooling Water(Savings)	15.418.251 MT/yr
	Hot Water(Savings)	36.118 MT/yr

I.	**Revenue/Savings Price**	
	Flash 2 (Revenue)	1,20 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,08 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,44 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	Lipids (Revenue)	1,00 $/kg
	EtOH ext. (Revenue)	7,00 $/kg
	Steam(Savings)	12,00 $/MT
	Cooling Water(Savings)	0,05 $/MT
	Hot Water(Savings)	0,05 $/MT

J.	**Revenues/Savings**	
	Flash 2 (Revenue)	79.727 $/yr
	BSFL_protein (Revenue)	2.629.268 $/yr
	Frass (Revenue)	4.553.020 $/yr
	Naphtha (Revenue)	94.833 $/yr
	HEFA (Main Revenue)	176.290 $/yr
	HVO (Revenue)	193.008 $/yr
	Flash 1 (Revenue)	51.820 $/yr
	BSFL_chitin_and_residue (Revenue)	1.270.079 $/yr
	Lipids (Revenue)	766.593 $/yr
	EtOH ext. (Revenue)	7.278.618 $/yr
	Steam(Savings)	1.827.862 $/yr

	Cooling Water(Savings)	770.913 $/yr
	Hot Water(Savings)	1.806 $/yr
1	Total Revenues	17.093.254 $/yr
2	Total Savings	2.600.580 $/yr

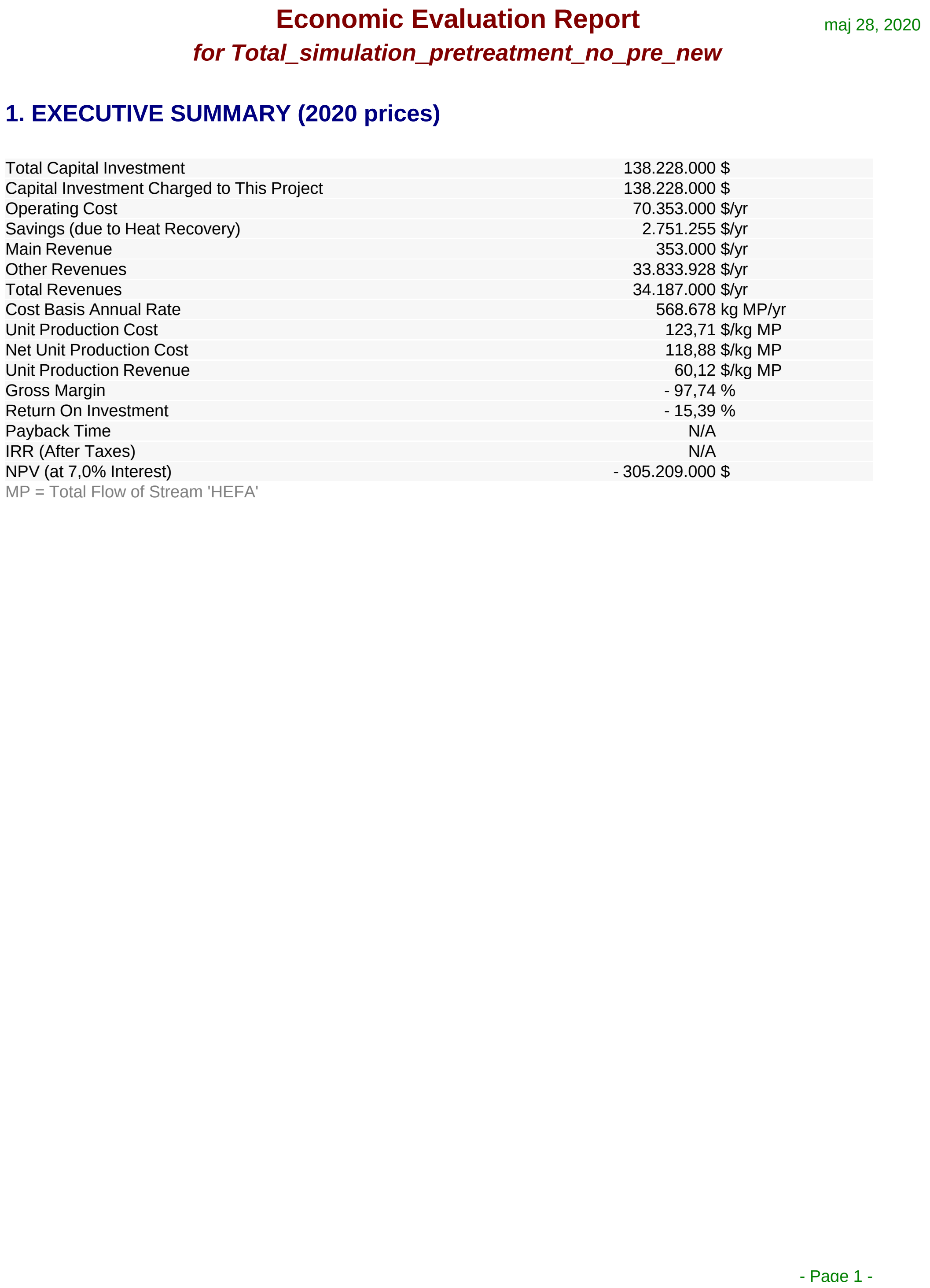
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	55.393.000 $/yr
2	Net AOC (K1-J2)	52.793.000 $/yr

L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	194,81 $/kg MP
	Net Unit Production Cost	185,67 $/kg MP
	Unit Production Revenue	60,12 $/kg MP

M.	Gross Profit (J-K)	- 35.700.000 $/yr
N.	Taxes (40%)	0 $/yr
O.	Net Profit (M-N + Depreciation)	- 25.386.000 $/yr

	Gross Margin	- 208,85 %
	Return On Investment	- 21,69 %
	Payback Time	N/A

MP = Total Flow of Stream 'HEFA'



Economic Evaluation Report

for Total_simulation_pretreatment_no_pre_new

maj 28, 2020

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	138.228.000 $
Capital Investment Charged to This Project	138.228.000 $
Operating Cost	70.353.000 $/yr
Savings (due to Heat Recovery)	2.751.255 $/yr
Main Revenue	353.000 $/yr
Other Revenues	33.833.928 $/yr
Total Revenues	34.187.000 $/yr
Cost Basis Annual Rate	568.678 kg MP/yr
Unit Production Cost	123,71 $/kg MP
Net Unit Production Cost	118,88 $/kg MP
Unit Production Revenue	60,12 $/kg MP
Gross Margin	- 97,74 %
Return On Investment	- 15,39 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7,0% Interest)	- 305.209.000 $

MP = Total Flow of Stream 'HEFA'

- Page 1 -

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor Vessel Volume = 624,89 m3	4.247.000	4.247.000
1 / 0 / 0	V-103	Flat Bottom Tank Vessel Volume = 4764,28 m3	1.237.000	1.237.000
1 / 0 / 0	V-111	Blending Tank Vessel Volume = 687,01 m3	1.186.000	1.186.000
1 / 0 / 0	V-104	Flat Bottom Tank Vessel Volume = 4466,75 m3	1.181.000	1.181.000
1 / 0 / 0	SP-101	Screw Press Throughput = 21536,12 kg/h	506.000	506.000
1 / 0 / 0	EC-102	Heat Exchanger Heat Exchange Area = 876,12 m2	463.000	463.000
1 / 0 / 0	ST-101	Heat Sterilizer Rated Throughput = 7028,30 L/h	456.000	456.000
1 / 0 / 0	V-115	Decanter Tank Vessel Volume = 65,79 m3	414.000	414.000
1 / 0 / 0	V-101	Blending Tank Vessel Volume = 1133566,97 L	397.000	397.000
1 / 0 / 0	BM-101	Bead Mill Bead Volume = 623,25 L	383.000	383.000
1 / 0 / 0	BC-104	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-103	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-105	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-101	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-106	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-102	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-107	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	EC-101	Heat Exchanger Heat Exchange Area = 358,52 m2	271.000	271.000
1 / 0 / 0	V-112	Flat Bottom Tank Vessel Volume = 534,26 m3	262.000	262.000
1 / 0 / 0	DDR-102	Drum Dryer Drum Area = 45,67 m2	246.000	246.000
1 / 0 / 0	EV-102	Multi-Effect Evaporator Mean Heat Transfer Area = 210,62 m2	221.000	221.000
1 / 0 / 0	SP-102	Screw Press Throughput = 7050,48 kg/h	207.000	207.000
1 / 0 / 0	SL-104	Silo Vessel Volume = 664174,17 L	204.000	204.000
1 / 0 / 0	V-102	Blending Tank	193.000	193.000

		Vessel Volume = 403537,17 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	102.000	102.000
		Mean Heat Transfer Area = 68,25 m2		
1 / 0 / 0	G-101	Centrifugal Compressor	75.000	75.000
		Compressor Power = 46,48 kW		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 19963,23 L		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 646,11 L		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 10368,36 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 285,98 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 164,84 L		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 164,08 kg/h		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 120,54 kg/h		
1 / 0 / 0	EH-102	Heat Exchanger	55.000	55.000
		Heat Exchange Area = 25,31 m2		
1 / 0 / 0	V-105	Flat Bottom Tank	51.000	51.000
		Vessel Volume = 53484,30 L		
1 / 0 / 0	DDR-101	Drum Dryer	50.000	50.000
		Drum Area = 0,74 m2		
1 / 0 / 0	V-114	Flat Bottom Tank	48.000	48.000
		Vessel Volume = 49733,82 L		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,09 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,65 kW		
1 / 0 / 0	EH-101	Heat Exchanger	40.000	40.000
		Heat Exchange Area = 14,53 m2		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 30,38 L		
1 / 0 / 0	V-108	Decanter Tank	34.000	34.000
		Vessel Volume = 68,15 L		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 0,95 L		
1 / 0 / 0	V-113	Decanter Tank	34.000	34.000
		Vessel Volume = 693,49 L		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,72 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000
		Pump Power = 0,68 kW		
1 / 1 / 0	PM-109	Centrifugal Pump	13.000	26.000
		Pump Power = 0,41 kW		
1 / 1 / 0	PM-103	Centrifugal Pump	11.000	22.000
		Pump Power = 0,28 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	10.000	20.000

		Pump Power = 0,08 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,03 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-113	Centrifugal Pump	10.000	20.000
		Pump Power = 0,06 kW		
1 / 1 / 0	PM-102	Centrifugal Pump	10.000	20.000
		Pump Power = 0,12 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,03 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 26,39 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 1,48 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 11,37 kW		
1 / 0 / 0	V-117	Flat Bottom Tank	4.000	4.000
		Vessel Volume = 1506,33 L		
1 / 0 / 0	V-118	Flat Bottom Tank	4.000	4.000
		Vessel Volume = 1487,26 L		
1 / 0 / 0	V-116	Flat Bottom Tank	4.000	4.000
		Vessel Volume = 1328,64 L		
1 / 0 / 0	V-106	Flash Drum	2.000	2.000
		Vessel Volume = 41,80 L		
1 / 0 / 0	V-109	Flash Drum	1.000	1.000
		Vessel Volume = 12,16 L		
1 / 0 / 0	SD-101	Solids Drum	1.000	1.000
		Vessel Volume = 14,21 L		
		Unlisted Equipment		3.935.000
			TOTAL	19.674.000

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)

1. Equipment Purchase Cost	19.674.000
2. Installation	12.771.000
3. Process Piping	6.886.000
4. Instrumentation	7.870.000
5. Insulation	590.000
6. Electrical	1.967.000
7. Buildings	8.853.000
8. Yard Improvement	2.951.000
9. Auxiliary Facilities	7.870.000
TPDC	69.432.000

3B. Total Plant Indirect Cost (TPIC)

10. Engineering	17.358.000
11. Construction	24.301.000
TPIC	41.659.000

3C. Total Plant Cost (TPC = TPDC+TPIC)

TPC	111.091.000

3D. Contractor's Fee & Contingency (CFC)

12. Contractor's Fee	5.555.000
13. Contingency	11.109.000
CFC = 12+13	16.664.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)

DFC	127.754.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	47,38	214.278	10.152.481	100,00
TOTAL		214.278	10.152.481	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Chicken feed	0,55	29.565.231	kg	16.260.877	72,35
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	3,83
Hexane	8,35	135.087	L(STP)	1.127.303	5,02
Hydrogen	2,00	116.315	kg	232.629	1,04
Lactobacillus	1,45	2.447.829	kg	3.549.351	15,79
Sal.juice	0,00	84.948.903	kg	0	0,00
Sal.pulp	0,00	23.323.372	kg	0	0,00
Water	2,50	177.306	m3(STP)	443.264	1,97
TOTAL				22.474.979	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	20.509.947	kW-h	2.050.995	16,65
Steam	12,00	606.975	MT	7.283.701	59,13
Steam (High P)	20,00	10	MT	208	0,00
Cooling Water	0,05	59.605.584	MT	2.980.279	24,19
Hot Water	0,05	72.236	MT	3.612	0,03
TOTAL				12.318.794	100,00

Note: Savings (2751255 $/yr) exist in the process due to heat recovery.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	**$**	**%**
Raw Materials	22.475.000	31,95
Labor-Dependent	10.152.000	14,43
Facility-Dependent	23.884.000	33,95
Laboratory/QC/QA	1.523.000	2,16
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	12.319.000	17,51
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	70.353.000	100,00

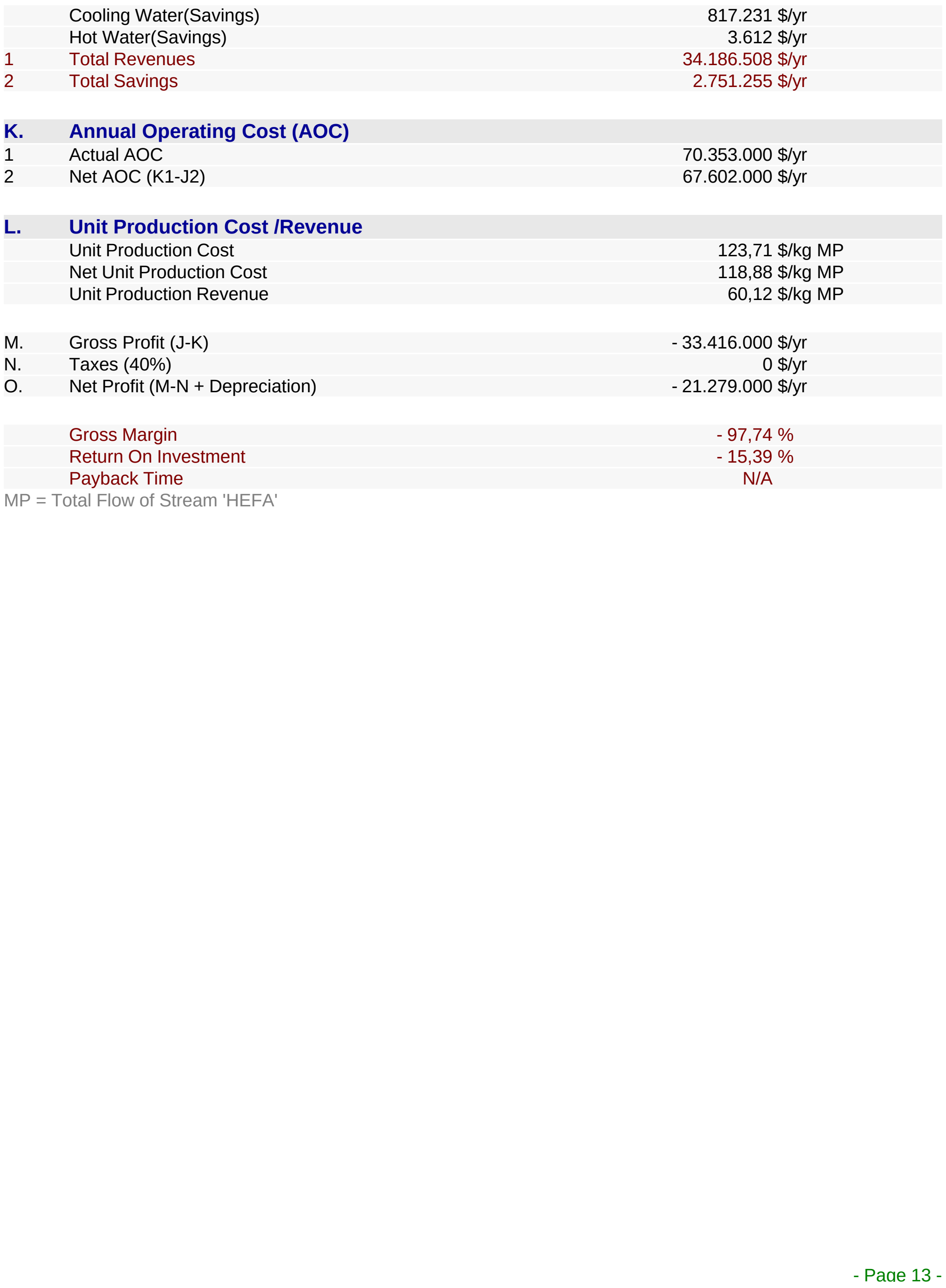
10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	127.754.000 $
B.	Working Capital	4.086.000 $
C.	Startup Cost	6.388.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	138.228.000 $
G.	Investment Charged to This Project	138.228.000 $

H.	**Revenue/Savings Rates**	
	Flash 2 (Revenue)	133.011 kg /yr
	BSFL_protein (Revenue)	2.440.357 kg /yr
	Frass (Revenue)	107.513.951 kg /yr
	Naphtha (Revenue)	344.847 kg /yr
	HEFA (Main Revenue)	568.678 kg /yr
	HVO (Revenue)	386.015 kg /yr
	Flash 1 (Revenue)	234.040 kg /yr
	BSFL_chitin_and_residue (Revenue)	3.479.668 kg /yr
	Lipids (Revenue)	1.533.185 kg /yr
	EtOH ext. (Revenue)	2.079.605 kg /yr
	Steam(Savings)	160.868 MT/yr
	Cooling Water(Savings)	16.344.618 MT/yr
	Hot Water(Savings)	72.236 MT/yr

I.	**Revenue/Savings Price**	
	Flash 2 (Revenue)	1,20 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,08 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,44 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	Lipids (Revenue)	1,00 $/kg
	EtOH ext. (Revenue)	7,00 $/kg
	Steam(Savings)	12,00 $/MT
	Cooling Water(Savings)	0,05 $/MT
	Hot Water(Savings)	0,05 $/MT

J.	**Revenues/Savings**	
	Flash 2 (Revenue)	159.454 $/yr
	BSFL_protein (Revenue)	5.258.536 $/yr
	Frass (Revenue)	9.106.039 $/yr
	Naphtha (Revenue)	189.666 $/yr
	HEFA (Main Revenue)	352.580 $/yr
	HVO (Revenue)	386.015 $/yr
	Flash 1 (Revenue)	103.640 $/yr
	BSFL_chitin_and_residue (Revenue)	2.540.158 $/yr
	Lipids (Revenue)	1.533.185 $/yr
	EtOH ext. (Revenue)	14.557.236 $/yr
	Steam(Savings)	1.930.412 $/yr



	Cooling Water(Savings)	817.231 $/yr
	Hot Water(Savings)	3.612 $/yr
1	Total Revenues	34.186.508 $/yr
2	Total Savings	2.751.255 $/yr
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	70.353.000 $/yr
2	Net AOC (K1-J2)	67.602.000 $/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	123,71 $/kg MP
	Net Unit Production Cost	118,88 $/kg MP
	Unit Production Revenue	60,12 $/kg MP
M.	Gross Profit (J-K)	- 33.416.000 $/yr
N.	Taxes (40%)	0 $/yr
O.	Net Profit (M-N + Depreciation)	- 21.279.000 $/yr
	Gross Margin	- 97,74 %
	Return On Investment	- 15,39 %
	Payback Time	N/A

MP = Total Flow of Stream 'HEFA'

Economic Evaluation Report

for Total_simulation_pretreatment_no_pre_new

maj 28, 2020

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	174.589.000 $
Capital Investment Charged to This Project	174.589.000 $
Operating Cost	99.204.000 $/yr
Savings (due to Heat Recovery)	3.049.514 $/yr
Main Revenue	705.000 $/yr
Other Revenues	67.667.856 $/yr
Total Revenues	68.373.000 $/yr
Cost Basis Annual Rate	1.137.356 kg MP/yr
Unit Production Cost	87,22 $/kg MP
Net Unit Production Cost	84,58 $/kg MP
Unit Production Revenue	60,12 $/kg MP
Gross Margin	- 40,70 %
Return On Investment	- 7,21 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7,0% Interest)	- 278.586.000 $

MP = Total Flow of Stream 'HEFA'

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor	6.422.000	6.422.000
		Vessel Volume = 1249,79 m3		
1 / 0 / 0	V-111	Blending Tank	1.706.000	1.706.000
		Vessel Volume = 1374,02 m3		
1 / 0 / 0	V-103	Flat Bottom Tank	1.236.000	1.236.000
		Vessel Volume = 4762,85 m3		
1 / 0 / 0	V-104	Flat Bottom Tank	1.181.000	1.181.000
		Vessel Volume = 4465,41 m3		
1 / 0 / 0	SP-101	Screw Press	882.000	882.000
		Throughput = 43072,25 kg/h		
1 / 0 / 0	V-115	Decanter Tank	773.000	773.000
		Vessel Volume = 186,08 m3		
1 / 0 / 0	ST-101	Heat Sterilizer	565.000	565.000
		Rated Throughput = 14056,59 L/h		
1 / 0 / 0	BM-101	Bead Mill	542.000	542.000
		Bead Volume = 1246,51 L		
1 / 0 / 0	EC-102	Heat Exchanger	462.000	462.000
		Heat Exchange Area = 875,86 m2		
1 / 0 / 0	V-112	Flat Bottom Tank	428.000	428.000
		Vessel Volume = 1068,53 m3		
1 / 0 / 0	V-101	Blending Tank	401.000	401.000
		Vessel Volume = 1150017,75 L		
1 / 0 / 0	SP-102	Screw Press	361.000	361.000
		Throughput = 14100,96 kg/h		
1 / 0 / 0	SL-104	Silo	358.000	358.000
		Vessel Volume = 1328348,34 L		
1 / 0 / 0	DDR-102	Drum Dryer	327.000	327.000
		Drum Area = 91,34 m2		
1 / 0 / 0	BC-102	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-104	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-106	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-105	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-103	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-101	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-107	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	EC-101	Heat Exchanger	271.000	271.000
		Heat Exchange Area = 358,42 m2		
1 / 0 / 0	EV-102	Multi-Effect Evaporator	219.000	219.000
		Mean Heat Transfer Area = 209,92 m2		
1 / 0 / 0	V-102	Blending Tank	195.000	195.000

		Vessel Volume = 409875,75 L		
1 / 0 / 0	G-101	Centrifugal Compressor	105.000	105.000
		Compressor Power = 92,95 kW		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101.000	101.000
		Mean Heat Transfer Area = 67,77 m2		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 1292,21 L		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 39926,45 L		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 20736,71 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 571,95 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 329,69 L		
1 / 0 / 0	DDR-101	Drum Dryer	59.000	59.000
		Drum Area = 1,49 m2		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 241,08 kg/h		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 328,17 kg/h		
1 / 0 / 0	EH-102	Heat Exchanger	56.000	56.000
		Heat Exchange Area = 26,12 m2		
1 / 0 / 0	V-105	Flat Bottom Tank	55.000	55.000
		Vessel Volume = 59454,89 L		
1 / 0 / 0	V-114	Flat Bottom Tank	52.000	52.000
		Vessel Volume = 54974,92 L		
1 / 0 / 0	V-113	Decanter Tank	50.000	50.000
		Vessel Volume = 1961,49 L		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,09 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,64 kW		
1 / 0 / 0	EH-101	Heat Exchanger	40.000	40.000
		Heat Exchange Area = 14,80 m2		
1 / 0 / 0	V-108	Decanter Tank	34.000	34.000
		Vessel Volume = 276,64 L		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 91,62 L		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 2,67 L		
1 / 1 / 0	PM-109	Centrifugal Pump	18.000	36.000
		Pump Power = 0,82 kW		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,73 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000
		Pump Power = 0,68 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-103	Centrifugal Pump	15.000	30.000
		Pump Power = 0,55 kW		
1 / 1 / 0	PM-102	Centrifugal Pump	10.000	20.000
		Pump Power = 0,23 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000

		Pump Power = 0,00 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	10.000	20.000
		Pump Power = 0,16 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	10.000	20.000
		Pump Power = 0,16 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,05 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,03 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	10.000	20.000
		Pump Power = 0,16 kW		
1 / 1 / 0	PM-113	Centrifugal Pump	10.000	20.000
		Pump Power = 0,12 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,05 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 2,96 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 22,74 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 52,79 kW		
1 / 0 / 0	V-118	Flat Bottom Tank	7.000	7.000
		Vessel Volume = 2974,51 L		
1 / 0 / 0	V-117	Flat Bottom Tank	7.000	7.000
		Vessel Volume = 3012,67 L		
1 / 0 / 0	V-116	Flat Bottom Tank	6.000	6.000
		Vessel Volume = 2657,29 L		
1 / 0 / 0	V-106	Flash Drum	2.000	2.000
		Vessel Volume = 83,59 L		
1 / 0 / 0	V-109	Flash Drum	1.000	1.000
		Vessel Volume = 24,32 L		
1 / 0 / 0	SD-101	Solids Drum	1.000	1.000
		Vessel Volume = 28,43 L		
		Unlisted Equipment		5.021.000
			TOTAL	25.105.000

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	25.105.000
2. Installation	14.881.000
3. Process Piping	8.787.000
4. Instrumentation	10.042.000
5. Insulation	753.000
6. Electrical	2.511.000
7. Buildings	11.297.000
8. Yard Improvement	3.766.000
9. Auxiliary Facilities	10.042.000
TPDC	87.184.000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	21.796.000
11. Construction	30.514.000
TPIC	52.310.000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	139.494.000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	6.975.000
13. Contingency	13.949.000
CFC = 12+13	20.924.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	160.418.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	47,38	214.278	10.152.481	100,00
TOTAL		214.278	10.152.481	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Chicken feed	0,55	59.130.463	kg	32.521.754	75,70
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	2,01
Hexane	8,35	135.087	L(STP)	1.127.303	2,62
Hydrogen	2,00	232.629	kg	465.258	1,08
Lactobacillus	1,45	4.895.657	kg	7.098.703	16,52
Sal.juice	0,00	169.897.805	kg	0	0,00
Sal.pulp	0,00	46.646.743	kg	0	0,00
Water	2,50	354.611	m3(STP)	886.528	2,06
TOTAL				42.961.101	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	39.988.919	kW-h	3.998.892	27,49
Steam	12,00	628.007	MT	7.536.079	51,81
Steam (High P)	20,00	21	MT	416	0,00
Cooling Water	0,05	60.039.054	MT	3.001.953	20,64
Hot Water	0,05	144.472	MT	7.224	0,05
TOTAL				14.544.562	100,00

Note: Savings (3049514 $/yr) exist in the process due to heat recovery.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	$	%
Raw Materials	42.961.000	43,31
Labor-Dependent	10.152.000	10,23
Facility-Dependent	30.023.000	30,26
Laboratory/QC/QA	1.523.000	1,54
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	14.545.000	14,66
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	99.204.000	100,00

10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	160.418.000 $
B.	Working Capital	6.151.000 $
C.	Startup Cost	8.021.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	174.589.000 $
G.	Investment Charged to This Project	174.589.000 $

H.	**Revenue/Savings Rates**	
	Flash 2 (Revenue)	266.023 kg /yr
	BSFL_protein (Revenue)	4.880.715 kg /yr
	Frass (Revenue)	215.027.902 kg /yr
	Naphtha (Revenue)	689.695 kg /yr
	HEFA (Main Revenue)	1.137.356 kg /yr
	HVO (Revenue)	772.030 kg /yr
	Flash 1 (Revenue)	468.081 kg /yr
	BSFL_chitin_and_residue (Revenue)	6.959.337 kg /yr
	Lipids (Revenue)	3.066.370 kg /yr
	EtOH ext. (Revenue)	4.159.210 kg /yr
	Steam(Savings)	177.778 MT/yr
	Cooling Water(Savings)	18.179.074 MT/yr
	Hot Water(Savings)	144.472 MT/yr

I.	**Revenue/Savings Price**	
	Flash 2 (Revenue)	1,20 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,08 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,44 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	Lipids (Revenue)	1,00 $/kg
	EtOH ext. (Revenue)	7,00 $/kg
	Steam(Savings)	12,00 $/MT
	Cooling Water(Savings)	0,05 $/MT
	Hot Water(Savings)	0,05 $/MT

J.	**Revenues/Savings**	
	Flash 2 (Revenue)	318.907 $/yr
	BSFL_protein (Revenue)	10.517.071 $/yr
	Frass (Revenue)	18.212.079 $/yr
	Naphtha (Revenue)	379.332 $/yr
	HEFA (Main Revenue)	705.161 $/yr
	HVO (Revenue)	772.030 $/yr
	Flash 1 (Revenue)	207.279 $/yr
	BSFL_chitin_and_residue (Revenue)	5.080.316 $/yr
	Lipids (Revenue)	3.066.370 $/yr
	EtOH ext. (Revenue)	29.114.472 $/yr
	Steam(Savings)	2.133.337 $/yr

	Cooling Water(Savings)	908.954 $/yr
	Hot Water(Savings)	7.224 $/yr
1	Total Revenues	68.373.017 $/yr
2	Total Savings	3.049.514 $/yr

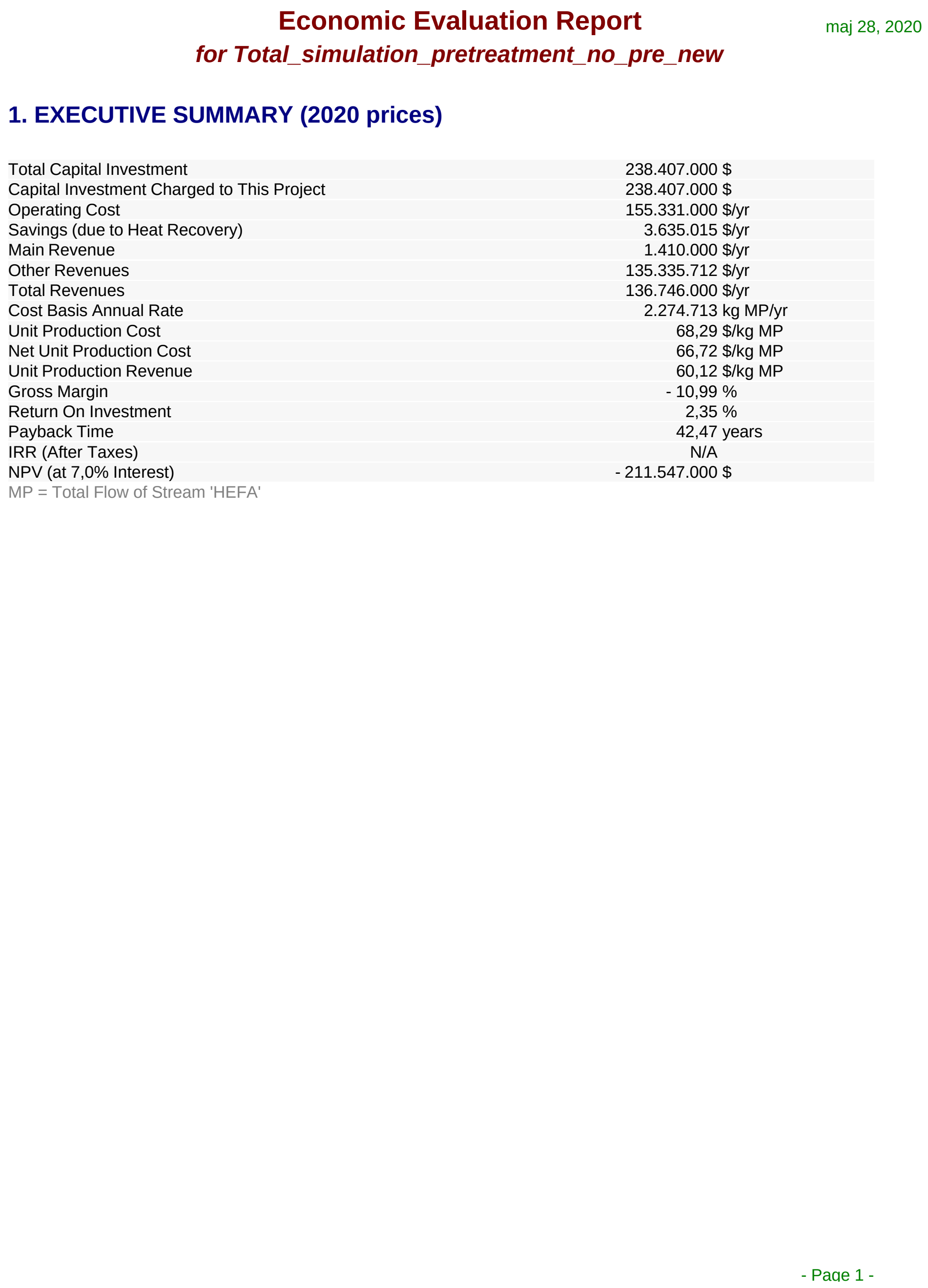
K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	99.204.000 $/yr
2	Net AOC (K1-J2)	96.203.000 $/yr

L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	87,22 $/kg MP
	Net Unit Production Cost	84,58 $/kg MP
	Unit Production Revenue	60,12 $/kg MP

M.	Gross Profit (J-K)	- 27.831.000 $/yr
N.	Taxes (40%)	0 $/yr
O.	Net Profit (M-N + Depreciation)	- 12.591.000 $/yr

	Gross Margin	- 40,70 %
	Return On Investment	- 7,21 %
	Payback Time	N/A

MP = Total Flow of Stream 'HEFA'



Economic Evaluation Report

for Total_simulation_pretreatment_no_pre_new

maj 28, 2020

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	238.407.000 $
Capital Investment Charged to This Project	238.407.000 $
Operating Cost	155.331.000 $/yr
Savings (due to Heat Recovery)	3.635.015 $/yr
Main Revenue	1.410.000 $/yr
Other Revenues	135.335.712 $/yr
Total Revenues	136.746.000 $/yr
Cost Basis Annual Rate	2.274.713 kg MP/yr
Unit Production Cost	68,29 $/kg MP
Net Unit Production Cost	66,72 $/kg MP
Unit Production Revenue	60,12 $/kg MP
Gross Margin	- 10,99 %
Return On Investment	2,35 %
Payback Time	42,47 years
IRR (After Taxes)	N/A
NPV (at 7,0% Interest)	- 211.547.000 $

MP = Total Flow of Stream 'HEFA'

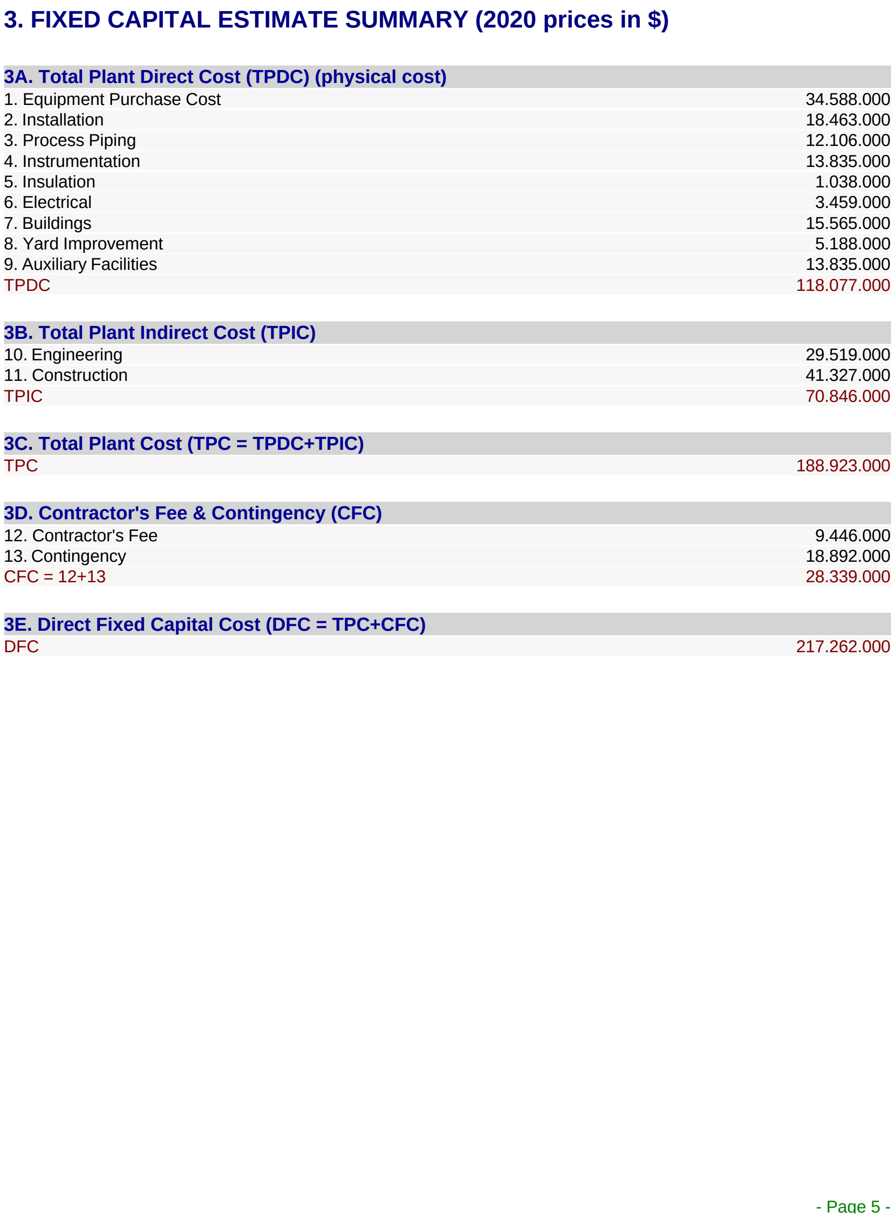
2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor Vessel Volume = 2499,57 m3	9.956.000	9.956.000
1 / 0 / 0	V-111	Blending Tank Vessel Volume = 2748,04 m3	2.493.000	2.493.000
1 / 0 / 0	SP-101	Screw Press Throughput = 86144,49 kg/h	1.535.000	1.535.000
1 / 0 / 0	V-115	Decanter Tank Vessel Volume = 526,32 m3	1.442.000	1.442.000
1 / 0 / 0	V-103	Flat Bottom Tank Vessel Volume = 4769,99 m3	1.238.000	1.238.000
1 / 0 / 0	V-104	Flat Bottom Tank Vessel Volume = 4470,32 m3	1.182.000	1.182.000
1 / 0 / 0	SL-104	Silo Vessel Volume = 2656696,68 L	973.000	973.000
1 / 0 / 0	BM-101	Bead Mill Bead Volume = 2493,01 L	766.000	766.000
1 / 0 / 0	ST-101	Heat Sterilizer Rated Throughput = 28113,18 L/h	701.000	701.000
1 / 0 / 0	V-112	Flat Bottom Tank Vessel Volume = 2137,05 m3	700.000	700.000
1 / 0 / 0	SP-102	Screw Press Throughput = 28201,93 kg/h	628.000	628.000
1 / 0 / 0	EC-102	Heat Exchanger Heat Exchange Area = 876,74 m2	463.000	463.000
1 / 0 / 0	DDR-102	Drum Dryer Drum Area = 182,69 m2	436.000	436.000
1 / 0 / 0	V-101	Blending Tank Vessel Volume = 1184740,71 L	409.000	409.000
1 / 0 / 0	BC-104	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-107	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-103	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-106	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-101	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-102	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-105	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	EC-101	Heat Exchanger Heat Exchange Area = 358,92 m2	271.000	271.000
1 / 0 / 0	G-101	Centrifugal Compressor Compressor Power = 185,90 kW	253.000	253.000
1 / 0 / 0	EV-102	Multi-Effect Evaporator	219.000	219.000

		Mean Heat Transfer Area = 208,86 m2		
1 / 0 / 0	V-102	Blending Tank	199.000	199.000
		Vessel Volume = 423295,64 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101.000	101.000
		Mean Heat Transfer Area = 66,94 m2		
1 / 0 / 0	V-113	Decanter Tank	94.000	94.000
		Vessel Volume = 5547,92 L		
1 / 0 / 0	DDR-101	Drum Dryer	79.000	79.000
		Drum Area = 2,97 m2		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 41473,42 L		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 2584,42 L		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 79852,90 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 1143,91 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 659,37 L		
1 / 0 / 0	V-105	Flat Bottom Tank	63.000	63.000
		Vessel Volume = 71491,09 L		
1 / 0 / 0	V-114	Flat Bottom Tank	59.000	59.000
		Vessel Volume = 65528,31 L		
1 / 0 / 0	EH-102	Heat Exchanger	58.000	58.000
		Heat Exchange Area = 27,78 m2		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 482,17 kg/h		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 656,33 kg/h		
1 / 0 / 0	V-108	Decanter Tank	48.000	48.000
		Vessel Volume = 1791,88 L		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,10 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,65 kW		
1 / 0 / 0	EH-101	Heat Exchanger	41.000	41.000
		Heat Exchange Area = 15,38 m2		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 7,56 L		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 290,39 L		
1 / 1 / 0	PM-109	Centrifugal Pump	24.000	48.000
		Pump Power = 1,64 kW		
1 / 1 / 0	PM-103	Centrifugal Pump	20.000	40.000
		Pump Power = 1,11 kW		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,76 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-102	Centrifugal Pump	14.000	28.000
		Pump Power = 0,47 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	12.000	24.000

		Pump Power = 0,33 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	12.000	24.000
		Pump Power = 0,32 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	12.000	24.000
		Pump Power = 0,32 kW		
1 / 0 / 0	V-117	Flat Bottom Tank	11.000	11.000
		Vessel Volume = 6025,33 L		
1 / 0 / 0	V-118	Flat Bottom Tank	11.000	11.000
		Vessel Volume = 5949,03 L		
1 / 1 / 0	PM-113	Centrifugal Pump	10.000	20.000
		Pump Power = 0,23 kW		
1 / 0 / 0	V-116	Flat Bottom Tank	10.000	10.000
		Vessel Volume = 5314,58 L		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,05 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,04 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,03 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,11 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,10 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 105,57 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 5,92 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 45,47 kW		
1 / 0 / 0	V-106	Flash Drum	3.000	3.000
		Vessel Volume = 167,19 L		
1 / 0 / 0	V-109	Flash Drum	2.000	2.000
		Vessel Volume = 48,64 L		
1 / 0 / 0	SD-101	Solids Drum	1.000	1.000
		Vessel Volume = 56,85 L		
		Unlisted Equipment		6.918.000
			TOTAL	34.588.000



3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	34.588.000
2. Installation	18.463.000
3. Process Piping	12.106.000
4. Instrumentation	13.835.000
5. Insulation	1.038.000
6. Electrical	3.459.000
7. Buildings	15.565.000
8. Yard Improvement	5.188.000
9. Auxiliary Facilities	13.835.000
TPDC	118.077.000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	29.519.000
11. Construction	41.327.000
TPIC	70.846.000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	188.923.000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	9.446.000
13. Contingency	18.892.000
CFC = 12+13	28.339.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	217.262.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	47,38	214.278	10.152.481	100,00
TOTAL		214.278	10.152.481	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Chicken feed	0,55	118.260.925	kg	65.043.509	77,49
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	1,03
Hexane	8,35	135.087	L(STP)	1.127.303	1,34
Hydrogen	2,00	465.258	kg	930.516	1,11
Lactobacillus	1,45	9.791.314	kg	14.197.406	16,92
Sal.juice	0,00	339.795.610	kg	0	0,00
Sal.pulp	0,00	93.293.487	kg	0	0,00
Water	2,50	709.222	m3(STP)	1.773.056	2,11
TOTAL				83.933.344	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	78.948.065	kW-h	7.894.806	41,52
Steam	12,00	671.099	MT	8.053.185	42,35
Steam (High P)	20,00	42	MT	831	0,00
Cooling Water	0,05	61.006.832	MT	3.050.342	16,04
Hot Water	0,05	288.944	MT	14.447	0,08
TOTAL				19.013.612	100,00

Note: Savings (3635015 $/yr) exist in the process due to heat recovery.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	$	%
Raw Materials	83.933.000	54,04
Labor-Dependent	10.152.000	6,54
Facility-Dependent	40.708.000	26,21
Laboratory/QC/QA	1.523.000	0,98
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	19.014.000	12,24
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	155.331.000	100,00

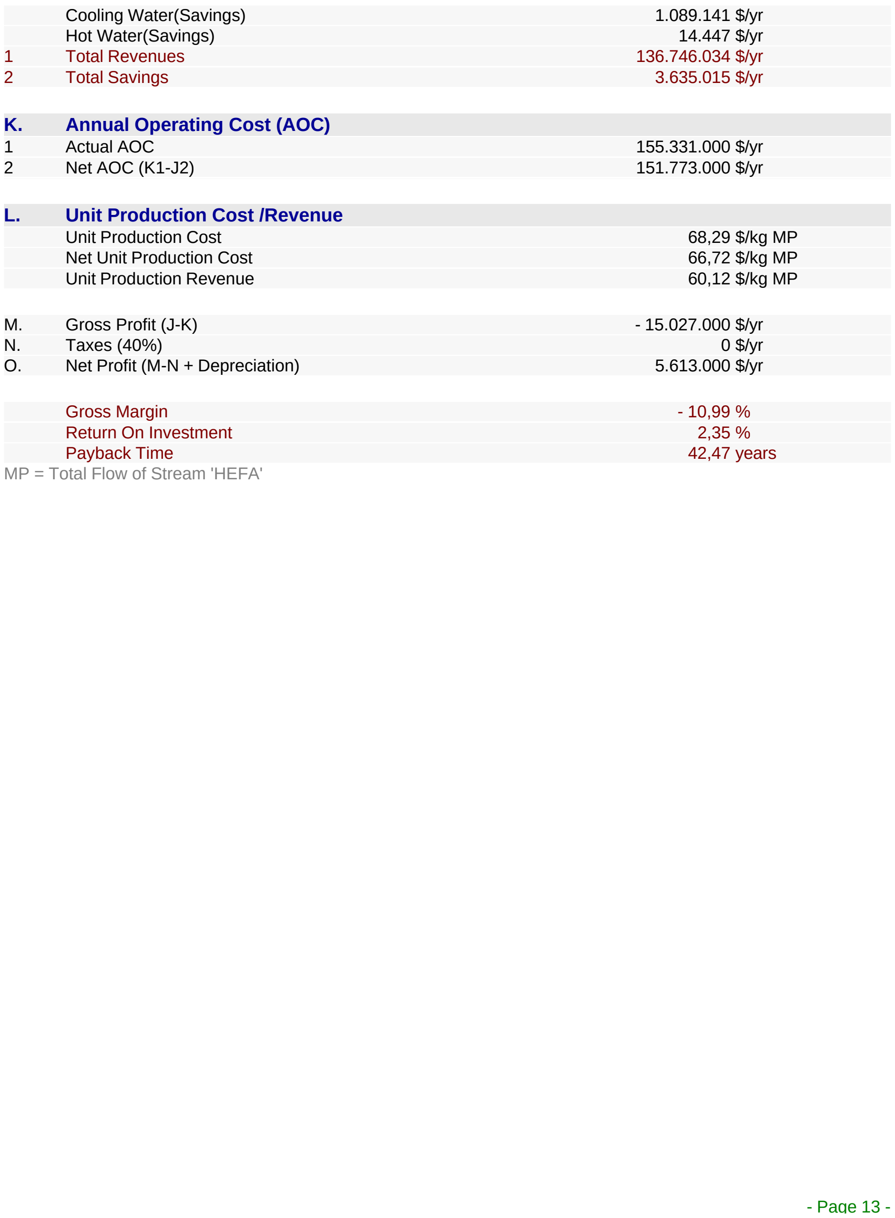
10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	217.262.000 $
B.	Working Capital	10.282.000 $
C.	Startup Cost	10.863.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	238.407.000 $
G.	Investment Charged to This Project	238.407.000 $

H.	**Revenue/Savings Rates**	
	Flash 2 (Revenue)	532.045 kg /yr
	BSFL_protein (Revenue)	9.761.429 kg /yr
	Frass (Revenue)	430.055.805 kg /yr
	Naphtha (Revenue)	1.379.389 kg /yr
	HEFA (Main Revenue)	2.274.713 kg /yr
	HVO (Revenue)	1.544.060 kg /yr
	Flash 1 (Revenue)	936.161 kg /yr
	BSFL_chitin_and_residue (Revenue)	13.918.674 kg /yr
	Lipids (Revenue)	6.132.741 kg /yr
	EtOH ext. (Revenue)	8.318.420 kg /yr
	Steam(Savings)	210.952 MT/yr
	Cooling Water(Savings)	21.782.816 MT/yr
	Hot Water(Savings)	288.944 MT/yr

I.	**Revenue/Savings Price**	
	Flash 2 (Revenue)	1,20 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,08 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,44 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	Lipids (Revenue)	1,00 $/kg
	EtOH ext. (Revenue)	7,00 $/kg
	Steam(Savings)	12,00 $/MT
	Cooling Water(Savings)	0,05 $/MT
	Hot Water(Savings)	0,05 $/MT

J.	**Revenues/Savings**	
	Flash 2 (Revenue)	637.814 $/yr
	BSFL_protein (Revenue)	21.034.142 $/yr
	Frass (Revenue)	36.424.158 $/yr
	Naphtha (Revenue)	758.664 $/yr
	HEFA (Main Revenue)	1.410.322 $/yr
	HVO (Revenue)	1.544.060 $/yr
	Flash 1 (Revenue)	414.559 $/yr
	BSFL_chitin_and_residue (Revenue)	10.160.632 $/yr
	Lipids (Revenue)	6.132.741 $/yr
	EtOH ext. (Revenue)	58.228.943 $/yr
	Steam(Savings)	2.531.427 $/yr



	Cooling Water(Savings)	1.089.141 $/yr
	Hot Water(Savings)	14.447 $/yr
1	Total Revenues	136.746.034 $/yr
2	Total Savings	3.635.015 $/yr

K.	**Annual Operating Cost (AOC)**	
1	Actual AOC	155.331.000 $/yr
2	Net AOC (K1-J2)	151.773.000 $/yr

L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	68,29 $/kg MP
	Net Unit Production Cost	66,72 $/kg MP
	Unit Production Revenue	60,12 $/kg MP

M.	Gross Profit (J-K)	- 15.027.000 $/yr
N.	Taxes (40%)	0 $/yr
O.	Net Profit (M-N + Depreciation)	5.613.000 $/yr

	Gross Margin	- 10,99 %
	Return On Investment	2,35 %
	Payback Time	42,47 years

MP = Total Flow of Stream 'HEFA'

Economic Evaluation Report

for Total_simulation_pretreatment_severe

maj 28, 2020

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	125.529.000 $
Capital Investment Charged to This Project	125.529.000 $
Operating Cost	62.022.000 $/yr
Main Revenue	470.000 $/yr
Other Revenues	27.330.341 $/yr
Total Revenues	27.800.000 $/yr
Cost Basis Annual Rate	757.663 kg MP/yr
Unit Production Cost	81,86 $/kg MP
Unit Production Revenue	36,69 $/kg MP
Gross Margin	- 123,10 %
Return On Investment	- 18,47 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7,0% Interest)	- 287.983.000 $

MP = Total Flow of Stream 'HEFA'

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor	2.948.000	2.948.000
		Vessel Volume = 320,92 m3		
1 / 0 / 0	V-103	Flat Bottom Tank	1.237.000	1.237.000
		Vessel Volume = 4767,14 m3		
1 / 0 / 0	V-104	Flat Bottom Tank	1.182.000	1.182.000
		Vessel Volume = 4468,98 m3		
1 / 0 / 0	V-111	Blending Tank	850.000	850.000
		Vessel Volume = 349,56 m3		
1 / 0 / 0	EC-102	Heat Exchanger	463.000	463.000
		Heat Exchange Area = 876,56 m2		
1 / 0 / 0	V-101	Blending Tank	395.000	395.000
		Vessel Volume = 1125326,82 L		
1 / 0 / 0	ST-101	Heat Sterilizer	356.000	356.000
		Rated Throughput = 3155,10 L/h		
1 / 0 / 0	V-119	Receiver Tank	343.000	343.000
		Vessel Volume = 549,48 m3		
1 / 0 / 0	V-120	Receiver Tank	343.000	343.000
		Vessel Volume = 549,48 m3		
1 / 0 / 0	LD-101	Blending Tank	310.000	310.000
		Vessel Volume = 22,95 m3		
1 / 0 / 0	SP-101	Screw Press	291.000	291.000
		Throughput = 10768,13 kg/h		
1 / 0 / 0	BC-103	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-101	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-106	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-105	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-102	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-107	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	EC-101	Heat Exchanger	271.000	271.000
		Heat Exchange Area = 358,74 m2		
1 / 0 / 0	BM-101	Bead Mill	270.000	270.000
		Bead Volume = 310,22 L		
1 / 0 / 0	HX-102	Heat Exchanger	234.000	234.000
		Heat Exchange Area = 280,70 m2		
1 / 0 / 0	EV-102	Multi-Effect Evaporator	221.000	221.000
		Mean Heat Transfer Area = 211,14 m2		
1 / 0 / 0	V-102	Blending Tank	191.000	191.000
		Vessel Volume = 400346,38 L		
1 / 0 / 0	EH-109	Heat Exchanger	189.000	189.000
		Heat Exchange Area = 197,22 m2		
1 / 0 / 0	V-112	Flat Bottom Tank	166.000	166.000

		Vessel Volume = 281,20 m3		
1 / 0 / 0	DDR-102	Drum Dryer	153.000	153.000
		Drum Area = 14,47 m2		
1 / 0 / 0	SL-104	Silo	130.000	130.000
		Vessel Volume = 347547,08 L		
1 / 0 / 0	SP-102	Screw Press	110.000	110.000
		Throughput = 3208,61 kg/h		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	102.000	102.000
		Mean Heat Transfer Area = 68,59 m2		
1 / 0 / 0	G-101	Centrifugal Compressor	78.000	78.000
		Compressor Power = 53,63 kW		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 11379,74 L		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 861,03 L		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 4718,54 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 219,34 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 382,21 L		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 160,54 kg/h		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 218,69 kg/h		
1 / 0 / 0	V-115	Decanter Tank	55.000	55.000
		Vessel Volume = 2268,77 L		
1 / 0 / 0	EH-102	Heat Exchanger	54.000	54.000
		Heat Exchange Area = 24,80 m2		
1 / 0 / 0	DDR-101	Drum Dryer	50.000	50.000
		Drum Area = 0,37 m2		
1 / 0 / 0	V-105	Flat Bottom Tank	49.000	49.000
		Vessel Volume = 50506,84 L		
1 / 0 / 0	V-114	Flat Bottom Tank	47.000	47.000
		Vessel Volume = 47116,94 L		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,09 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,65 kW		
1 / 0 / 0	EH-101	Heat Exchanger	39.000	39.000
		Heat Exchange Area = 14,37 m2		
1 / 0 / 0	V-108	Decanter Tank	34.000	34.000
		Vessel Volume = 75,41 L		
1 / 0 / 0	V-113	Decanter Tank	34.000	34.000
		Vessel Volume = 137,63 L		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 1,29 L		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 43,44 L		
1 / 0 / 0	SLDR-101	Sludge Dryer	33.000	33.000
		Evaporative Capacity = 11,95 MT/h		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,72 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000

		Pump Power = 0,67 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-109	Centrifugal Pump	15.000	30.000
		Pump Power = 0,55 kW		
1 / 1 / 0	PM-103	Centrifugal Pump	12.000	24.000
		Pump Power = 0,37 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	10.000	20.000
		Pump Power = 0,04 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	10.000	20.000
		Pump Power = 0,04 kW		
1 / 1 / 0	PM-128	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-111	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-129	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	10.000	20.000
		Pump Power = 0,04 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-127	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-102	Centrifugal Pump	10.000	20.000
		Pump Power = 0,15 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 14,38 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 35,19 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000

		Electric Power = 1,71 kW		
1 / 0 / 0	V-117	Flat Bottom Tank	5.000	5.000
		Vessel Volume = 2006,92 L		
1 / 0 / 0	V-118	Flat Bottom Tank	5.000	5.000
		Vessel Volume = 1979,74 L		
1 / 0 / 0	V-116	Flat Bottom Tank	5.000	5.000
		Vessel Volume = 1774,18 L		
1 / 0 / 0	V-106	Flash Drum	2.000	2.000
		Vessel Volume = 55,75 L		
1 / 0 / 0	V-109	Flash Drum	1.000	1.000
		Vessel Volume = 16,17 L		
1 / 0 / 0	SD-101	Solids Drum	1.000	1.000
		Vessel Volume = 18,94 L		
		Unlisted Equipment		3.549.000
			TOTAL	17.743.000

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	17.743.000
2. Installation	12.054.000
3. Process Piping	6.210.000
4. Instrumentation	7.097.000
5. Insulation	532.000
6. Electrical	1.774.000
7. Buildings	7.984.000
8. Yard Improvement	2.661.000
9. Auxiliary Facilities	7.097.000
TPDC	63.152.000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	15.788.000
11. Construction	22.103.000
TPIC	37.891.000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	101.044.000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	5.052.000
13. Contingency	10.104.000
CFC = 12+13	15.157.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	116.200.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	47,38	226.348	10.724.386	100,00
TOTAL		226.348	10.724.386	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Air	0,00	844.735.704	kg	0	0,00
Cellulase	1,50	617.178	kg	925.766	5,37
Chicken feed	0,55	14.711.786	kg	8.091.482	46,90
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	4,99
Hexane	8,35	135.087	L(STP)	1.127.979	6,54
Hydrogen	2,00	134.212	kg	268.423	1,56
Lactobacillus	1,45	3.804.046	kg	5.515.867	31,97
Sal.juice	0,00	42.282.124	kg	0	0,00
Sal.pulp	0,00	11.608.881	kg	0	0,00
Water	2,50	183.960	m3(STP)	459.900	2,67
TOTAL				17.250.972	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	12.016.896	kW-h	1.201.690	11,20
Steam	12,00	594.446	MT	7.133.347	66,49
Steam (High P)	20,00	14	MT	277	0,00
Cooling Water	0,05	45.019.979	MT	2.250.999	20,98
TOTAL				10.727.965	100,00

Note: The utilities cost is reduced by 5747236 $/yr due to heat recovered in the process.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	**$**	**%**
Raw Materials	17.251.000	27,81
Labor-Dependent	10.724.000	17,29
Facility-Dependent	21.710.000	35,00
Laboratory/QC/QA	1.609.000	2,59
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	10.728.000	17,30
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	62.022.000	100,00

10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	116.200.000 $
B.	Working Capital	3.518.000 $
C.	Startup Cost	5.810.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	125.529.000 $
G.	Investment Charged to This Project	125.529.000 $

H.	**Revenue Rates**	
	Flash 2 (Revenue)	168.337 kg /yr
	BSFL_protein (Revenue)	3.247.057 kg /yr
	Frass (Revenue)	61.286.715 kg /yr
	Naphtha (Revenue)	460.486 kg /yr
	HEFA (Main Revenue)	757.663 kg /yr
	HVO (Revenue)	513.839 kg /yr
	Flash 1 (Revenue)	290.204 kg /yr
	BSFL_chitin_and_residue (Revenue)	4.637.182 kg /yr
	EtOH sol. (Revenue)	1.035.094 kg /yr
	Lipids (Revenue)	763.121 kg /yr

I.	**Revenue Price**	
	Flash 2 (Revenue)	1,16 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,13 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,39 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	EtOH sol. (Revenue)	7,00 $/kg
	Lipids (Revenue)	1,00 $/kg

J.	**Revenues**	
	Flash 2 (Revenue)	194.583 $/yr
	BSFL_protein (Revenue)	6.996.829 $/yr
	Frass (Revenue)	7.863.842 $/yr
	Naphtha (Revenue)	253.267 $/yr
	HEFA (Main Revenue)	469.751 $/yr
	HVO (Revenue)	513.839 $/yr
	Flash 1 (Revenue)	114.057 $/yr
	BSFL_chitin_and_residue (Revenue)	3.385.143 $/yr
	EtOH sol. (Revenue)	7.245.660 $/yr
	Lipids (Revenue)	763.121 $/yr
	Total Revenues	27.800.092 $/yr

K.	**Annual Operating Cost (AOC)**	
	AOC	62.022.000 $/yr

L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	81,86 $/kg MP
	Unit Production Revenue	36,69 $/kg MP

M.	Gross Profit (J-K)	- 34.223.000 $/yr
N.	Taxes (40%)	0 $/yr
O.	Net Profit (M-N + Depreciation)	- 23.184.000 $/yr

	Gross Margin	- 123,10 %
	Return On Investment	- 18,47 %
	Payback Time	N/A

MP = Total Flow of Stream 'HEFA'

Economic Evaluation Report

maj 28, 2020

for Total_simulation_pretreatment_severe

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	151.355.000 $
Capital Investment Charged to This Project	151.355.000 $
Operating Cost	84.311.000 $/yr
Main Revenue	940.000 $/yr
Other Revenues	54.660.682 $/yr
Total Revenues	55.600.000 $/yr
Cost Basis Annual Rate	1.515.325 kg MP/yr
Unit Production Cost	55,64 $/kg MP
Unit Production Revenue	36,69 $/kg MP
Gross Margin	- 51,64 %
Return On Investment	- 10,23 %
Payback Time	N/A
IRR (After Taxes)	N/A
NPV (at 7,0% Interest)	- 256.909.000 $

MP = Total Flow of Stream 'HEFA'

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor Vessel Volume = 641,84 m3	4.313.000	4.313.000
1 / 0 / 0	V-103	Flat Bottom Tank Vessel Volume = 4766,18 m3	1.237.000	1.237.000
1 / 0 / 0	V-111	Blending Tank Vessel Volume = 699,13 m3	1.197.000	1.197.000
1 / 0 / 0	V-104	Flat Bottom Tank Vessel Volume = 4468,09 m3	1.181.000	1.181.000
1 / 0 / 0	SP-101	Screw Press Throughput = 21536,25 kg/h	506.000	506.000
1 / 0 / 0	EC-102	Heat Exchanger Heat Exchange Area = 876,39 m2	463.000	463.000
1 / 0 / 0	ST-101	Heat Sterilizer Rated Throughput = 6310,21 L/h	441.000	441.000
1 / 0 / 0	V-119	Receiver Tank Vessel Volume = 1098,95 m3	430.000	430.000
1 / 0 / 0	V-120	Receiver Tank Vessel Volume = 1098,95 m3	430.000	430.000
1 / 0 / 0	V-101	Blending Tank Vessel Volume = 1133074,44 L	397.000	397.000
1 / 0 / 0	BM-101	Bead Mill Bead Volume = 620,43 L	382.000	382.000
1 / 0 / 0	LD-101	Blending Tank Vessel Volume = 45,90 m3	377.000	377.000
1 / 0 / 0	HX-102	Heat Exchanger Heat Exchange Area = 561,41 m2	354.000	354.000
1 / 0 / 0	EH-109	Heat Exchanger Heat Exchange Area = 394,43 m2	287.000	287.000
1 / 0 / 0	BC-106	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-105	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-102	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-107	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-103	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-101	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	EC-101	Heat Exchanger Heat Exchange Area = 358,67 m2	271.000	271.000
1 / 0 / 0	V-112	Flat Bottom Tank Vessel Volume = 562,40 m3	271.000	271.000
1 / 0 / 0	EV-102	Multi-Effect Evaporator Mean Heat Transfer Area = 210,87 m2	221.000	221.000
1 / 0 / 0	SL-104	Silo	211.000	211.000

		Vessel Volume = 695092,50 L		
1 / 0 / 0	DDR-102	Drum Dryer	203.000	203.000
		Drum Area = 28,94 m2		
1 / 0 / 0	V-102	Blending Tank	192.000	192.000
		Vessel Volume = 403310,17 L		
1 / 0 / 0	SP-102	Screw Press	192.000	192.000
		Throughput = 6417,22 kg/h		
1 / 0 / 0	G-101	Centrifugal Compressor	151.000	151.000
		Compressor Power = 107,25 kW		
1 / 0 / 0	V-115	Decanter Tank	102.000	102.000
		Vessel Volume = 6417,05 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	102.000	102.000
		Mean Heat Transfer Area = 68,41 m2		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 1722,07 L		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 22759,48 L		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 9437,08 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 764,42 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 438,67 L		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 321,09 kg/h		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 437,37 kg/h		
1 / 0 / 0	EH-102	Heat Exchanger	55.000	55.000
		Heat Exchange Area = 25,08 m2		
1 / 0 / 0	V-105	Flat Bottom Tank	51.000	51.000
		Vessel Volume = 53476,21 L		
1 / 0 / 0	DDR-101	Drum Dryer	50.000	50.000
		Drum Area = 0,74 m2		
1 / 0 / 0	V-114	Flat Bottom Tank	48.000	48.000
		Vessel Volume = 49723,37 L		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,09 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,65 kW		
1 / 0 / 0	EH-101	Heat Exchanger	39.000	39.000
		Heat Exchange Area = 14,46 m2		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 23,20 L		
1 / 0 / 0	V-113	Decanter Tank	34.000	34.000
		Vessel Volume = 389,26 L		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 3,64 L		
1 / 0 / 0	V-108	Decanter Tank	34.000	34.000
		Vessel Volume = 43,83 L		
1 / 0 / 0	SLDR-101	Sludge Dryer	33.000	33.000
		Evaporative Capacity = 23,89 MT/h		
1 / 1 / 0	PM-109	Centrifugal Pump	20.000	40.000
		Pump Power = 1,10 kW		
1 / 1 / 0	PM-103	Centrifugal Pump	17.000	34.000

		Pump Power = 0,74 kW		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,72 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000
		Pump Power = 0,67 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-102	Centrifugal Pump	12.000	24.000
		Pump Power = 0,31 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-127	Centrifugal Pump	10.000	20.000
		Pump Power = 0,16 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-128	Centrifugal Pump	10.000	20.000
		Pump Power = 0,16 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-111	Centrifugal Pump	10.000	20.000
		Pump Power = 0,16 kW		
1 / 1 / 0	PM-129	Centrifugal Pump	10.000	20.000
		Pump Power = 0,16 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 0 / 0	V-118	Flat Bottom Tank	8.000	8.000
		Vessel Volume = 3959,49 L		
1 / 0 / 0	V-117	Flat Bottom Tank	8.000	8.000
		Vessel Volume = 4013,84 L		
1 / 0 / 0	V-116	Flat Bottom Tank	8.000	8.000

		Vessel Volume = 3548,36 L		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 28,75 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 70,38 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 3,42 kW		
1 / 0 / 0	V-106	Flash Drum	2.000	2.000
		Vessel Volume = 111,51 L		
1 / 0 / 0	V-109	Flash Drum	2.000	2.000
		Vessel Volume = 32,34 L		
1 / 0 / 0	SD-101	Solids Drum	1.000	1.000
		Vessel Volume = 37,88 L		
		Unlisted Equipment		4.314.000
			TOTAL	21.570.000

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	21.570.000
2. Installation	13.553.000
3. Process Piping	7.549.000
4. Instrumentation	8.628.000
5. Insulation	647.000
6. Electrical	2.157.000
7. Buildings	9.707.000
8. Yard Improvement	3.236.000
9. Auxiliary Facilities	8.628.000
TPDC	75.675.000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	18.919.000
11. Construction	26.486.000
TPIC	45.405.000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	121.080.000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	6.054.000
13. Contingency	12.108.000
CFC = 12+13	18.162.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	139.242.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	47,38	226.348	10.724.386	100,00
TOTAL		226.348	10.724.386	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Air	0,00	1.689.471.408	kg	0	0,00
Cellulase	1,50	1.234.355	kg	1.851.533	5,69
Chicken feed	0,55	29.423.572	kg	16.182.965	49,77
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	2,65
Hexane	8,35	135.087	L(STP)	1.127.979	3,47
Hydrogen	2,00	268.423	kg	536.847	1,65
Lactobacillus	1,45	7.608.093	kg	11.031.734	33,93
Sal.juice	0,00	84.564.247	kg	0	0,00
Sal.pulp	0,00	23.217.762	kg	0	0,00
Water	2,50	367.920	m3(STP)	919.800	2,83
TOTAL				32.512.412	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	23.002.515	kW-h	2.300.252	17,14
Steam	12,00	748.756	MT	8.985.074	66,93
Steam (High P)	20,00	28	MT	554	0,00
Cooling Water	0,05	45.564.566	MT	2.278.228	16,97
TOTAL				13.423.821	100,00

Note: The utilities cost is reduced by 9005674 $/yr due to heat recovered in the process.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	$	%
Raw Materials	32.512.000	38,56
Labor-Dependent	10.724.000	12,72
Facility-Dependent	26.041.000	30,89
Laboratory/QC/QA	1.609.000	1,91
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	13.424.000	15,92
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	84.311.000	100,00

10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	139.242.000 $
B.	Working Capital	5.151.000 $
C.	Startup Cost	6.962.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	151.355.000 $
G.	Investment Charged to This Project	151.355.000 $

H.	**Revenue Rates**	
	Flash 2 (Revenue)	336.675 kg /yr
	BSFL_protein (Revenue)	6.494.113 kg /yr
	Frass (Revenue)	122.573.429 kg /yr
	Naphtha (Revenue)	920.971 kg /yr
	HEFA (Main Revenue)	1.515.325 kg /yr
	HVO (Revenue)	1.027.678 kg /yr
	Flash 1 (Revenue)	580.407 kg /yr
	BSFL_chitin_and_residue (Revenue)	9.274.363 kg /yr
	EtOH sol. (Revenue)	2.070.189 kg /yr
	Lipids (Revenue)	1.526.243 kg /yr

I.	**Revenue Price**	
	Flash 2 (Revenue)	1,16 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,13 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,39 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	EtOH sol. (Revenue)	7,00 $/kg
	Lipids (Revenue)	1,00 $/kg

J.	**Revenues**	
	Flash 2 (Revenue)	389.166 $/yr
	BSFL_protein (Revenue)	13.993.658 $/yr
	Frass (Revenue)	15.727.683 $/yr
	Naphtha (Revenue)	506.534 $/yr
	HEFA (Main Revenue)	939.502 $/yr
	HVO (Revenue)	1.027.678 $/yr
	Flash 1 (Revenue)	228.114 $/yr
	BSFL_chitin_and_residue (Revenue)	6.770.285 $/yr
	EtOH sol. (Revenue)	14.491.320 $/yr
	Lipids (Revenue)	1.526.243 $/yr
	Total Revenues	55.600.184 $/yr

K.	**Annual Operating Cost (AOC)**	
	AOC	84.311.000 $/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	55,64 $/kg MP
	Unit Production Revenue	36,69 $/kg MP
M.	Gross Profit (J-K)	- 28.711.000 $/yr
N.	Taxes (40%)	0 $/yr
O.	Net Profit (M-N + Depreciation)	- 15.483.000 $/yr
	Gross Margin	- 51,64 %
	Return On Investment	- 10,23 %
	Payback Time	N/A

MP = Total Flow of Stream 'HEFA'

Economic Evaluation Report

for Total_simulation_pretreatment_severe

maj 28, 2020

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	194.499.000 $
Capital Investment Charged to This Project	194.499.000 $
Operating Cost	127.782.000 $/yr
Main Revenue	1.879.000 $/yr
Other Revenues	109.321.356 $/yr
Total Revenues	111.200.000 $/yr
Cost Basis Annual Rate	3.030.650 kg MP/yr
Unit Production Cost	42,16 $/kg MP
Unit Production Revenue	36,69 $/kg MP
Gross Margin	- 14,91 %
Return On Investment	0,13 %
Payback Time	774,83 years
IRR (After Taxes)	N/A
NPV (at 7,0% Interest)	- 184.413.000 $

MP = Total Flow of Stream 'HEFA'

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor Vessel Volume = 1283,68 m3	6.529.000	6.529.000
1 / 0 / 0	V-111	Blending Tank Vessel Volume = 1398,25 m3	1.722.000	1.722.000
1 / 0 / 0	V-103	Flat Bottom Tank Vessel Volume = 4765,23 m3	1.237.000	1.237.000
1 / 0 / 0	V-104	Flat Bottom Tank Vessel Volume = 4467,64 m3	1.181.000	1.181.000
1 / 0 / 0	SP-101	Screw Press Throughput = 43072,50 kg/h	882.000	882.000
1 / 0 / 0	V-120	Receiver Tank Vessel Volume = 2197,91 m3	605.000	605.000
1 / 0 / 0	V-119	Receiver Tank Vessel Volume = 2197,90 m3	605.000	605.000
1 / 0 / 0	ST-101	Heat Sterilizer Rated Throughput = 12620,42 L/h	547.000	547.000
1 / 0 / 0	BM-101	Bead Mill Bead Volume = 1240,86 L	540.000	540.000
1 / 0 / 0	HX-102	Heat Exchanger Heat Exchange Area = 1122,82 m2	537.000	537.000
1 / 0 / 0	LD-101	Blending Tank Vessel Volume = 91,80 m3	480.000	480.000
1 / 0 / 0	EC-102	Heat Exchanger Heat Exchange Area = 876,30 m2	463.000	463.000
1 / 0 / 0	V-112	Flat Bottom Tank Vessel Volume = 1124,80 m3	444.000	444.000
1 / 0 / 0	EH-109	Heat Exchanger Heat Exchange Area = 788,87 m2	434.000	434.000
1 / 0 / 0	V-101	Blending Tank Vessel Volume = 1148910,40 L	401.000	401.000
1 / 0 / 0	SL-104	Silo Vessel Volume = 1390184,89 L	373.000	373.000
1 / 0 / 0	SP-102	Screw Press Throughput = 12834,43 kg/h	335.000	335.000
1 / 0 / 0	G-101	Centrifugal Compressor Compressor Power = 214,51 kW	288.000	288.000
1 / 0 / 0	BC-101	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-102	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-103	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-105	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-106	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-107	Belt Conveyor	276.000	276.000

		Belt Length = 100,00 m		
1 / 0 / 0	DDR-102	Drum Dryer	271.000	271.000
		Drum Area = 57,88 m2		
1 / 0 / 0	EC-101	Heat Exchanger	271.000	271.000
		Heat Exchange Area = 358,59 m2		
1 / 0 / 0	EV-102	Multi-Effect Evaporator	221.000	221.000
		Mean Heat Transfer Area = 210,39 m2		
1 / 0 / 0	V-102	Blending Tank	194.000	194.000
		Vessel Volume = 409311,64 L		
1 / 0 / 0	V-115	Decanter Tank	191.000	191.000
		Vessel Volume = 18150,15 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	102.000	102.000
		Mean Heat Transfer Area = 68,06 m2		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 3444,13 L		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 45518,95 L		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 18874,17 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 1528,84 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 877,34 L		
1 / 0 / 0	DDR-101	Drum Dryer	59.000	59.000
		Drum Area = 1,48 m2		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 874,74 kg/h		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 642,17 kg/h		
1 / 0 / 0	EH-102	Heat Exchanger	56.000	56.000
		Heat Exchange Area = 25,66 m2		
1 / 0 / 0	V-105	Flat Bottom Tank	55.000	55.000
		Vessel Volume = 59424,44 L		
1 / 0 / 0	V-114	Flat Bottom Tank	52.000	52.000
		Vessel Volume = 54949,57 L		
1 / 0 / 0	SLDR-101	Sludge Dryer	46.000	46.000
		Evaporative Capacity = 47,78 MT/h		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,09 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,65 kW		
1 / 0 / 0	EH-101	Heat Exchanger	40.000	40.000
		Heat Exchange Area = 14,65 m2		
1 / 0 / 0	V-113	Decanter Tank	36.000	36.000
		Vessel Volume = 1101,01 L		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 10,30 L		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 69,28 L		
1 / 0 / 0	V-108	Decanter Tank	34.000	34.000
		Vessel Volume = 162,90 L		
1 / 1 / 0	PM-109	Centrifugal Pump	27.000	54.000
		Pump Power = 2,20 kW		
1 / 1 / 0	PM-103	Centrifugal Pump	23.000	46.000

		Pump Power = 1,47 kW		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,73 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000
		Pump Power = 0,68 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-102	Centrifugal Pump	16.000	32.000
		Pump Power = 0,62 kW		
1 / 0 / 0	V-118	Flat Bottom Tank	13.000	13.000
		Vessel Volume = 7918,97 L		
1 / 0 / 0	V-117	Flat Bottom Tank	13.000	13.000
		Vessel Volume = 8027,69 L		
1 / 1 / 0	PM-111	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-127	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-128	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 0 / 0	V-116	Flat Bottom Tank	12.000	12.000
		Vessel Volume = 7096,72 L		
1 / 1 / 0	PM-129	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,03 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,04 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	10.000	20.000
		Pump Power = 0,17 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,05 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,04 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	10.000	20.000
		Pump Power = 0,17 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,03 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	10.000	20.000
		Pump Power = 0,17 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000

		Pump Power = 0,01 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 140,77 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 57,50 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 6,84 kW		
1 / 0 / 0	V-106	Flash Drum	3.000	3.000
		Vessel Volume = 223,01 L		
1 / 0 / 0	V-109	Flash Drum	2.000	2.000
		Vessel Volume = 64,68 L		
1 / 0 / 0	SD-101	Solids Drum	1.000	1.000
		Vessel Volume = 75,76 L		
		Unlisted Equipment		5.576.000
			TOTAL	27.878.000

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	27.878.000
2. Installation	16.009.000
3. Process Piping	9.757.000
4. Instrumentation	11.151.000
5. Insulation	836.000
6. Electrical	2.788.000
7. Buildings	12.545.000
8. Yard Improvement	4.182.000
9. Auxiliary Facilities	11.151.000
TPDC	96.296.000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	24.074.000
11. Construction	33.704.000
TPIC	57.778.000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	154.074.000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	7.704.000
13. Contingency	15.407.000
CFC = 12+13	23.111.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	177.185.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	47,38	226.348	10.724.386	100,00
TOTAL		226.348	10.724.386	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Air	0,00	3.378.942.557	kg	0	0,00
Cellulase	1,50	2.468.710	kg	3.703.065	5,87
Chicken feed	0,55	58.847.140	kg	32.365.927	51,35
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	1,37
Hexane	8,35	135.087	L(STP)	1.127.979	1,79
Hydrogen	2,00	536.847	kg	1.073.693	1,70
Lactobacillus	1,45	15.216.184	kg	22.063.467	35,00
Sal.juice	0,00	169.128.482	kg	0	0,00
Sal.pulp	0,00	46.435.520	kg	0	0,00
Water	2,50	735.840	m3(STP)	1.839.599	2,92
TOTAL				63.035.285	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	44.973.937	kW-h	4.497.394	23,37
Steam	12,00	1.057.673	MT	12.692.080	65,97
Steam (High P)	20,00	55	MT	1.107	0,01
Cooling Water	0,05	46.683.601	MT	2.334.180	12,13
TOTAL				19.240.525	100,00

Note: The utilities cost is reduced by 15520237 $/yr due to heat recovered in the process.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	$	%
Raw Materials	63.035.000	49,33
Labor-Dependent	10.724.000	8,39
Facility-Dependent	33.173.000	25,96
Laboratory/QC/QA	1.609.000	1,26
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	19.241.000	15,06
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	127.782.000	100,00

10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	177.185.000 $
B.	Working Capital	8.455.000 $
C.	Startup Cost	8.859.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	194.499.000 $
G.	Investment Charged to This Project	194.499.000 $

H.	**Revenue Rates**	
	Flash 2 (Revenue)	673.350 kg /yr
	BSFL_protein (Revenue)	12.988.226 kg /yr
	Frass (Revenue)	245.146.839 kg /yr
	Naphtha (Revenue)	1.841.942 kg /yr
	HEFA (Main Revenue)	3.030.650 kg /yr
	HVO (Revenue)	2.055.356 kg /yr
	Flash 1 (Revenue)	1.160.815 kg /yr
	BSFL_chitin_and_residue (Revenue)	18.548.725 kg /yr
	EtOH sol. (Revenue)	4.140.377 kg /yr
	Lipids (Revenue)	3.052.485 kg /yr

I.	**Revenue Price**	
	Flash 2 (Revenue)	1,16 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,13 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,39 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	EtOH sol. (Revenue)	7,00 $/kg
	Lipids (Revenue)	1,00 $/kg

J.	**Revenues**	
	Flash 2 (Revenue)	778.332 $/yr
	BSFL_protein (Revenue)	27.987.315 $/yr
	Frass (Revenue)	31.455.365 $/yr
	Naphtha (Revenue)	1.013.068 $/yr
	HEFA (Main Revenue)	1.879.003 $/yr
	HVO (Revenue)	2.055.356 $/yr
	Flash 1 (Revenue)	456.229 $/yr
	BSFL_chitin_and_residue (Revenue)	13.540.569 $/yr
	EtOH sol. (Revenue)	28.982.637 $/yr
	Lipids (Revenue)	3.052.485 $/yr
	Total Revenues	111.200.359 $/yr

K.	**Annual Operating Cost (AOC)**	
	AOC	127.782.000 $/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	42,16 $/kg MP
	Unit Production Revenue	36,69 $/kg MP
M.	Gross Profit (J-K)	- 16.582.000 $/yr
N.	Taxes (40%)	0 $/yr
O.	Net Profit (M-N + Depreciation)	251.000 $/yr
	Gross Margin	- 14,91 %
	Return On Investment	0,13 %
	Payback Time	774,83 years

MP = Total Flow of Stream 'HEFA'

Economic Evaluation Report

for Total_simulation_pretreatment_severe

maj 28, 2020

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	269.206.000 $
Capital Investment Charged to This Project	269.206.000 $
Operating Cost	213.236.000 $/yr
Main Revenue	3.758.000 $/yr
Other Revenues	218.642.720 $/yr
Total Revenues	222.401.000 $/yr
Cost Basis Annual Rate	6.061.301 kg MP/yr
Unit Production Cost	35,18 $/kg MP
Unit Production Revenue	36,69 $/kg MP
Gross Margin	4,12 %
Return On Investment	10,58 %
Payback Time	9,45 years
IRR (After Taxes)	2,99 %
NPV (at 7,0% Interest)	- 59.361.000 $

MP = Total Flow of Stream 'HEFA'

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor Vessel Volume = 2567,37 m3	10.130.000	10.130.000
1 / 0 / 0	V-111	Blending Tank Vessel Volume = 2796,51 m3	2.518.000	2.518.000
1 / 0 / 0	SP-101	Screw Press Throughput = 86145,01 kg/h	1.535.000	1.535.000
1 / 0 / 0	V-103	Flat Bottom Tank Vessel Volume = 4769,99 m3	1.238.000	1.238.000
1 / 0 / 0	V-104	Flat Bottom Tank Vessel Volume = 4470,32 m3	1.182.000	1.182.000
1 / 0 / 0	SL-104	Silo Vessel Volume = 2780369,89 L	997.000	997.000
1 / 0 / 0	V-119	Receiver Tank Vessel Volume = 4395,81 m3	954.000	954.000
1 / 0 / 0	V-120	Receiver Tank Vessel Volume = 4395,81 m3	954.000	954.000
1 / 0 / 0	HX-102	Heat Exchanger Heat Exchange Area = 2245,63 m2	814.000	814.000
1 / 0 / 0	BM-101	Bead Mill Bead Volume = 2481,72 L	764.000	764.000
1 / 0 / 0	V-112	Flat Bottom Tank Vessel Volume = 2249,59 m3	726.000	726.000
1 / 0 / 0	ST-101	Heat Sterilizer Rated Throughput = 25240,84 L/h	678.000	678.000
1 / 0 / 0	EH-109	Heat Exchanger Heat Exchange Area = 1577,73 m2	658.000	658.000
1 / 0 / 0	LD-101	Blending Tank Vessel Volume = 183,60 m3	635.000	635.000
1 / 0 / 0	SP-102	Screw Press Throughput = 25668,87 kg/h	583.000	583.000
1 / 0 / 0	G-101	Centrifugal Compressor Compressor Power = 429,02 kW	550.000	550.000
1 / 0 / 0	EC-102	Heat Exchanger Heat Exchange Area = 876,74 m2	463.000	463.000
1 / 0 / 0	V-101	Blending Tank Vessel Volume = 1181383,81 L	408.000	408.000
1 / 0 / 0	DDR-102	Drum Dryer Drum Area = 115,75 m2	361.000	361.000
1 / 0 / 0	V-115	Decanter Tank Vessel Volume = 51336,38 L	357.000	357.000
1 / 0 / 0	BC-103	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-106	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-101	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-107	Belt Conveyor	276.000	276.000

		Belt Length = 100,00 m		
1 / 0 / 0	BC-102	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-105	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	EC-101	Heat Exchanger	271.000	271.000
		Heat Exchange Area = 358,92 m2		
1 / 0 / 0	EV-102	Multi-Effect Evaporator	219.000	219.000
		Mean Heat Transfer Area = 209,60 m2		
1 / 0 / 0	V-102	Blending Tank	199.000	199.000
		Vessel Volume = 421809,95 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101.000	101.000
		Mean Heat Transfer Area = 67,47 m2		
1 / 0 / 0	DDR-101	Drum Dryer	79.000	79.000
		Drum Area = 2,96 m2		
1 / 0 / 0	SLDR-101	Sludge Dryer	74.000	74.000
		Evaporative Capacity = 95,57 MT/h		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 91037,90 L		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 37748,34 L		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 6888,27 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 3057,69 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 1754,69 L		
1 / 0 / 0	V-113	Decanter Tank	66.000	66.000
		Vessel Volume = 3114,12 L		
1 / 0 / 0	V-105	Flat Bottom Tank	63.000	63.000
		Vessel Volume = 71382,69 L		
1 / 0 / 0	V-114	Flat Bottom Tank	59.000	59.000
		Vessel Volume = 65433,14 L		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 1284,35 kg/h		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 1749,48 kg/h		
1 / 0 / 0	EH-102	Heat Exchanger	57.000	57.000
		Heat Exchange Area = 26,83 m2		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,10 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,65 kW		
1 / 0 / 0	EH-101	Heat Exchanger	40.000	40.000
		Heat Exchange Area = 15,06 m2		
1 / 1 / 0	PM-109	Centrifugal Pump	36.000	72.000
		Pump Power = 4,41 kW		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 216,15 L		
1 / 0 / 0	V-108	Decanter Tank	34.000	34.000
		Vessel Volume = 811,55 L		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 29,13 L		
1 / 1 / 0	PM-103	Centrifugal Pump	30.000	60.000

		Pump Power = 2,95 kW		
1 / 0 / 0	V-118	Flat Bottom Tank	22.000	22.000
		Vessel Volume = 15837,95 L		
1 / 0 / 0	V-117	Flat Bottom Tank	22.000	22.000
		Vessel Volume = 16055,37 L		
1 / 1 / 0	PM-102	Centrifugal Pump	21.000	42.000
		Pump Power = 1,24 kW		
1 / 0 / 0	V-116	Flat Bottom Tank	20.000	20.000
		Vessel Volume = 14193,45 L		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,75 kW		
1 / 1 / 0	PM-129	Centrifugal Pump	16.000	32.000
		Pump Power = 0,66 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-127	Centrifugal Pump	16.000	32.000
		Pump Power = 0,65 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000
		Pump Power = 0,70 kW		
1 / 1 / 0	PM-111	Centrifugal Pump	16.000	32.000
		Pump Power = 0,65 kW		
1 / 1 / 0	PM-128	Centrifugal Pump	16.000	32.000
		Pump Power = 0,65 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,07 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,07 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,09 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,05 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000

		Pump Power = 0,01 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 115,01 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 281,54 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 13,67 kW		
1 / 0 / 0	V-106	Flash Drum	5.000	5.000
		Vessel Volume = 446,03 L		
1 / 0 / 0	V-109	Flash Drum	3.000	3.000
		Vessel Volume = 129,37 L		
1 / 0 / 0	SD-101	Solids Drum	2.000	2.000
		Vessel Volume = 151,52 L		
		Unlisted Equipment		7.739.000
			TOTAL	38.695.000

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)

1. Equipment Purchase Cost	38.695.000
2. Installation	20.075.000
3. Process Piping	13.543.000
4. Instrumentation	15.478.000
5. Insulation	1.161.000
6. Electrical	3.870.000
7. Buildings	17.413.000
8. Yard Improvement	5.804.000
9. Auxiliary Facilities	15.478.000
TPDC	131.517.000

3B. Total Plant Indirect Cost (TPIC)

10. Engineering	32.879.000
11. Construction	46.031.000
TPIC	78.910.000

3C. Total Plant Cost (TPC = TPDC+TPIC)

TPC	210.427.000

3D. Contractor's Fee & Contingency (CFC)

12. Contractor's Fee	10.521.000
13. Contingency	21.043.000
CFC = 12+13	31.564.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)

DFC	241.991.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	47,38	226.348	10.724.386	100,00
TOTAL		226.348	10.724.386	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Air	0,00	6.757.885.373	kg	0	0,00
Cellulase	1,50	4.937.420	kg	7.406.130	5,97
Chicken feed	0,55	117.694.285	kg	64.731.857	52,17
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	0,69
Hexane	8,35	135.087	L(STP)	1.127.979	0,91
Hydrogen	2,00	1.073.693	kg	2.147.387	1,73
Lactobacillus	1,45	30.432.370	kg	44.126.936	35,56
Sal.juice	0,00	338.256.977	kg	0	0,00
Sal.pulp	0,00	92.871.043	kg	0	0,00
Water	2,50	1.471.680	m3(STP)	3.679.199	2,97
TOTAL				124.081.042	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	88.917.411	kW-h	8.891.741	28,26
Steam	12,00	1.676.586	MT	20.119.033	63,94
Steam (High P)	20,00	111	MT	2.214	0,01
Cooling Water	0,05	49.030.209	MT	2.451.510	7,79
TOTAL				31.464.420	100,00

Note: The utilities cost is reduced by 28539596 $/yr due to heat recovered in the process.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	**$**	**%**
Raw Materials	124.081.000	58,19
Labor-Dependent	10.724.000	5,03
Facility-Dependent	45.357.000	21,27
Laboratory/QC/QA	1.609.000	0,75
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	31.464.000	14,76
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	213.236.000	100,00

10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	241.991.000 $
B.	Working Capital	15.115.000 $
C.	Startup Cost	12.100.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	269.206.000 $
G.	Investment Charged to This Project	269.206.000 $

H.	Revenue Rates	
	Flash 2 (Revenue)	1.346.700 kg /yr
	BSFL_protein (Revenue)	25.976.453 kg /yr
	Frass (Revenue)	490.293.697 kg /yr
	Naphtha (Revenue)	3.683.885 kg /yr
	HEFA (Main Revenue)	6.061.301 kg /yr
	HVO (Revenue)	4.110.713 kg /yr
	Flash 1 (Revenue)	2.321.630 kg /yr
	BSFL_chitin_and_residue (Revenue)	37.097.451 kg /yr
	EtOH sol. (Revenue)	8.280.754 kg /yr
	Lipids (Revenue)	6.104.971 kg /yr

I.	Revenue Price	
	Flash 2 (Revenue)	1,16 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,13 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,39 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	EtOH sol. (Revenue)	7,00 $/kg
	Lipids (Revenue)	1,00 $/kg

J.	Revenues	
	Flash 2 (Revenue)	1.556.664 $/yr
	BSFL_protein (Revenue)	55.974.631 $/yr
	Frass (Revenue)	62.910.732 $/yr
	Naphtha (Revenue)	2.026.137 $/yr
	HEFA (Main Revenue)	3.758.006 $/yr
	HVO (Revenue)	4.110.713 $/yr
	Flash 1 (Revenue)	912.457 $/yr
	BSFL_chitin_and_residue (Revenue)	27.081.139 $/yr
	EtOH sol. (Revenue)	57.965.276 $/yr
	Lipids (Revenue)	6.104.971 $/yr
	Total Revenues	222.400.726 $/yr

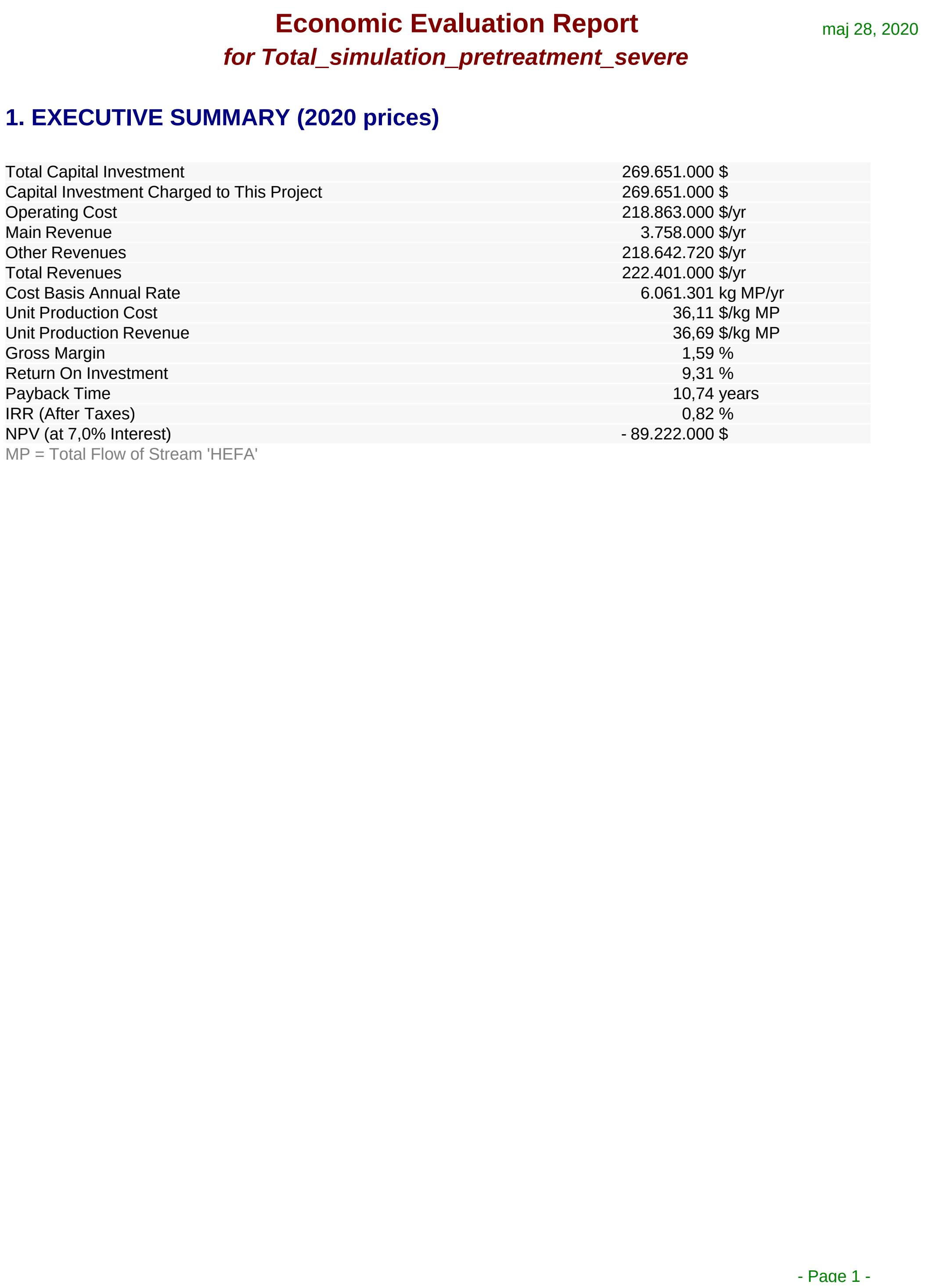
K.	**Annual Operating Cost (AOC)**	
	AOC	213.236.000 $/yr

L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	35,18 $/kg MP
	Unit Production Revenue	36,69 $/kg MP

M.	Gross Profit (J-K)	9.165.000 $/yr
N.	Taxes (40%)	3.666.000 $/yr
O.	Net Profit (M-N + Depreciation)	28.488.000 $/yr

	Gross Margin	4,12 %
	Return On Investment	10,58 %
	Payback Time	9,45 years

MP = Total Flow of Stream 'HEFA'



Economic Evaluation Report

for Total_simulation_pretreatment_severe

maj 28, 2020

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	269.651.000 $
Capital Investment Charged to This Project	269.651.000 $
Operating Cost	218.863.000 $/yr
Main Revenue	3.758.000 $/yr
Other Revenues	218.642.720 $/yr
Total Revenues	222.401.000 $/yr
Cost Basis Annual Rate	6.061.301 kg MP/yr
Unit Production Cost	36,11 $/kg MP
Unit Production Revenue	36,69 $/kg MP
Gross Margin	1,59 %
Return On Investment	9,31 %
Payback Time	10,74 years
IRR (After Taxes)	0,82 %
NPV (at 7,0% Interest)	- 89.222.000 $

MP = Total Flow of Stream 'HEFA'

- Page 1 -

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor	10.130.000	10.130.000
		Vessel Volume = 2567,37 m3		
1 / 0 / 0	V-111	Blending Tank	2.518.000	2.518.000
		Vessel Volume = 2796,51 m3		
1 / 0 / 0	SP-101	Screw Press	1.535.000	1.535.000
		Throughput = 86145,01 kg/h		
1 / 0 / 0	V-103	Flat Bottom Tank	1.238.000	1.238.000
		Vessel Volume = 4769,99 m3		
1 / 0 / 0	V-104	Flat Bottom Tank	1.182.000	1.182.000
		Vessel Volume = 4470,32 m3		
1 / 0 / 0	SL-104	Silo	997.000	997.000
		Vessel Volume = 2780369,89 L		
1 / 0 / 0	V-119	Receiver Tank	954.000	954.000
		Vessel Volume = 4395,81 m3		
1 / 0 / 0	V-120	Receiver Tank	954.000	954.000
		Vessel Volume = 4395,81 m3		
1 / 0 / 0	HX-102	Heat Exchanger	814.000	814.000
		Heat Exchange Area = 2245,63 m2		
1 / 0 / 0	BM-101	Bead Mill	764.000	764.000
		Bead Volume = 2481,72 L		
1 / 0 / 0	V-112	Flat Bottom Tank	726.000	726.000
		Vessel Volume = 2249,59 m3		
1 / 0 / 0	ST-101	Heat Sterilizer	678.000	678.000
		Rated Throughput = 25240,84 L/h		
1 / 0 / 0	EH-109	Heat Exchanger	658.000	658.000
		Heat Exchange Area = 1577,73 m2		
1 / 0 / 0	LD-101	Blending Tank	635.000	635.000
		Vessel Volume = 183,60 m3		
1 / 0 / 0	SP-102	Screw Press	583.000	583.000
		Throughput = 25668,87 kg/h		
1 / 0 / 0	G-101	Centrifugal Compressor	550.000	550.000
		Compressor Power = 429,02 kW		
1 / 0 / 0	EC-102	Heat Exchanger	463.000	463.000
		Heat Exchange Area = 876,74 m2		
1 / 0 / 0	V-101	Blending Tank	408.000	408.000
		Vessel Volume = 1181383,81 L		
1 / 0 / 0	DDR-102	Drum Dryer	361.000	361.000
		Drum Area = 115,75 m2		
1 / 0 / 0	V-115	Decanter Tank	357.000	357.000
		Vessel Volume = 51336,38 L		
1 / 0 / 0	BC-103	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-106	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-101	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-107	Belt Conveyor	276.000	276.000

		Belt Length = 100,00 m		
1 / 0 / 0	BC-102	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-105	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	EC-101	Heat Exchanger	271.000	271.000
		Heat Exchange Area = 358,92 m2		
1 / 0 / 0	EV-102	Multi-Effect Evaporator	219.000	219.000
		Mean Heat Transfer Area = 209,60 m2		
1 / 0 / 0	V-102	Blending Tank	199.000	199.000
		Vessel Volume = 421809,95 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101.000	101.000
		Mean Heat Transfer Area = 67,47 m2		
1 / 0 / 0	DDR-101	Drum Dryer	79.000	79.000
		Drum Area = 2,96 m2		
1 / 0 / 0	SLDR-101	Sludge Dryer	74.000	74.000
		Evaporative Capacity = 95,57 MT/h		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 91037,90 L		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 37748,34 L		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 6888,27 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 3057,69 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 1754,69 L		
1 / 0 / 0	V-113	Decanter Tank	66.000	66.000
		Vessel Volume = 3114,12 L		
1 / 0 / 0	V-105	Flat Bottom Tank	63.000	63.000
		Vessel Volume = 71382,69 L		
1 / 0 / 0	V-114	Flat Bottom Tank	59.000	59.000
		Vessel Volume = 65433,14 L		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 1284,35 kg/h		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 1749,48 kg/h		
1 / 0 / 0	EH-102	Heat Exchanger	57.000	57.000
		Heat Exchange Area = 26,83 m2		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,10 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,65 kW		
1 / 0 / 0	EH-101	Heat Exchanger	40.000	40.000
		Heat Exchange Area = 15,06 m2		
1 / 1 / 0	PM-109	Centrifugal Pump	36.000	72.000
		Pump Power = 4,41 kW		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 216,15 L		
1 / 0 / 0	V-108	Decanter Tank	34.000	34.000
		Vessel Volume = 811,55 L		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 29,13 L		
1 / 1 / 0	PM-103	Centrifugal Pump	30.000	60.000

		Pump Power = 2,95 kW		
1 / 0 / 0	V-118	Flat Bottom Tank	22.000	22.000
		Vessel Volume = 15837,95 L		
1 / 0 / 0	V-117	Flat Bottom Tank	22.000	22.000
		Vessel Volume = 16055,37 L		
1 / 1 / 0	PM-102	Centrifugal Pump	21.000	42.000
		Pump Power = 1,24 kW		
1 / 0 / 0	V-116	Flat Bottom Tank	20.000	20.000
		Vessel Volume = 14193,45 L		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,75 kW		
1 / 1 / 0	PM-129	Centrifugal Pump	16.000	32.000
		Pump Power = 0,66 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-127	Centrifugal Pump	16.000	32.000
		Pump Power = 0,65 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000
		Pump Power = 0,70 kW		
1 / 1 / 0	PM-111	Centrifugal Pump	16.000	32.000
		Pump Power = 0,65 kW		
1 / 1 / 0	PM-128	Centrifugal Pump	16.000	32.000
		Pump Power = 0,65 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,07 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,07 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,09 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,05 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000

		Pump Power = 0,01 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 115,01 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 281,54 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 13,67 kW		
1 / 0 / 0	V-106	Flash Drum	5.000	5.000
		Vessel Volume = 446,03 L		
1 / 0 / 0	V-109	Flash Drum	3.000	3.000
		Vessel Volume = 129,37 L		
1 / 0 / 0	SD-101	Solids Drum	2.000	2.000
		Vessel Volume = 151,52 L		
		Unlisted Equipment		7.739.000
			TOTAL	38.695.000

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)	
1. Equipment Purchase Cost	38.695.000
2. Installation	20.075.000
3. Process Piping	13.543.000
4. Instrumentation	15.478.000
5. Insulation	1.161.000
6. Electrical	3.870.000
7. Buildings	17.413.000
8. Yard Improvement	5.804.000
9. Auxiliary Facilities	15.478.000
TPDC	131.517.000

3B. Total Plant Indirect Cost (TPIC)	
10. Engineering	32.879.000
11. Construction	46.031.000
TPIC	78.910.000

3C. Total Plant Cost (TPC = TPDC+TPIC)	
TPC	210.427.000

3D. Contractor's Fee & Contingency (CFC)	
12. Contractor's Fee	10.521.000
13. Contingency	21.043.000
CFC = 12+13	31.564.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)	
DFC	241.991.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	69,00	226.348	15.618.038	100,00
TOTAL		226.348	15.618.038	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Air	0,00	6.757.885.373	kg	0	0,00
Cellulase	1,50	4.937.420	kg	7.406.130	5,97
Chicken feed	0,55	117.694.285	kg	64.731.857	52,17
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	0,69
Hexane	8,35	135.087	L(STP)	1.127.979	0,91
Hydrogen	2,00	1.073.693	kg	2.147.387	1,73
Lactobacillus	1,45	30.432.370	kg	44.126.936	35,56
Sal.juice	0,00	338.256.977	kg	0	0,00
Sal.pulp	0,00	92.871.043	kg	0	0,00
Water	2,50	1.471.680	m3(STP)	3.679.199	2,97
TOTAL				124.081.042	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	88.917.411	kW-h	8.891.741	28,26
Steam	12,00	1.676.586	MT	20.119.033	63,94
Steam (High P)	20,00	111	MT	2.214	0,01
Cooling Water	0,05	49.030.209	MT	2.451.510	7,79
TOTAL				31.464.498	100,00

Note: The utilities cost is reduced by 28539596 $/yr due to heat recovered in the process.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	$	%
Raw Materials	124.081.000	56,69
Labor-Dependent	15.618.000	7,14
Facility-Dependent	45.357.000	20,72
Laboratory/QC/QA	2.343.000	1,07
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	31.464.000	14,38
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	218.863.000	100,00

10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	241.991.000 $
B.	Working Capital	15.560.000 $
C.	Startup Cost	12.100.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	269.651.000 $
G.	Investment Charged to This Project	269.651.000 $

H.	**Revenue Rates**	
	Flash 2 (Revenue)	1.346.700 kg /yr
	BSFL_protein (Revenue)	25.976.453 kg /yr
	Frass (Revenue)	490.293.697 kg /yr
	Naphtha (Revenue)	3.683.885 kg /yr
	HEFA (Main Revenue)	6.061.301 kg /yr
	HVO (Revenue)	4.110.713 kg /yr
	Flash 1 (Revenue)	2.321.630 kg /yr
	BSFL_chitin_and_residue (Revenue)	37.097.451 kg /yr
	EtOH sol. (Revenue)	8.280.754 kg /yr
	Lipids (Revenue)	6.104.971 kg /yr

I.	**Revenue Price**	
	Flash 2 (Revenue)	1,16 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,13 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,39 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	EtOH sol. (Revenue)	7,00 $/kg
	Lipids (Revenue)	1,00 $/kg

J.	**Revenues**	
	Flash 2 (Revenue)	1.556.664 $/yr
	BSFL_protein (Revenue)	55.974.631 $/yr
	Frass (Revenue)	62.910.732 $/yr
	Naphtha (Revenue)	2.026.137 $/yr
	HEFA (Main Revenue)	3.758.006 $/yr
	HVO (Revenue)	4.110.713 $/yr
	Flash 1 (Revenue)	912.457 $/yr
	BSFL_chitin_and_residue (Revenue)	27.081.139 $/yr
	EtOH sol. (Revenue)	57.965.276 $/yr
	Lipids (Revenue)	6.104.971 $/yr
	Total Revenues	222.400.726 $/yr

K.	**Annual Operating Cost (AOC)**	
	AOC	218.863.000 $/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	36,11 $/kg MP
	Unit Production Revenue	36,69 $/kg MP
M.	Gross Profit (J-K)	3.537.000 $/yr
N.	Taxes (40%)	1.415.000 $/yr
O.	Net Profit (M-N + Depreciation)	25.112.000 $/yr
	Gross Margin	1,59 %
	Return On Investment	9,31 %
	Payback Time	10,74 years

MP = Total Flow of Stream 'HEFA'

Economic Evaluation Report

maj 28, 2020

for Total_simulation_pretreatment_severe

1. EXECUTIVE SUMMARY (2020 prices)

Total Capital Investment	268.472.000 $
Capital Investment Charged to This Project	268.472.000 $
Operating Cost	203.956.000 $/yr
Main Revenue	3.758.000 $/yr
Other Revenues	218.642.720 $/yr
Total Revenues	222.401.000 $/yr
Cost Basis Annual Rate	6.061.301 kg MP/yr
Unit Production Cost	33,65 $/kg MP
Unit Production Revenue	36,69 $/kg MP
Gross Margin	8,29 %
Return On Investment	12,69 %
Payback Time	7,88 years
IRR (After Taxes)	5,95 %
NPV (at 7,0% Interest)	- 16.147.000 $

MP = Total Flow of Stream 'HEFA'

2. EQUIPMENT SPECIFICATION AND FOB COST (2020 prices)

Main Equipment

Quantity/ Standby/ Staggered	Name	Description	Unit Cost ($)	Cost ($)
1 / 0 / 0	FR-101	Fermentor Vessel Volume = 2567,37 m3	10.130.000	10.130.000
1 / 0 / 0	V-111	Blending Tank Vessel Volume = 2796,51 m3	2.518.000	2.518.000
1 / 0 / 0	SP-101	Screw Press Throughput = 86145,01 kg/h	1.535.000	1.535.000
1 / 0 / 0	V-103	Flat Bottom Tank Vessel Volume = 4769,99 m3	1.238.000	1.238.000
1 / 0 / 0	V-104	Flat Bottom Tank Vessel Volume = 4470,32 m3	1.182.000	1.182.000
1 / 0 / 0	SL-104	Silo Vessel Volume = 2780369,89 L	997.000	997.000
1 / 0 / 0	V-119	Receiver Tank Vessel Volume = 4395,81 m3	954.000	954.000
1 / 0 / 0	V-120	Receiver Tank Vessel Volume = 4395,81 m3	954.000	954.000
1 / 0 / 0	HX-102	Heat Exchanger Heat Exchange Area = 2245,63 m2	814.000	814.000
1 / 0 / 0	BM-101	Bead Mill Bead Volume = 2481,72 L	764.000	764.000
1 / 0 / 0	V-112	Flat Bottom Tank Vessel Volume = 2249,59 m3	726.000	726.000
1 / 0 / 0	ST-101	Heat Sterilizer Rated Throughput = 25240,84 L/h	678.000	678.000
1 / 0 / 0	EH-109	Heat Exchanger Heat Exchange Area = 1577,73 m2	658.000	658.000
1 / 0 / 0	LD-101	Blending Tank Vessel Volume = 183,60 m3	635.000	635.000
1 / 0 / 0	SP-102	Screw Press Throughput = 25668,87 kg/h	583.000	583.000
1 / 0 / 0	G-101	Centrifugal Compressor Compressor Power = 429,02 kW	550.000	550.000
1 / 0 / 0	EC-102	Heat Exchanger Heat Exchange Area = 876,74 m2	463.000	463.000
1 / 0 / 0	V-101	Blending Tank Vessel Volume = 1181383,81 L	408.000	408.000
1 / 0 / 0	DDR-102	Drum Dryer Drum Area = 115,75 m2	361.000	361.000
1 / 0 / 0	V-115	Decanter Tank Vessel Volume = 51336,38 L	357.000	357.000
1 / 0 / 0	BC-103	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-106	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-101	Belt Conveyor Belt Length = 100,00 m	276.000	276.000
1 / 0 / 0	BC-107	Belt Conveyor	276.000	276.000

		Belt Length = 100,00 m		
1 / 0 / 0	BC-102	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	BC-105	Belt Conveyor	276.000	276.000
		Belt Length = 100,00 m		
1 / 0 / 0	EC-101	Heat Exchanger	271.000	271.000
		Heat Exchange Area = 358,92 m2		
1 / 0 / 0	EV-102	Multi-Effect Evaporator	219.000	219.000
		Mean Heat Transfer Area = 209,60 m2		
1 / 0 / 0	V-102	Blending Tank	199.000	199.000
		Vessel Volume = 421809,95 L		
1 / 0 / 0	EV-101	Multi-Effect Evaporator	101.000	101.000
		Mean Heat Transfer Area = 67,47 m2		
1 / 0 / 0	DDR-101	Drum Dryer	79.000	79.000
		Drum Area = 2,96 m2		
1 / 0 / 0	SLDR-101	Sludge Dryer	74.000	74.000
		Evaporative Capacity = 95,57 MT/h		
1 / 0 / 0	SL-101	Silo	73.000	73.000
		Vessel Volume = 91037,90 L		
1 / 0 / 0	SL-102	Silo	73.000	73.000
		Vessel Volume = 37748,34 L		
1 / 0 / 0	SL-103	Silo	73.000	73.000
		Vessel Volume = 6888,27 L		
1 / 0 / 0	PFR-101	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 3057,69 L		
1 / 0 / 0	PFR-102	Plug Flow Reactor	71.000	71.000
		Vessel Volume = 1754,69 L		
1 / 0 / 0	V-113	Decanter Tank	66.000	66.000
		Vessel Volume = 3114,12 L		
1 / 0 / 0	V-105	Flat Bottom Tank	63.000	63.000
		Vessel Volume = 71382,69 L		
1 / 0 / 0	V-114	Flat Bottom Tank	59.000	59.000
		Vessel Volume = 65433,14 L		
1 / 0 / 0	GBX-104	Generic Box	58.000	58.000
		Rated Throughput = 1284,35 kg/h		
1 / 0 / 0	GBX-103	Generic Box	58.000	58.000
		Rated Throughput = 1749,48 kg/h		
1 / 0 / 0	EH-102	Heat Exchanger	57.000	57.000
		Heat Exchange Area = 26,83 m2		
1 / 1 / 0	PM-101	Centrifugal Pump	44.000	88.000
		Pump Power = 7,10 kW		
1 / 1 / 0	PM-107	Centrifugal Pump	43.000	86.000
		Pump Power = 6,65 kW		
1 / 0 / 0	EH-101	Heat Exchanger	40.000	40.000
		Heat Exchange Area = 15,06 m2		
1 / 1 / 0	PM-109	Centrifugal Pump	36.000	72.000
		Pump Power = 4,41 kW		
1 / 0 / 0	V-107	Decanter Tank	34.000	34.000
		Vessel Volume = 216,15 L		
1 / 0 / 0	V-108	Decanter Tank	34.000	34.000
		Vessel Volume = 811,55 L		
1 / 0 / 0	V-110	Decanter Tank	34.000	34.000
		Vessel Volume = 29,13 L		
1 / 1 / 0	PM-103	Centrifugal Pump	30.000	60.000

		Pump Power = 2,95 kW		
1 / 0 / 0	V-118	Flat Bottom Tank	22.000	22.000
		Vessel Volume = 15837,95 L		
1 / 0 / 0	V-117	Flat Bottom Tank	22.000	22.000
		Vessel Volume = 16055,37 L		
1 / 1 / 0	PM-102	Centrifugal Pump	21.000	42.000
		Pump Power = 1,24 kW		
1 / 0 / 0	V-116	Flat Bottom Tank	20.000	20.000
		Vessel Volume = 14193,45 L		
1 / 1 / 0	PM-125	Centrifugal Pump	17.000	34.000
		Pump Power = 0,75 kW		
1 / 1 / 0	PM-129	Centrifugal Pump	16.000	32.000
		Pump Power = 0,66 kW		
1 / 1 / 0	PM-114	Centrifugal Pump	16.000	32.000
		Pump Power = 0,71 kW		
1 / 1 / 0	PM-127	Centrifugal Pump	16.000	32.000
		Pump Power = 0,65 kW		
1 / 1 / 0	PM-126	Centrifugal Pump	16.000	32.000
		Pump Power = 0,70 kW		
1 / 1 / 0	PM-111	Centrifugal Pump	16.000	32.000
		Pump Power = 0,65 kW		
1 / 1 / 0	PM-128	Centrifugal Pump	16.000	32.000
		Pump Power = 0,65 kW		
1 / 1 / 0	PM-112	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-106	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-105	Centrifugal Pump	12.000	24.000
		Pump Power = 0,33 kW		
1 / 1 / 0	PM-118	Centrifugal Pump	10.000	20.000
		Pump Power = 0,07 kW		
1 / 1 / 0	PM-117	Centrifugal Pump	10.000	20.000
		Pump Power = 0,07 kW		
1 / 1 / 0	PM-116	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-110	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-121	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-104	Centrifugal Pump	10.000	20.000
		Pump Power = 0,09 kW		
1 / 1 / 0	PM-122	Centrifugal Pump	10.000	20.000
		Pump Power = 0,05 kW		
1 / 1 / 0	PM-108	Centrifugal Pump	10.000	20.000
		Pump Power = 0,08 kW		
1 / 1 / 0	PM-124	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-115	Centrifugal Pump	10.000	20.000
		Pump Power = 0,00 kW		
1 / 1 / 0	PM-120	Centrifugal Pump	10.000	20.000
		Pump Power = 0,01 kW		
1 / 1 / 0	PM-119	Centrifugal Pump	10.000	20.000
		Pump Power = 0,02 kW		
1 / 1 / 0	PM-123	Centrifugal Pump	10.000	20.000

		Pump Power = 0,01 kW		
1 / 0 / 0	EH-103	Electric Heater	8.000	8.000
		Electric Power = 115,01 kW		
1 / 0 / 0	EH-104	Electric Heater	8.000	8.000
		Electric Power = 281,54 kW		
1 / 0 / 0	EH-108	Electric Heater	8.000	8.000
		Electric Power = 13,67 kW		
1 / 0 / 0	V-106	Flash Drum	5.000	5.000
		Vessel Volume = 446,03 L		
1 / 0 / 0	V-109	Flash Drum	3.000	3.000
		Vessel Volume = 129,37 L		
1 / 0 / 0	SD-101	Solids Drum	2.000	2.000
		Vessel Volume = 151,52 L		
		Unlisted Equipment		7.739.000
			TOTAL	38.695.000

3. FIXED CAPITAL ESTIMATE SUMMARY (2020 prices in $)

3A. Total Plant Direct Cost (TPDC) (physical cost)

1. Equipment Purchase Cost	38.695.000
2. Installation	20.075.000
3. Process Piping	13.543.000
4. Instrumentation	15.478.000
5. Insulation	1.161.000
6. Electrical	3.870.000
7. Buildings	17.413.000
8. Yard Improvement	5.804.000
9. Auxiliary Facilities	15.478.000
TPDC	131.517.000

3B. Total Plant Indirect Cost (TPIC)

10. Engineering	32.879.000
11. Construction	46.031.000
TPIC	78.910.000

3C. Total Plant Cost (TPC = TPDC+TPIC)

TPC	210.427.000

3D. Contractor's Fee & Contingency (CFC)

12. Contractor's Fee	10.521.000
13. Contingency	21.043.000
CFC = 12+13	31.564.000

3E. Direct Fixed Capital Cost (DFC = TPC+CFC)

DFC	241.991.000

4. LABOR COST - PROCESS SUMMARY

Labor Type	Unit Cost ($/h)	Annual Amount (h)	Annual Cost ($)	%
Operator	11,73	226.348	2.655.066	100,00
TOTAL		226.348	2.655.066	100,00

5. MATERIALS COST - PROCESS SUMMARY

Bulk Material	Unit Cost ($)	Annual Amount		Annual Cost ($)	%
Air	0,00	6.757.885.373	kg	0	0,00
Cellulase	1,50	4.937.420	kg	7.406.130	5,97
Chicken feed	0,55	117.694.285	kg	64.731.857	52,17
Ethyl Alcohol	6,75	127.638	L(STP)	861.554	0,69
Hexane	8,35	135.087	L(STP)	1.127.979	0,91
Hydrogen	2,00	1.073.693	kg	2.147.387	1,73
Lactobacillus	1,45	30.432.370	kg	44.126.936	35,56
Sal.juice	0,00	338.256.977	kg	0	0,00
Sal.pulp	0,00	92.871.043	kg	0	0,00
Water	2,50	1.471.680	m3(STP)	3.679.199	2,97
TOTAL				124.081.042	100,00

NOTE: Bulk material consumption amount includes material used as:
- Raw Material
- Cleaning Agent
- Heat Transfer Agent (if utilities are included in the operating cost)

6. VARIOUS CONSUMABLES COST (2020 prices) - PROCESS SUMMARY

THE CONSUMABLES COST IS ZERO.

7. WASTE TREATMENT/DISPOSAL COST (2020 prices) - PROCESS SUMMARY

THE TOTAL WASTE TREATMENT/DISPOSAL COST IS ZERO.

8. UTILITIES COST (2020 prices) - PROCESS SUMMARY

Utility	Unit Cost ($)	Annual Amount	Ref. Units	Annual Cost ($)	%
Std Power	0,10	88.917.411	kW-h	8.891.741	28,26
Steam	12,00	1.676.586	MT	20.119.033	63,94
Steam (High P)	20,00	111	MT	2.214	0,01
Cooling Water	0,05	49.030.209	MT	2.451.510	7,79
TOTAL				31.464.498	100,00

Note: The utilities cost is reduced by 28539596 $/yr due to heat recovered in the process.

9. ANNUAL OPERATING COST (2020 prices) - PROCESS SUMMARY

Cost Item	**$**	**%**
Raw Materials	124.081.000	60,84
Labor-Dependent	2.655.000	1,30
Facility-Dependent	45.357.000	22,24
Laboratory/QC/QA	398.000	0,20
Consumables	0	0,00
Waste Treatment/Disposal	0	0,00
Utilities	31.464.000	15,43
Transportation	0	0,00
Miscellaneous	0	0,00
Advertising/Selling	0	0,00
Running Royalties	0	0,00
Failed Product Disposal	0	0,00
TOTAL	203.956.000	100,00

10. PROFITABILITY ANALYSIS (2020 prices)

A.	Direct Fixed Capital	241.991.000 $
B.	Working Capital	14.382.000 $
C.	Startup Cost	12.100.000 $
D.	Up-Front R&D	0 $
E.	Up-Front Royalties	0 $
F.	Total Investment (A+B+C+D+E)	268.472.000 $
G.	Investment Charged to This Project	268.472.000 $

H.	**Revenue Rates**	
	Flash 2 (Revenue)	1.346.700 kg /yr
	BSFL_protein (Revenue)	25.976.453 kg /yr
	Frass (Revenue)	490.293.697 kg /yr
	Naphtha (Revenue)	3.683.885 kg /yr
	HEFA (Main Revenue)	6.061.301 kg /yr
	HVO (Revenue)	4.110.713 kg /yr
	Flash 1 (Revenue)	2.321.630 kg /yr
	BSFL_chitin_and_residue (Revenue)	37.097.451 kg /yr
	EtOH sol. (Revenue)	8.280.754 kg /yr
	Lipids (Revenue)	6.104.971 kg /yr

I.	**Revenue Price**	
	Flash 2 (Revenue)	1,16 $/kg
	BSFL_protein (Revenue)	2,15 $/kg
	Frass (Revenue)	0,13 $/kg
	Naphtha (Revenue)	0,55 $/kg
	HEFA (Main Revenue)	0,62 $/kg
	HVO (Revenue)	1,00 $/kg
	Flash 1 (Revenue)	0,39 $/kg
	BSFL_chitin_and_residue (Revenue)	0,73 $/kg
	EtOH sol. (Revenue)	7,00 $/kg
	Lipids (Revenue)	1,00 $/kg

J.	**Revenues**	
	Flash 2 (Revenue)	1.556.664 $/yr
	BSFL_protein (Revenue)	55.974.631 $/yr
	Frass (Revenue)	62.910.732 $/yr
	Naphtha (Revenue)	2.026.137 $/yr
	HEFA (Main Revenue)	3.758.006 $/yr
	HVO (Revenue)	4.110.713 $/yr
	Flash 1 (Revenue)	912.457 $/yr
	BSFL_chitin_and_residue (Revenue)	27.081.139 $/yr
	EtOH sol. (Revenue)	57.965.276 $/yr
	Lipids (Revenue)	6.104.971 $/yr
	Total Revenues	222.400.726 $/yr

K.	**Annual Operating Cost (AOC)**	
	AOC	203.956.000 $/yr
L.	**Unit Production Cost /Revenue**	
	Unit Production Cost	33,65 $/kg MP
	Unit Production Revenue	36,69 $/kg MP
M.	Gross Profit (J-K)	18.445.000 $/yr
N.	Taxes (40%)	7.378.000 $/yr
O.	Net Profit (M-N + Depreciation)	34.056.000 $/yr
	Gross Margin	8,29 %
	Return On Investment	12,69 %
	Payback Time	7,88 years

MP = Total Flow of Stream 'HEFA'