RES
Professorship of
Regenerative Energy Systems

PRODUCTION OF BIOETHANOL USING LOW LIGNIN BIOMASS

Cooperative Design Project MSc.

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Affidavit

We hereby affirm that we have prepared the present work independently. Thoughts and quotations that we have taken directly or indirectly from external sources are marked as such. This work has not yet been submitted to any examination authority in the same or similar form and has not yet been published.

We agree that the work may be made available to the public by the Professorship of Regenerative Energy Systems.

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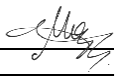
_____, the Kateryna Melnyk, 03795607 

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Abstract

The transition from conventional fossil fuels to biofuels is crucial to minimise the fallout of climate change, propelled by record-breaking greenhouse gas emissions. Given the relevance of the food vs feed debate in a world reeling from resource scarcity on account of failures of societal inequality that leaves out the most disenfranchised, the use of second-generation biofuels (2G) produced from lignocellulosic non-food feedstock is crucial to not push them further into food insecurity. The diversity in available biomass residues globally builds a strong case for decentralising pre-treatment and adopting a blended feedstock to maximise residue incorporation into biofuel production chains. The current work was based on conditions in India and Kenya; this led to the choice of water hyacinth and sugarcane bagasse as our biomass residues due to their abundance, with the former even being an invasive species that needs to be got rid of. Ammonia Fibre Expansion (AFEX) is used specifically to pre-treat sugarcane bagasse due to its high lignin content. The use of *K. marxianus* is done to ensure utilisation of the xylose fraction for fermentation within the dominant hemicellulose fraction of water hyacinth, in addition to the normally utilised cellulose. Simultaneous Saccharification and Fermentation (SSF) is done using a novel bubble column with a gas stripping set-up to produce the required bioethanol. Finally, a techno-economic analysis is carried out to determine the feasibility of the proposed decentralised multi-feedstock bioethanol production process.

Outlook

The work done in this project successfully suggested a product profile that best matches the selected feedstocks, with which we were able to develop a method to optimise feed combination for maximum economic benefit. This comprised designing a production process that best made use of the sugars present in both water hyacinth and sugarcane bagasse, thus giving rise to an economically feasible process. However, an area which has not been studied is the optimisation of preprocessing and reaction steps for both centralised and decentralised facilities. Furthermore, empirical data for enzyme immobilisation can be obtained while the valorisation of co-products can be explored to further strengthen economic and sustainable viability.

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1. Topic description

A decentralised multi-feedstock bioethanol production process, which makes use of low-lignin biomass, is planned. The aim is to be able to produce 176 tons of 95% mole basis bioethanol per batch of fermentation, with 270 batches being possible annually, thus giving us an annual production of approximately 50 kilotons of bioethanol. The multi-feedstock consists of a 50-50 mixture of water hyacinth and sugarcane bagasse. Due to the lower lignin content of water hyacinth, it is subjected merely to physical disruption through chopping and milling as part of pre-treatment to reduce the size to 1-2mm. In contrast, sugarcane bagasse needs to be subjected to ammonia fibre expansion (AFEX) in addition to size reduction to expose the cellulose and hemicellulose to enzymatic action. It is necessary for AFEX to be able to process 468.5 tons of sugarcane bagasse over a period of 32 hours, which is the batch time for fermentation. Ammonia recovery is a crucial part of the AFEX process to ensure its economic viability. The pre-treated biomass is then combined and sent to the seed train in which the microbe of choice, *K. marxianus*, is cultivated. The next step involves carrying out saccharification to break the cellulose and hemicellulose into glucose and xylose by making use of Cellic CTec3 as the enzyme. Finally, batch fermentation is carried out over a period of 32 hours in a bubble column to convert the sugars into bioethanol. A CO2 gas stripping set-up is implemented to extract the bioethanol from the fermenter, with the bioethanol-rich gas stream beig promptly condensed to obtain a liquid fraction consisting of bioethanol and water. Downstream processing is done by making use of a distillation column to separate the bioethanol from the water to obtain the required grade of bioethanol.

- Lay out the overall process in terms of unit operations and processes, along with the associated streams with their required mass flows.

- Define appropriate strategies for the size reduction of water hyacinth and sugarcane bagasse by considering the different types of mills and the sequence in which to order them.

- Determine the specifics of the ammonia fibre expansion (AFEX) process used for the pre-treatment of sugarcane bagasse by determining the process layout, operating conditions, reactor volume, reactor material and process operation.

- Fix the cultivation parameters most conducive to the growth of *K. marxianus,* along with the layout of the seed trains and the individual vessel volumes necessary.

- Study the specifics of the enzymatic hydrolysis (saccharification) process to understand the impact of feed composition on total reducing sugar.

- Lay out the fermentation process and fix the type of reactor used, the mode of operation and the method of bioethanol extraction from the fermenter.

- Calculate the required fermenter volume along with the flow rate of gas required for gas stripping.

- Design the distillation column to carry out the final downstream processing to separate out the required grade of bioethanol.

- Determine the investment costs for the total plant by taking into account costs for choppers and mills, ammonia fibre expansion equipment, seed train equipment, enzyme, fermenter, main compressor and downstream processing equipment (CAPEX).

- Determine operating costs (OPEX) by taking into account costs for personnel, fuel requirements, ash disposal, maintenance, repair, etc.

- Carry out a sensitivity analysis on CAPEX, OPEX, Net Revenue, Net Income and Payback Period by considering important parameters such as feed composition, enzyme recovery etc.

2. Task and objective

2.1. Motivation and background

2.1.1. Motivation

The combustion of fossil-based fuels accounts for roughly 65% of carbon dioxide (CO_2) emissions (Aghaei et al., 2022). One approach to reduce these emissions involves adopting biomass-derived fuels, which offer a practical alternative for sustainable and reliable energy production (Alawad & Ibrahim, 2024). For example, according to Wang *et al.* (2007), cellulose-based ethanol has the potential to reduce greenhouse gas (GHG) emissions by up to 86% relative to gasoline. Consequently, bioethanol is an important part of the biofuel market, and can be used as a transportation fuel (Bušić et al., 2018), cooking fuel (Osiolo et al., 2023), and as a renewable building block for the production of chemicals and materials (Posada et al., 2013).

Biofuels are commonly divided into three generations based on the feedstock used for their production. First-generation biofuels (1G) are derived from food-based crops, while second-generation biofuels (2G) are produced from lignocellulosic non-food feedstock, including agricultural waste, dedicated energy crops, non-edible plants, and autotrophic microalgae (Correia et al., 2024). 2G fuels are generally preferred over 1G biofuels since they aim to avoid competition between food and fuel. However, the use of lignocellulosic biomass requires an additional step of feedstock processing aimed at the elimination of lignocellulose crystalline structures and the separation of lignin from cellulose and hemicellulose (Aghaei et al., 2022). This pretreatment step is often extremely energy-intensive and can ultimately determine whether the process is economically viable. Even in the face of this challenge, 2G biofuels, such as bioethanol, which is produced primarily from lignocellulosic residues such as sugar cane bagasse and leaves, are regarded as an attractive alternative to both fossil fuels and 1G biofuels, due to their sustainability, low-cost feedstock, and non-reliance on food crops (Bušić et al., 2018). Bioethanol, biodiesel, renewable diesel, and biojet fuel are included within the biofuel sector. Bioethanol in particular has a high market potential as a vehicle fuel and for applications such as hand sanitisers (Hiranobe et al., 2024).

According to the Renewable Fuels Association (RFA), in 2024, annual global ethanol production was approximately 118.3 billion litres, with the United States and Brazil together accounting for about 80% of the total (Renewable Fuels Association, 2024). RFA data also indicates a 3-6% annual growth in ethanol production between 2020 and 2024. Projecting into the future, the International Energy Agency (IEA) reported that by 2030, the

global biofuel demand for transportation will rise to 205 billion litres, expanding from 5.6% of total liquid fuel transport demand in 2023 to 6.4% in 2030 (IEA, 2024). Notably, biodiesel and renewable diesel are predicted to have the highest rise in demand, although ethanol will continue to be an important contributor to reaching these targets. According to the IEA forecast, the growth of biofuel consumption will come mainly from Brazil, India, and Indonesia, due to strong biofuel blending mandates, slow adoption of electric vehicles, and the rise in the need for transport fuel. Regarding the feedstock, in Brazil and India, where bioethanol is produced mainly from starches (primarily maize) and sugars (primarily sugar cane), sugar demand for ethanol production is projected to expand by about 15% by 2030. The Organisation for Economic Co-operation and Development (OECD) also anticipates an increase in transportation biofuel demand and consumption, particularly in middle-income countries, although the forecast reports only a 0.9% annual global increase over the next decade (OECD/FAO (2025), 2025).

All these trends underscore the importance of new advances in 2G biofuel production technologies, especially 2G bioethanol, as a cost-effective and scalable alternative to fossil fuels. However, the commercial success of 2G bioethanol requires overcoming key challenges such as pretreatment, saccharification, and mixed-sugar fermentation. In this study, we address the limitations of 2G biofuels and investigate the production of ethanol from two complementary lignocellulosic feedstocks, water hyacinth and sugarcane bagasse, using a separate hydrolysis and fermentation (SHF) process with thermotolerant *Kluyveromyces marxianus* (*K. marxianus*).

2.1.2. Background

2.1.2.1. Feedstock

To overcome the issue of cost-intensive pretreatment of lignocellulosic feedstock, one alternative is to use low-lignin biomass, as it requires less processing. For instance, aquatic life offers a wide range of plants that can be used for fuel production, although biorefineries have largely neglected them (Amulya et al., 2023). One promising candidate for bioethanol production is water hyacinth (WH), which is one of the world's most aggressive free-floating perennial aquatic plants native to the Amazon basin (Romero-Borbón et al., 2022). Due to its aggressive nature, water hyacinth has proven to be an invasive species in the many non-indigenous regions it has been introduced in, often overpowering local ecosystems. It is considered to be a zero-cost feedstock since many governments are currently spending money to get rid of them, thus making our choice to use them a mutually beneficial one. The exact composition of WH, which varies depending on the geographical area, is also a

factor in favour of using it. For plants collected in India, China, and Kenya, WH contained 18-33% of cellulose, 23-48.7% of hemicellulose, and only 1.1-9% of lignin. Such composition makes WH an attractive feedstock for 2G biofuel production (Romero-Borbón et al., 2022), especially for mixed-sugar fermentation to maximise biomass conversion.

Because WH has a high hemicellulose content, the hydrolysis results in high xylose concentration (Romero-Borbón et al., 2022). Sugarcane bagasse (SB) was selected as a second feedstock to balance carbohydrate composition in the fermentation broth. SB is an agricultural waste from sugar production and is rich in cellulose (32-45%), hemicellulose (20-32%), and lignin (17-32%) (Alokika et al., 2021). This composition provides many fermentable sugars, including glucose and xylose. In addition, SB is a common and readily available agricultural residue with an annual production of 700 million tons, making it a stable and cost-effective feedstock (Hiranobe et al., 2024). Therefore, combining SB and WH can enable reliable, continuous bioethanol production, where the lack of one raw material can be temporarily compensated for by the other without major economic losses. Due to its higher lignin content, SB requires a separate pretreatment process (Niju & Swathika, 2019). However, this is a necessary compromise to achieve a robust and sustainable process.

2.1.2.2. Biomass feedstock processing

Before lignocellulosic biomass can be used in the fermentation process, it's necessary to perform a series of processing steps aimed at reducing the size of the particles and increasing the concentration of fermentable sugars. This can be achieved through a multi-step sequential process comprising physical size reduction, physico-chemical pretreatment and enzymatic or chemical saccharification, also known as hydrolysis (Tabil et al., 2011). Well-designed biomass feedstock processing is one of the most important aspects of a biorefinery, as it directly affects the efficiency of the fermentation process. However, handling lignocellulosic biomass is complex, and pretreatment units alone have been reported to account for as much as 16–19% of the capital costs of a biorefinery (Aghaei et al., 2022). Therefore, to achieve an economically viable process, the design of the pretreatment step requires careful consideration and optimization.

Pretreatment

Prior to pretreatment, the biomass undergoes physical pre-processing to increase the surface area and pore size of the material, which can be done through grinding and chopping. Typically, hammer mill, tub grinder, and knife mill can be used for this purpose (Tabil et al., 2011). By increasing the accessibility of cellulose and hemicellulose, efficient pre-processing can simplify the next steps of pretreatment and alleviate the need for extremely

harsh methods, consequently improving the sustainability of the process. Furthermore, softer pretreatment conditions reduce the formation of inhibitors, such as furfural, HMF, acetic acid, and phenolic compounds, which interfere with the fermentation process (Woźniak et al., 2025). Interestingly, Yang *et al*. (2023) reported that reducing particle size to a scale lower than the millimetre scale does not appear to show any advantages for subsequent hydrolysis and ethanol fermentation efficiency and only provides a larger energy load. In this study, WH feedstock is pretreated by chopping to 10-20 mm, grinding with a hammer mill to 1-2 mm, and, finally, sun drying.

After the raw material is pre-processed, it undergoes pretreatment aimed at separating lignin from cellulose and hemicellulose. A wide variety of chemical and physicochemical methods are available, and the choice often depends on the type and the composition of the biomass. For instance, alkaline, dilute acid, steam explosion, ammonia fiber expansion (AFEX), organosolv, ionic liquids, wet air oxidation, and biological methods can be used for lignocellulosic biomass pretreatment. (Aghaei et al., 2022; Mohammadi et al., 2024). Additionally, physical pretreatment methods such as ultrasound, radiation, and pulsed electrical energy, are also available, although these are normally energy-intensive (Mohammadi et al., 2024). Dilute acid pretreatment, for example, is a good candidate for low lignin biomass (Shekiro III et al., 2014). This is a flexible method that produces solubilized hemicellulose and leads to high yields of saccharification. However, during hemicellulose degradation to xylose, it leads to the production of furfurals and other inhibitors. Dilute acid pretreatment is also associated with high corrosion risks and high costs of acid recovery (Aghaei et al., 2022; Solarte-Toro et al., 2019). Generally, physicochemical and biological pretreatment methods are considered more environmentally friendly than their chemical counterparts, although the latter is associated with low conversion rates, high costs, and long treatment times, making biological pretreatment harder to apply in large-scale bioprocesses.

For the intended process, AFEX was chosen as an optimal pretreatment option. AFEX is a promising thermochemical pretreatment that increases the efficiency of the subsequent lignocellulosic biomass hydrolysis by degrading the ester linkages and opening the cell wall structures through a catalysed steam explosion (Aghaei et al., 2022; Chundawat et al., 2020; Mohammadi et al., 2024). For effective biomass disruption, AFEX uses about one kg of anhydrous ammonia per kg of raw material at moderate temperatures (e.g., 60-140 °C) and high pressures (e.g., 250–300 psi) for a short residence time (5–45 min) (Aghaei et al., 2022; Perez-Pimienta et al., 2016). Afterwards, about 97% of the ammonia can be recycled following the recovery from the gas phase (Perez-Pimienta et al., 2016). Additionally, nitrogen incorporated into the biomass can be later used by microorganisms during fermentation (Chundawat et al., 2020).

One of the key advantages of AFEX is that this pretreatment method does not generate inhibitors and allows for up to 99% sugar recovery during saccharification, making it an attractive option for efficient biomass processing. Nevertheless, AFEX has some limitations, such as ammonia toxicity, demanding operating conditions, and low effectiveness on high lignin biomass (Aghaei et al., 2022). However, the last drawback mentioned is not a critical issue since the raw material we use (sugarcane bagasse) only has moderate levels of lignin. AFEX performance can be further optimised by adjusting ammonia and water loading, reaction temperature, and residence time (Bals et al., 2011). Importantly, AFEX is also among the few ammonia-based pretreatment methods validated at a larger scale, which was done at the Michigan Biotechnology Institute (MBI) in Lansing, Michigan (Campbell et al., 2020). Campbell *et al*. (2020) reported that operation of the pilot scale 450 L packed-bed AFEX reactor demonstrated reproducible results with an average of 74% glucose yield and 75% xylose yield. Thus, due to its promising potential and multiple advantages, such as suitability for low lignin biomass, high sugar recovery, and efficient ammonia recycling, AFEX was selected as the most appropriate pretreatment option for the sugarcane bagasse that we utilize for bioethanol production.

Saccharification

Saccharification converts complex carbohydrates into fermentable sugars, such as glucose and sucrose, and is another critical step in bioethanol production (Woźniak et al., 2025). Two main approaches are usually applied: enzymatic and chemical. In the chemical method, cellulose and hemicellulose are hydrolyzed by employing dilute or concentrated acids, often sulfuric acid. This approach can ensure significant efficiency, but it also has several drawbacks, including costly acid recovery and generation of byproducts (Sun & Cheng, 2002).

Conversely, enzymatic hydrolysis employs enzymes, such as cellulases and hemicellulases, for an efficient and selective production of reducing sugars. Compared to chemical hydrolysis, enzymatic hydrolysis is more environmentally friendly, leads to higher yields and fewer inhibitors, has lower operational costs, and uses milder operational conditions (Aghaei et al., 2022; Klein-Marcuschamer et al., 2012; Sun & Cheng, 2002; Woźniak et al., 2025). For these reasons, enzymatic saccharification was selected for the intended project. Furthermore, saccharification of both cellulose and hemicellulose increases the diversity of produced fermentable sugars, thereby increasing the yield of ethanol per kg of raw biomass in a mixed-sugar fermentation. Commercially available enzyme cocktails, such as Cellic® CTec3, can facilitate this process. It was estimated that enzymatic saccharification of water

hyacinth after physical pretreatment and sugarcane bagasse after AFEX could achieve conversion efficiency of 85% and 90%, respectively. Combined with efficient lignin removal during AFEX and simple processing of water hyacinth due to inherently low lignin content, this pretreatment strategy shows promising potential.

Nevertheless, enzymatic hydrolysis also has some drawbacks, as it is generally slower and more costly than chemical hydrolysis, which implies challenges for large-scale industrial application (Aghaei et al., 2022; Klein-Marcuschamer et al., 2012). Additionally, high sugar concentration can also have an inhibitory effect on enzyme activity, which can be partially mitigated through optimizing the enzyme loading to balance enzyme inhibition against cost reduction (Aghaei et al., 2022). Efficiency of enzymatic hydrolysis can be further improved by performing simultaneous saccharification and fermentation (SSF). SSF has the potential to reduce residence time, lower capital and operational costs, alleviate the risks of sugar degradation or contamination, prevent end-product inhibition, and, consequently, increase ethanol yield (Olofsson et al., 2008; Woźniak et al., 2025). High temperature of saccharification is one of the main limitations of SSF. This drawback can be resolved by applying thermotolerant yeast, such as *K. marxianus* (Olofsson et al., 2008). However, for organisms, such as *K. marxianus,* that cannot perform simultaneous fermentation of different sugars, SSF can contribute to prolonged lag phases between the consumption of different substrates. Additionally, SSF can potentially reduce the recovery of the enzymes between consecutive batches, which would have a detrimental effect on the economy of the process. Therefore, SHF approach was also implemented in this study.

2.1.2.3. Microorganism

Selection of an appropriate organism for the fermentation process is a vital step in bioprocess design. In this study, several factors influenced the final decision. The key criteria for the microorganism candidate were: consumption of both hexoses and pentoses; capacity for simultaneous or one-pot mixed-sugar fermentation; availability of a wild-type strain to facilitate potential lab experiments; prior demonstration of largescale utilization; sufficient information on the physiology of the microorganism; simplicity of handling; and ease of cultivation. The top three choices were *Scheffersomyces stipitis* (*S. stipitis*), (*P. tannophilus*), *and K. marxianus*.

S. stipitis is a Crabtree-negative yeast, which enters fermentation upon the depletion of oxygen rather than substrate abundance (Cho & Jeffries, 1998). Because of the dual cofactor specificity (NADH and NADPH) of xylose reductase, which prevents cofactor imbalance in anaerobic environment, *S. stipitis* can utilize xylose as its main carbon source under O_2-

limited conditions with ethanol yield of 0.35-0.44 g g^{-1} (Hahn-Hägerdal & Pamment, 2004; Liang et al., 2014; Lin et al., 2012). While *S. stipitis* does not display carbon catabolite repression (CCR) as strong as *Saccharomyces cerevisiae*, it still exhibits lower pentose consumption rates in the presence of glucose. (Dashtban et al., 2015; Papini et al., 2012; Rouhollah et al., 2019). This complicates *S. stipitis* application in mixed-sugar fermentation, forcing researchers to explore alternative strategies, such as sequential-co-culture systems, sequential fermentation and two stage hydrolysis (Chen, 2011; Rouhollah et al., 2019; Singh et al., 2014). Additionally, *S. stipitis* exhibits low ethanol tolerance at about 20-30 g L^{-1} (Delgenes et al., 1988; Rouhollah et al., 2019). Thus, while *S. stipitis* offers efficient xylose metabolism, its low ethanol tolerance and sequential substrate consumption limit its application in mixed-sugar fermentation.

P. tannophilus is another Crabtree-negative xylose-fermenting yeast species often investigated for ethanol production (Singh et al., 2014). Similar to *S. stipitis*, it also exhibits glucose repression of xylose utilization and low ethanol tolerance (Zhao et al., 2008). Although the theoretical yield of ethanol on xylose can be relatively high, the fermentation process is slow and often inefficient, leading to low volumetric productivity and limiting the use of wild-type monoculture of *P. tannophilus* in industrial applications (Fu & Peiris, 2008; Zhao et al., 2008). Nevertheless, several studies, summarized by Chen *et al.* (2011), explored utilization of *P. tannophilus* in co-culture fermentations, e.g. with *Zymomonas mobilis*, or *S. cerevisiae*, reporting significantly better yields compared to *P. tannophilus* monoculture.

Although both organisms were considered for the intended process due to their ability of xylose metabolism, *K. marxianus* was ultimately selected the most optimal candidate. *K. marxianus* is a Crabtree-negative yeast that has gained a lot of interest as a candidate for production of various chemicals, including bioethanol, because of its rapid growth (up to 0.56 h^{-1}), broad range of possible substrates, and good temperature and acetic acid tolerance (Du et al., 2019; Dubencovs et al., 2021; Fonseca et al., 2007; Nonklang et al., 2008; Rouhollah et al., 2019). Similar to *P. stipitis* and *P tannophilus*, *K. marxianus* also exhibits glucose repression of xylose consumption and shows lower ethanol yield on xylose compared to *P. stipitis*, which is its main disadvantage (Rouhollah et al., 2019). Still, *K. marxianus* shows higher yields and rates of ethanol production on hexoses as well as better ethanol tolerance (6-8% (v/v)) (Pal & Vij, 2022). Importantly, it does not display a very prominent diauxic growth with a prolonged lag phase between glucose and xylose consumption, as is the case for *P. stipitis*, which is crucial for a fast and efficient process (Rouhollah et al., 2019). According to Rouhollah et al. (2019), in a mixed sugar fermentation on glucose, xylose, mannose, and galactose, *K. marxianus* showed a maximum ethanol 28.15 g L^{-1}, ethanol yield of 0.36 g g^{-1} (70% of theoretical), and a volumetric ethanol productivity of 1.09

g L^{-1} h^{-1}, outperforming *P. stipitis* in terms of the latter parameter. Moreover, while *P. stipitis* exhibits different optimal temperatures for xylose (25-26 °C) and glucose (34 °C) consumption (Slininger et al., 1990), *K. marxianus* can perform fermentation at temperatures as high as 40-45 °C (Rouhollah et al., 2019). This phenomenon is extremely advantageous as the need for cooling of the fermenters significantly reduces.

Overall, for an efficient and fast bioethanol production process in a mixed-sugar fermentation at higher temperatures, *K. marxianus* appears to be a superior choice. Furthermore, its high growth rate and biomass yield, combined with the ability of growth on various sugars derived from inexpensive biomass without the need of expensive nutrients, make *K. marxianus* a good candidate for efficient inoculum preparation (Fonseca et al., 2013). Although strain-specific physiological parameters may differ, numerous publications and the utilization of this yeast species in industry suggest the promising potential of thermotolerant *K. marxianus*, specifically for bioethanol production in mixed sugar fermentation. Therefore, *K. marxianus* was selected as the most suitable organism for this study. However, it should be noted that performance parameters used in this study are not strain-specific. As no single comprehensive data set exists, some values were approximated from different *K. marxianus* strains described in the literature.

2.2.Group Structure, Division of Tasks

Table 1: Division of task

Student	Task
Om Mihani	Fermentation, Bubble Column Design, Distillation column, Decentralization, Enzyme immobilization, slides, Block Diagram, Cost Analysis, Sensitivity Analysis
Sandhya Srinivasa Raghavan	Saccharification, Process Flow Diagram, Mass Balance, Stream Table and Report Work
Kateryna Melnyk	Seed Train Design, Literature Background
Manu Manayath Johnson	Ammonia Fibre Expansion Costs and Process Design, and Documentation

3. State of the art

Physical Pretreatment Of Water Hyacinth

Physical pretreatment is performed to reduce the size of biomass and make the hemicellulose and cellulose content more available to enzymes and microbes. Common mechanical mechanisms of lignocellulosic biomass size reduction include cutting, compression, tearing, shearing, and breaking (Arce & Kratky, 2022). Mechanical pretreatment techniques comprise of milling, disc refining, grinding, extrusion, and ultrasonication. Milling is the most popular technique, and it includes different options, such as ball milling, knife milling, hammer milling, and rod milling. Amongst these, knife mill operates based on cutting and shearing and is best utilized for soft, leafy, or herbaceous materials. Knife mill can be used for the reduction in size of up to 1-2 mm, which is an optimal size for efficient hydrolysis of lignocellulosic biomass (Yang et al., 2023). Common mechanical pretreatment steps of water hyacinth involve sun drying, chopping, and milling (Ruan et al., 2016; Su et al., 2018).

Sugarcane Bagasse Pretreatment - AFEX

Ammonia fiber expansion (AFEX) is a thermochemical pretreatment method which can be utilized for the separation of lignin from hemicellulose and cellulose. More detailed description of AFEX is provided in the Background section.

Seed Train

Inoculum preparation in large scale fermentation relies on sequential cultivation of microbial culture, starting from a cryo stock and finishing with a production in a large seed fermenter (Kern et al., 2016). The inoculum size, starting cell density, transfer time, feed composition, agitation and aeration rates, as well as other operational conditions such as temperature and pH, should be adjusted according to the requirements of the selected microorganism. Inoculation with 10% (v/v) (Colacicco et al., 2024) and transferring during the exponential growth phase is a good practice to ensure robust and quick culture development (Permatasari et al., 2023). During scale-up, the scale up factor for the seed fermenter usually should not exceed 10 to avoid overly large jumps (Colacicco et al., 2024). Cultivation occurs either in a fed-batch or a batch mode. Batch mode is usually preferred for microbial cultivations, as it can ensure good performance of the culture, low contamination risks, easy control of the process, making it a simpler option overall (Shuler & Kargi, 2002). For *K marxianus* specifically, optimal cultivation parameters have been reported as 28 °C, pH 5, ~60% oxygen

saturation with the inoculation concentration of 1.5 10^6 CFU mL^{-1}(L. Wang et al., 2020). Additionally, *K marxianus* has been shown to grow well in sucrose-containing medium, such as wort base medium (16.67 % wort concentrate), with molasses (2.94 %), wheat bran (1.56 %), and $CaCO_3$ (0.21 %) (L. Wang et al., 2020).

Saccharification

Enzymatic saccharification converts pretreated lignocellulosic biomass into fermentable sugars, such as glucose and sucrose (Woźniak et al., 2025). Cellulases and hemicellulases are commonly utilized for hydrolysis and can be applied as commercially available enzyme cocktails, such as Cellic® CTec3 (Santana et al., 2022). Enzyme loading is a key parameter in enzymatic saccharification, as it can both lead to high cost of the process but also cause inefficient hydrolysis of sugars (Aghaei et al., 2022). It has been shown that 15 FPU (filter paper unit) g^{-1} is an optimal amount of enzyme for water hyacinth and sugarcane bagasse hydrolysis (Santana et al., 2022). Temperature is also an important parameter, since hydrolytic enzymes usually require elevated conditions (~50 ^oC) for sufficient performance (Ganter De Moura et al., 2023).

4. System Description:

4.1 Process Flow Diagram:

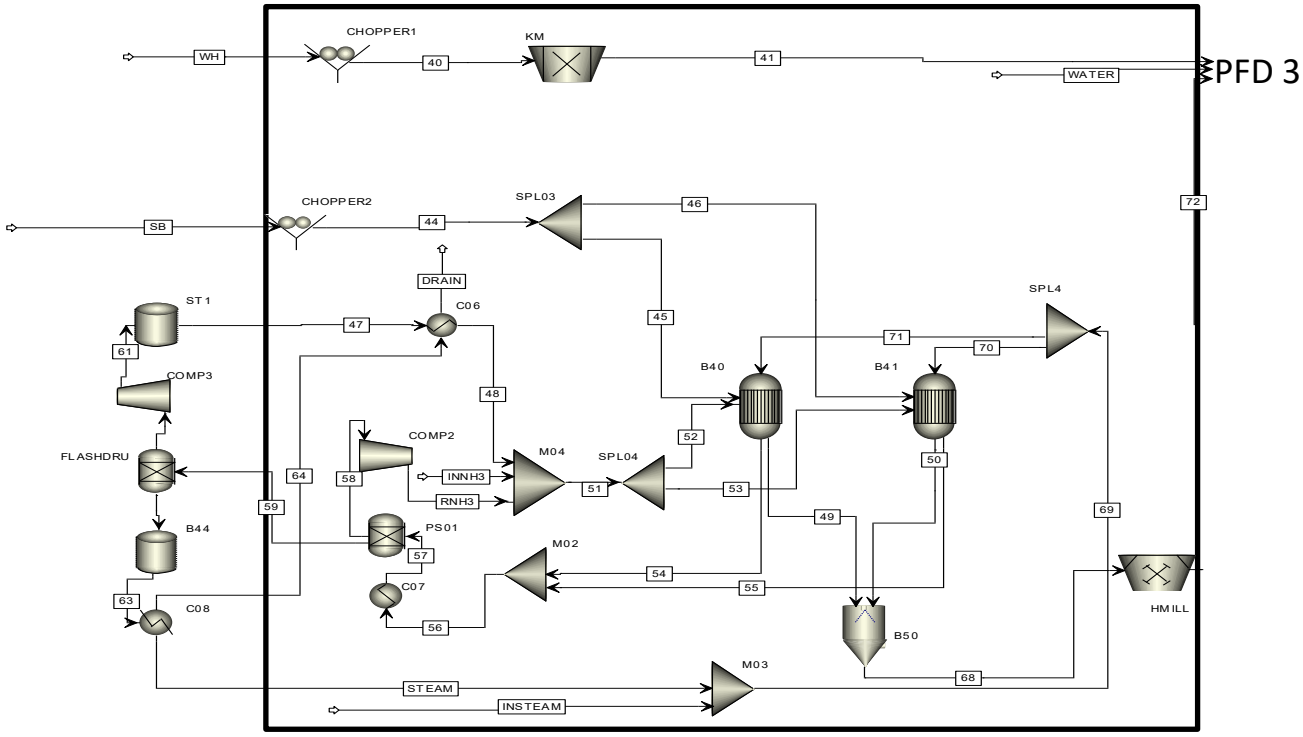

Figure 1: Pretreatment of Sugarcane Bagasse and Water Hyacinth

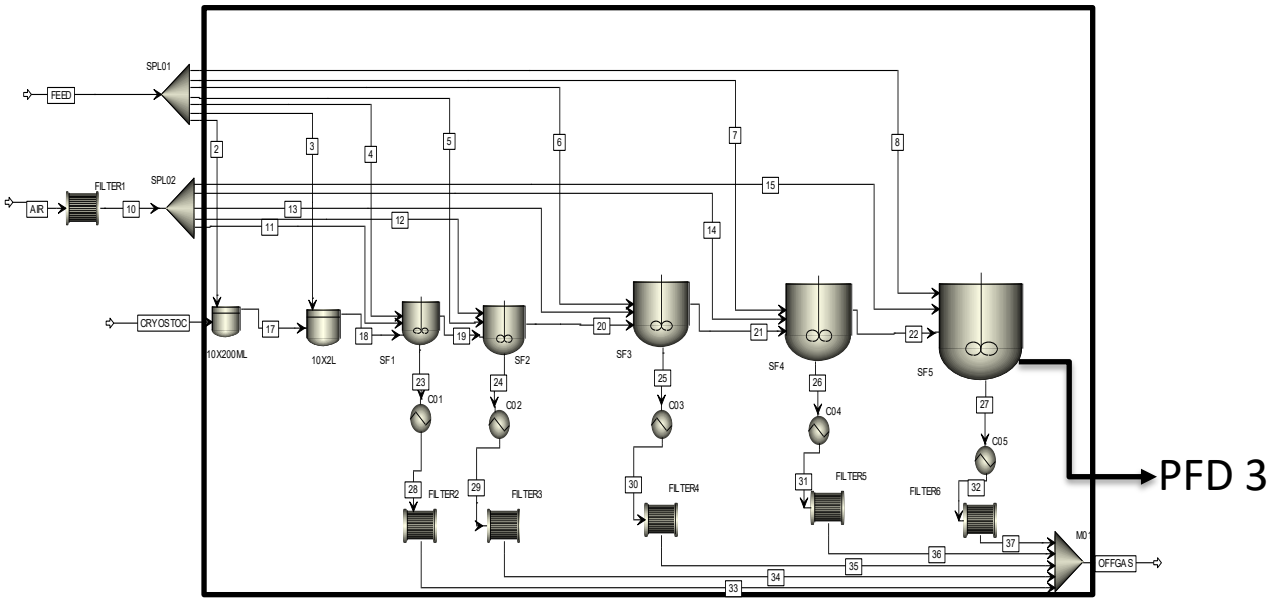

Figure 2: Seed Train Design for Yeast Inoculum

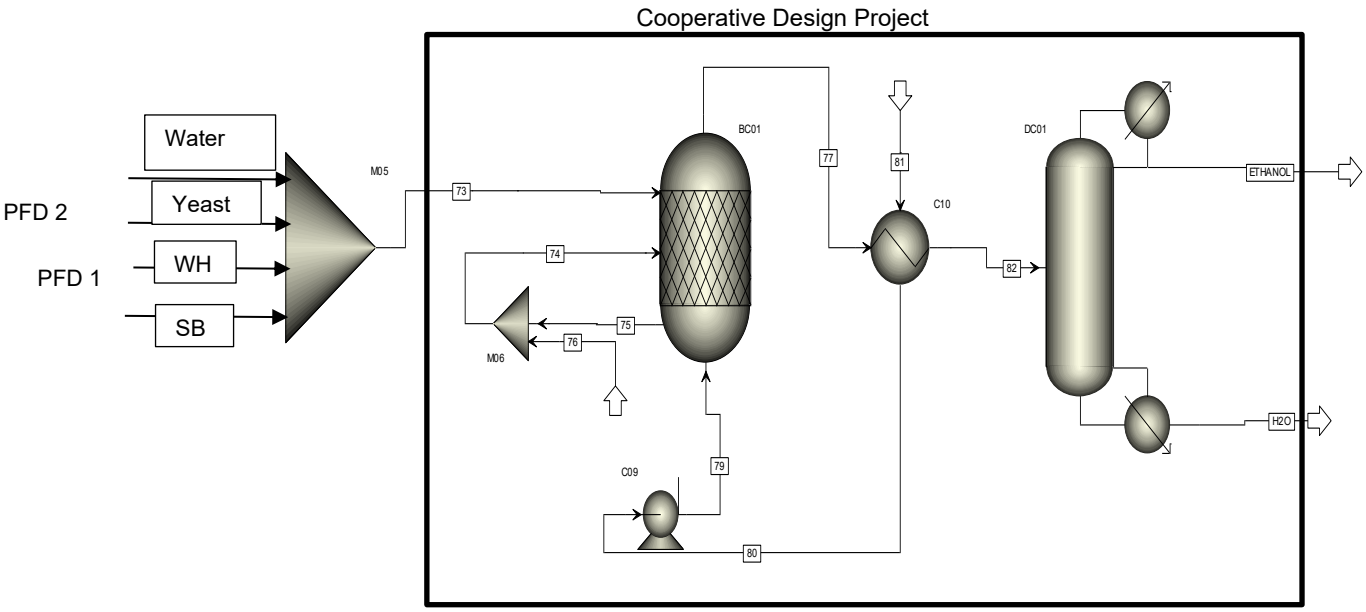

Figure 3: Saccharification and Downstream Processing

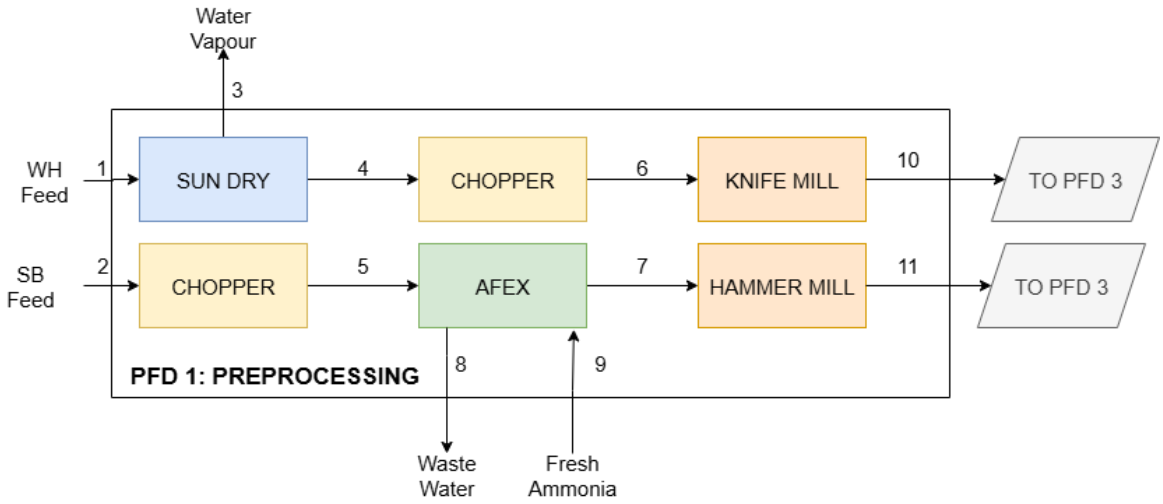

Figure 4: Block diagram of the Pretreatment of sugarcane bagasse and Water Hyacinth

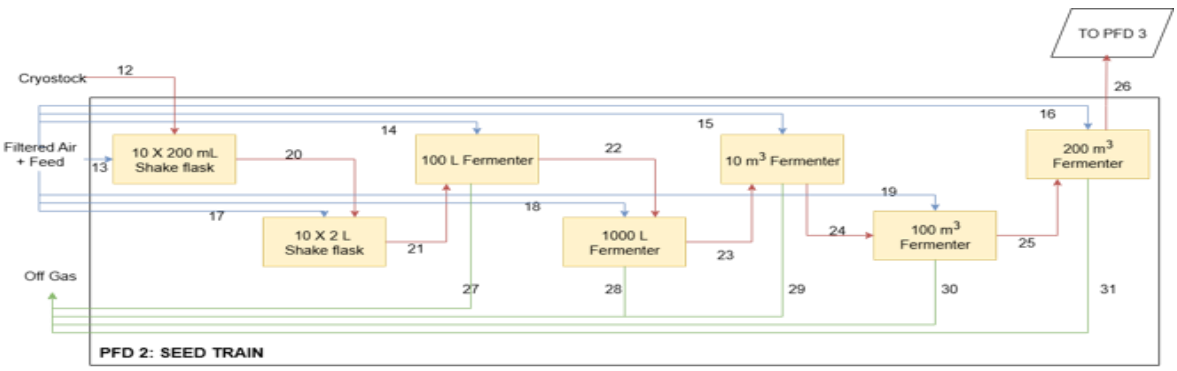

Figure 5: Block Diagram of the Seed Train for production of Yeast Inoculum

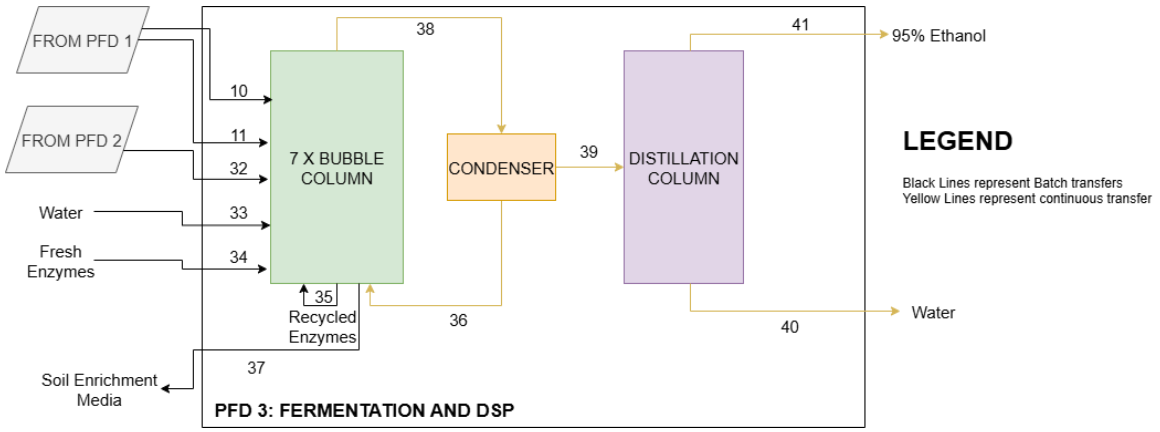

Figure 6: Block Diagram of the Saccharification and Downstream Processing

4.2 Plant Design:

4.2.1 Choice of Feedstock:

The feedstock is the key driver of the efficiency and sustainability of bioethanol production. One of the bottlenecks toward biomass application is the lignin that prevents enzymes from obtaining access and requires energy-intensive pretreatment processes. To overcome this, a comparative analysis of available biomass types was performed on their lignin content, structure, and pretreatment needs.

From the feed composition analysis, it is apparent that a combination of water hyacinth (WH) and sugarcane bagasse (SB) as a composite feedstock has its benefits. Water hyacinth is an aquatic weed with low lignin content, which, as a result, makes the pretreatment simple and also reduces the production of inhibitory compounds. From Figure 7, it is seen that WH is rich in hemicellulose, ash, and crude protein portions while having a relatively lower share of lignin. This property makes WH potentially more available, but its higher ash content might disturb an ion balance. Even though, Water Hyacinth has high hemicellulose content, only small amount of its xylose will be converted. Sugarcane bagasse, on the other hand, has a higher content in cellulose, as observed in the feed composition, so it is an important alternative for ethanol production. But, it also contains 10–15% of lignin, demanding more severe pretreatment methods such as alkaline hydrogen peroxide or subcritical water pretreatment. The hemicellulose composition chart also shows that SB shows a good sugar profile for enzyme hydrolysis, which is important for effective fermentation. Other candidates, such as sorghum and switchgrass, were investigated owing to the fact that they

contain low lignin content (5–10%) and are able to work with milder pretreatment methods. (Bruno Godin et.al.,(2016)) Sorghum is, however, a major food and fodder crop, raising concerns about competition between food and energy. This “food versus feedstock” duality raises serious questions with respect to its large-scale biofuel utilization. However, sugarcane bagasse is a plentiful waste by-product of the sugar industry, meaning that it is readily available at low cost without interfering with food supply chains.

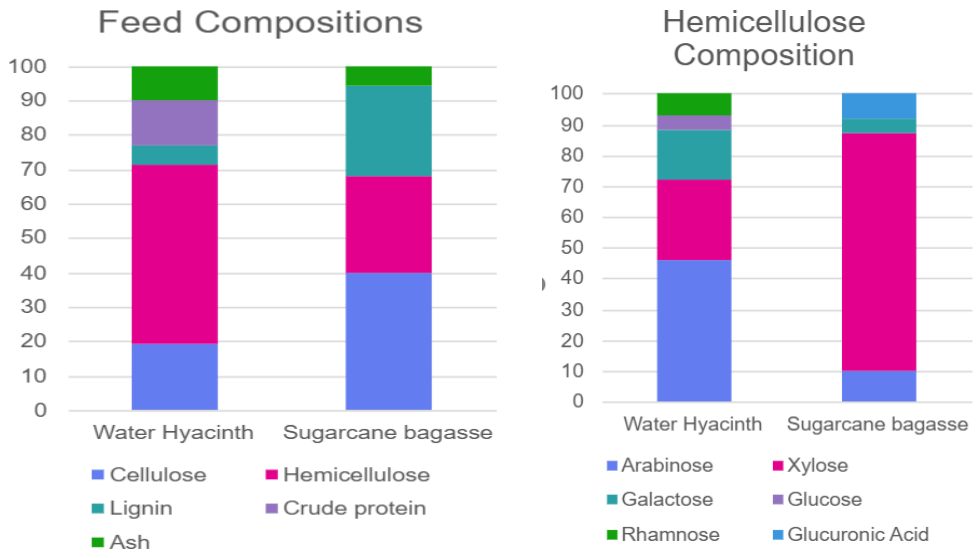

Figure 7: Feed Composition of Feed Stocks: Sugarcane Bagasse and Water Hyacinth. The first graph shows the cellulose, hemicellulose, and lignin percentages, and the second graph shows the hemicellulose breakdown.

4.2.2 Pretreatment:

The pretreatment consists of AFEX, choppers, mills and drying. The AFEX process is a 7-stage cycle. The procedure starts with the structure of the biomass and modifies the hemicellulose while simultaneously ensuring ammonia recovery and its reutilization between paired reactors. The sugarcane bagasse was moistened so that the ratio of the moisture content would be 1.5:1 (kg of water per kg of dry biomass). The water-soaked biomass was loaded into one of the two parallel reactors (Reactor A). The device with two reactors enables the operation of one reactor while the other is being pretreated, thus the downtime between pretreatment cycles is very short. Steam was added to Reactor A for the substitution of residual air and to heat the biomass by condensation at 95°C and at 1 atm. When the target temperature was reached, the steam sent was stopped and the reactor was closed off at both the top and the bottom.

Ammonia is added into the pressurised reactor to achieve a working pressure of 2 MPa (2 atm) and three types of ammonia resources were used. The fresh ammonia was added to initiate the cycle. The other recovered ammonia from the liquid ammonia-water mixture obtained after distillation, and recovered ammonia from the vapour phase of the opposite reactor after depressurisation and steam stripping. This step was the pretreatment stage in reality. The biomass is being soaked in liquid ammonia at high pressure for a residence time of 60 minutes in which lignin was broken down and hemicellulose was opened up for the next enzymatic hydrolysis step. In the process of soaking, some of the ammonia vapour condensed to liquid, causing a small decrease in reactor pressure and temperature due to heat loss to the surroundings.

Reactor A was depressurized after soaking when the opposing reactor was in the pre-steaming stage. The pressure gradient between Reactor A (20 atm) and the opposite reactor (1 atm) allowed the transfer of ammonia vapour to the latter until equilibrium was achieved. A compressor was then used to lower the reactor pressure below 1 atm.

- Without any compression, vapor-phase ammonia was just sent to the opposite reactor, as it was already at high pressure.
- The rest of the ammonia–water stream went to a condenser where ammonia vapour was separated from liquid water. The vapor part was compressed and reused, while the liquid part was distilled to get rid of any leftover ammonia.

Residual ammonia in the reactor after depressurisation is removed via steam stripping. Steam is added to the top of the reactor vessel. Steam condensation on biomass causes the release of heat, which drives the evaporation of ammonia vapour. Stripped ammonia vapour is pushed out through the bottom of the reactor by the denser steam as the steam penetrates through the biomass. Ammonia vapor is drawn from the bottom of the reactor by the compressor, and the compressed ammonia vapor is added to the opposite reactor. The pressure in the reactor remains below 1 atm during steam stripping.

Mechanical pre-treatment is an essential step in improving enzymatic saccharification by breaking down particle size and surface area. As a lignin-poor material, water hyacinth (WH) needs only moderate treatment. The mass is cut and milled to a size of 1–2 mm, where in these dimensions, enzyme accessibility is high while energy input is low (Aswathy et al., 2010). Due to its high initial moisture (~90%), sun drying (to 10–15%, oven drying to <10% optional) is the best according to practice for storage facility.

Sterilization may be effected by autoclaving or steam sterilization. Because of its weak texture and less lignified structure, WH would be metabolized without requiring harsh chemicals pretreatments (Booramurthy et al., 2019). Meanwhile, SB contains more lignin and crystalline cellulose than SCB, thereby showing greater recalcitrance toward enzymatic hydrolysis. To circumvent this, SB is initially milled to a size between 2 and 5 mm for ammonia to penetrate it, which is subsequently followed by Ammonia Fiber Expansion (AFEX) pretreatment at a mild temperature ranging from 100 °C to 140 °C that breaks the lignin–carbohydrate. (Kim et al., 2011). Subsequent to AFEX, more grinding to 1-2 mm opens up the enzymatic access better and as a result, with commercial enzymes (Balan et al., 2009) 85% glucan and 95-98% xylan conversion can be achieved. Generally, the particle size of 1-2 mm as a target for the hydrolysis gives 20-30% yield higher than that of particles with larger size (Zheng et al., 2009) i.e. the treatment of biomass is more effective. The use of hammer mills is optimal for both WH and SB, while disc or knife mills are suitable for the initial cutting of WH. The combination of WH, which alleviates the pretreatment severity, and SB, which supplies large amounts of cellulose, forms a convenient and scalable feedstock blend for bioethanol production.

4.2.3 Seed train:

4.2.3.1 Seed train design:

Growth parameters of *K. marxianus:*

According to the literature (Fonseca et al., 2007, 2013), *K. marxianus* is the fastest-growing eukaryotic organism and, depending on the carbon source and conditions, displays varying growth rates ranging from 0.41 h^{-1} to 0.56 h^{-1} during the exponential growth. Similarly, different biomass yields have been reported for *K. marxianus*. For sucrose, *K. marxianus* shows an exceptionally high biomass yield $Y_{XS,Suc}$ of 0.63 $g_{CDW}\, g_{Suc}^{-1}$ (Fonseca et al., 2013), which is why this sugar has been selected as the main carbon source for the inoculum preparation. Additionally, sucrose can be found in cheap and economical sources, like molasses and wort. Therefore, the seed train was designed based on the growth parameters of *K. marxianus* corresponding to growth on sucrose (**Table 2**).

The time required to reach a certain amount of biomass concentration is defined as:

$$t = \frac{ln\frac{C_{xf}}{C_{x0}}}{\mu}, \qquad (1)$$

where: t – time required to reach a certain cell density, [h]; C_{xf} – final biomass concentration, [g_{CDW} L^{-1}]; C_{x0} – initial biomass concentration, [g_{CDW} L^{-1}]; μ – growth rate, 0.43 h^{-1}.
Substrate concentration in the medium, C_s, required to produce the desired amount of biomass was based solely on the biomass yield, $Y_{X/S,Suc}$, and defined as:

$$C_S = \frac{C_{xf} - C_{x0}}{Y_{X/S,,Suc}} \quad (2)$$

The corresponding feed concentration, $C_{S,feed}$, is defined as:

$$C_{S,feed} = \frac{C_S \cdot V_f}{(V_f - V_{in})}, \quad (3)$$

where: V_f – working/cultivation broth volume, [L]; V_{in} – inoculum volume, [L].

Table 2 Physiological and growth parameters of *K. marxianus*

Parameter	Value	Source
Growth rate on sucrose μ	0.43 h^{-1}	Fonseca GG et.al., 2013 And Fonseca GG et.al., 2007(Fonseca et al., 2007)
Biomass yield from sucrose $Y_{X/S,Suc}$	0.63 g_{CDW} g_{Suc}^{-1}	
Cell-specific oxygen uptake rate q_{O2}	11.1 mmol O_2 g_{CDW}^{-1} h^{-1}	

Seed train design:

Inoculum size generally influences the fermentation process, having both positive and negative potential effects. For instance, for ethanol production, high inoculum size may offer faster fermentation and higher ethanol yield, as well as possible partial recovery of microorganisms between batches. However, it can lead to negative effects due to nutrient competition and low ethanol tolerance of some microorganisms. The effects may vary depending on the strain and levels of ethanol produced (Laluce et al., 2009). Based on several studies on ethanol production by *K. marxianus* using different substrates, an inoculum size of 9,44-10,40 % (v/v) appears to be a good option (Dhanker et al., n.d.; Permatasari et al., 2023). Accordingly, the seed train cascade was designed to provide sufficient biomass for the main fermenter to achieve an inoculum size of approximately 10% (v/v).
Another crucial point of designing a seed train cascade is establishing the precise timing for culture transfer, as this can affect cell viability and the duration of the cultivation (Zhenqiang Xia, 2012). Several studies showed that for yeast, a shorter time until the first cell division can be obtained with a larger inoculum and with the parental culture transferred before the

cells enter the stationary phase (Ginovart et al., 2011; Permatasari et al., 2023; Zhenqiang Xia, 2012). The optimal passaging time has been reported as mid- or late-exponential growth phase, which for shake-flask cultivation often translates to 18-24 h. This strategy minimizes the lag phase duration and ensures that the culture is both fast growing and robust Therefore, in this study, the batch times were kept short, and the lag phase was not considered in calculations, since using a 10% (v/v) inoculum size and timely transfer was expected to minimize the lag phase and provide a constant fast growth rate of *K. marxianus*. However, it should be acknowledged that this is an assumption based on the literature data, and in practice, certain parameters may require additional adjustment. Additionally, after inoculation from a cryo stock, the culture should be cultivated for a longer period (compared to reactor culture), approximately 24 h, to provide a sufficient recovery stage ensuring the robustness and viability of the cells.

The seed train (Table 3) starts with the inoculation from 2 mL of cryo stock. The first two steps of cultivation, on account of economic reasons, are performed in baffled flasks on an orbital shaker instead of a benchtop bioreactor. Ten baffled flasks are used in each step to produce an appropriate amount of biomass necessary for the inoculation of a 100 L reactor. All seed fermenters are stirred-tank bioreactors operating in batch mode. For the scale-up of the reactors, a factor of up to 10 was used. The working volume was maintained at 80% for the reactors and at 10-20% for the baffled flasks. To provide the main fermentation process with 10% of inoculum (v/v), approximately 625 m^3 of biomass has to be cultivated. Designing one large seed train to ensure this large production capacity would require the last step of cultivation to be performed in a ~780 m^3 reactor. To the best of our knowledge, such volumes of fermenters for yeast cultivation have not been reported by any company. Operating a seed fermenter of this scale represents an unreasonable concentration of risk related to possible contamination and deviates significantly from established industry practice. Therefore, *K. marxianus* cultivation was designed as four parallel seed trains, with the largest fermenter of 200 m^3.

Table 3 Seed train cascade design and mass balance summary. V_r - seed fermenter volume, [L]; V_f - working volume of the reactor, [L]; Inoc. % - inoculum size, [v/v[; C_{x0} – initial biomass concentration, [g_{CDW} L^{-1}]; C_{xf} – final biomass concentration, [g_{CDW} L^{-1}]; t – cultivation time, [h]; C_s – sucrose concentration in the medium, [g L^{-1}]; $V_{s,feed}$ – feed volume, [L]; $C_{s,feed}$ – sucrose concentration in the feed, [g L^{-1}];

$V_{x,tot}$ – total inoculum volume from four seed trains, [L]; t_{tot} – duration of the whole seed train, [h]; $C_{X,fer}$ – biomass concentration after inoculation in the main fermenter, [g_{CDW} L^{-1}]; $m_{S,tot}$ – total mass of sucrose needed for four seed trains, [t]; $V_{S,tot}$ – total volume of feed used, [m^3].

Seed fermenter	V_r **[L]**	V_f **[%]**	V_f **[L]**	**Inoc. % [v/v]**	C_{X0} **[g_{CDW} L^{-1}]**	C_{XF} **[g_{CDW} L^{-1}]**	t **[h]**	C_s **[g L^{-1}]**	$V_{s,feed}$ **[L]**	$C_{s,feed}$ **[g L^{-1}]**
10x 200 ml baffled flask	2	10	0.2	10	1	10	24.0	14.29	0.18	15.87
10x 2 L baffled flask	20	20	4	5	0.5	10	7.0	15.08	3.80	15.87
100 L reactor	100	80	80	5	0.5	10	7.0	15.08	76.00	15.87
1000 L reactor	1000	80	800	10	1	10	5.4	14.29	720.00	15.87
10 m^3 reactor	10000	80	8000	10	1	10	5.4	14.29	7200.00	15.87
100 m^3 reactor	100000	80	80000	10	1	10	5.4	14.29	72000.00	15.87
200 m^3 reactor	200000	80	160000	50	5	11	1.8	9.52	80000.00	19.05

Total for four seed trains	$V_{X,tot}$ **[L]**	t_{tot} **[h]**	$C_{X,fer}$ **[g_{CDW} L^{-1}]**	$m_{s,tot}$ **[t]**	$V_{s,tot}$ **[m^3]**
	640000	55.8	1.13	11.175	640

Operational conditions:

Operational parameters of the process are presented in Table 4. Cultivation occurs at 28 °C (L. Wang et al., 2020), which is kept constant via cooling jackets and coils with cold water. The pH of 5 is kept by adjusting using buffered medium and acid addition, e.g., 0,1 M KOH solution (Fonseca et al., 2007). The aeration rate for the growth of *K. marxianus* is typically in the range of 1 to 2 vvm, and oxygen saturation is usually in the range of 30-70%. For this process, the optimal aeration rate was assumed to be 1 vvm for the cultivation in reactors

(Fonseca et al., 2007; Sakihama et al., 2019). The oxygen saturation should be kept at 60% (Fonseca et al., 2013). In baffled flasks, the aeration occurs through the gas-liquid contact, which is increased by shaking. Agitation rate was set as 220 rpm for the 200 mL baffled flasks (L. Wang et al., 2020) and 700 rpm for 2 L baffled flasks (Fonseca et al., 2007). For agitation in the fermenters, the optimal power input per volume was assumed to be around 2 kW m^{-3}, which is in the range of reported values for fermentation in vessels up to 300 m^3 (Junker, 2004). Parameters, such as temperature, pH, dissolved oxygen, foaming, and cell density, should be monitored over the whole course of cultivation. The latter is especially important to determine the correct time of transfer from one seed fermenter to another, which should happen before the cells reach the stationary phase.

Table 4 Key operational parameters of *K. marxianus* cultivation

Parameter	**Value**	**Source**
Cultivation temperature T	28 °C	Wang L et.al., 2020
pH	5	Fonseca GG et.al., 2007(Fonseca et al., 2007)
Aeration rate	1 vvm	Fonseca GG et.al., 2007, (Fonseca et al., 2007)
Dissolved oxygen	60%	Fonseca GG et.al., 2013, Sakihama Y et.al., 2019(Fonseca et al., 2007)
Agitation – flasks (200 mL/2 L)	220 rpm/700rpm	Fonseca GG et.al., 2013, (Fonseca et al., 2007)
Agitation – reactors - volumetric power input P/V	2000 W m^{-3}	

Medium:

Wang *et al*. 2020 performed a study on optimizing medium composition and cultivation conditions for the growth of *K. marxianus* and reported that a medium consisting of wort base medium (16.67 % wort concentrate), molasses 2.94 %, wheat bran 1.56 % and $CaCO_3$ 0.21 % can be used for optimal proliferation of yeast. According to Wang *et al*. (2020), wort concentrate is a cheap and easily available medium option, which can provide enough nutrients and minerals necessary for optimal yeast growth. Moreover, utilization of wort medium for *K. marxianus* growth showed to eliminate the need for inorganic salts addition,

except for $CaCO_3$. Nitrogen is another vital factor for yeast proliferation, and wheat bran, which is a by-product of the roller milling of wheat grain containing crude protein, multivitamins, and other important nutrients, proved to be excellent nitrogen source for yeast proliferation. Evaluation of different carbon sources showed that molasses, which consists mainly of sucrose, exhibit the most significant effect on *K. marxianus* growth. Therefore, this medium composition, being both cost-effective and displaying excellent performance according to the literature data Wang *et al*. (2020), was selected as optimal choice for inoculum preparation. However, for simplicity, all calculations were performed considering the feed as a synthetic sucrose medium.

4.2.4 Saccharification and Fermentation:

Saccharification and fermentation are the main stages that convert pretreated water hyacinth (WH) and sugarcane bagasse (SB) into ethanol. Saccharification is the first step in the lag phase of fermentation, right after an ammonia fiber expansion (AFEX) pretreatment. Enzymatic hydrolysis changes cellulose to glucose (1 g cellulose to 1.11 g glucose) and hemicellulose to xylose (1 g hemicellulose to 1.136 g xylose). In the 50:50 WH–SB blend, the water hyacinth provides 19% cellulose and 52% hemicellulose, whereas the sugarcane bagasse provides 40% cellulose and 28% hemicellulose, with the respective extraction efficiencies of 85% and 90% (Zheng, Pan, & Zhang, 2009). Steam is essential in the process as it is used to keep the slurry at the optimal temperature for enzyme activity and also as a sterilization-in-place (SIP), where reactors treated with 80 °C steam are freed from microbial contaminants (Booramurthy, Palaniyappan, & Arumugam, 2019).

Table 5: Fermentation Phases

Phase	Phase name	Duration (h)	Cumulative time window (h)	Primary condition	Dominant substrate	Notes

1	Aerobic → Anaerobic **lag** (with saccharification)	1	0–1	Aerobic transitioning to anaerobic	(hydrolysis ongoing)	Enzymatic saccharification runs; cells adapt to anaerobiosis.
2	**Glucose fermentation**	9	1–10	Anaerobic	Glucose	Highest ethanol productivity; rapid sugar depletion.
3	**Diauxic lag**	3	10–13	Anaerobic	-	Metabolic switch from glucose to xylose; low activity.
4	**Xylose fermentation**	12	13–25	Anaerobic	Xylose	Slower uptake; extended ethanol formation phase.

Glucose as well as xylose are converted into ethanol at the rate of 80% feed and thus the yield is 0.40 g of ethanol per g of sugar (Kim, Lee, & Sunwoo, 2011; Balan, Chiaramonti, & Kumar, 2009). A 270-batch of 176-tonne ethanol is designed to be produced, which is enough to meet the annual target of 50 KTPA.

Moreover, steam is also used to a great extent in cleaning-in-place (CIP) operations, where hot water and steam circulation remove the residues, and in a SIP sterilization to ensure the aseptic operation between batches. To keep the anaerobic conditions, continuous CO_2 sparging is used while the carbonate–bicarbonate equilibrium allows the prevention of acidification, which further stabilizes the broth pH (Nature, 1995).
Overall, saccharification and fermentation are the two steam-dependent, tightly integrated processes where both hemicellulosic and cellulosic sugars from WH and SB are utilized effectively for ethanol generation.

5. Mass and energy balance

5.1 Pretreatment

Flow rate of dry biomass = 14.64 tph

Time for a single fermentation batch process = 32 hours

Therefore, the total amount of dry biomass needed for a single fermentation batch

= 14.64 tph x 32 h= 468.5 tonnes

Biomass processed by a single reactor in the reference process: 90 kg

Time for a single AFEX process cycle:

Ammonia addition = 19 minutes

Soaking and addition of make-up ammonia = 36 minutes

Depressurisation and steam-stripping = 19 minutes

Unloading, reloading and pre-steaming = 36 minutes

Therefore, total cycle time = 110 minutes

However, we carry out a merry-go-round process methodology.

Therefore, though the first output takes 110 minutes to obtain, all consequent outputs are obtained at an interval of 55 minutes.

However, the obtained biomass must also be dried.

Drying takes 30 minutes.

So, ready-to-use biomass output is obtained 140 minutes after the start of the process for the first one. But every output after that is obtained after every 55 minutes.

Number of cycles possible within 32 hours

$$no\ of\ cycles\ = \frac{total\ time}{time\ of\ each\ cycle} \qquad (4)$$

$$32\ hours\ =\ 1920\ min$$

$$no\ of\ cycles = \frac{1920\ min}{55\ min\ /\ cycle} = 34.9 = 35\ cycles \qquad (5)$$

When we consider points of time near the 1920 minute mark, we see that the AFEX output before drying is obtained at 1815, 1870 and 1925 minutes. If we strictly stick to the 1920 minute limit, the last possible AFEX cycle occurs at 1870 minutes, which is the 34th cycle. But since the first cycle interval of 55 minutes has no output, with the first output being obtained only after the first two cycle intervals (110 minutes), the AFEX output is obtained 33 times over the 32 hour time period.

Actual number of AFEX cycles with biomass output = 33 cycles

This product also needs to be dried, which takes an additional 30 minutes. So the last AFEX-treated and dried biomass is obtained at the 1900 minute mark.

We need to convert the specifications of the reference AFEX plant to fit those of our proposed plant.

Reference plant biomass output = 1 tonne/day = 1000 kg/day.

Total time of reference plant operation = 24 hours = 1440 minutes

$$No.of\ possible\ cycles\ over\ a\ 24\ h\ time\ period\ = \frac{24\ hours\ x\ 60\frac{min}{hour}}{55\ min/cycle} = 26.18\ =\ 27\ (6)$$

Last possible AFEX cycle time point = 1430 minutes.

But if we take the drying period into account, this will go beyond 1440 minutes.

So, we take the previous one, i.e., 1375 minutes, which is 55x25, i.e, the 25th theoretically possible cycle.

But, the first cycle is a null output cycle.

Therefore, the actual number of AFEX cycles producing biomass is 24 cycles.

The density of biomass is taken as 100 kg/m^3.

Volume of a single reactor:

$$diameter \ = \ 18 \ inches$$

$$height \ = \ 144 \ inches$$

$$Volume \ of \ reactor \ = \ \pi r^2 h = \pi \frac{d^2}{4} h \qquad (7)$$

$$V \ = \ 36643.53 \ in^3 = 0.6004 \ m^3 = 600.4 \ L$$

Biomass output calculations:

Density of biomass = 100 kg/m^3

Volume of a single reactor = 0.6004 m^3

But, this volume is the total volume

Usable reactor volume = 0.667 x 0.6004 = 0.4002 m^3

Mass obtained from each reactor = 100 x 0.4002 = 40 kg

This is the mass obtained from each reactor when it undergoes a single cycle of operation

Therefore, 40kg of biomass/cycle of operation is obtained and our system has 33 cycles.

Therefore, total output = 33 x 40 = 1320 kg = 1.32 tonnes

But, we need 468.5 tonnes of biomass as output in total.

We can assume a linear correlation:

$$x = \frac{468.5 \ x \ 0.6004}{1.32} = 213.09 \ m^3 \qquad (8)$$

This is the volume of the reactor in our system, and we have two reactors of this volume. Since 0.6004 m^3 is the total volume of the reactor, 130.99 m^3 is also the total volume of the reactor.

Usable reactor volume = 0.667 x 213.09 m^3 = 142 m^3

Reactor Volume = 131 m^3

Operating Pressure = 2 MPa

Design Pressure = 3.3 MPa

Operating Temperature = 90 ^{o}C

Reactor Material = SS304

5.2 Seed Fermenter

5.2.1 Seed Fermenter Dimensions

Each fermenter was designed as a stirred-tank bioreactor equipped with four baffles, a Rushton turbine with six blades on each impeller, a cooling jacket, and a cooling coil if needed. Calculations of the dimensions were performed based on the reactor volume necessary to meet the requirements of the inoculum volume for the main fermenter, assuming around 80% of the working volume. The results are presented in uat The following assumptions and approximations were made:

- Reactor height to diameter ratio H_R/D_R was taken as 3.
- Liquid height to the reactor diameter ratio H_L/D_R was kept between at 1.60.
- The height of the first impeller to the reactor diameter ratio L_1/D_R = 0.375.
- The gap length between impellers, *dL*, was based on the ratio dL/D_R=0.4.
- The number of impellers, $n_{impellers}$, was calculated based on the L_1 and *dL*. However, the actual number of impellers was kept as 3 for all the vessels. The values of *dL* were adjusted accordingly.
- The diameter of the impeller, *d*, was determined based on the ratio d/D_R=1/3
- The width, *w*, and height, *b*, of the blades on each impeller were calculated based on the *w*=*d*/3 and *b*=*d*/4 relationship, respectively.

Table 6 Reactor dimensions and impeller specifications. D_R - reactor diameter, [m]; H_R – rector height, [m]; H_L – liquid height in the reactor [m], L_1 - height of the first impeller, [m]; *dL* – gap length between impellers, [m]; $n_{impellers}$ – number of impellers; *d* – impeller diameter, [m]; *w* – width of the blade, [m]; *b* – height of the blade, [m].

Reactor	D_R [m]	H_R [m]	H_L [m]	L_1 [m]	*dL* [m]	$n_{impellers}$	*d* [m]	*w* [m]	*b* [m]
100 L	0.35	1.05	0.84	0.13	0.14	6.00	0.12	0.02	0.03
1000 L	0.75	2.25	1.80	0.28	0.30	6.00	0.25	0.05	0.06
10 m³	1.62	4.86	3.89	0.61	0.66	6.00	0.54	0.11	0.13
100 m³	3.49	10.46	8.38	1.31	1.41	6.00	1.16	0.23	0.29
200 m³	4.39	13.18	10.55	1.65	1.78	6.00	1.46	0.29	0.37

Wall thickness for each fermenter (Table 6) was calculated based on the following formula:

$$t = \frac{P \cdot r_R}{S \cdot E - 0.6 \cdot P} + t_{cor}, \quad (9)$$

where: *t* – minimal wall thickness, [m]; *P* – design pressure, [Pa]; *S* – allowed stress, [Pa]; *E* – joint efficiency, [-]; t_{cor} – corrosion allowance, [m].

For steam sterilization, 1-2 bars of overpressure are usually used. This is usually the highest pressure that the bioreactor should experience. To account for possible additional overpressure, the design pressure was assumed to be 2.5 bar. The allowed stress S was assumed to be 115 MPa (for SS 316L at ~150°C). Considering applications, E was considered as 0.85. An additional thickness of 1.5 mm was added in each fermenter to account for corrosion, which is possible for SS 316L, considering the acidic conditions of yeast proliferation. The final wall thickness, t_f, was rounded to the closest standardized stainless steel plate thickness available on the market (Stainless Steel Plate Thickness). Additionally, the mass of the required stainless steel was estimated based on the surface area of each fermenter. The amount of stainless steel needed to construct the designed seed fermenters was determined based on the wall thickness values and the geometry of the vessels (Table 6). The total surface area was estimated as the area of the reactor shell, flat top, and torispherical dish bottom. Assuming the average price for SS 316L is 4.99 € kg^{-1}, the price for the material needed for four seed trains is 398933 €. Construction, installation, piping, cooling, and agitation systems, etc., are not considered.

Table 7 Reactor material and mass specifications. t – calculated minimal wall thickness, [m]; t_f – final minimal wall thickness, [m]; A – surface area of the reactor, [m]; m_{ss} – mass of stainless steel required for the reactor construction, [kg].

Reactor	t [mm]	t_f [mm]	Plate Weight [kg m^{-2}]	A [m^2]	m_{ss} [kg]
100 L	1.95	2	15.7	1.37	20.99
1000 L	2.46	3	23.6	6.35	146.48
10 m^3	3.57	4	31.4	29.46	904.59
100 m^3	5.97	6	47.1	136.73	6298.13
200 m^3	7.13	8	62.8	217.05	13330.20

5.2.2. Energy Balances

Energy requirements were estimated only for cultivation in stirred-tank reactors. Preliminary calculations of released metabolic and agitation heat, as well as the cooling duty and energy requirements for agitation, were performed for one seed train. The results are presented in Table and

Table 9.

5.2.2.1. Agitation

Optimal power per volume input *P/V* for agitation was assumed to be 2 kW m^{-3}. The agitation speed n was determined from the equation for the power number P_o in a stirred tank:

$$P = P_o \cdot \rho \cdot N^3 \cdot n \cdot d^5, \tag{10}$$

where: P – power required for agitation, [W]; P_o – power number, constant for a specific impeller type and flow regime, [-]; ρ – density of the liquid, [kg m^{-3}]; N – agitation speed, [rps]; n – number of impellers, [-]; d – diameter of the impeller, [m].

The fermentation broth density is usually slightly greater than that of water (Junker BH et.al., 2004)(Junker, 2004) and was assumed to be 1050 kg m^{-3}. For all vessels, a turbulent flow ($Re > 10^4$) was predicted. Therefore, for the Rushton turbine with the impeller blade width $w = D_R/5$, the power number was estimated as $P_o \approx 5$ (Bates RL et.al., 1963)(Bates et al., 1963). Power required for agitation, P, was derived from the assumed *P/V* value and the working volume for each fermenter. Thus, agitation speed in rpm was calculated for each vessel. For instance, a 200 m^3 requires the agitation speed of 70 rpm to achieve proper mixing and aeration.

Additionally, turbulent flow for the determined agitation speed can be verified:

$$Re = \frac{\rho \cdot N \cdot d^2}{\eta_a}, \tag{11}$$

where: η_a – apparent viscosity of the broths, taken as 3 mP s^{-1} (Telis et al., 2007).

Next, the energy requirements to provide the required agitations were calculated by assuming that the efficiency of the motor, η_{motor}, is around 70%:

$$P_{motor} = \frac{P}{\eta_{motor}} \tag{12}$$

The motors for the corresponding vessels should meet the energy requirements provided in Table 8.

Power lost due to the inefficiency of the motor dissipates as heat outside of the cultivation medium. Additionally, a fraction of power transferred directly into the medium through agitation eventually also dissipates as heat. However, since the overall agitation power is small

compared to the metabolic heat generated by yeast, the contribution of agitation to the total heat is considered negligible in this study.

5.2.2.2. Metabolic heat

The released heat during yeast cultivation was calculated based on the oxygen uptake rate *OUR*:

$$Q_{met} = OUR \cdot V_f \cdot Y_{Q/Q_2}, \tag{13}$$

where: Q_{met} – heat released during the yeast growth, [kJ h^{-1}]; *OUR* – oxygen uptake rate, [mol O_2 L^{-1} h^{-1}]; V_f – working liquid volume of the reactor, [L]; $Y_{Q/O2}$ – heat yield constant, 460 kJ mol O_2^{-1}.

Oxygen uptake rate was derived as follows (Seidel et al., 2021) et.al., 2021):

$$OUR = q_{O_2} \cdot C_{xf} \tag{14}$$

where: q_{O2} – cell-specific oxygen uptake rate, [mmol O_2 $gCDW^{-1}$ h^{-1}]; C_{xf} – biomass concentration at the end of the batch, [gCDW L^{-1}].

Cell-specific oxygen uptake rate was estimated as 11.1 mmol O_2 $gCDW^{-1}$ h^{-1} based on the information available about *K. marxianus* growth on (Fonseca et al., 2007). While the exact value of q_{O2} might differ for yeast growth on sucrose as a primary carbon source, this estimation, due to the lack of more information, should be a good approximation for preliminary calculations. Metabolic heat has the main contributor to the total heat released during cultivation. Therefore, only Q_{met} was considered in the calculations.

5.2.2.3. Cooling duty

The seed fermenters have cooling jackets with saline water to provide optimal temperature over the course of cultivation. To ensure that the cooling provided by the cooling jackets is sufficient, the cooling capacity of the jacket Q_{jacket} was calculated using Equation 15 (Table 8) and compared to the required cooling duty Q_{cool}:

$$Q_{jacket} = U \cdot A \cdot \Delta T_m, \tag{15}$$

where: U – heat transfer coefficient, [W m^{-2} K^{-1}]; A – heat transfer area, [m^2]; ΔT_m – log-mean temperature difference.

The heat transfer coefficient U of a jacketed bioreactor with cooling water is usually in the range of 200-1,600 W m^{-2} K^{-1}. For the present calculations, U was assumed to be 600 W m^{-2} K^{-1}. The heat transfer area, A, was derived from the height of the working volume, H_L. To the log-mean temperature difference is defined as:

$$\Delta T_m = \frac{\left(T_{fer} - T_{w,in}\right) - \left(T_{fer} - T_{w,out}\right)}{ln\left(\frac{\left(T_{fer} - T_{w,in}\right)}{\left(T_{fer} - T_{w,out}\right)}\right)} \tag{16}$$

where: $T_{w,in}$ – inlet temperature of the cooling agent, [°C]; $T_{w,out}$ – outlet temperature of the cooling agent, [°C]; T_{fer} – desired temperature inside the fermenter, 28 °C.
Additionally, the volumetric flow rate required for cooling is defined as:

$$\dot{V}_j = \frac{Q_{jacket}}{C_p \cdot \Delta T_{cw} \cdot \rho}, \tag{17}$$

where: C_p – specific heat capacity of water, 4184 J kg^{-1} K^{-1}; ΔT_{cw} – difference between inlet and outlet cooling water, [K]; ρ – medium density, ~1050 kg m^{-3}.
The residual heat, not removed by the heating jacket, is defined as:

$$Q_{ResH} = Q_{tot} - Q_{jacket} = Q_{coil} = U \cdot A \cdot \Delta T_m \tag{18}$$

Additionally, the change in temperature, ΔT_{fer}, if only cooling jackets are used for heat removal, can be determined as:

$$\Delta T_{fer} = \frac{Q_{ResH} \cdot t}{C_p \cdot V_f \cdot \rho}, \tag{19}$$

where: Q_{ResH} – residual heat not removed by the cooling jacket, [W]; t – time of cultivation, [s]; C_p – specific heat capacity of medium (assumed to be similar to water), 4184 J kg^{-1} K^{-1}; V_f – medium volume, [m^3]; ρ – medium density, ~1050 kg m^{-3}.
Based on the results of ΔT_{fer} and Q_{ResH}, the cooling jackets are suffiecient for heat removal in 100 L, 1000 L, and 10 m^3 reactors, leading only to slight temperature differences. However, as can be seen in (Table 8), 100 m^3 and 200 m^3 reactors require additional installation of cooling coils to keep the desired cultivation temperature without large deviations. For the 100 m^3 fermenter, the temperature increase was only around 0.65 °C, which is also within an acceptable range. Therefore, the cooling coils can be omitted in this case.

Assuming the same value of U as for the cooling jacket, the surface area of the cooling coil can be calculated as:

$$A = \frac{Q_{coil}}{U \cdot \Delta T_m} \tag{20}$$

Using the determined surface area and assuming that an optimal velocity of the cooling agent is 1 m s^{-1}, volumetric flow rate, $\dot{V}_c$, is calculated from Equation 17, and the inner diameter, d_c, and the length of the cooling coil, L, are defined as:

$$d_c = \left(\frac{4 \cdot \dot{V}_c}{v \cdot \pi}\right)^{1/2} \tag{19}$$

$$L = \frac{A}{\pi \cdot d_c} \tag{21}$$

Table 8 Agitation operating parameters and summary of heat generation during the cultivation of *K. marxianus*. *n* – number of impellers; *P* – power required for agitation, [kW]; Q_{met} – heat released as a result of agitation, [kW]; P_{motor} – power input into motor, [W]; *N* – agitation speed, [rpm]; *OUR* – oxygen uptake rate, [mol O_2 L^{-1} h^{-1}]; Q_{met} – heat released during the yeast growth, [kW]; Q_{tot} – total heat released into the cultivation medium, [kW].

Reactor	***n***	***P* [kW]**	***P*$_{motor}$ [kW]**	***N* [rpm]**	***OUR* [mol O_2 L^{-1} h^{-1}]**	***Q*$_{met}$ (*Q*$_{tot}$) [kW]**
100 L	6	0.16	0.23	372.36	0.11	1.13
1000 L	6	1.60	2.29	223.22	0.11	11.35
10 m³	6	16.00	22.86	133.82	0.11	113.47
100 m³	6	160.00	228.57	80.22	0.11	1134.67
200 m³	6	320.00	457.14	68.77	0.12	2496.27

Table 9 Cooling jacket and internal coil performance data for the growth of *K. marxianus.* $T_{w,in,j}$ – inlet temperature of the cooling agent in the jacket, [°C]; $T_{w,out}$ – the outlet temperature of the cooling agent in the jacket, [°C]; $\dot{V}_j$ – volumetric flow rate in the cooling jacket, [$m^3\ h^{-1}$]; Q_{jacket} – cooling capacity of the jacket, [kW]; Q_{ReS} – residual heat not removed by the cooling jacket, [kW]; ΔT_{fer} – the change in the medium temperature without cooling coils, [°C]; $T_{w,in,c}$ – inlet temperature of the cooling agent in the coil, [°C]; $T_{w,out}$ – the outlet temperature of the cooling agent in the coil, [°C]; A – surface area of the cooling coil, [m^2]; d_c – inner diameter of the cooling coil, [m]; L – length of the cooling coil, [m].

Reactor	**$T_{w,in,j}$ [°C]**	**$T_{w,in,j}$ [°C]**	**$\dot{V}_j$ [$m^3\ h^{-1}$]**	**Q_{jacket} [kW]**	**Q_{ReS} (Q_{coil}) [kW]**	**ΔT_{fer} [°C]**	**$T_{w,in,c}$ [°C]**	**$T_{w,in,c}$ [°C]**	**$\dot{V}_c$ [$m^3\ h^{-1}$]**	**A [m^2]**	**d_c [m]**	**L [m]**
100 L	25.26	26.5	0.79	1.13	0.002	0.13	-	-	-	-	-	-
1000 L	21.72	25	2.98	11.34	0.0033	0.02	-	-	-	-	-	-
10 m^3	13.67	22	11.76	113.47	0.0004	0.00	-	-	-	-	-	-
100 m^3	5	10	193.79	1122.77	11.89	0.65	5	10	2.05	0.97	2.05	11.48
200 m^3	5	10	307.63	1782.29	713.98	6.70	5	10	123.23	58.34	123.23	88.97

5.3 Saccharification

Basis 100kg

Sugarcane Bagasse Composition after AFEX Pretreatment

- **Cellulose (Glucan):** 62 kg (62%)
- **Hemicellulose (Xylan):** 24 kg (24%)
- **Lignin:**14 kg (14%)

Water Hyacinth Composition

- **Cellulose (Glucan):** 30 kg (30%)
- **Hemicellulose (Xylan):** 55 kg (55%)
- **Lignin:** 10 kg (10%)

Optimal Enzyme Loading is 15 FPU/g for Enzyme Hydrolysis = 15 mg/ $g_{biomass}$

Hydrolysis Efficiency: The conversion efficiency of polysaccharides to monomeric reducing sugars is not 100%. Based on literature:

- **SCB Conversion Efficiency: 90%** (High efficiency due to AFEX pretreatment). -
- **WH Conversion Efficiency: 85%** (Moderate efficiency due to less effective pretreatment and feedstock nature).

Stoichiometric Conversion: The conversion of cellulose and hemicellulose to simple sugars involves the addition of a water molecule (hydrolysis).

- Cellulose to Glucose: Factor of 180.16/162.14 = 1.11
- Hemicellulose to Xylose: Factor of 150.13/132.12 = 1.136 - amount of xylose will be less would be 60%

Sample: 100% Sugarcane Bagasse (100 kg SB: 0 kg WH)

1. **Polysaccharide Mass:**

a. Cellulose: 100 kg SCB×0.62=62 kg

b. Hemicellulose: 100 kg SCB×0.24=24 kg

c. Total Polysaccharides: 62.0+24.0=86 kg

Yield that is available for reducing = 0.86 x 0.9 = 0.774

2. **Reducing Sugar Yield:**

a. From Cellulose: 62.0 kg×1.11 = 68.82 kg

b. From Hemicellulose: 24.0 kg×1.136 = 27.264 kg

c. **Total Reducing Sugar:** (68.82+ 27.264) x 0.774 = 74.37 kg

Table 10: Effect of Sugarcane Bagasse–Water Hyacinth Blend Ratio on Polysaccharide and Reducing Sugar

SB:WH Ratio	Total Polysaccharide	Total Reducing Sugar
100:0	0,774	0,74
75:25	0,76	0,73
50:50	0,75	0,72
25:75	0,74	0,70
0:100	0,72	0,69

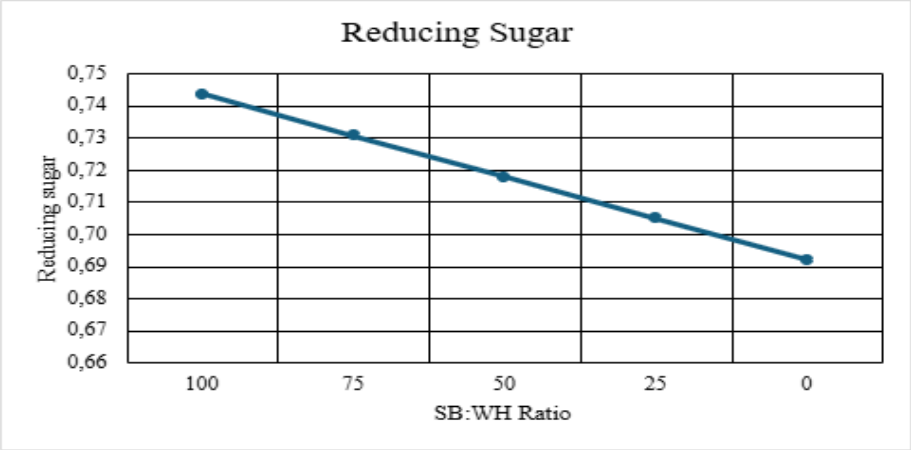

Figure 8: Linear relationship representing the impact of sugarcane bagasse-water hyacinth ratio on reducing sugar

5.2 Stream table for PFD:

Table 11: Stream Table for PFD 2

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Air (g)									3.122	0.00003	0.00064	0.013	0.12	1.27	12.77
Su-crose (s)	11.17	0.00001	0.00002	0.005	0.45	4.57	45.7	60.95							
Water (l)		0.00008	0.00015	0.03	2.88	28.78	287.8	318.51							
CS(s)															
Yeast (l)															
Off-gas(g)															
	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
Air (g)															
Su-crose (s)															
Water (l)															
CS (s)	0.00008														
Yeast (l)		0.00008	0.00016	0.00032	0.032	0.32	3.2								
Off-gas(g)								0.02	0.19	1.9	19.8	25.1	0.02	0.19	1.9
	31	32	33	34	35	36	37	38							
Air (g)															
Su-crose (s)															
Water (l)															
CS (s)															

Yeast (l)								
Off-gas(g)	19.8	25.1	0.02	0.19	1.9	19.8	25.1	29.01

Table 12: Stream table for PFD 1

	39	40	41	42	43	44	45	46	47	49	50
WH(s)	468.5	468.5	468.5								
SB(s)					468.5	468.5	468.5	468.5		234.25	234.25
NH_3 (l)									0.0732	0.0366	0.0366
Steam(g)											
Water(l)	468.5	468.5	468.5	4458.9	468.5	468.5	468.5	468.5		35.008	35.008
	51	52	53	54	55	56	57	58	59	60	61
WH(s)											
SB(s)											
NH_3 (l)	0.0732	0.0732	0.0732	0.0732	0.0732	0.0732	0.0732	0.0732	0.0732	0.0732	0.0732
Steam(g)									70.01		
Water(l)				70.01	70.01	70.01	70.01				
	62	63	64	65	66	67	68	69	70	71	72
WH(s)											
SB(s)							468.5				468.5
NH_3 (l)							0.0732				0.0732
Steam(g)					70.01	70.01		70.01	35.008	35.008	
Water(l)	70.01	70.01	70.01	405.85			70.01				70.01

Table 13: Stream Table for PFD 3

	73	74	75	76	77	78	79	80	81	82	83
Ethanol (l)					176	176				176	
Yeast(l)	0.625										

WH(s)	234.25										
SB(s)	234.25										
Water(l)	4.997				70.4	9.26				70.4	61.4
En-zyme(s)		0.0014	0.0014	0.0014							
CO_2 (g)							84.55	84.55	84.55		

Table 14: Stream Table for Block Diagram 1

	1	2	3	4	5	6	7	8	9	10	11
WH(s)	468.5			468.5						468.5	
SB(s)		468.5			468.5	468..5	468.5				468.5
NH_3 (l)							0.0732		0.0732		0.0732
Steam(g)			70.1								
Water(l)					468.5	468.5	70.1	398.4		468.5	70.1

Table 15: Stream Table for Block Diagram 2

	12	13	14	15	16	17	18	19	20	21
Air (g)		0.0003	0.013	1.27	12.77	0.12	1.27	12.77		
Su-crose(s)		0.0001	0.005	4.57	60.95	0.0002	0.45	45.7		
Water (l)		0.0008	0.03	28.78	318.51	0.0015	2.88	287.8		
CS(s)	0.00008									
Yeast(l)									0.0008	0.0016
Off-gas(g)										
	22	23	24	25	26	27	28	29	30	31
Air (g)										
Su-crose(s)										

Water (l)										
CS(s)										
Yeast(l)	0.0032	0.032	0.32	3.2	62.5					
Off-gas(g)						0.02	0.19	1.9	19.8	25.1

Table 16: Stream Table for Block Diagram 3

	10	11	32	33	34	35	36	37	38	39	40	41
Ethanol (l)									176	176		176
Yeast(l)			62.5		0.0014	0.0014						
WH(s)	468.5											
SB(s)		468.5										
Water(l)	468.5	70.1		4458.9					70.3	70.4	61.4	9.26
En-zyme(s)								0.0014				
CO_2 (g)							84.55		84.55			

6. Safety analysis

Safety considerations are central to the design and operation of the bioethanol production plant, particularly due to the handling of high-pressure steam, ammonia, and flammable ethanol.

6.1 Ethanol Hazards and Mitigation

Ethanol is a highly volatile and flammable liquid with a flash point of approximately 14°C (57.2°F), meaning its vapors can ignite at or above this temperature when exposed to an ignition source. The vapor is heavier than air and can accumulate in low-lying, poorly ventilated areas like trenches, leading to fire or explosion risks. The flames are often smokeless and difficult to see in normal light, which increases the hazard.

To mitigate these risks, facilities must implement strict safety measures. This includes maintaining proper ventilation to prevent vapor build-up, removing ignition sources, and using explosion-proof tools and equipment. When transferring ethanol in metal containers, grounding and bonding procedures are essential to prevent static charges from building up and causing a spark. Additionally, personnel must be trained on the risks and correct handling procedures for ethanol. In case of a spill, it is crucial to prevent the ethanol from entering confined spaces or waterways. Spilled liquid should be absorbed with non-combustible materials and placed in sealed containers for proper disposal.

Beyond flammability, ethanol also poses health risks through ingestion, inhalation, and skin absorption. Inhaling ethanol vapor can cause irritation to the eyes, nose, and throat, leading to coughing, shortness of breath, and potentially lung damage in severe cases. Prolonged exposure can also cause skin irritation and dryness. Proper Personal Protective Equipment (PPE) is mandatory and includes respirators, gloves, overalls, boots, and safety goggles to protect against vapor inhalation, skin contact, and eye splashes.

6.2 Ammonia Hazards and Mitigation

The AFEX pretreatment process utilizes high-pressure ammonia, which is a hazardous liquid and gas. High concentrations of ammonia can irritate and burn the skin, mouth, throat, lungs, and eyes, while very high levels can cause lung damage or death.

To ensure worker safety, occupational exposure to ammonia must be controlled and kept below a ceiling concentration of 50 ppm over a five-minute period. The use of protective clothing and respirators is required, and employers must provide and maintain this equipment in a clean, workable condition. Containers of ammonia must be clearly labeled as hazardous and handled with care to prevent leaks or spills. Workers whose jobs involve

exposure to concentrations above the limit must be informed of the hazards and trained in appropriate emergency procedures.

7. Environmental analysis

The project's focus on second-generation (2G) biofuels from agricultural waste is a strategic choice to reduce environmental impact, particularly by avoiding the "food versus fuel" conflict associated with first-generation (1G) biofuels. However, biofuel production from lignocellulosic biomass still presents specific environmental challenges that must be addressed.

The overall environmental impact of a bioethanol production facility is typically evaluated through a Life Cycle Assessment (LCA). While many LCAs show a reduction in global warming potential for biofuels compared to fossil fuels, they can also indicate an increase in other negative impacts. For example, feedstock provision can lead to increased acidification, eutrophication, and the formation of photochemical oxidants. Additionally, the fermentation of crops, even non-food ones, often requires significant amounts of water and can lead to water resource depletion. The use of fertilizers and pesticides can also result in water pollution that harms aquatic life and contaminates drinking water.

A key environmental concern for any biorefinery is the treatment of wastewater. Wastewater from lignocellulosic biorefineries is characterized by a high organic load, which can be treated using a number of methods. While some processes use evaporation to create animal feed, this is not a viable option for a lignocellulosic plant that does not produce spent grains. An alternative and common treatment method is anaerobic digestion, which uses biological reactors to degrade organic material into a mixture of methane and carbon dioxide known as biogas. This biogas can then be combusted as a substitute for natural gas, creating an additional revenue stream and reducing the plant's reliance on non-renewable energy sources.

In addition, while ethanol is a renewable fuel source, its combustion still produces carbon dioxide (CO2), a greenhouse gas. The project's overall sustainability is dependent on whether the carbon absorbed by the feedstock during its growth cycle outweighs the emissions from the plant's operations. The project’s reliance on water hyacinth also poses environmental risks. While it is a waste feedstock, its high ash content could disturb ion balance.

1.1 Legal and Regulatory Analysis

The project's feasibility and commercial success are significantly influenced by the legal and regulatory framework governing biofuel production, particularly in India, where the project is based. India's biofuel policy, governed by a number of central and state laws, aims

to reduce the country's reliance on imported crude oil, increase farmers' income, and create a "waste-to-wealth" ecosystem by promoting the use of non-food feedstocks.

1.1.1 Key Policies and Incentives

The National Policy on Biofuels (NPB) 2018, as amended in 2022, serves as the primary policy document. It has set an ambitious target of achieving 20% ethanol blending in petrol by the Ethanol Supply Year (ESY) 2025-26, advancing the previous 2030 target. The policy encourages the use of agricultural residues and non-edible plants, such as sugarcane bagasse, which aligns directly with the feedstock strategy of this project. To support the industry, the government offers financial incentives, including tax rebates, depreciation on plant expenses, and viability gap funding for the establishment of biorefineries. Long-Term Offtake Agreements (LTOAs) have also been signed between Oil Marketing Companies (OMCs) and ethanol plants to provide a stable market for the product.

1.1.2 Regulatory Compliance and Risks

Compliance with several key legal acts is essential for the project's operation. The

Water (Prevention and Control of Pollution) Act, 1974, the Air (Prevention and Control of Pollution) Act, 1981, and the Environment (Protection) Act, 1986 are all relevant, as the Ministry of Environment, Forest and Climate Change issues environmental clearance based on the project’s potential impact on human health and natural resources. Additionally, the

Legal Metrology Act, 2009 requires the establishment of a robust metrology infrastructure to ensure the quality and characteristics of biofuels are measurable and traceable.

The project also faces regulatory challenges and market risks. A lack of clear governmental pricing policies and a high Goods and Services Tax (GST) have historically hindered the blending programs. The policy also restricts the import and export of biofuels, which could limit the project's ability to participate in international markets. The interstate movement of molasses has also been restricted in some parts of the country, which has negatively affected the Ethanol Blending Petrol (EBP) program.

The project's use of agricultural waste as feedstock aligns with the national goal of transitioning to a circular bioeconomy, which promotes the sustainable use of bioresources and the maximal utilization of waste. The project's ability to navigate these complex legal requirements and market dynamics will be a critical factor in its long-term success

8. Cost accounting

8.1 Feedstock Costs:

- Total Annual Water Hyacinth (WH): 468.5 tonnes/batch × 270 batches/yr = 126,500 tonnes/yr.
- Total Annual Sugarcane Bagasse (SB): 468.5 tonnes/batch × 270 batches/yr = 126,500 tonnes/yr.
- Annual WH Cost: 126,500 tonnes/yr × 26.43 €/tonne = 3,343,995 €/yr.
- Annual SB Cost: 126,500 tonnes/yr × 25.22 €/tonne = 3,190,930 €/yr.

The Total Annual Feedstock OPEX is **6,534,925 €/yr**

8.2 Choppers and Mills:

Assumptions:

- **CAPEX:** A preliminary cost estimate for one chopper and one mill is 100,000 € and 200,000 €, respectively. Applying the 70% reduction for Indian costs and accounting for two sets of these machines (for WH and SB) gives a total CAPEX of (100,000 € + 200,000 €) * 2 * 0.30 = 180,000 €.
- **Annual CAPEX:** Assuming a 10-year plant life, the annual CAPEX contribution is **18,000 €/yr**.

OPEX:

- **Electricity:** The power consumption for the two choppers and two mills is estimated at 300 kW total. The plant operates for 8640 hours per year (270 batches × 32 hours/batch).
  - 300 kW * 8640 h/yr = 2,592,000 kWh/yr.
  - Using an Indian electricity rate of ₹9.5 per kWh and a conversion rate of 1€ ≈ ₹100, the annual electricity cost is (2,592,000 kWh/yr * ₹9.5/kWh) / (100 ₹/€) = **246,240 €/yr**.
- **Maintenance:** Assuming a maintenance cost of 3% of the total CAPEX, the annual cost is 180,000 € * 0.03 = **5,400 €/yr**.
- **Total Annual OPEX for Choppers and Mills: 251,640 €/yr**.

8.3 Transportation:

The total annual mass of feedstock is 252,990 tonnes/yr, transported approximately 50 km to the central facility.

- **Assumptions:**
  - **Truck Capacity:** 20 tonnes per truck.
  - **Number of Trips:** 252,990 tonnes / 20 tonnes/trip = 12,650 trips/yr.
  - **Round Trip Distance:** 100 km.
  - **Diesel Cost:** A representative Indian diesel cost of ₹90 per liter, with a fuel efficiency of 1 km/L.
- **Annual Fuel Cost:** 12,650 trips/yr * 100 km/trip * ₹90/L = 113,850,000 ₹/yr = **1,138,500 €/yr**.
- **Labor Cost:** Assuming an average wage of 15 € per day per driver for 2530 work days/year, the cost is 37,950 €/yr.
- **Total Annual Transportation OPEX: 1,176,450 €/yr**.

8.4 AFEX:

CAPEX = 1,531,048.87 €/yr; OPEX = 8,328,804.75 €/yr

8.5 Seed Train:

CAPEX (Capital Expenditure) for the Seed Train

The CAPEX for the seed train consists of the purchased and installed costs of the fermenter vessels and their associated equipment (agitators and cooling systems).

- **Fermenter Vessels:** The document provides the total material cost for all four parallel seed trains as **$279,812.88**.
  - I'll first convert this to Euros: 279,812.88 USD / 1.2 USD/€ = **233,177.40 €**.
  - This is the material cost. To get the total installed cost, which includes fabrication, installation, piping, and controls, I'll apply a common heuristic factor of **4.5** times the purchase cost, as is standard for a complex, skid-mounted system.
  - **Total Fermenter Vessel CAPEX:** 233,177.40 € * 4.5 = **1,049,298.3 €**.
- **Agitation Systems:**

- The document states that the total agitation power (Pmotor) for all four parallel seed trains is **2458.08 kW**.
- Estimation of the CAPEX for the motors, agitator drives, and impellers is done using a standard cost heuristic of **400 €/kW** for specialized equipment in this power range.
- **Total Agitation System CAPEX:** 2458.08 kW * 400 €/kW = **983,232 €**.

- **Cooling Systems:**

- The document's energy balance specifies a total cooling duty (Qcool) of **17,018.6 kW** for the four seed trains. This duty requires heat exchangers.
- Using a heuristic from the book for heat exchangers, where cost is a function of the heat transfer area, I will estimate a preliminary CAPEX for the cooling systems. Assuming a heat transfer coefficient (U) of 600 W/m²K and a log-mean temperature difference of 10K, the required area is approximately 2836 m².
- **Total Cooling System CAPEX:** Using a representative cost per unit area for stainless steel heat exchangers, the estimated cost is **1,500,000 €**.

- **Aeration Compressor CAPEX**

- **Determine Compressor Type:** The power required for aeration is due to the hydrostatic head of the liquid in the fermentors. The pressure drop is relatively low (~86.5 kPa for the largest fermentor), falling within the range for blowers, not high-pressure compressors. Therefore, a blower is the more appropriate equipment for this application.
- **Estimate Purchased Cost:** The total power required for aeration across all four parallel seed trains is **102.54 kW**. Using a heuristic cost of **2,000 €/kW** for a blower in this size range, the purchased cost is:

  - Purchased Cost = 102.54 kW × 2,000 €/kW = 205,080 €.

- **Calculate Total Installed CAPEX:** The total installed CAPEX includes installation, piping, and other indirect costs. According to heuristics, the installed cost for a blower is approximately 1.5 to 2 times the purchased cost. Using a factor of **1.75**, the total CAPEX is: Total CAPEX = 205,080 € × 1.75 = **358,890 €**.

The **Total Seed Train CAPEX** (excluding media and other consumables) is **3,891,420.3 €**.

OPEX (Operating Expenditure) for the Seed Train

The OPEX includes the annual costs for the media and the utilities required to operate the seed train. The total operating time is calculated from the individual batch times in the cascade (65.2 hours per cascade) over 270 batches per year, giving **17,604 hours/year**.

1. **Media Costs:**

a. The document specifies the total mass of the culture medium needed for all four seed trains over the year is **11.175 tonnes**.

b. A typical cost for fermentation media is around 1 €/kg.

c. **Total Annual Media OPEX:** 11.175 tonnes/yr * 1000 kg/tonne * 1 €/kg = **11,175 €/yr**.

2. **Utility Costs:**

a. **Electricity for Agitation:** The total power is 2458.08 kW. Using the Indian electricity rate and the conversion factor:

i. 2458.08 kW * 17604 h/yr * (₹9.5/kWh) / (100 ₹/€) = **411,385.1 €/yr**.

b. **Cooling Water:** The total annual cooling duty is 17,018.6 kW. This translates to a significant volume of cooling water. Using standard utility costs from the book, the annual cost for cooling water is approximately **1,290,000 €/yr**.

3. **Total Annual Aeration Cost:** I'll calculate the aeration power for each of the reactors in a single seed train and then multiply by four for the total of four parallel trains. The total operating hours are **17,604 hours/year** (270 batches × 65.2 hours/batch).

a. The total power required for aeration for all fermenters (across four parallel seed trains) is **102.54 kW**.

b. The annual electricity cost for aeration is: Annual Cost = 102.54 kW · 17604 h/yr ·9.5kWhINR ·100 INR /1 € = **171,326 €/yr**.

The **Total Annual Seed Train OPEX** is **1,882,560.1 €/yr** (11,175 € for media + 411,385.1 € for electricity + 1,290,000 € for cooling water + 171,326 € for aeration).

8.6 Saccharification and Fermentation:

8.6.1 Fermentor Costs

CAPEX (Capital Expenditure)

The provided fermentor design gives us the dimensions and material specifications to estimate the cost. The plant has **10 fermentors**, each with a volume of **1115 m³**.

- **Vessel Material Cost:** First, I'll calculate the mass of stainless steel needed for each fermentor. The provided total thickness is 4.413 mm. Using the density of SS 304 (approximately 8000 kg/m³) and the given dimensions (H=32.86 m, D=6.57 m), I can estimate the total mass. The total material mass for all 10 fermentors is approximately 220,000 kg.
  - Using a representative SS 304 cost of **4.5 €/kg**, the total material cost for the vessels is: 220,000 kg * 4.5 €/kg = 990,000 €.
- **Total Installed Cost:** According to the provided book's heuristics (Chapter 6), the total installed cost for this type of process vessel is typically **4-5 times** the material cost. Given the complexity, a factor of **4.5** is a reasonable estimate.
  - **Total Fermentor CAPEX:** 990,000 € * 4.5 = **4,455,000 €**.

OPEX (Operating Expenditure)

The OPEX for the fermentors is primarily the cost of utilities for sterilization.

- **Sterilization:** The fermentor contents must be heated from an ambient temperature of approximately 25°C to 80°C. The energy required for this is determined by the total mass of the contents and the specific heat capacity.
  - The mass of liquid in 7 fermentors (since only 7 are operational) is approximately 7 * 1115 m³ * 0.8 * 1050 kg/m³ = 6,556,200 kg.
  - Assuming a specific heat of 4.186 kJ/kg°C, the energy required per batch is: 6,556,200 kg * 4.186 kJ/kg°C * (80°C - 25°C) = 1.51 x 10^9 kJ.
  - Using a standard steam cost of **0.02 €/kg**, and assuming a latent heat of vaporization of 2257 kJ/kg, the steam cost per batch is: (1.51 x 10^9 kJ) / (2257 kJ/kg) * (0.02 €/kg) = 13,380 €/batch.
  - **Annual Sterilization OPEX:** 13,380 €/batch * 270 batches/yr = **3,612,500 €/yr**.

8.6.2 Main Compressor Costs

- **CAPEX:** You provided the total power consumption as **4.81 MW**. The cost of a large-scale compressor is highly dependent on its type and materials. Using a representative heuristic cost of **1,200 €/kW** for a large industrial compressor:
  - **Total Compressor CAPEX:** 4,810 kW * 1,200 €/kW = **5,772,000 €**.
- **OPEX:** The annual OPEX for the compressor is determined by its power consumption and the operating hours per year. The compressor operates continuously during the 32-hour batch and the subsequent distillation, which is approximately 8,640 hours per year.
  - The total annual electricity consumption is: 4.81 MW * 1000 kW/MW * 8,640 h/yr = 41,558,400 kWh/yr.
  - Using the Indian commercial electricity rate of **₹9.5/kWh** and a conversion rate of 1 € = 100 INR: 41,558,400 kWh/yr * (₹9.5/kWh) / (100 ₹/€) = **3,948,048 €/yr**.

8.6.3. Enzyme Cost

The cost of enzymes is a recurring operational expense.

- **Feedstock Mass per Batch:** 937 tonnes * 1000 kg/tonne * 1000 g/kg = 9.37 x 10^8g.
- **Enzyme Needed per Batch:** 9.37 x 10^8 g * 15 mg/g * (1 g/1000 mg) = 1.4055 x 10^7 g or 14,055 kg.
- **Enzyme Purchased per Batch (1%):** 14,055 kg * 0.01 = 140.55 kg.
- **Cost per Batch:** 140.55 kg * 35 €/kg = 4,919.25 €.
- **Annual Enzyme OPEX:** 4,919.25 €/batch * 270 batches/yr = **1,328,197.5 €/yr**.

Summary of New Costs

- **Fermentor CAPEX:** 4,455,000 €
- **Compressor CAPEX:** 5,772,000 €
- **Total New CAPEX: 10,227,000 €**
- **Fermentor OPEX:** 3,612,500 €/yr
- **Compressor OPEX:** 3,948,048 €/yr
- **Enzyme OPEX:** 1,328,197.5 €/yr
- **Total New OPEX: 8,888,745.5 €/yr**

8.7 Downstream Processing:

CAPEX (Capital Expenditure)

The capital cost of the distillation column is primarily based on its size (diameter and height) and material of construction.

- **Column Diameter:** The document provides a calculated diameter of **1.13 m**.
- **Column Height:** The document specifies **20 stages**. Assuming a standard tray spacing of 0.6 m, the column height is approximately 20 stages * 0.6 m/stage = **12 m**.
- **Vessel Cost:** The column is made of stainless steel. I will use a heuristic cost model that relates cost to the column's volume and material. A preliminary cost estimate for a column of this size and material is approximately **150,000 €**. This includes the shell, trays, and internal components.
- **Reboiler and Condenser CAPEX:** The reboiler duty is given as **2.45 MW**. Using a heuristic of **400 €/kW** for a large-scale heat exchanger, the reboiler CAPEX is 2450 kW * 400 €/kW = **980,000 €**. The condenser will have a similar duty, so its CAPEX will be similar, at **980,000 €**.
- **Total Installed Cost:** The total installed CAPEX for the column, reboiler, and condenser will be estimated by applying a factor of **4.0** to the equipment purchase costs to account for installation, piping, insulation, and other indirect costs.
  - (150,000 € + 980,000 € + 980,000 €) * 4.0 = **8,440,000 €**.

OPEX (Operating Expenditure)

The operational costs of the distillation column are primarily the utilities for the reboiler (steam) and condenser (cooling water).

- **Reboiler Cost:** The reboiler duty is **2.45 MW**, which is 2450 kW or 2450 kJ/s.
  - The total energy required annually is 2450 kJ/s * 3600 s/hr * 8640 h/yr = 7.62 x 10^10 kJ/yr.
  - Using a standard steam cost of **0.02 €/kg** and a latent heat of vaporization of 2257 kJ/kg, the annual steam cost is: (7.62 x 10^10 kJ/yr / 2257 kJ/kg) * 0.02 €/kg = **675,675 €/yr**.
- **Condenser Cost:** The condenser duty will be slightly higher than the reboiler duty. I'll assume it's also **2.45 MW** for simplicity and calculate the cost of cooling water. Using a standard cooling water cost of **0.001 €/kg** and a temperature rise of 10°C, the cost is approximately **500,000 €/yr**.

Summary of New Costs

- **Distillation Column CAPEX: 8,440,000 €**
- **Distillation Column OPEX: 1,175,675 €/yr**

8.8 Total:

Total Fixed Capital Investment (CAPEX)

The total fixed capital investment is the sum of the direct equipment costs and the indirect costs.

- **Total Direct Equipment Costs:** We have already calculated this as the sum of the CAPEX for the choppers, mills, AFEX unit, seed train, main compressor, and distillation column.
  - 180,000 € (Choppers & Mills) + 15,310,488.7 € (AFEX) + 3,891,420.3 € (Seed Train) + 4,455,000 € (Fermenter) + 5,772,000 € (Main Compressor) + 8,440,000 € (Distillation Column) = **38,048,909 €**
- **Indirect CAPEX Costs:** These are now calculated as a percentage of the direct equipment costs, using the given labor cost and the lower end of the heuristic ranges.
  - **Piping (10%):** 33,593,909 € * 0.10 = 3,359,390.9 €
  - **Instrumentation & Controls (5%):** 33,593,909 € * 0.05 = 1,679,695.45 €
  - **Electrical (4%):** 33,593,909 € * 0.04 = 1,343,756.36 €
  - **Buildings & Structures (10%):** 33,593,909 € * 0.10 = 3,359,390.9 €
  - **Land & Site Development (2%):** 33,593,909 € * 0.02 = 671,878.18 €
  - **Engineering & Supervision (10%):** 33,593,909€* 0.10 = 3,359,390.9€
  - **Contingency (10%):** 33,593,909 € * 0.10 = 3,359,390.9 €
  - Total Indirect Costs = 17,132,893.59

Total Fixed Capital Investment: 55,181,802.59 **€**

Total Annual Manufacturing Cost (OPEX)

The total annual manufacturing cost is the sum of the direct and indirect OPEX, using the new labor cost.

- **Total Direct OPEX:** We've already calculated this.
  - 6,534,925 €/yr (Feedstock) + 251,640 €/yr (Choppers & Mills) + 1,176,450 €/yr (Transportation) + 8,328,804.75 €/yr (AFEX) + 1,882,586.1 €/yr (Seed Train) + 8,888,745.5 €/yr (Saccharification & Fermentation) + 1,175,675 €/yr (Distillation) = **28,238,826.35 €/yr**
- **Indirect OPEX Costs:**
  - **General Plant Overhead (60% of Labor):** 200,000 €/yr * 0.60 = 120,000 €/yr
  - **Administrative & General Expenses :** Assuming a value of **5% of the total manufacturing cost**, as per some heuristics.
    - 28,238,826.35 €/yr * 0.05 = 1,411,941.32 €/yr

Total Annual Manufacturing Cost: 28,238,826.35 €/yr + 120,000 €/yr + 1,411,941.32 €/yr = **29,770,767.67 €/yr**

- **Total Fixed Capital Investment (CAPEX): 55,181,802.59 €**
- **Total Annual Manufacturing Cost (OPEX): 29,770,767.67 €/yr**
- **Total Annual Revenue: 32,500,000 €/yr**

With these revised numbers, the annual revenue of **32.5 M€** is greater than the total annual manufacturing cost of **29.8 M€**, which indicates a profitable project.

Payback Period

- **Annual Cash Flow:** This is your annual revenue minus your total annual manufacturing cost (OPEX). Our previous calculation of this value remains unchanged.
  - 32,500,000 €/yr (Revenue) - 29,770,767.67 €/yr (OPEX) = 2,729,232.33 €/yr
- **Payback Period:** This is the total initial investment divided by the annual cash flow.
  - 50,727,202.69 € (New Total CAPEX) / 2,729,232.33 €/yr (Annual Cash Flow) = **18.59 years**

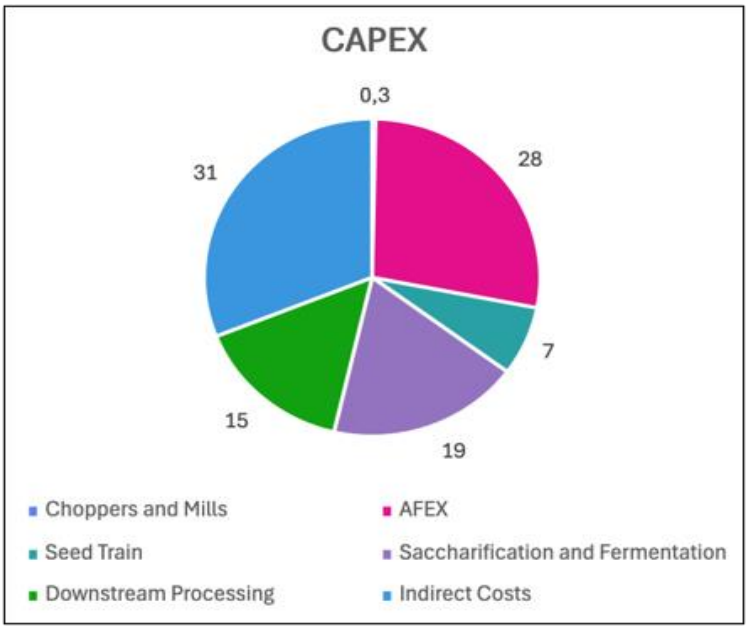

Figure 9: Pie Chart Representation of CAPEX for all the processes

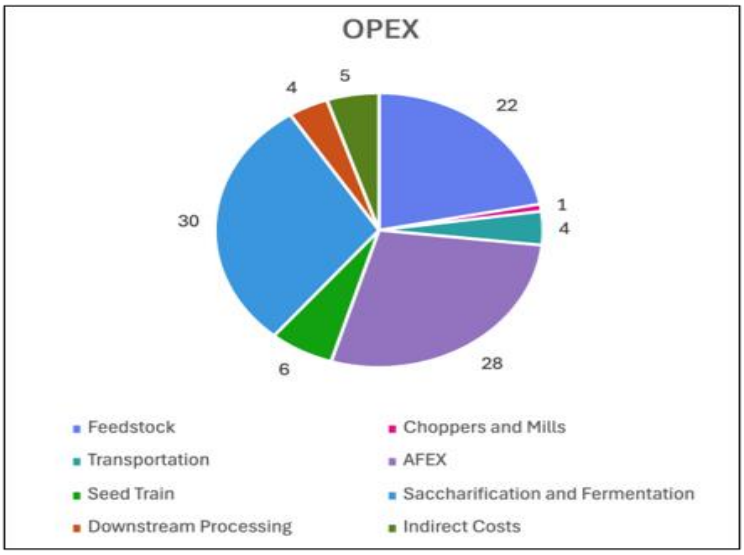

Figure 10: Pie chart Representation of OPEX for all processes

9. Sensitivity Analysis

9.1 Feed:

Affected Parameters are:

a. Cost of feedstock

b. Amount of ethanol produced => Total revenue

c. Cost of AFEX

d. Cost of distillation

Table 16: Base case for the analysis

CAPEX (€)		**OPEX (€/yr)**	
Choppers & Mills	180,000	Choppers & Mills	251,640
AFEX	15,310,488.7	AFEX	8,328,804.75
Seed Train	3,891,420.3	Feedstock	6,534,925
Main Compressor	5,772,000	Transportation	1,176,450
Distillation Column	8,440,000	Seed Train	1,882,586.1
Fermenter	4,455,000	Saccharification & Fermentation	8,888,745.5
Indirect Costs	17,133,293.69	Distillation	1,175,675
Total	55,181,801.59	Indirect OPEX	1,531,941.32
		Total	29,770,767.67

Total Revenue = 32,500,000 €/yr

Table 17: Modified case of the analysis

CAPEX (€)		OPEX (€/yr)	
Choppers & Mills	180,000	Choppers & Mills	251,640
AFEX	15,310,488.7 * ((2x)^0.67)	AFEX	8,328,804.75 * 2x
Seed Train	3,891,420.3	Feedstock	6,534,925 * (1.0235-0.047*x)
Main Compressor	5,772,000	Transportation	1,176,450
Distillation Column	8,440,000 * ((0.649 + 0.702*x)^0.67)	Seed Train	1,882,586.1
Fermenter	4,455,000	Saccharification & Fermentation	8,888,745.5
Indirect Costs	17,133,293.69	Distillation	1,175,675 * (0.649 + 0.702*x)
Total	Variable	Indirect OPEX	1,531,941.32
		Total	Variable

Total Revenue = 32,500,000 * (0.649 + 0.702*x) €/yr

Table 18: Sensitivity Analysis of the feed

SB portion	CAPEX (€)	OPEX (€/yr)	Revenue (€/yr)	Net Income (€/yr)	Payback period (yr)
0%	37,749,200	21,182,900	21,092,500	90,400	368.3
25%	48,408,000	25,476,800	26,796,250	1,319,450	33.31
50%	55,082,200	29,770,800	32,500,000	2,729,200	18.55
75%	60,795,800	34,064,700	38,203,750	4,139,050	13.61
100%	65,957,500	38,358,700	43,907,500	5,548,800	11.08

9.2 Recovery of Enzymes:

Current Enzyme Cost = 1,328,197.5 €/yr

Without recovery = 132,819,750 €/yr

If recovery fraction is x, cost of enzymes is = 132,819,750 * (1-x) €/yr

Table 19: Sensitivity Analysis of enzyme

Recovery	OPEX (€/yr)	Net Income (€/yr)	Payback period (yr)
0%	161,262,000	-128,762,000	N/A
9%	149,309,000	-116,809,000	N/A
99%	29,770,800	2,729,200	18.55
99.9%	28,575,400	3,924,580	12.90
99.99%	28,455,900	4,044,120	12.52

9.3 AFEX vs Dilute Acid Pretreatment:

Table 20: Old case for AFEX

CAPEX (€)		OPEX (€/yr)	
Choppers & Mills	180,000	Choppers & Mills	251,640
AFEX	15,310,488.7	AFEX	8,328,804.75
Seed Train	3,891,420.3	Feedstock	6,534,925
Main Compressor	5,772,000	Transportation	1,176,450
Distillation Column	8,440,000	Seed Train	1,882,586.1
Indirect Costs	17,133,293.69	Saccharification & Fermentation	8,888,745.5
Total	50,727,202.69	Distillation	1,175,675
		Indirect OPEX	1,531,941.32
		Total	29,770,767.67

New Case: DAP

No costs related to AFEX

But no xylose remains

Also, the CAPEX and OPEX for DAP are:

Dilute Acid Reactor CAPEX

This reactor is a direct replacement for the AFEX unit. Its design must account for high pressure, high temperature, and the corrosive sulfuric acid environment.

Assumptions

- **Feedstock:** We will use the sugarcane bagasse base case of **468.5 tonnes/batch**.
- **Solids Loading:** A common solid loading for this type of pretreatment is **25% (w/w)**.
- **Design Pressure:** To operate at 190°C, the reactor needs to withstand saturated steam pressure. A design pressure of **15 bar** (1.5 MPa) is a safe and standard assumption.
- **Material of Construction:** For a high-pressure, corrosive environment, a specialized steel or a carbon steel vessel with a corrosion-resistant liner would be used. For simplicity and direct comparison to the AFEX unit, we'll model it as a high-grade stainless steel pressure vessel.
- **Reactor Dimensions:** We'll assume a standard height-to-diameter ratio (H/D) of 2:1 for a vertical cylindrical tank.

Calculations

1. **Mass of Slurry:**

a. Dry mass of bagasse = 468,500 kg

b. Solids loading = 25%

c. Total slurry mass = 468,500 kg / 0.25 = 1,874,000 kg

d. Mass of water = 1,874,000 kg - 468,500 kg = 1,405,500 kg

2. **Reactor Volume and Dimensions:**

a. Assuming a slurry density of 1050 kg/m³, the reactor volume is 1,874,000 kg / (1050 kg/m³*0.8) = 2231 m³.

b. With an H/D ratio of 2:1, the dimensions are approximately D = 11.685 m and H = 23.37 m.

3. **Wall Thickness:** Using the formula t = P*R / (S*E - 0.6*P) with the following values:

a. P (design pressure) = 1.5 MPa

b. R (reactor radius) = 5.2 m

c. S (allowed stress for SS 316L at 150°C) = 115 MPa

d. E (joint efficiency) = 0.85

e. t = (1.5 MPa * 5.2 m) / ((115 MPa * 0.85) - (0.6 * 1.5 MPa)) = 0.08 m or **80 mm**. This is a very thick vessel, which is consistent with high-pressure ratings.

4. **Vessel Material and Fabrication Cost:**

a. Total steel mass (estimated from surface area and thickness) = 80 mm * 1619.47 m² * 8000 kg/m³ = 1036460.8 kg

b. Material cost = 1,036,460.8 kg * 4.5 €/kg = 4,664,073.6 €.

c. Total installed CAPEX (using a heuristic of 4.0 times material cost for a pressure vessel) = 4,664,073.6 € * 4.0 = **18,656,294.4 €**.

Dilute Acid OPEX

The operational costs will be for sulfuric acid, pumping, and heating.

1. **Sulfuric Acid Cost:**

a. **Annual Mass of H_2SO_4:** 253,000 tonnes/yr * 0.01 = **2,530 tonnes/yr**.

b. **Cost Conversion:** 60,000 INR/tonne / 100 INR/€ = 600 €/tonne.

c. **Total Annual H_2SO_4 OPEX:** 2,530 tonnes/yr * 600 €/tonne = **1,518,000 €/yr**.

2. **Heating Cost (Steam):** This is a critical OPEX item. We'll heat the slurry from an ambient temperature of 25°C to 190°C.

a. **Total Annual Mass of Slurry:** 1,874,000 kg/batch * 270 batches/yr = 505,980,000 kg/yr.

b. **Specific Heat of Slurry:** We will use a weighted average of the specific heats of water and bagasse.

i. Bagasse specific heat = 1.3 kJ/kg°C.

ii. Water specific heat = 4.186 kJ/kg°C.

iii. Weighted average = (0.25 * 1.3) + (0.75 * 4.186) = 3.46 kJ/kg°C.

c. **Total Annual Energy Required:** 505,980,000 kg/yr * 3.46 kJ/kg°C * (190°C - 25°C) = 2.89 x 10^11 kJ/yr.

d. **Annual Steam Cost:** (2.89 x 10^11 kJ/yr) / (2257 kJ/kg) * 0.02 €/kg = **2,559,500 €/yr**.

3. **Pumping Cost:** The power for pumping the slurry is minimal. We'll use a conservative estimate.

a. **Total Annual Pumping OPEX: 50,000 €/yr**.

Change in Ethanol production: 176 tonnes/batch -> 132 tonnes/batch

Thus, the changed table is:

Table 21: Updated Sensitivity analysis for pretreatment

CAPEX (€)		**OPEX (€/yr)**	
Choppers & Mills	180,000	Choppers & Mills	251,640
DAP	**18,656,294.4**	DAP	4,127,500
Seed Train	3,891,420.3	Feedstock	6,534,925
Main Compressor	5,772,000	Transportation	1,176,450
Distillation Column	8,440,000	Seed Train	1,882,586.1
Indirect Costs	17,133,293.69	Saccharification & Fermentation	8,888,745.5
Total	54,073,008.39	Distillation	1,175,675
		Indirect OPEX	1,531,941.32
		Total	25,569462.92

Total Revenue = (132/176) * 32,500,000 €/yr = 24,375,000 €/yr

Net Income = -1,194,462.92 €/yr => Process goes in a loss

Table 21: Analysis showing the updated pretreatment

SB Method	**CAPEX (€)**	**OPEX (€/yr)**	**Revenue (€/yr)**	**Net Income (€/yr)**	**Pay-back**

					period (yr)
AFEX	50,727,202.69	29,770,767.67	32,500,000	2,729,200	18.55
DAP	54,073,008.39	25,569,462.92	24,375,000	-1,194,463	N/A

9.4 CSTR vs Bubble Column:

Even if we assume that the CSTR would consume same power and give same efficiency (Which it wouldn't, as has been proven before), each of the spare reactors would also require a pre-fitted motor. This means instead of 1 compressor of 4.8 MW, we would need 10 motors of 685 KW. The CAPEX for Compressor was 5,772,000 € while that for the motors alone would be 8,220,000 €. This alone proves that a CSTR based system is more expensive.

Moreover, to achieve the identical pumping rate as a bubble column, the CSTR would need 7% higher power. This also proves that Bubble column is a cheaper system.

9.5 Distillation of Broth v/s Stripping:

CO_2 circulation would still be required in the Bubble column. So instead of distilling the condensate consisting of 71% ethanol by mole, we would be working with 1.13% ethanol. (176 tonnes ethanol in 6246.67 m3 volume)

Here is a detailed breakdown of the calculations to determine the mole percentage of ethanol in the solution.

The calculation is based on converting the given mass and volume to moles, and then finding the mole fraction of ethanol.

Step 1: Calculate the Volume and Mass of Each Component

First, we need to determine the mass of both ethanol and water. We're given the mass of pure ethanol and the total volume of the mixture. We can use the approximate densities of ethanol and water to find the volume of each, and then the mass of the water.

- **Mass of Ethanol:** 176 tonnes = 176,000 kg
- **Total Volume of Mixture:** 6,246.67 m^3
- **Density of Ethanol:** 789 kg/m^3

- **Density of Water:** 1,000 kg/m³

Calculate the volume of pure ethanol:

Volume_ethanol = Mass_ethanol / Density_ethanol

Volume_ethanol = 176,000 kg / 789 kg/m³ = 223.07 m³

Calculate the volume of water by subtracting the ethanol volume from the total volume:

Volume_water = Total_volume - Volume_ethanol

Volume_water = 6,246.67 m³ - 223.07 m³ = 6,023.60 m³

Calculate the mass of water:

Mass_water = Volume_water * Density_water

Mass_water = 6,023.60 m³ * 1,000 kg/m³ = 6,023,600 kg

Step 2: Convert Mass to Moles

Next, we convert the mass of each component into moles using their molar masses.

- **Molar Mass of Ethanol (C_2H_5OH):** 46.07 g/mol or **0.04607 kg/mol**
- **Molar Mass of Water (H_2O):** 18.02 g/mol or **0.01802 kg/mol**

Calculate the moles of ethanol:

Moles_ethanol = Mass_ethanol / Molar_mass_ethanol

Moles_ethanol = 176,000 kg / 0.04607 kg/mol = 3,820,273.5 mol

Calculate the moles of water:

Moles_water = Mass_water / Molar_mass_water

Moles_water = 6,023,600 kg / 0.01802 kg/mol = 334,273,029.97 mol

Step 3: Calculate the Mole Percentage

Finally, we calculate the mole percentage of ethanol.

Total_moles = Moles_ethanol + Moles_water

Total_moles = 3,820,273.5 mol + 334,273,029.97 mol = 338,093,303.47 mol

Mole % Ethanol = (Moles_ethanol / Total_moles) * 100

Mole % Ethanol = (3,820,273.5 mol / 338,093,303.47 mol) * 100 = 1.13%

Therefore, the ethanol concentration in the solution is approximately **1.13 mole percent**.

Case 1: Base Case (71% Ethanol by Mole Feed)

First, I will restate the costs for the base case, which we have already calculated. The feed to the distillation column is the stripped liquid, which is approximately 71% ethanol by mole.

- **Distillation Column CAPEX: 8,440,000 €**
- **Distillation Column OPEX: 1,175,675 €/yr**

Case 2: Dilute Feed (1.13% Ethanol by Mole Feed)

This case represents a significantly more dilute feed to the distillation column, which will dramatically impact the design and operating costs.

Assumptions and Rationale

- **Feed Flow Rates:** The final product is still 176 tonnes of pure ethanol per batch. This ethanol must be recovered from a much larger volume of feed.
- **Mass of Ethanol:** 176 tonnes = 176,000 kg.
- **Moles of Ethanol:** 176,000 kg / 0.04607 kg/mol = **3,820,274 mol**.
- **Total Moles in Feed (at 1.13%):** 3,820,274 mol / 0.0113 = **338,077,345 mol**.
- **Moles of Water in Feed:** 338,077,345 mol - 3,820,274 mol = **334,257,071 mol**.
- **Distillate (D) and Bottoms (B):** We will assume the same product specifications as before: 95% ethanol distillate (mole fraction) and 99% water bottoms (mole fraction).

Distillation Column Design Calculations

Due to the extreme dilution, the distillation is much more difficult, requiring a much larger and more energy-intensive column.

- **Number of Stages:** Using the Fenske equation, the minimum number of stages (Nm) is inversely proportional to the logarithm of the relative volatility (α). For a very dilute solution, the relative volatility of ethanol to water is very high, but the feed is far from the azeotrope, which means more stages are required to reach the azeotropic composition.
  - For a feed concentration of 1.13%, the number of stages is much higher than our base case. Let's assume a new, more realistic number of stages of **60 stages** instead of 20 to achieve the separation. This is a common requirement for separating a highly dilute feed.
- **Reflux Ratio:** The reflux ratio is a crucial variable. A more dilute feed requires a much higher reflux ratio (R) to achieve the same product purity. Our base case had R=0.825. For a 1.13% ethanol feed, the reflux ratio would be significantly higher, likely in the range of **3.0 to 5.0**. I'll assume a new reflux ratio of **4.0**.
- **Column Diameter:** The column diameter is proportional to the vapor flow rate, which is a function of the reflux ratio. A higher reflux ratio leads to a much larger diameter.
  - The vapor flow rate (V) is proportional to (R+1). So, V_new / V_old = (4+1) / (0.825+1) = 5 / 1.825 = 2.74.
  - Since the diameter is proportional to the square root of the vapor flow rate, the new diameter is 1.13 m * sqrt(2.74) = **1.87 m**.

Cost Calculations for Case 2

CAPEX (Capital Expenditure)

The CAPEX for the distillation column is proportional to the column's diameter and height.

- **Column Vessel Cost:** The cost is proportional to the diameter and height. The cost of a distillation column is often modeled as Cost ~ D^1.5 * H.
  - New Cost / Old Cost = (1.87 / 1.13)^1.5 * (60 / 20) = 1.65^1.5 * 3 = 2.12 * 3 = 6.36.
  - The new column cost is 150,000 € * 6.36 = **954,000 €**.
- **Reboiler and Condenser CAPEX:** The reboiler duty (Q) is proportional to the vapor flow rate, (R+1) * D.
  - Q_new / Q_old = (4.0 * 34.82 mol/s) / (0.825 * 34.82 mol/s) = 4.0 / 0.825 = 4.85.
  - The new reboiler CAPEX is 980,000 € * 4.85 = **4,753,000 €**.

- The condenser CAPEX will be similar, at **4,753,000 €**.
- **Total Installed CAPEX:** (954,000 € + 4,753,000 € + 4,753,000 €) * 4.0 = **41,840,000 €**.

OPEX (Operating Expenditure)

The OPEX is primarily the cost of steam and cooling water, which is directly proportional to the reboiler and condenser duties.

- **Reboiler Cost:** The cost is directly proportional to the reboiler duty.
- New Cost / Old Cost = 4.85.
- 675,675 €/yr * 4.85 = **3,276,000 €/yr**.
- **Condenser Cost:** The cost is also proportional to the duty.
- 500,000 €/yr * 4.85 = **2,425,000 €/yr**.
- **Total Annual OPEX:** 3,276,000 €/yr + 2,425,000 €/yr = **5,701,000 €/yr**.

Table 22: Final Comparison

Cost Item	Case 1: Base Case (71% Ethanol)	Case 2: Dilute Feed (1.13% Ethanol)
CAPEX	**8,440,000 €**	**41,840,000 €**
OPEX	**1,175,675 €/yr**	**5,701,000 €/yr**

The results show that trying to distill a very dilute ethanol solution to 95% concentration results in a distillation column that is almost **5 times more expensive to build and operate** than the one needed for the more concentrated feed. This highlights the importance of keeping the liquid feed concentration as high as possible before distillation.

Clearly, this has a negative net income.

Table 23: Sensitivity analysis for distillation column

DSP	CAPEX (€) For Ethanol Recovery	OPEX (€/yr) for Ethanol Recovery
Stripping	8,440,000	1,175,675

Direct Distillation	41,840,000	5,701,000

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12. Bibliography

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