UNIVERSITY of SAN CARLOS
SCIENTIA • VIRTUS • DEVOTIO

USC Chemical Engineering Student

Chemical Product Development and Process Design 1

Department of Chemical Engineering
Talamban, Cebu City, Philippines 6000

Term/Academic Year:
First Semester AY 2021-2022

INDUSTRIAL SCALE PRODUCTION OF SURFACTIN VIA FERMENTATION USING *Bacillus subtilis LB5a* and CASSAVA WASTEWATER SUBSTRATE

A basic engineering document (BED) submitted in partial fulfillment of the requirements in the course

CHE 4115
(Chemical Product Development and Process Design I)

BRANZUELA, JAMES DAREL M.
CABRERA, BLANCHE MARIE M.
CANAMA, GIBSON JAKE C.
DELCO, MONICA CLAIRE L.
FICK, STEVENSON RAYNE T.
TALANDRON, RHOEL A.

December 13, 2021
Revised: May 28, 2022

TABLE OF CONTENTS

Chapter I: INTRODUCTION

1.1 Product Description and Applications

Surfactants are additives that lower the surface tension between a liquid and an immiscible liquid, between a gas and a liquid, or between a liquid and a solid. The term “surfactant” is an abbreviation of “surface active agent” which means it is active at the surface. Thus, in a solution, the surfactant concentration is higher at the surface than in the bulk of the liquid (Porter, 1993). They serve various applications in various industries.

Surfactants are active ingredients in soaps and detergents that have the ability to concentrate at the air-water interface. They are commonly used to separate oily materials from a specific media because they can increase aqueous solubility of Non-Aqueous Phase Liquids (NAPLS) by lowering surface/interfacial tension at the air-water and water-oil interfaces (Fakruddin, 2012).

1.1.1 Shift to Biosurfactants

There has been a growing concern over the long-term effects of environmentally problematic industrial processes. The recent and significant call for ecological and environmental protection has prompted the development of “green” or eco-accommodating technologies and products. Thus, biosurfactants have seen an increase in global interest due to their many advantages over their chemical counterparts. Many of such advantages involve being a more sustainable, eco-friendlier, and less toxic choice as a surfactant particularly in applications that entail direct human contact. Biosurfactant produc tion by microorganisms is then an essential innovation for environmental sustainability in the coming generations (Jimoh & Lin, 2019).

1.1.2 Advantages of Biosurfactants

Surfactants are widely employed in industrial, agricultural, food, cosmetics, and pharmaceutical applications; nevertheless, because of its recalcitrant and persistent character, most of these molecules are chemically produced and may pose environmental and toxicological problems**.** On the other hand, biosurfactants have several advantages over its chemically synthesized counterparts.

A. Biodegradability

One of the prominent advantages of biological surfactants over their chemical surfactants is their biodegradability. While current literature regarding the biodegradability of biosurfactants are still limited, evidence of high biodegradation rate has been observed in some studies. Rodríguez-López et al. (2020) reported 50% biodegradation of the biosurfactant obtained from a corn wet-milling industry in as soon as 35 days at a pH of 5. This can be an ideal biodegradation rate as it is faster than chemical surfactants but also not too immediate for it to be rendered unusable in just a short amount of time. On the other hand, chemical-based surfactants need to undergo biodegrading treatments so as not to remain in the environment and cause harmful effects to other organisms.

B. Low Toxicity

Biosurfactants demonstrate lower toxicity than the chemically synthesized surfactants. Furthermore, it has also been reported that biosurfactants show higher EC50 (effective concentration to decrease 50% of test population) values than synthetic dispersants.

Chemical-based surfactants have been known to have potential toxic impacts on the environment and other organisms. Biosurfactants, on the contrary, have been promising in exhibiting little to no toxicity to human or animal beings. A lipopeptide biosurfactant produced from *Bacillus subtilis* SPB1 was tested for its toxicity against mice. During a 28-day period, no unusual behaviorial changes and no toxicity was observed among the species. This study by Sahnoun et al. (2014) concluded that the investigated lipopeptide biosurfactant is suitable for food, cosmetic, and pharmaceutical applications.

Marine-derived biosurfactants investigated by Voulgaridou et al. (2021) showed no cytotoxicity to human skin and liver. There was also an absence of mutagenic or anti-mutagenic potential, making biosurfactants an environmentally friendly choice for commercialization.

C. Availability of Raw Materials

Biosurfactants can be made from a variety of low-cost raw ingredients that are readily available in large quantities. The carbon source might be hydrocarbons, carbohydrates, and/or lipids, which could be employed individually or in combination. These can be found in renewable resources such as agricultural wastes (cellulosic, lignocellulosic, and other organic by-products) or non-toxic inert materials. Agricultural wastes that evidently produce high sophorolipids (SLs) yield are rice husks and wheat straw. Meanwhile, chemical surfactants are synthesized from petroleum and other derivatives, making them not very environmentally ideal (Rodríguez et al., 2020).

D. Surface and Interface Activity

Surfactants are additives that can lower the surface and interfacial tension of a product. Biosurfactants, specifically, possess a high performance in doing this. Surfactin from *Bactillus subtilis*, in particular, can lower the surface tension from 70 to 36 mN/m at a concentration of around 15.6 mg/l (Ahmad Mohammad Abdel-Mawgoud et al., 2008).

E. Other Advantages

Other advantages are biocompatibility and digestibility which allow for their application in cosmetic, pharmaceuticals, and as functional food additives. Due to the bio-utilizing method of synthesizing biosurfactants, they have a wide range of compatibility with other organisms and their cells. These allow them to be safely used in food, cosmetic, and pharmaceutical industries (Haque et al., 2020). With regards to their more advanced applications, biosurfactants have even been investigated for their potential as a drug delivery system (Gudiña et al., 2013).

1.1.3 Biosurfactants in the Cosmetics Industry

Biosurfactants are an emerging innovation in the cosmetics industry, primarily because of their low toxicity and their biocompatibility. In this section, the role and significance of biosurfactants in this industry is discussed.

A. Skin Microbiome

The skin is the biggest organ in the human body and has a complicated structure. Its principal role is to act as a barrier, preventing excessive moisture loss from the body while also blocking the entry of hazardous substances and viruses from the outside. The epidermis, dermis, and hypodermis are the three layers of the human skin, according to histology. Each of these layers plays an important role in the skin's function (Adu et al., 2020).

A complex network of interactions exists between epidermal cells and skin microbes which enables the colonization of skin surfaces by a wide array of microorganisms, both commensal and mutualistic. In addition, different skin types selectively cater and facilitate the growth of diverse groups of microorganisms. Therefore, to prevent the growth of pathogenic microorganisms in the skin’s surface, cosmetic and personal care products are formulated (Adu et al., 2020).

B. Cosmetic Applications of Biosurfactants

In addition to strengthening barrier functions, limiting pathogen growth, and cleansing and hydrating skin surfaces, cosmetic and personal care products are frequently formulated to offer nutrients and protection to the skin. Chemical surfactants are currently used by many skincare product manufacturers as emulsifiers and foaming agents in their formulations. About half of all chemical surfactants in the market are generated from petrochemicals which are non-renewable resources. Despite the effectiveness of these chemical surfactants in formulations, they could be detrimental to the skin and its microbiome. For this reason, there has been an arising impetus for the replacement of chemical surfactants with other compounds that can be alternatively produced from cheaper and sustainable resources. In addition, the substitutes should have low toxicity, biodegradability, and compatibility with the human skin, which eventually promotes less or negative effects on the health of consumers and the environment (Adu et al., 2020).

Biosurfactants have key physiochemical qualities that are vital for skin health maintenance. Their fatty acid ends, for example, are useful in hydrating rough and dry skin. Additionally, *Cutibacterium acnes* (*C. acnes*) hydrolysis of triglycerides in the fatty acid chain of microbial biosurfactants may aid in the maintenance of the acidic pH of the skin. This results to the adherence of resident skin flora and to the impediment of the growth of pathogenic skin microbes, encouraging a healthy skin microbiome (Adu et al., 2020).

Furthermore, the emulsification, foaming, wetting, and solubilizing functions and properties of biosurfactants are dependent on their chemical structure, which make them a desirable alternative ingredient for chemical surfactants for the production of creams, lotions, powder, shampoos, and other essential cosmetic products on the skin (Adu et al., 2020). Current commercial cosmetic and skincare products that contain microbial biosurfactant include Relipidium™ body and face moisturizers, Sopholiance™ deodorant, face cleanser and shower gels, and Kanebo™ moisturizer, cleansing, and UV filter enhanced skincare.

1.1.4. Surfactin

Surfactin is one of the, if not the most, popular biosurfactant produced from *Bacillus subtilis* used in the manufacture of cosmetic products. It is a secondary metabolite lipopeptide whose peptide chain is ring-shaped and contains seven amino acids. As a lipopeptide, lipid tail linked to a short linear or cyclic

oligopeptide. It also contains a β-hydroxy fatty acid chain of 13 to 16 carbon atoms. Additionally, it possesses a wide range of antibacterial activity among other properties (Hu et al., 2019).

Compared to other biosurfactants, surfactin is the most effective that is known thus far. This is exhibited in its ability to reduce the surface tension (ST) of water to as low as 27 mN/m and its very low critical micelle concentration of 0.01 g/L. It has also shown high emulsifying activity and has exhibited antimicrobial, antiviral, and anti-tumor activities (Gudiña et al., 2015; Hu et al., 2019; Janek et al., 2021).

A. Surfactin in the Cosmetics Industry

Surfactin is a family of lipopeptides. Among the lipopeptides known, surfactin is considered as the most effective in terms of interfacial properties and has foaming capabilities comparable to those of sodium dodecyl sulfate and bovine serum albumin. This biosurfactant has been more popularly used in the cosmetic industry as an emulsifying, foaming, cleansing, whitening, sequestering, and moisturizing agent. Furthermore, this biosurfactant is also used as an antiwrinkle, anti-aging, and antibacterial ingredient in some cosmetics. It also possesses good skin compatibility with low irritation, making it safe for topical dermatological applications. Due to its biocompatibility, it can be used as an ingredient in natural and organic cosmetic products (Kanlayavattanakul & Lourith, 2010).

B. Bacillus subtilis

Bacillus subtilis (or B. subtilis), shown in Figure 1, are Gram-positive rod-shaped bacteria that produce dormant spores that are heat resistant. This bacterium is not harmful and is used in the manufacturing of a variety of commercial items. Also, it can thrive in a minimum media that only contains essential salts, as well as carbon, nitrogen, and phosphorus sources. Carbon sources include mono-, di-, oligo-, and polysaccharides as well as sugar-derived alcohols, amino acids, peptides, and 2-, 3-, and 4-carbon compounds. Nitrogen can be obtained from nitrate, ammonium ions, urea, amino acids, peptides, and nucleosides (Schaechter, 2009).

Figure 1. *Bacillus subtilis obtained from (Goya et al., 2020)*

A single lipid bilayer membrane acts as a permeability barrier such that is bordered by a thick cell wall. A number of transport systems pick up nutrients from the medium. At least 15 phosphototransferase systems (PTS) are thought to be involved in the absorption of various sugars. They are the primary sugar absorption mechanisms for glucose, sucrose, and fructose (Schaechter, 2009). With this, *B. subtillis* is often used in the production of surfactin.

The production of surfactin utilizes four different strains of bacillus subtilis, namely ATCC 21332, LAMI005, LAMI008 MTCC2423, and LB5a. The cultivation techniques utilized and operating conditions for each of the bacteria strains are stipulated in Table 1.

C. Role of Bacillus subtilis in Surfactin Production

The target biosurfactant product in this study is surfactin. This biosurfactant is produced by various strains of *B. subtilis* (Yeh et al., 2006). This bacterium produces a broad spectrum of lipopeptide biosurfactants, which are cyclic molecules consisting of variable-length fatty acid (hydrophobic fraction) bound to a short peptide chain (hydrophilic fraction) of seven or ten amino acids (Camylla et al., 2018). Furthermore, *B. subtilis* produces three families of biosurfactant lipopeptides namely surfactin, iturin, and fengycin. The production of these lipopeptides depend on the strain of *B. subtilis* used (Coutte et al., 2010).

According to Coutte et al. (2010), *B. subtilis* is known to be able to thrive and grow even with a severely limited oxygen supply. In the case of surfactin production, the supply of sufficient dissolved oxygen (DO) plays a crucial role in the efficiency of the lipopeptide production. In a laboratory scale experiment study conducted by Ha, et al (2018b), an observed correlation between the oxygen uptake rate (OUR) and dissolved oxygen tension (DOT) was generated upon production of surfactin using *B. subtilis* (Y9 strain). In

Figure 2, it was observed that the change in agitation speeds can cause varied results in terms of DOT and produced surfactin production. It can be inferred from the plot that at a maintained agitation of 400 rpm, the surfactin produced is high. Maintaining the DOT at a certain threshold can lead to an increase of OUR. Thus, the synthesis of surfactin using *B. subtilis* depends closely on the oxygen supply (Coutte et al., 2010).

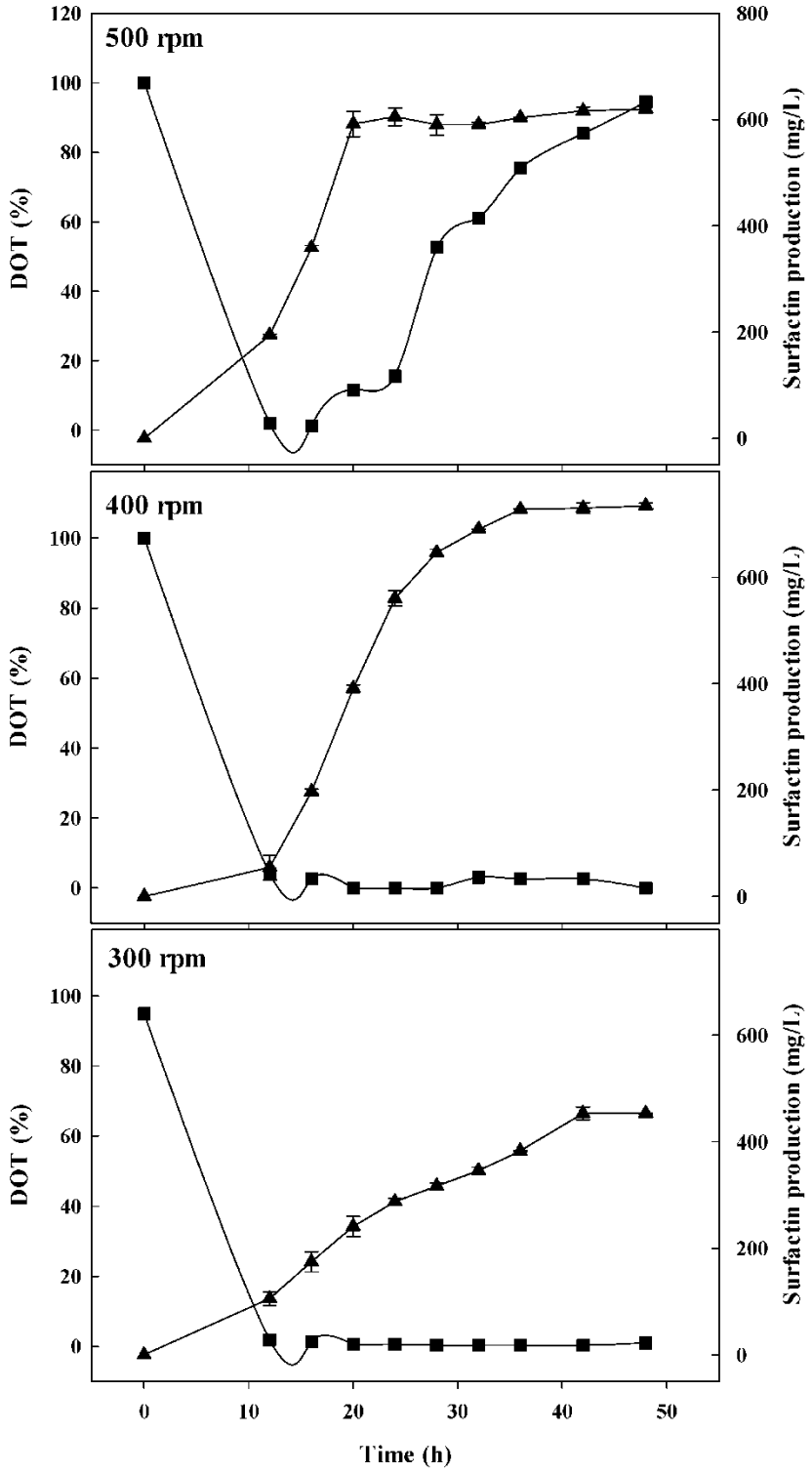


Figure 2. *Dissolved Oxygen Tension (DOT), Surfactin Production Concentration vs. Time graph of the Surfactin Production using Bacillus subtilis (Y9 strain) and 3% w/v galactose fermentation medium at agitation speeds of 300 rpm, 400 rpm, and 500 rpm.* (Ha et al., 2018a)

1.2. Production History and Current Strategies

To understand the production scale of surfactin, it is ideal to dwell on the history of the production of surfactants, in general, and on biosurfactants, in particular. Furthermore, a market study is also employed to get a grasp on the production capacity that would be feasible for such type of production. Finally, the strategies made in the production of surfactin what possible materials can be used is reviewed.

1.2.1. General History and Market Scope of the Production of Surfactants

In the 19th century, the production of the first synthetic detergents were produced from castor and almond oils for textile dyeing (Knepper & Berna, 2003). Common surfactant products produced through oils and fats are alkyl sulfonates (AS). Conventionally, the produced surfactants consist of an amphiphilic property to which it has a hydrophobic and hydrophilic substituent. It is primarily used in the production of soaps as it aids in micellar action to remove grease and oils. Interestingly, the said production are earlier sourced from natural fats and oils through saponification process with a strong base.

With the aim to produce a more environmentally friendly approach of producing surfactants, greener ways are introduced such as the use of microorganisms to produce biosurfactants, including surfactin from *B. subtilis*. In the following subsections, a brief background of the history and development of industrial scale production of surfactants is discussed.

A. History of Surfactant Production

In the industrial scale, owing to the rise of petrochemical production, surfactants are mainly sourced from crude oil and natural gas and is used for oil recovery assay. According to Knepper and Berna (2003), the production of surfactants is estimated to use 1.5% of the petrochemicals produced with starting materials such as ethylene, paraffins, and benzene. Also coined as synthetic surfactants, chemical surfactants have greater advantage than soap production due to the presence of insoluble scum from insoluble calcium and magnesium salts formed with hard water or from dirt.

The history of synthetic surfactant production is traced back in 1898 through an American patent claimed by Ernst Twitchell (Knepper & Berna, 2003; Twitchell, 1987). The said patent composed of the manufacture of sodium salts of petroleum sulfonates. Due to the shortage of fats at the time of World War, the option of using short-chain alkyl naphthalene sulfonates was sought to be a better substitute towards

the production of synthetic surfactant from petroleum products. This further led to the commercial rise of the first synthetic detergent with petrochemical feedstocks as its feedstock in the early 1930's.

B. Application of Surfactants

Among the functions of a surfactant are as a detergent, a wetting agent, an emulsifier, a foaming or anti-foaming agent, or as a dispersant. It is found in products like paints, emulsions, adhesives, inks, biocides or sanitizers, shampoos, toothpastes, firefighting foams, detergents, insecticides, deinking products for recycled papers, ski waxes, and spermicides (Hirsch, 2015).

C. History of the Production of Biosurfactants

Biosurfactants have been around in the industry as an alternative for chemical surfactants as it poses as a greener production which is beneficial to the environment and the community. However, before such potential arose, its discovery started with the results of the isolation of the first known biosurfactant which is surfactin from *B. subtilis* as reported in the study by Arima et al. (1968). Such discovery opened its doors to other several applications.

The produced biosurfactants are generally characterized into five major groups namely glycolipids, phospholipids and fatty acids, lipopeptides and lipoproteins, polymeric surfactants, and particulate surfactants (Randhawa & Rahman, 2014). Surfactin is characterized as a lipopeptide. Moreover, the production of these biosurfactants is commonly done extracellularly, and various bacteria, fungi, and yeast have been employed to aid in the production. With this, the quantity and quality (or nature) of carbon source is an influential factor to the production process. In the production of rhamnolipid (a glycolipid), nutrient concentration, pH, and culture age are key factors to the production (Rahman & Gakpe, 2008).

However, industrial scale production of surfactants with the use of microorganisms is yet to be done. Several studies, rather, have conducted laboratory-scale or small pilot-scale reaction process in the pursuit of producing biosurfactants such as surfactin.

D. Market Study of the Production of Biosurfactants

At present, several consumer products have utilized surfactants as an active ingredient in their respective production. According to Mordor Intelligence (2020), much of the use of biosurfactants is directed towards domestic care and cosmetics. These two industrial applications encompass detergents,

soaps, make-up accessories, and the like. The market share of biosurfactants according to industrial application is illustrated in Figure 3.

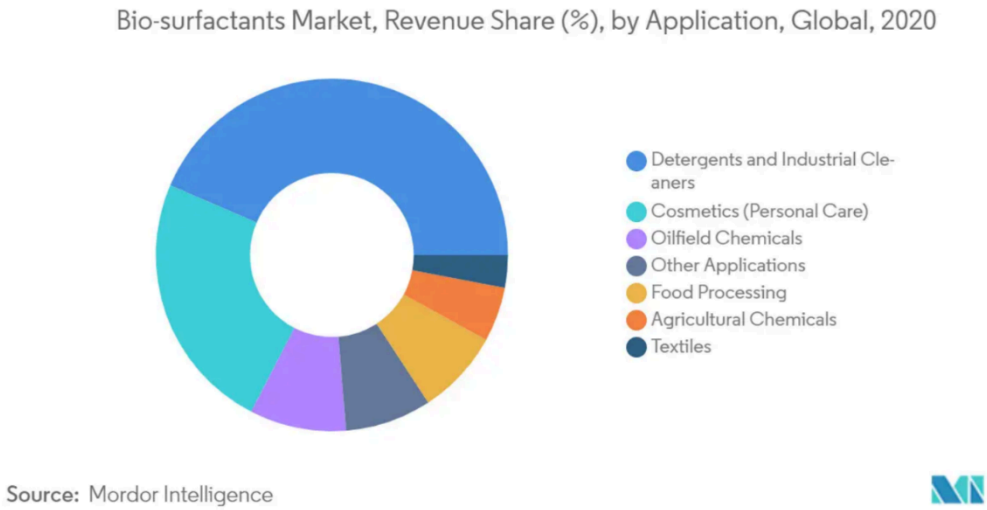


Figure 3. *Market Share of Produced Biosurfactant According to Application* (Mordor Intelligence, 2020)

In connection, much of the produced biosurfactants is intended for detergents and industrial cleaners. This is observed in the market study conducted by Mordor Intelligence (2020). Furthermore, the use of biosurfactants in cosmetics and personal care comes second. The same observation is observed by Vandeputte (2012) wherein 55% of the produced biosurfactants is utilized for beauty soaps, foundations, lipsticks, and other make-up materials and tools for personal care.

In the global scale of application, Walsh (2017) projected that the production of surfactants will soar to about 16.6 million tons in the year 2022. However, the production of biosurfactants, including surfactin, is still done on small, batch-scale production with only 0.1% produced out of the projected annual capacity (Vandeputte, 2012). Furthermore, in terms of production by region, Europe leads with a market share of 22%. This is then followed by North America and China with 21% and 18%, respectively (More, 2021). Specific to surfactin, the production cost is very expensive with a value of about $45.70 per gram.

On another study, Czinkoczy and Nemeth (2020a) conducted a pilot scale surfactin production via fermentation of *B. subtilis*. Through foam collection after batch fermentation, the amount of surfactin recovered from the system simulated at around 18,242 kg per annum (20 tons per annum). This production coincides with the idea that biosurfactant product has not yet been conducted at very large scales, and further studies and optimization procedures must be made to produce enough surfactin that can meet the demands of the consumers.

1.2.2. Current Production Strategies of Biosurfactants Usage in Cosmetic Industry

Chemical surfactants commonly used in the cosmetic industry include sodium lauryl sulfate (SLS), ammonium lauryl sulphate (ALS), sodium stearate, stearic acid, cetyl alcohol, cocamidopropylamine oxide, and polysorbate ester. SLS and ALS are used as cleansers and foaming agents. Sodium stearate is used in soap. Stearic acid is used in deodorants and antiperspirants. Cetyl alcohol is used as an emulsifier and thickening agent. Cocamidopropylamine oxide acts as a foaming agent, and polysorbate ester is used as a solubilizing agent (Mukherjee, 2021). Generally, these chemical surfactants are manufactured with the use of starting materials like petrochemicals that are obtained from oil, gas, and chemical processing. The resulting chemicals and synthetic alcohols are further processed with additional chemical reactions like alkylation, ethoxylation, or sulphation to produce different types of chemical surfactants (Chapman, 2016). Moreover, due to their synthetic nature, they can be mixed with other chemicals to produce chemical surfactants according to their proprietary purpose (in this case, cosmetics). However, as previously mentioned, chemical surfactants are not desirable mainly because they bring harmful effects to the environment. Thus, biosurfactants (particularly in the form of surfactin) are a better option especially in the cosmetic industry.

Biosurfactants are generally produced by bacteria, yeasts, and filamentous fungi under a wide range of different growth and environmental conditions (Rivera et al., 2019). Although they offer numerous advantages as mentioned in *Section 1.1.2.*, the production of biosurfactants poses a major problem as it is more costly compared to the production of chemical surfactants (Singh et al., 2019). This is actually a major reason why biosurfactants have not been widely employed in the industry. Several factors causing the costly production of biosurfactants include the use of refined raw materials, low biosurfactant product yield, by-product formation, and foam formation during fermentation (Nurfarahin et al., 2018). To address this issue, less expensive renewable substrates like agricultural and industrial wastes have been used to reduce the overall cost of biosurfactant production as substrates are responsible for about 10-30% of total production cost. (Ahmad et al., 2016; Tan & Li, 2018). The use of these certain wastes offers other advantages such as high availability of numerous renewable substrates, basic functionality of the product (biosurfactant) is maintained, and the product is safe and not harmful to the environment (Banat et al., 2014). Moreover, several researchers have reported that these wastes are viable sources of carbon and nitrogen and that the use of these wastes makes the production of biosurfactants more feasible at an

industrial scale (Moldes, 2020; Gudiña et al., 2015; Meneses et al., 2017; Bustos et al., 2004; Rivas et al., 2006; Moldes et al., 2007).

On a similar note, surfactin is currently produced by *B. subtilis* with different agricultural or industrial wastes used as substrate. Various studies have been conducted to examine and evaluate the use of certain agricultural and industrial wastes in the production of surfactin. One of the wastes commonly used is cassava wastewater. This type of carbohydrate-rich waste is commonly produced by cassava flour processing plants. Cosmann et al (2004) investigated the production of surfactin by *B. subtilits* LB5a strain from cassava wastewater. The researchers produced crude surfactin in a pilot bioreactor with 56 L of cassava wastewater and 4 L of inoculum. The medium was prepared by heating the cassava wastewater until boiling to remove the solids. Then, after cooling, it was centrifuged at 8000g for 20 min. The supernatant was placed into flasks and sterilized in an autoclave at 1 atm and 121°C for 15 mins while maintaining a pH of 5.9. As for the inoculum and culture preparation and conditions, the bacterial strain was incubated for 24 h at 30°C while two loops of culture were inoculated in 20 mL of nutrient broth and incubated in a rotary shaker at 150 rpm and 30°C for 8-12 hours. After three growing days, it was found that the crude surfactin concentration was 0.3 mg/mL, the surface tension (ST) reduction was 25 mN/mL, and the critical micelle concentration (CMC) was 28.3 mg/L. Another waste used is cashew apple juice (CAJ) wherein it is usually used as component of a carbon mixture. This type of waste was generally used as a supplement in a mineral medium. (Rocha et al.,2009). In the study by Oliveira et al (2013), CAJ was mixed with distilled water and ammonium sulfate. Furthermore, Table 1 below shows cassava wastewater and other agricultural and industrial waste substrates and microorganisms (*B. subtilis* strains) used in the pilot-scale production of surfactin and the available data on their corresponding operating conditions, cultivation modes, reactor volume, product concentration, and product yield.

Table 1*. The Use of Agricultural and Industrial Wastes as Substratres in the Pilot-Scale Production of Surfactin*

Substrate	Microorganism	Cultivation Mode	Operating Conditions	Product Concentration (g/L)	Product Yield (mg surfactin/ g substrate)	Volume (L)	Reference
Cassava wastewater	*Bacillus subtilits* LB5a	Batch shake flask	150 rpm, 30˚C	3.0	-	80	Nitschke & Pastore (2006)
		Batch bioreactor	150 rpm, 35˚C, 0.38 vvm (first 12 h) and 0.63 vvm [volume of air sparged/(volume medium.minute] (rest of period) aeration rate, pH 5.40-7.63	2.42	0.68[a]	40	Barros et al. (2008)
Potato processing wastewater	*Bacillus subtilis* ATC 21332	Batch shake flask	pH 7.0, 150 rpm, 30˚C	0.44	61.7[b]	0.126	Thompson et al. (2000)
		Chemostat	pH 7.0, 250 rpm (w/o baffle) and 400 rpm (w/ baffle), 30˚C, 0.5 vvm aeration rate,	0.9	-	2.2	Noah et al. (2005)
Rice mill polishing residue (RMPR)	*Bacillus subtilis* MTCC 2423	Batch shake flask	150 rpm, 30˚C (pH and DO conc were not indicated)	4.8	4.17	0.2	Gurjar & Sengupta (2015)

	Bacillus subtilis LAMI008	Batch shake flask	-	0.0035	-	-	Rocha et al. (2009)
Cashew apple juice	*Bacillus subtilis* LAMI005	Batch bioreactor	-	0.123	-	-	Giro et al. (2009)
		Batch shake flask	180 rpm, 30˚C	0.319	-	0.055	Freitas de Oliveira et al. (2013)

[a]Yield is expressed as g of surfactin/L of cassava wastewater

[b]Yield is per gram of carbohydrate carbon consumed.

1.3. Process Design Constraints

For this design project, the constraints considered are:

1.3.1. Economic Constraints

- Cassava Wastewater supply;
- Cost of raw materials, and auxiliaries;
- Availability of equipment in the market.
- Installation, maintenance, transport, and operation costs of equipment

1.3.2. Health, Safety, and Environmental Constraints

- Worker and workplace safety from recognized hazards (e.g. exposure to corrosive chemicals such as ammonia, excessive noise levels, mechanical dangers, fire hazard due to heating processes);
- Effluent standards set by DENR for wastewater

1.3.3. Legal Constraints

- RA 9275: *Philippine Clean Water Act of 2004;*
- RA 9003: *Ecological Solid Waste Management Act of 2000*
- DAO 2021 – 19: *Updated Water Quality Guidelines (WQG) and General Effluent Standards (GES)* partially amending to DENR Administrative Order (DAO) 2016 – 08;
- RA 3720: *Food, Drug, and Cosmetic Act;*
- Labor Laws such as the *Labor Code of the Philippines* and RA 11058: *Occupational Safety and Health Standards Act.*

1.3.4. Manufacturability Constraints

- The raw material source must contain enough carbohydrates, sugar, nitrogen, iron, and ammonia;
- Presence of Cyanide and other toxic substances in Cassava wastewater
- Availability of data in literature (e.g. kinetic data, thermodynamic, physical and chemical properties);

Consumer these days look for natural ingredients in their cosmetic products, which have an equal, greater, or complementary advantage over chemical-based ingredients. In this sense, biosurfactants are natural compounds with high potential in cosmetic product formulation due to their biodegradability and impact on health. In fact, these biosurfactants, a diverse class of biomolecules are already exploited in many fields, including the cosmetic industry (Vecino et al., 2017). Furthermore, in the same study by Vecino et al. (2017), it was reported that, although biosurfactants are more eco-friendly compared to their chemical

counterpart which is petroleum-derived surfactants, they both have to follow the same restrictions and regulations.

In the current industry, the use of synthetic culture media causes a highly expensive biosurfactant production. To compensate for the production cost, two basic strategies have been adopted by the industry: an emphasis on the procurement of cheap agro-industrial waste substrates as raw materials to lower the initial feed cost and the development of efficient and successfully optimized bioprocesses including the optimization of the culture conditions and cost-effective recovery processes for maximum biosurfactant production and recovery (Saharan et al., 2011).

Chapter II: PROCESS OPTIONS AND SELECTION

When designing a process, the most important step is to translate the customer's needs into the design basis. The information presented in this section is necessary to evaluate the most feasible route for the design project.

2.1. Traditional Production of Surfactin using Potato Starch Wastewater

Biosurfactants are classified mainly by their chemical composition and are generally produced by microbial fermentation process using cheap agro-based substrates and waste materials. Surfactin, a cyclic lipopeptide produced by *B. subtilis* consisting of seven amino-acid ring structure combined with a fatty acid chain, is considered to be one of the most potent biosurfactants. It is classified as a low molecular weight biosurfactant that has an effect of lowering surface tension and interfacial tension (Muthusamy et al., 2008).

In a pilot scale design by Noah et al. (2005), four (4) liters (L) of effluent is heated in an autoclave for 90 minutes (mins) and is left overnight for the pre-treatment of low-solids potato process effluent. This process ensures that organisms associated with relatively high amounts of solids are removed. The treated potato effluent is then poured into a bioreactor, operated in batch conditions. Sterilized probes put via the head plate were used to measure dissolved oxygen (DO) and pH. Within a thermowell, a thermocouple was put below the liquid level and utilized to maintain a temperature of 30 °C.

A stirred tank bioreactor, utilizing two Rushton impellers, is used to ferment two (2) liters (L) of potato process effluent, which is heated for 60 minutes (mins), before it is inoculated with 200 mL of *B. subtilis*. Dissolved oxygen (DO) and pH are monitored during this process. The batch run operates for 72 hours (hrs), with impellers running at 400 rpm.

The medium used to culture the *B. subtilis* is SPE (simulated potato effluent) which is composed of the following components:

***Table 2**. Composition of the Simulated Potato Effluent (Noah et al., 2005b)*

Component	Amount in g L^{-1}
Potato Starch	5.0
Glucose	0.5
Sucrose	1.0
Maltose	1.0
Peptone	3.5
Tryptone	3.5
$MgSO_4$ $7H_2O$	0.2
Yeast Extract	0.1
$(NH_4)_2SO_4$	0.8
$FeSO_4$	0.03
$MnSO_4$ H_2O	0.0022

Surfactin is recovered by collecting the contents from the bioreactor and centrifuging it at 10,000 gravities (g) for 14 minutes (mins) at 4 °C. Afterwards, concentrated HCl will be added to the supernatant until pH is about 2.0 then it is refrigerated for 24 hours (hrs). At this point, the precipitate contains the surfactin. Finally, contents is subjected to centrifuging at 11,000 gravities (g) for 20 minutes (mins) at 4 °C, recovering surfactin pellets.

2.1.1 Characteristics of Produced Surfactin

The recovery of surfactin depends on the pH condition of the process. The study by Noah et al. (2005) verified that pH at the recovery step has to be less than 2.5 to have a full recovery of surfactin from solution. Furthermore, dissolved oxygen (DO) limited surfactin production at its initial parts. Increasing the impeller speed significantly decreased the batch run time, consequently, improving the production of surfactin. However, such speed must also not exceed a specific level that depends on the scale of production.

At a pH of 2.1, the amount of surfactin produced was 10,988 g L^{-1}. The addition of industrial scale HCl solution poses a challenge when such process is to be brought in a larger scale. At 400 rpm, surfactin

was produced between 17-24 hours (hrs) with a concentration of 0.6 g L^{-1}, greatest yield observed in varied impeller speed. At this impeller speed, DO dropped to 0-3.2% within 0-17 hours (hrs) and at the 23rd hour rose to 47%.

Alternatively, recent studies have shown that cassava wastewater could also yield surfactin as it contains sugar content which is used to feed the microorganisms and produce the surfactant. As presented in the previous section, cassava wastewater would yield surfactin at desirable properties and conditions. Furthermore, the demand for usage of cassava wastewater is minimal as compared to other wastewater substrate used in industries. Thus, this design shall focus its attention on using cassava wastewater as substrate for the propagation of *B. subtilis LB5a* strain for the production of surfactin.

2.2. Method of Quantitative Selection of Process and Equipment: Analytic Hierarchy Process (AHP)

To properly compare and select the options to be used in the industrial production of surfactin from cassava wastewater, the Analytic Hierarchy Process (AHP) is used. Developed by T.L. Saaty in the1970s, the AHP is a multi-criteria decision-making approach wherein factors are arranged in a hierarchic structure. AHP is divided into three sections: (1) the problem to be solved, (2) possible solutions or the alternatives, and (3) the criteria that will be used to evaluate the alternatives. AHP provides a rational framework for a necessary decision by quantifying its criteria and alternative options and linking these elements to the problem (Kostagiolas, 2012). In addition, Triantaphyllou and Mann (1995) described the AHP as a “support tool” that can be used to solve difficult and complex engineering problems wherein the objectives, criteria, subcriteria, and options are organized in multi-level hierarchical structure. They then stated that the relevant data are derived by using pairwise comparisons. These comparisons are used to determine the relative performance measures of the alternatives of each individual decision criterion, as well as the weights of importance of the decision criteria. The AHP provides a way for improving consistency if the comparisons are not entirely consistent.

The Analytical Hierarchy Process is performed in five steps: (1) Define the options needed to be evaluated, (2) Define the problem and criteria, (3) Construct a set of pairwise comparison matrices and compute the option scores, (4) Check consistency, and lastly, (5) get relative weights and rank the options. It should be noted that the values of the pairwise comparisons in the AHP are determined according to the scale developed by Saaty as presented in Table 3.

Table 3. *Scale of Relative Importance (Triantaphyllou and Mann, 1995)*

Intensity of Importance	Definition	Explanation
1	Equal importance	Two options contribute equally to the objective
3	Weak importance of one over another	Experience and judgment slightly favor one option over another
5	Essential or strong importance	Experience and judgment strongly favor one option over another
7	Demonstrated importance	An option is strongly favored, and its dominance is demonstrated in practice
9	Absolute importance	The evidence is favoring one activity over another is of the highest possible of affirmation
2,4,6,8	Intermediate values between the two adjacent judgments	When compromise is needed

For an *f* number of criteria, which importance are to be determined, a matrix of *f* × *f* dimensions is formulated. The criteria are subjectively compared in pairs according to the intensity of importance. Cells of the matrix are specified as the closest integer approximations of weight ratio (Palcic & Lalic, 2009).

$$A = \begin{bmatrix} a_{11} & a_{12} & \cdots & a_{1f} \\ a_{21} & a_{22} & \cdots & a_{2f} \\ \vdots & \vdots & \vdots & \vdots \\ a_{f1} & a_{f2} & \cdots & a_{ff} \end{bmatrix}$$

Where *A* is the scalar matrix. The element of the matrix *a* is a ratio between compared criteria.

2.3 Process Options and Selection for the Production of Surfactin using Cassava Wastewater Substrate

In this section, the options for the process steps involved in the manufacture of surfactin are evaluated. The AHP evaluation tool shall be used in most of the process selection for the setup of the surfactin production plant.

From the summary of related literature on surfactin production in Table 1, *B. subtilis* LB5a is a strain that yields one of the highest surfactin product concentration. While *B. subtilis* MTCC 2423 generated the most sizeable yield, it is not as supported in literature as LB5a is. Furthermore, LB5a being able to be grown in wastewater is economically and environmentally ideal.

2.3.1. Determination of Overall Mode of Operation

According to Douglas (1988), a hierarchical procedure can be done in the selection process of a plant design to wrap the heads around how the process and equipment procedures are determined from a wide array of options available. At utmost priority is the determination of what mode of operation the system will run throughout the system. However, a disclaimer to this process option is that although the process is selected for a particular mode of operation, a specific process step may opt not to strictly follow the overall mode given that the factors that lie within the system is justified according to its feasibility and economic constraints.

In industries, the two major modes of operation done in process operations are **batch process** and **continuous process.** Both methods are possible in any industry sectors, may it be in food production, in pharmaceuticals manufacture, in mining operations, petroleum and petrochemical extraction, or in any other manufacturing systems. Despite the process step functions the same for both operations, the difference lie on the technological maturity and feasibility, and economic factors which can affect the decision-making process.

The core process of this plant design is to conduct a growth and fermentation process of the *B. subtilis Lb5a* microbe to produce surfactin with cassava wastewater substrate. Thus, it is highly valued that the mode of operation for this design is dependent on the control of these microorganisms throughout the reaction cycle. For batch process of fermentation, the input materials are processed on a limited amount of substance per day. Here, around one to two runs can be made in a day depending on the cycle time allotted for the entire production. Furthermore, the fermentation is done under a closed system and a discontinuous upstream and downstream process is observed with the inclusion of start-up and shut-down operations. On the other hand, continuous fermentation allows the nutrients to contiously be added into the system with a continuous output of products (Magar, 2021).

In the selection process, the AHP tool is unapplicable since there is only 2 options considered. Thus, the selection process quantifies the scoring using the same scale presented in Table 3 but with great

emphasis on the justification. The selection considers 3 main criteria for selection: Technological Maturity, Economic Considerations, and Safety, Health, and Environment (SHE). Each would weigh in the overall score as 40%, 30%, and 30%, respectively.

Table 4 presents the quantitative scoring for the selection of the overall mode of operation for the production of surfactin. Based on collated literatures and assessment, it is then considered that the overall process will be under a batch mode of operation.

Table 4. *Evaluation and Selection Procedures in the Determination of Overall Mode of Operation for the Production of Surfactin using B. subtilis Lb5a strain and Cassava Wastewater*

CRITERIA (% WEIGHT)	BATCH PROCESS SCORE	CONTINUOUS PROCESS SCORE	JUSTIFICATION
Technological Maturity (40%)	6	3	The production of surfactin is considered a process to obtain a secondary metabolite. This favors the use of batch processes compared to continuous types. Further, the use of microorganisms may cause contamination and mutations should the process be in continuous setup. Thus, it is much more complicated to operate and maintain despite high product yield. This leads to most industries using microorganisms to conduct operations under a batch process (BYJU, 2022; Magar, 2021).
Economic Considerations (30%)	5	3	Batch process require lower investment value as compared to continuous process. Furthermore, the operation of continuous fermentation may lead to a very complicated downstream process in obtaining the product (Magar, 2021). In the reaction of surfactin, the foam product is scraped off from the broth to yield as much surfactin as possible. Thus, continuous process is difficult to be done upon acidification and flotation steps. This implies higher cost is such process to get the necessary product yield and purity.
Safety, Health, and Environment (30%)	6	4	Though it would produce a high product yield, continuous process operations may occur contamination and mutation of culture throughout the reaction run (Magar, 2021).
TOTAL SCORE	**5.7**	**3.3**	**DECISION/REMARKS:** The plant design process will be conducted under an **overall batch process** design.

2.3.2. Determination of Unit Operation Processes

Upon determining that the general process design is done under a batch system, the next subsections stipulate and evaluate the possible unit operation process for each step done in the upstream, core, and downstream lines in the production of surfactin using cassava wastewater substrate and *B. subtilis LB5a* strain microorganism. As stated, the AHP selection tool will be used as the quantitative scoring method in determining which specific unit process will be done. Furthermore, the use of various references such as innovation literatures, patents, case studies, and research papers will be the basis for justification on the use of each candidate process.

A. Solids Removal from Cassava Wastewater

Cassava wastewater is composed of solids such as sediments, and these must be removed before it is used for fermentation. Sediment composition in cassava wastewater is 0.16 g/100 L.

i. Option 1: Centrifugal Sedimentation

Centrifugal sedimentation is a separation process that exploits the density difference between the solids and the liquid. By centrifugal force, the solids or sediments found initially in the cassava wastewater are removed from the liquid supernatant. The supernatant is the desired product for the following process steps. This type of sedimentation boasts of great clarifying power (“Front-Matter,” 2016).

Centrifugation will be performed at 5,000 rpm for 10 minutes as performed by Costa et al. (2010). The centrifuge type will be tubular as it is also operable and common in industrial applications. Furthermore, the operation requires only a simple separation of solids from the liquid (i.e. no need to classify particle sizes). A simple tubular centrifuge consists of a vertical tube with a large length-to-diameter ratio. Its rotation is about its vertical axis.

Centrifuges require the monitoring for excessive vibrations. This phenomenon can be detrimental to workers’ safety. Thus, this equipment must be given an appropriate enclosure.

Centrifuges are widely used in the industry to remove fine particles from the liquid. Its cost mostly lies only in the initial investment or installation of the centrifuge. It does not require any chemical reagent or any other expensive material to operate (Pawliszyn, 2012).

ii. Option 2: Gravitational Sedimentation

Gravitational sedimentation is performed in a sedimentation tank. Here, the suspended solids are meant to settle to the bottom of the tank after a time, leaving the supernatant at the top. Sedimentation was performed by Lawal et al. (2019) for 6 hours.

This process, being a less complex option, poses less risk to the safety of the workers. It also does not require any chemical reagent or any other expensive or toxic material to operate.

***Table 5**. Selection of Process Option for Solids Removal from Cassava Wastewater*

Option	Clarifying Efficiency (41%)	Process Time (39%)	Ease of Operability and Technological Maturity (11%)	Health and Safety (5%)	Environment (4%)	**Composite Impact**
Centrifugal Sedimentation	**0.308**	**0.347**	**0.035**	**0.01**	**0.034**	**0.734**
Gravitational Sedimentation	0.102	0.043	0.075	0.04	0.006	0.266
Total	0.41	0.39	0.11	0.05	0.04	1.000

As shown in Table 5, centrifugal sedimentation garnered a higher composite score of 0.734. The centrifuge is a superior equipment in terms of process time (10 minutes as opposed to 6 hours) and clarifying efficiency. Furthermore, in this industrial process, the centrifuge equipment is more economical as it can be used for several other process steps as discussed in the subsections below. The settling tank takes more time to perform the necessary process and serves in only this process step. The input-output diagram for solids removal from cassava wastewater is then presented in Figure 4.

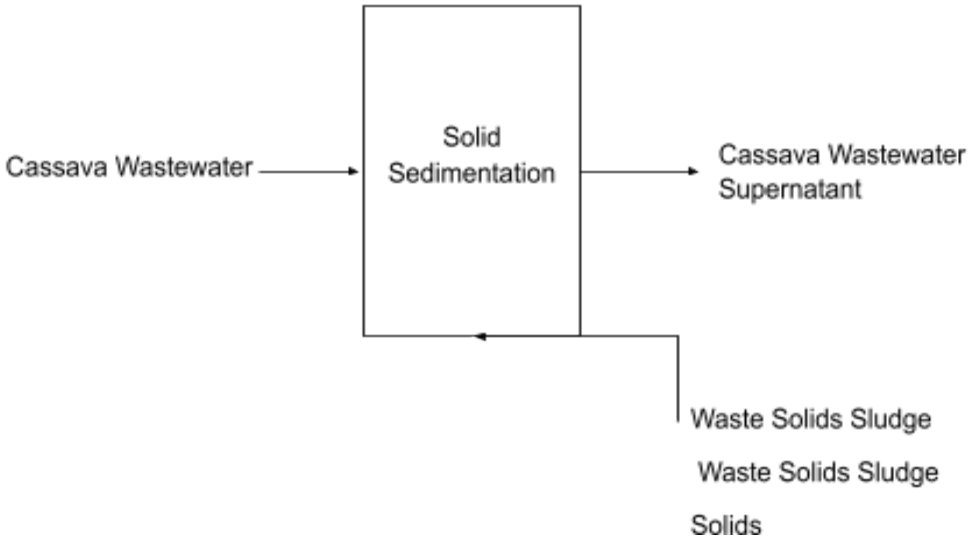


Figure 4*. Input-Output Diagram for Solids Removal from Cassava Wastewater*

B. Substrate Pre-treatment (Removal of Cyanide)

Cyanide is toxic to living organisms even in low concentration. Free cyanide exists in two different species, hydrogen cyanide (HCN) or ionized cyanide, depending on the pH and temperature of the environmental medium. Cassava plants contain cyanides ranging from 75 to 1000 mg/kg of cassava depending on the plant variety and the soil condition. During the production of starch and cassava tubers, large amount of natural cyanogenic glycosides is released. In order to protect the bacterial cell, these cyanides should be removed before the cassava wastewater is mixed with the B. subtilis.

i. Adsorption by Activated Oyster Shell Ash

One of the most economic and efficient processes of cyanide removal from wastewater is adsorption (Chen et al., 2004). In terms of cost, simplicity, ease of operation, and insensitivity to toxic substances, this technique is found to be superior among others. One of the adsorbents that are found to be effective in adsorbing cyanides are oyster shells. These shells are made of 95% calcium carbonate, while the remaining 5% is composed of traces of manganese, aluminum, iron, sulphate and magnesium (Maduka, 2013). These shells are non-biodegradable and are continuously accumulating due to increase consumption of seafood. Insert statistics of oyster shell waste. Insert where activated oyster shells will be bought

In a laboratory scale study of Akpan and Etuk (2019), calcined ash (COSA) and activated ash (AOSA) adsorbents were prepared from oyster shell. It was found that AOSA removes cyanide from cassava wastewater better than COSA. They also investigated the adsorption of cyanide as a function of pH, adsortbent dosage, contact time, temperature, and initial cyanide concentration. It was found that for a batch adsorption process, it is strongly pH dependent, and the maximum cyanide removal was found at pH 10, where 91.48% cyanide removal for AOSA was observed. For the contact time and temperature, highest cyanide removal was found at 80 minutes and 30^{o} C, respectively. The mechanism of adsorption is physical adsorption.

ii. Oxidation of cyanides

Cyanide destruction, often known as oxidation, is the process of breaking down the carbon-nitrogen triple link in WAD cyanides (Weak Acid Dissociables such as free cyanide, hydrogen cyanide, and other cyanide species where the cyano group shares weak bonds with metals such as cadmium, copper, nickel, and zinc) by a chemical oxidant (or other agent).

Chemical method is the most popular method amoth the different types of cyanide destruction methods. Among the options of commonly used chemical oxidants are hydrogen peroxide, chlorine, oxygen, hypochlorite, and sulfur dioxide. These oxidants have a common characteristic of having high electron affinity, which enables them to attract electrons away from the cyanide anion resulting in cyanate formation. Chemical oxidation is an excellent fit for greater volume streams with WAD cyanides, especially those with cyanide levels that exceed the limits supported by biological treatment alternatives, due to its efficacy and relative technical simplicity.

In a study of Khun-anake et al. (2000), sodium hypochlorite and calcium hypochlorite were used to remove cyanide contaminants from laboratory wastewater at the reaction time of 30 minutes. The hypochlorite ion, formed when chlorine dissolves in water, is the active reagent for chlorine oxidation of free and complexed cyanide. Hypochlorite ions can also be made by dissolving appropriate salts in water, such as sodium or calcium hypochlorite. In aqueous solution, free cyanide reacts quickly with hypochlorite (OCl) to generate cyanogen chloride, sometimes known as tear gas. Cyanide reacts quickly with free chlorine as well. However, cyanogen chloride is readily hydrolyzed to cyanate and chloride ions at high pH (oxidation process usually carried at pH 10 to 11). If the feed to chlorination is inadequately buffered, more alkali must

be provided since the hydrolysis step consumes hydroxide. Alkali addition may be avoided by using sodium or calcium hypochlorite (Liu & Liptak, 1999).

The use of electromagnetic vortex layer devices for cyanide wastewater treatment allows for the oxidation of cyanides to cyanates while concurrently forming non-toxic carbonates and ammonia. The procedure is carried out in an alkaline medium with pH ranging from 9 to 10. Lime, soda, in the form of a 5 to 10 percent water solution, is used as an alkaline agent, while a 5 to 10 percent solution of lime chloride with calcium hypochlorite or chlorine is used as an oxidizing agent. At an initial concentration of 30 to 350 mg/L, the residual quantity of cyanide after treatment ranges from 0.005 to 0.09 mg/L, having a cyanide reduction of 99.98% (Hubar, 2021).

One major downside of applying oxidation by hypochlorite for cyanide removal in the CWW is the reaction between starch and hypochlorite. In a study by Eisenbraun and Purves (1961), starch was oxidized by calcium hypochlarate at near pH 12 at 20 ^{0}C. Moreover, despite its effectiveness, chemical oxidation is costly due to the chemicals being consumed for oxidation and pH control, and the effluent discharge costs.

Table 6. *Selection of Process Option for the Removal of Cyanide*

Option	Removal Performance (54%)	Ease of Operability and Technological Maturity (24%)	Health and Safety (10%)	Environment (6%)	Economy (6%)	**Composite Impact**
Adsorption	**0.36**	**0.120**	**0.075**	**0.045**	**0.050**	**0.65**
Oxidation	0.18	0.12	0.025	0.015	0.010	0.35
Total	0.54	0.24	0.10	0.06	0.06	1.000

After the AHP selection process as summarized in Table 6, adsorption by activated oyster shell ash obtained a higher composite impact score of 0.65. Adsorption process outscores the oxidation process in terms of equipment configuration, health and environment criterion, and economic value. In terms of material requirements and conversion efficiency, although the abovementioned literature data suggests that oxidation process removes cyanide better, but due to the reaction of hypochlorite with sugar makes it

unaligned with the overall purpose of the process. The goal is to remove cyanides while retain the sugar content of the CWW. Regarding auxiliary equipment, the oxidation produces precipitates that would further need another solid separation equipment which adds additional capital cost. For the reaction process, unwanted sugar and hypochlorite reactions take place in the oxidation process. In terms of Material Hazards, high exposures to hypochlorite may cause respiratory complications. In terms of operational costs, oxidation process costs more expensive due to high dependence in chemicals.

C. Cell Propagation

Currently, the production of surfactin is limited only to lab-scale experiments. Thus, there are still minimal studies and patents investigating this fermentation process. Usually, biomass growth and the actual surfactin production (in Section 2.3.4) are performed in the same vessel, in a single process step. However, in this design for a larger scale of production, these two processes will be carried out in two separate batch fermenters. Thus, even in this bioreactor, surfactin is already produced albeit in minimal amounts.

Cell propagation involves the feeding of glucose substrate to the *B. subtilis* in a growth medium. The goal in this stage is to shorten the lag phase and to increase the specific growth rate. Ultimately, it is desired to reduce the production time and, consequently, the cost of production (Stamenković-Stojanović et al., 2020a).

Typically, the growth medium is a nutrient broth that contains mineral salts and the glucose substrate (Cooper et al., 1981; Moshtagh et al., 2019). Glucose was chosen as it is the most appropriate substrate feed for *B. subtilis* (Stamenković-Stojanović et al., 2020a). Other less common broth mediums that can also culture *B. subtilis* are Luria Bertani and Landy's mediums (Coutte et al., 2010).

For this process design, the medium will consist of the treated cassava wastewater whose composition can be found in Table 16. Furthermore, the glucose comes from cassava wastewater which is also rich in trace metals. According to Cooper et al. (1981), the presence of metal cations improves both biomass and surfactin production. Optimum trace elements at these respective concentrations were identified by Mohammad Abdel-Mawgoud et al. (2008): $ZnSO_4 \cdot 7H_2O$ (0.16 g/L), $FeCl \cdot 6H_2O$ (0.27 g/L), $MnSO_4 \cdot H_2O$ (0.017 g/L) and $MgSO_4 \cdot 7H_2O$ (0.4 g/L). Combining optimizing elements increase surfactin productivity (Mohammad Abdel-Mawgoud et al, 2008). These elements are not consumed and are thus carried onto the surfactin production stage.

The carbon and nitrogen concentrations is based on the carbon-to-nitrogen (C:N) ratio needed for *B. subtilis* to grown and produce surfactin. For surfactin, the C:N ratio is 10. Nitrogen will be sourced from NH_4NO_3. For cell propagation, Stamenković-Stojanović et al (2020a) recommends 10 g glucose/L as the carbon source concentration and a cultivation time of 24 hours. After this cultivation time, a biomass yield of 6.2 g/L can be achieved (Stamenković-Stojanović et al., 2020a). Accordingly, a glucose concentration of 25.05 g/L can be generated from the treated cassava wastewater. Thus, the medium will be diluted with water to reduce glucose concentration to 10 g glucose/L.

Surfactin fermentation is performed under aerobic conditions. Thus, oxygen concentration and the oxygen transfer rate (OTR) must be monitored and adjusted for successful cultivation of the microorganisms. (Stamenković-Stojanović et al., 2020a). Air must then be supplied in the bioreactor through aeration. Biomass yield of *B. subtilis* significantly increases at an OTR of 10 mol/(m^3h). The OTR values lower than this did not significantly differ in their biomass yield. Stamenković-Stojanović et al. (2020) was able to yield a biomass concentration of 6.2 g/L.

Stojanovic (2020a), for *B. subtilis* biomass growth, used an agitation speed of 150 rpm and 37˚C. Initial pH will be maintained between 6.5-9.0 (with 6.8 as the optimum) as high levels of cell growth are seen here (Mohammad Abdel-Mawgoud et al., 2008). The pH in the medium is said to decrease possibly due to the addition of NH_4NO_3 and Iron as observed in the studies by Wei & Chu (1998) & Yeh et al. (2006). Thus adding NaOH pellets as pH buffer will be added at a concentration of 3,000 mg/L to achieve the desired pH range as performed by Wei & Chu (1998).

One popular *B. subtilis* strain that produces surfactin with cassava wastewater as substrate source is *B. subtilis* LB5a. This was also utilized by Barros et al. (2008) and Nitschke & Pastore (2005). In the cell propagation stage, an inoculum size of 2% v/v will be fed as this was considered optimal for surfactin production (Mohammad Abdel-Mawgoud et al., 2008).

The input-output diagram for cell propagation in the production of surfactin is illustrated in Figure 5.

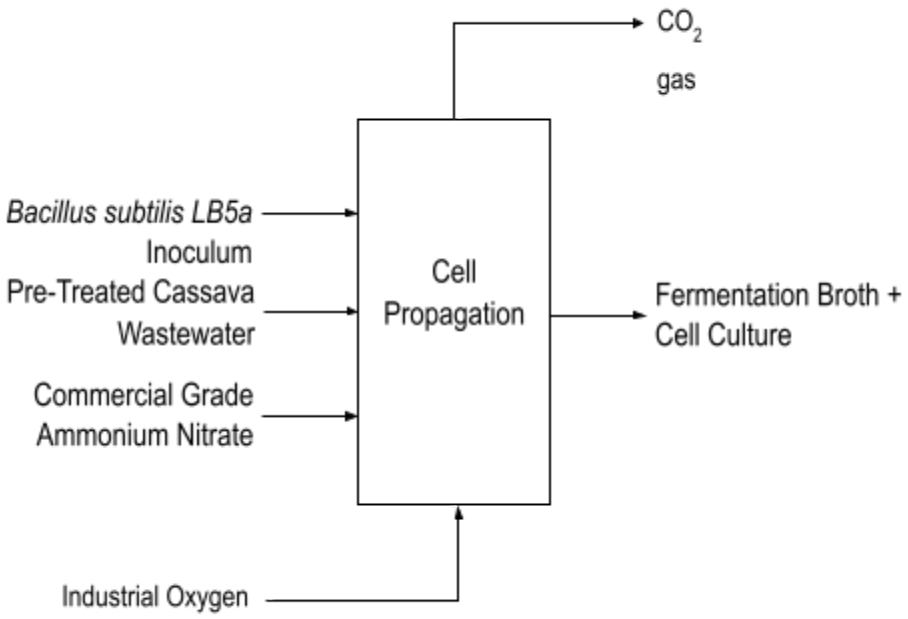


***Figure** 5. Input-Output Diagram for Cell Propagation*

D. Surfactin Production

Surfactin is produced from *B. subtilis* fed with a glucose substrate in a nutrient medium. The microorganism is grown in and comes from the cell propagation batch fermenter in Section 2.3.2. The nutrient medium, which is treated cassava wastewater fed with trace elements as nutrients, and the glucose are also taken from the previous fermenter. Furthermore, ammonium nitrate is also supplied in this stage still according to the C:N ratio of 10 needed for surfactin.

Moshtagh et al. (2019), for biosurfactant production from *B. subtilis*, found the following as the "optimum" conditions: an an agitation speed of 150 rpm and a temperature of 27˚C for the "best" surface tension and emulsification index for the surfactin product. Surfactin production can occur at an initial pH range of 6.0 to 9.0 and will be maintained in that range throughout. However, initial pH will be controlled at 6.8 as highest levels of of surfactin production are obtained here (Mohammad Abdel-Mawgoud et al., 2008). As this fermentation stage must also be aerobic, the bioreactor must also be aerated. Oxygen will be supplied according to stoichiometric requirements for the surfactin production reaction.

One innovation that improves surfactin production is by the addition of metal cations. Cooper et al. (1981) found that metal cations such as Iron and Magenisum induce a second and more dramatic exponential growth phase for both the surfactin and the biomass. In this design, trace metal elements are not consumed from the first fermentor and thus are used for this second fermentor.

Since materials consumed from the first fermentor are replenished in this stage, the equipment to be used in this process step is a fed-batch bioreactor.

i. Base Case: Foaming Fed-Batch Bioreactor

Typically, surfactin is produced by feeding glucose to *B. subtilis* in a batch fermentation process. From the reaction, foaming occurs, and surfactin, being a surface-active agent, accumulates in the foam. Thus, further collection of foam on top should be considered. Furthermore, with the addition of Iron and other metal cations, surfactin yield can be significantly improved. Cooper et al. (1981) performed fermentation with metal additivies and, afterwards, collected the foam produced into another vessel. Fermentation ran for 48 hours.

ii. Selection 2: Fed-Batch Bubbleless Membrane Bioreactor

Although surfactin can mostly be extracted from the fermentation foam, too much foam may not always be ideal. In scaled-up processes for surfactin production, the amount of foam to be collected may be too overwhelming, and there would possibly be a need for intense cooling processes (Coutte et al., 2010). Thus, Coutte et al., (2010) introduced a bubbleless membrane bioreactor system that prevents air sparging. The bioreactor utilizes a hollow fiber membrane as air-liquid contractor, and fermentation runs for 72 hours.

One usual problem demonstrated by utilizing a membrane bioreactor is the adsorption of surfactin onto the membrane. Surfactin has the strong ability to adsorb onto surfaces, and much of the surfactin produced is rapidly adsorbed on the aeration membranes (Shakerifard et al., 2009). Although, this issue can easily be solved by saturating the membrane surface so that surfactin can accumulate in the medium (Coutte et al., 2010). Thus, the bubbleless membrane bioreactor system also has the ability to keep the surfactin in the broth.

The input-output diagram for cell surfactin production is shown in Figure 6.

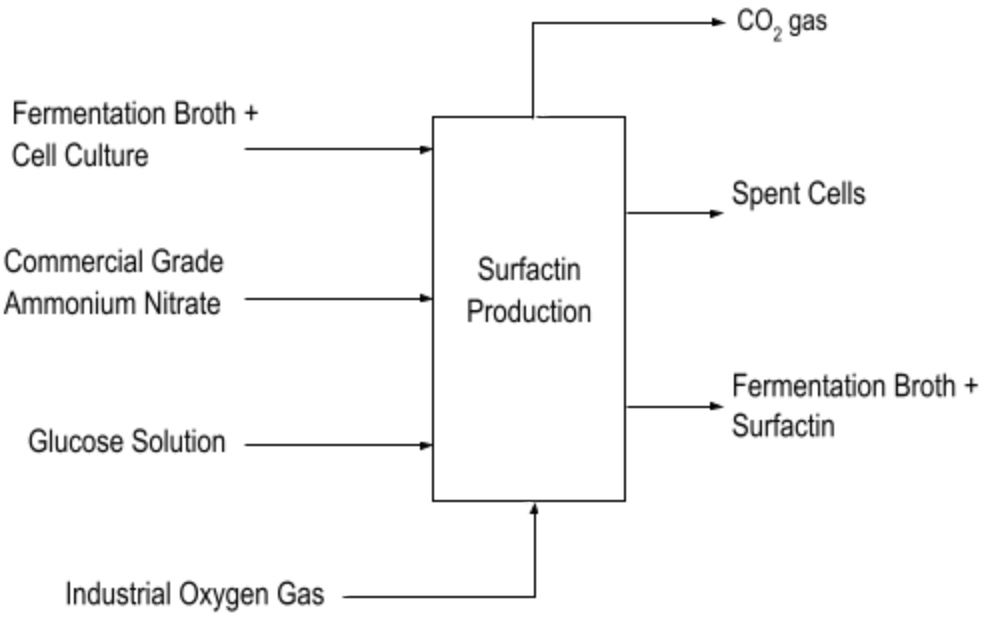


***Figure** 6. Input-Output Diagram for Surfactin Production*

Two options have been determined: the traditional bioreactor system and the bubbleless bioreactor system. To compare these options, the AHP was implemented. The selection process is shown in Table 7.

***Table 7.** Selection of Process Option for Surfactin Production*

Option	Product Yield and Purity (52%)	Ease of Operability and Technological Maturity (22%)	Health and Safety (17%)	Environment (7%)	Economy (3%)	**Composite Impact**
Foaming Fed-Batch Bioreactor	**0.430**	**0.146**	**0.085**	**0.035**	**0.026**	**0.722**
Fed-Batch Bubbleless Membrane Bioreactor	0.090	0.074	0.085	0.035	0.004	0.278
Total	0.52	0.22	0.17	0.07	0.03	1.000

With a composite impact score of 0.722, the foaming batch bioreactor is chosen. Coutte et al. (2010), although introduced the bubbleless bioreactor system, discovered that the foamed system generates a better surfactin yield. Furthermore, the selected system has already been widely used especially in lab-scale processes, allowing for future operations to be more familiar and of ease. The bubbleless bioreactor system, however, is a novel and more complicated technology. This can thus be cause for greater operational, installation, and maintenance costs.

Additionally, the detailed justifications of the selection process can be found in Appendix III.

E. Acid Precipitation and Foam Recovery

After the fermentation process, surfactin will be initially recovered from the fermentation broth by in-situ acid precipitation. According to Dlamini (2017), acid precipitation is one of the most commonly used and cost-efficient methods for recovering biosurfactants, like surfactin, from fermentation cultures. Kusaric and Sukan (2014) stated the the low pH neturalizes the negative charges of surfactin molecules which in turn, reduces their water solubility. Liu et al. (2007) then added that when the pH of the culture is decreased below a pH level of 6.5, macromolecular impurities and surfactin begin to precipitate. Moreover, acid precipitation carried out in the range of pH 2.0 to pH 4.0 leads to a high recovery factor of at least 97% and a surfactin purity of approximately 60% (Chen & Juang, 2008). The fermentation broth will be acidified to a pH level of 2.0 by adding commercial grade hydrochloric acid (HCl). The desired pH level is in line with other surfactin production studies that also set the pH of the resultant acid precipitation of surfactin at 2.0 (Andrade et al., 2016; Barros et al., 2008; Cooper et al., 1981; Czinkóczky & Németh, 2020b; Isa et al., 2008; Joshi et al., 2015). Afterwards, foam recovery will take place.

Foaming is an important process to consider in the lab-scale and industrial-scale production of biosurfactants, especially during the fermentation process. Foaming is not desired as it can disrupt the fermentation process, lead to carryover of fermentation broth with the off gas, and present difficulties in the process control of the system (Etoc et al., 2006). However, foaming can be exploited in a way that it can be recovered to help intensify biosurfactant production, and one way to do so is by foam fractionation. Winterburn & Martin (2012) defined foam fractionation as a process for enriching solutions of surface-active species like biosurfactants. This process involves the input of gas to the biosurfactant solution which creates a new gas-liquid interface onto which the biosurfactant molecules adsorb and stabilize. This then

generates a rising foam. Over time, the foam will overflow, and it will be collapsed in order to recover the adsorbed biosurfactant.

Certain studies have already utilized foam fractionation in the production of biosurfactants specifically surfactin. Cooper et al. (1981) was able to conduct foam fractionation in their study, and it was found that fermentations done without foam fractionation produced very poor product yield while fermentations done with foam fractionation produced high amount of surfactin, producing a surfactin concentration of 0.8 g/L. Davis et al. (2001) utilized in situ batch foam fractionation in the production of surfactin by *B. subtilis* ATCC 21332 which resulted to 90% surfactin recovery. Moreover, Barros et al. (2008) was able to use the foam recovery system designed by Davis et al. (2001) in their study on the pilot scale production of surfactin by *Bacillus subtilis* LB5a using cassava wastewater as substrate. It was found in their study that the foam recovery system led to a yield of 0.64 g of surfactin per liter of substrate. They then described such system as an efficient method to recover surfactin. Additionally, in situ recovery of surfactin by foam fractionation is able to achieve high surfactin recovery rates of at least 90% (Dlamini, 2017).

In this pilot-industrial scale production of surfactin, the foam fractionation process will be adapted from the study of Davis et al. (2001) where the in situ recovery of surfactin will be implemented such that a foam column will be inserted through the vessel headplate of the bioreactor, and it will be positioned in such a way that the bottom of the column will be just above the fermentation broth surface. Figure 7 shows the input-output diagram for acid precipitation and foam fractionation.

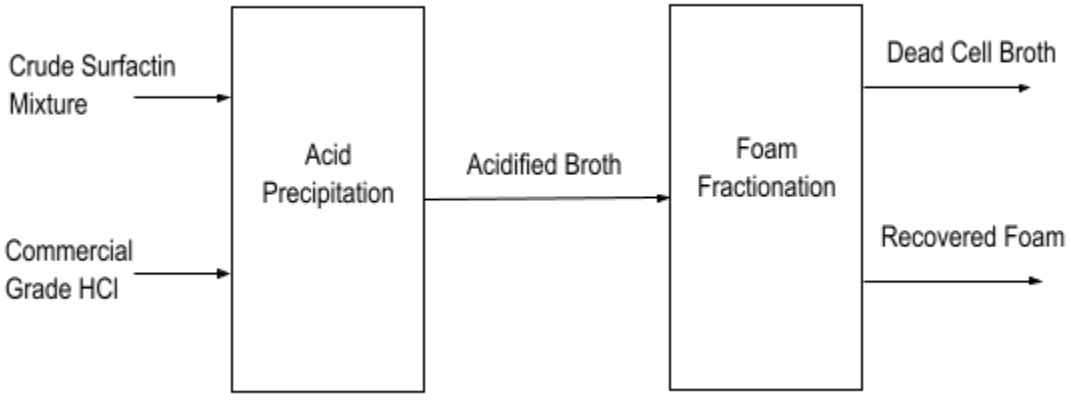


Figure 7. *Input-Output Diagram for In situ Acid Precipitation and Foam Recovery*

F. Moisture Removal of Wet Surfactin Mixture

After the foam recovery step, the collected permeate is then subjected to further recovery and purification in order to obtain the final product: white and dry powder surfactin. Generally, the processes involved are neutralization, centrifugation, and lyophilization (Andrade et al., 2016; Barros et al., 2008; Cooper et al., 1981; Czinkóczky & Németh, 2020b; Hoffmann et al., 2021; Joshi et al., 2015; Nitschke & Pastore, 2006).

To obtain the desirable product, which is in powder form, the surfactin mixture must be dried to remove excess moisture from the centrifugation step conducted previously. It is frequently necessary to prepare the completed product for particular applications (e.g., cosmetics) after the final high – performance purification stages (i.e., acidification and flotation).

i. Option 1: Freeze Drying or Lyophilization

The removal of water from frozen state by sublimation is known as freeze drying or lyophilization. Freeze drying consists of three independent steps: freezing of product solution, water removal by sublimation in a primary drying stage, removal of residual moisture by heating in its secondary drying stage. Since surfactin produced by *B.subtilits* consists of seven amino-acid ring structures with a fatty acid chain, we can take surfactin's composition like protein, which is also under food-grade products, the goal of this further purification process. To start-up the lyophilizer (apparatus used for lyophilization), it will be set at a temperature of -60 ℃ for 30 mins. After starting-up, the lyophilizer will be filled with the surfactin. To ensure effective freeze drying, high ratio of surface area to volume must be observed. It is usually best that a solid content of about 10% w/w or depth of material filled into the lyophilizer does not exceed 20 mm (Matejtschuk, 2007). After loading, the freezing process may begin which involves bringing the temperature below its freezing temperature which is about – 25 ℃ (Lewis et al., 2010). Temperature will be allowed to fall further until it steadies at – 40 ℃, at this point vacuum will be started. Once surfactin inside the lyophilizer achieves good appearance, which is mostly achieved after a minimum of 72 hours of lyophilization, air will be admitted as gentle as possible. Surfactin can now be removed for storage at refrigerated temperature (Matejtschuk, 2007).

ii. Option 2: Spray Drying

Spray drying is a well-known method of particle production that involves converting a liquid substance into dried particles using a gaseous hot drying medium. The spray drying mechanism works by removing moisture from the feed product by exposing it to a hot environment. Three primary steps can be identified in the process: atomization, droplet-to-particle conversion, and particle collection (Santos et al., 2018). In a study of the impact of using spray drying as purification process for three lipopeptide biosurfactants produced by *B.subtilis*, the spray dryer had an inlet temperature of 140 °C and outlet temperature of 70 °C. The spray drying air pressure was set at 40 mm water column, and the pumping flow was kept at 8.5 ml•min^{-1} (Vassaux et al., 2021). Table 8 below then shows the summarized selection process.

Table 8. *Selection of Process Options for Drying Process*

Option	Product Quality (51%)	Process Time (22%)	Ease of Operability and Technological Maturity (17%)	Health and Safety (5%)	Economy (5%)	**Composite Impact**
Freeze Drying	**0.380**	**0.070**	**0.110**	**0.030**	**0.010**	**0.600**
Spray Drying	0.130	0.150	0.060	0.020	0.040	0.400
Total	0.51	0.22	0.17	0.05	0.05	1.000

With a composite score of 0.600, Freeze drying is chosen for moisture removal of the surfactin mixture. Although both processes can be considered in the removal of moisture, the product's quality is of greatest concern. Spray drying poses great advantage on the economical aspect and can also be ran on an almost continuous type of operation compared to freeze drying. The problem with spray drying that greatly degrades the quality of the product would be on its operating temperature that starts at 40 °C. With that, to ensure that the product obtain will be of quality, freeze drying is chosen. Freeze drying is often carried out to obtain a final moisture content of 1 – 3% (Berk, 2018). The input-output diagram for lyophilization is presented below.

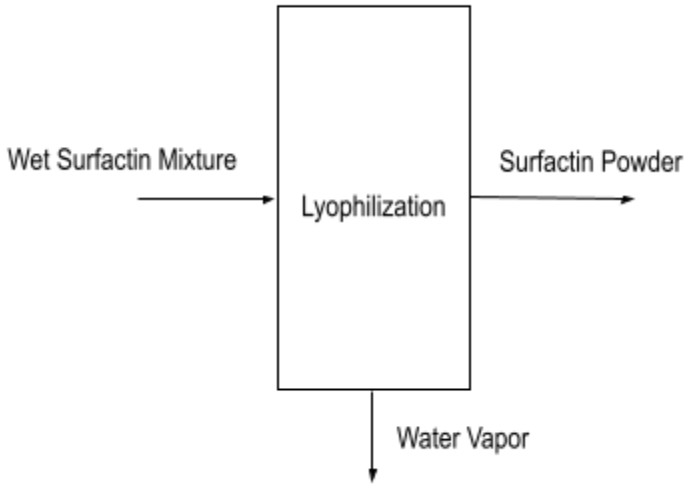


***Figure** 8.* Input-Output Diagram of Lyophilization

Chapter III: BASIS OF DESIGN

3.1. Design Objectives

In line with the abovementioned information, the objectives of the design are:

- To design a feasible foam industrial scale, fed-batch core process in producing surfactin from cassava wastewater using *B. subtilis;*
- To propose appropriate industrial scale upstream and downstream processes to produce quality substrate from waste and to achieve high purity product.
- To maximize production at minimum cost without compromising safety, health, and the environment; and
- To employ automated control systems to maintain product quality.

3.2. Battery Limits

In the design of processes, battery limits are specified to define the scope and limitations of the design. Table 9 summarizes the inside and outside battery limits for the conceptual process design of the pilot-industrial production of surfactin.

Table 9*. Battery Limits in the Process Plant Design*

Inside Battery Limits	Outside Battery Limits
● Perform overall material and energy balance for the entire surfactin production plant. ● Perform material and component balance around each process equipment. ● Chemical engineering design for substrate pre-treatment, cell propragation, surfactin production, separation of growth medium and foam, surfactin extraction ● Chemical engineering design for utilities and equipment auxiliaries used in the overall surfactin production plant ● Mechanical engineering design of the equipment and auxiliaries used in the overall production plant. ● Conduct HAZOP and FMEA analysis on the entire plant. ● Prepare Process and Instrumentation Diagram (P&ID) for the entire plant. ● Perform economic analysis based on the input and output streams.	● Preparation of *B. subtillis* inoculum. This includes the pre-cultivation of the bacteria and preparation of the growth/nutrient medium. ● Develop a process scheme for the packaging of the surfactin product. ● Recovery of ammonia from cell separation process. ● Recovery of cells from the cell separation process. ● Recovery of carbon dioxide from the fermenter ● Separation of metals from cassava wastewater. ● Waste Treatment design process for Solid Sludge, Dead Cell Broth, and Cyanide Wastes

3.3. Description of Design and Process Definition

In this section, the initial block scheme is constructed to identify the input and ouput streams that are expected from the entire plant design. The values employed are observed based on the preliminary assumptions from the selected process techniques included assumptions from the overall reaction, physical and chemical properties of materials used, and the location at which the process will take place.

3.3.1. Process Chosen

The pre-treatment of cassava wastewater will involve solids removal through centrifugal sedimentation and cyanic removal through adsorption. This is to prepare the cassava wastewater for its role as the substrate source in the succeeding process steps.

For the surfactin production stage, the bubbled fed-batch reactor was selected for the production of surfactin by *B. subtilis*. As mentioned, such system has been successfully used in lab-scale production already. Thus, it is a promising system to use for future upscaled operations. Surfactin production will be performed for 48 hours in a feb-batch fermenter. Prior to this, cell propagation will be performed in a batch fermenter for 24 hours.

For the downstream processes, foam recovery will be done through in-situ foam fractionation. When the foam has been separated, centrifugation will be utilized to obtain a cell-free broth. Then, acid precipitation using commercial grade (~33%) hydrochloric acid and flotation process will be done *in situ*. This will separate the dead cells from the surfactin product. Finally, the wet surfactin foam from the flotation process – to which was scraped off the system – is freeze dried at vacuum pressure via batch process to obtain white powder at very low moisture content (of about 3%-5% w/w).

A. Input-Output Diagram

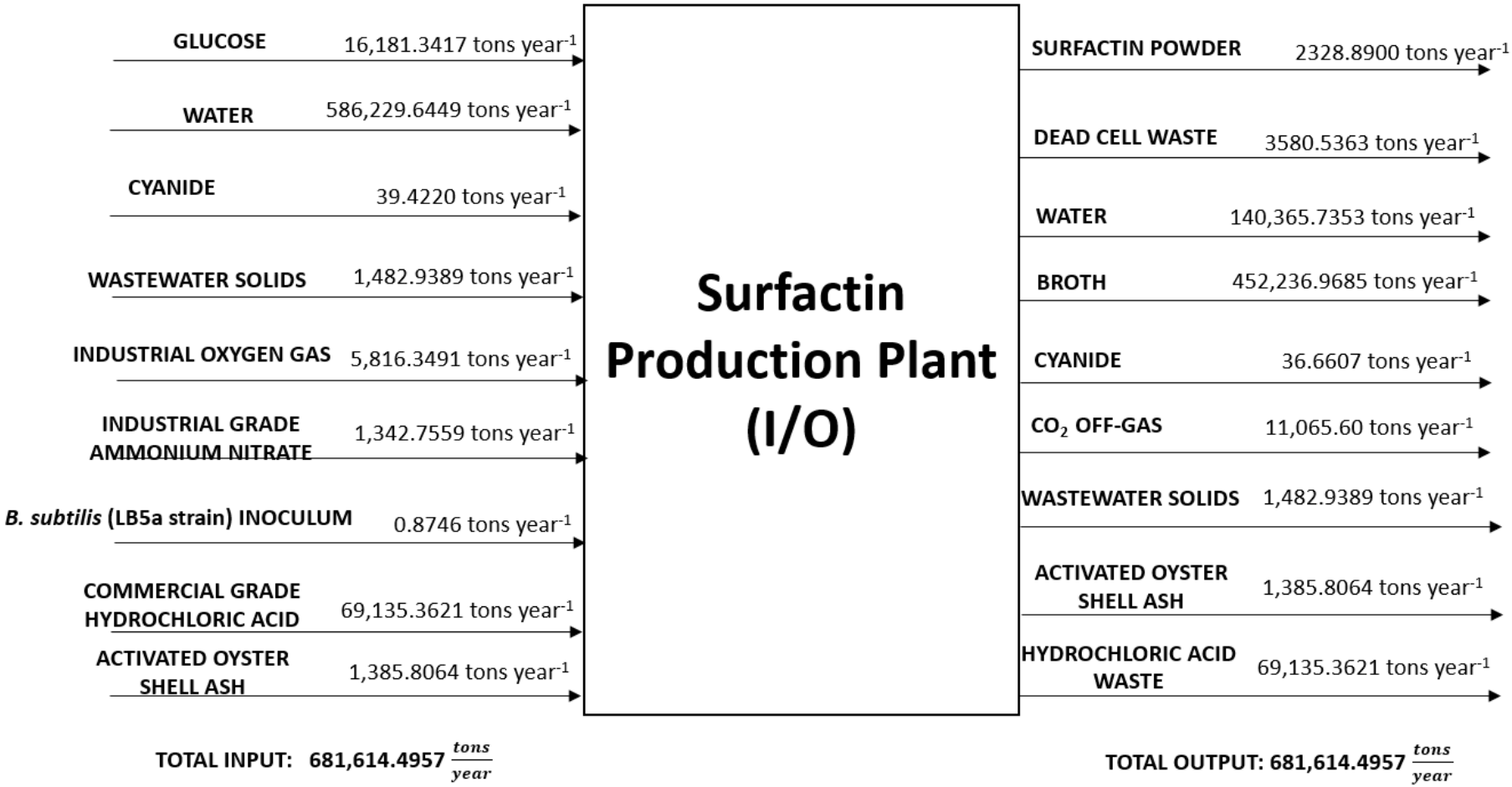


Figure 9. *Input-output diagram of the surfactin production plant using B. subtilis (LB5a strain) and Cassava Wastewater Substrate*

B. Functional Block Diagram

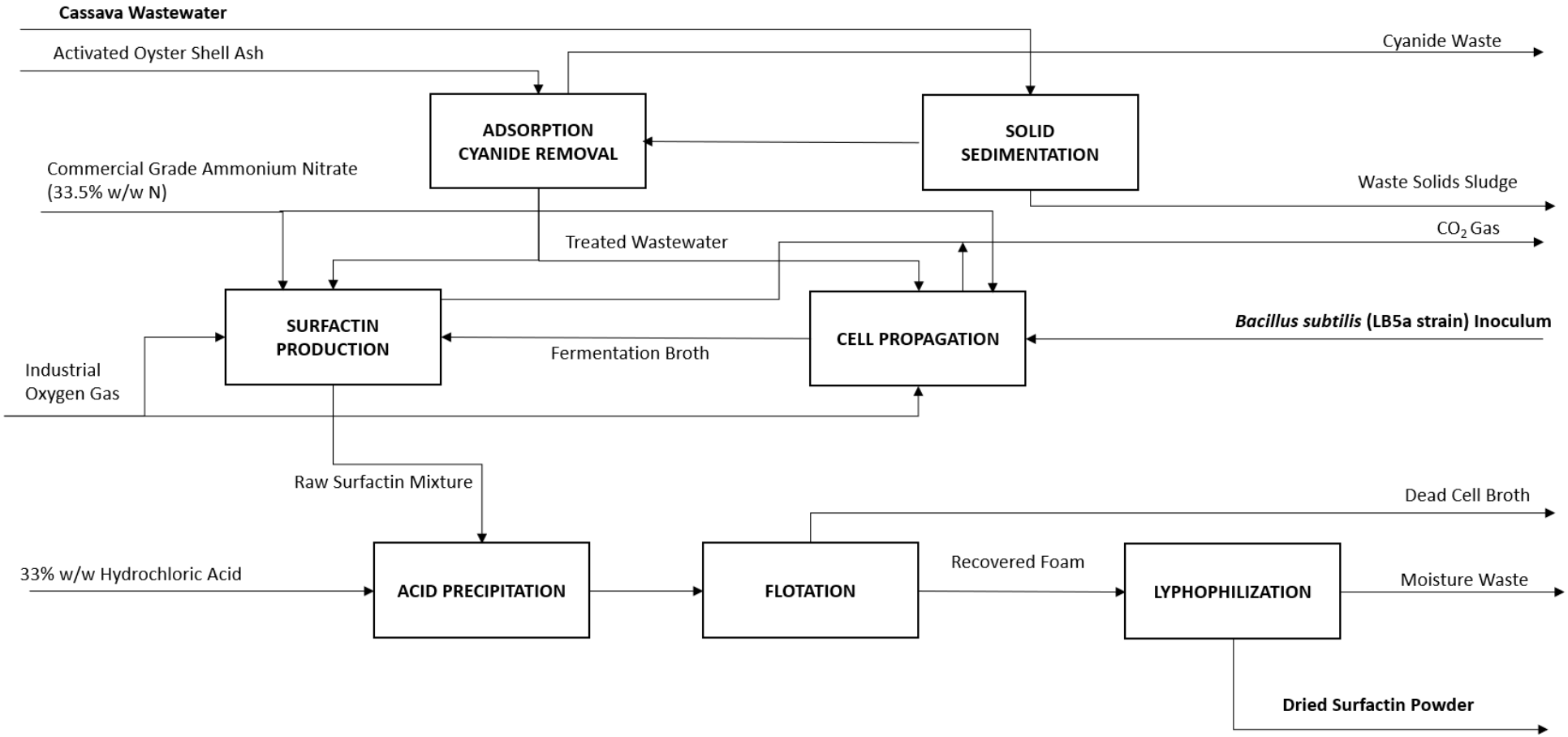
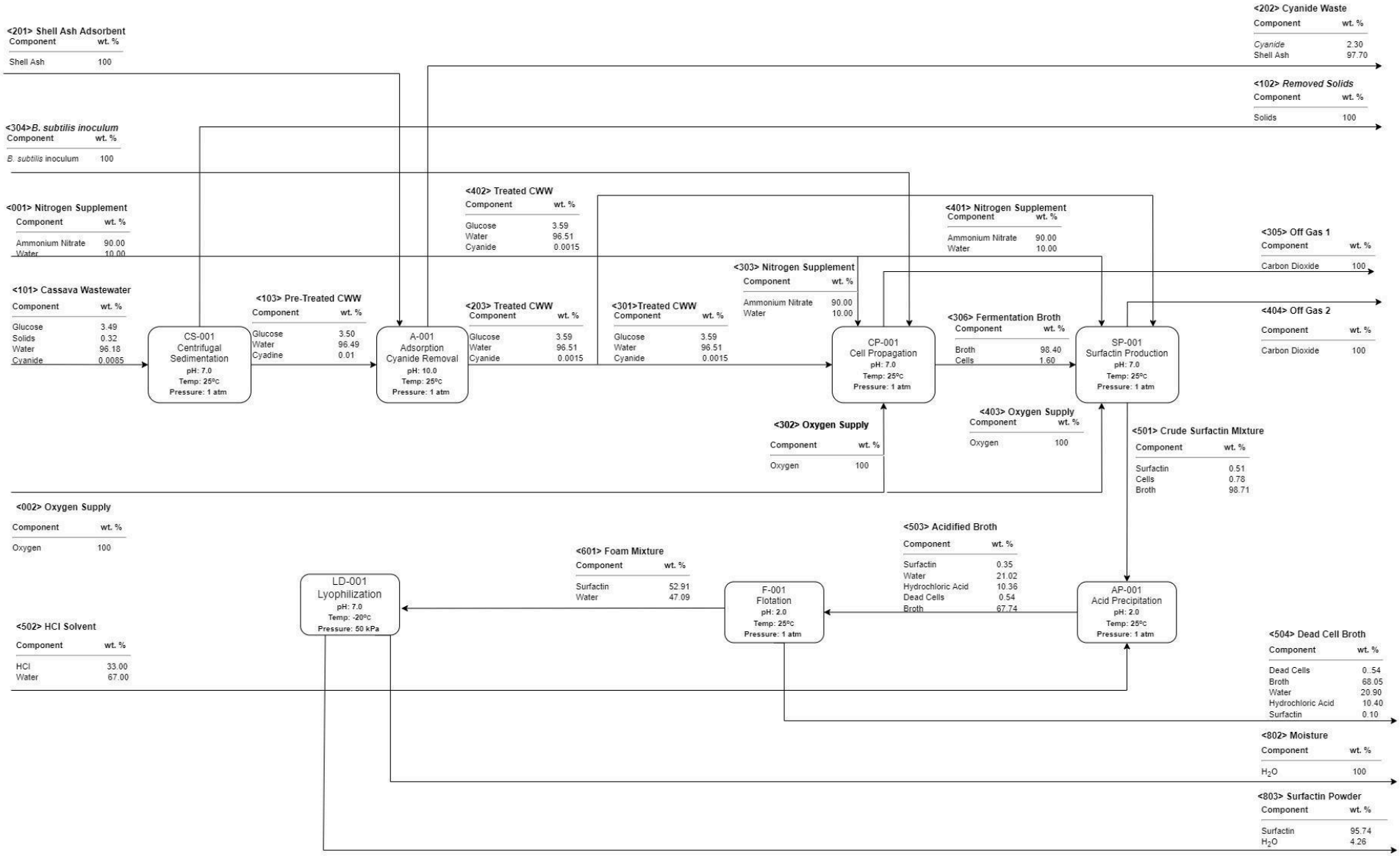


Figure 10. *Functional Block Flow Diagram of the Industrial Scale Surfactin Production Plant*

C. Process Flow Diagram with Stream Specifications and Preliminary Component Concentrations



3.3.2. Thermodynamic Properties, Transport and Kinetic Data

A. Thermodynamic Properties

Stipulated in Table 10 are the thermodynamic properties of pure compounds that are involved in the process system. This include the critical pressure and temperature, heat of fusion, heat of vaporization, and heat of formation of each substance. Meanwhile, Table 11 presents the gaseous heat capacities of the materials involved and its respective range of temperature. Furthermore, the equation of ammonia in the determination of heat capacity is different from other compounds involved.

Table 10. *Thermodynamic Properties of Pure Compounds (Flickinger, 2017; Green & Perry, 2008)*

Component	Formula	Critical Temperature T_c (K)	Critical Pressure $P_c \times 10^5$ (Pa)	Heat of fusion (kJ/mol)	Heat of Vaporization (kJ/mol)	Heat of formation at 25°C (kJ/mol)
Glucose	$C_6H_{12}O_6$	1034.42	66.30	31.406	117.459	-45.857
Ammonium Nitrate	NH_4NO_3	405.65	11.30	6.1086	---	-338.444
B. subtilis	$CH_{1.66}O_{0.27}N_{0.20}$	374.15	50.00	---	---	-91.00
Water	H_2O	647.13	21.94	6.008	40.706	-285.840
Hydrochloric Acid	HCl	324.65	8.36	1.992	16.150	-166.732
Oxygen Gas	O_2	154.58	5.02	0.4485	6.816	---
Carbon Dioxide	CO_2	304.25	73.76	7.9496	25.2295	-393.5136
Hydrogen Cyanide	HCN	456.85	53.90	8.4057	25.2170	101.2528

Table 11. *Heat Capacities and Temperature Range of Pure Compounds (Green & Perry, 2008)*

Component	Formula	Gas Phase Heat Capacity (C_p) Parameters[a,b]					Range of Temperature (K)
		C_1	C_2	C_3	C_4	C_5	
Ammonium Nitrate	NH_4NO_3	31.8	---	---	---	---	273-298
Water	H_2O	276,370	-2,090.1	8.125	-0.014116	9.370×10^{-6}	273.16-533.15
Hydrogen Cyanide	HCN	95,398	-197.52	0.3883	---	---	259.83-298.85
Carbon Dioxide	CO_2	10.34	0.00274	---	---	195,500	273-1200
Hydrochloric Acid	HCl	32.124	-13.459	19.869	-6.854	-0.050	100-700

[a] Temperature values are in Kelvin (K) units

[b] All substances uses the equation for heat capcacity (in J/mol.K) which is $C_p\,[=]\frac{J}{mol.K} = C_1 + C_2T + C_3T^2 + C_4T^3 + \frac{C_5}{T^2}$

B. Kinetic Property Data

The chemical equations involved in the reaction process of producing surfactin is divided into two which are the growth phase and fermentation phase. Each phase has a specific chemical equation based on the calculation of the pseudo equation for biomass and surfactin production.

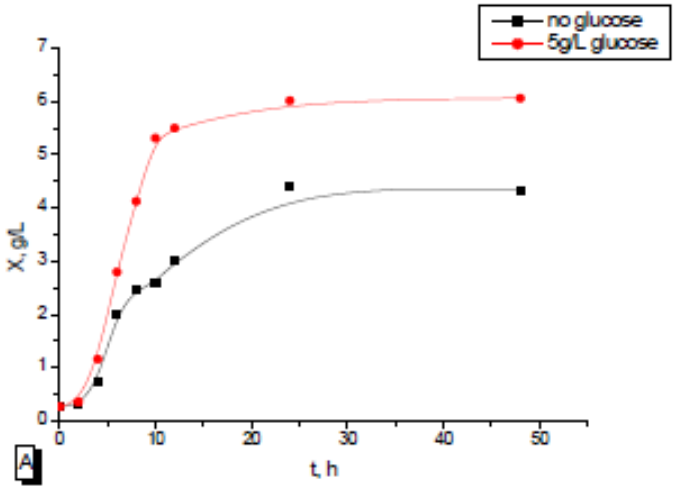
REACTOR 1: GROWTH PHASE | BACTERIA PROPAGATION

REACTOR 2: FERMENTATION PHASE | SURFACTIN PRODUCTION

For the batch growth kinetic data, Table 12 presents the concentration of *B. subtilis* in g/L at a measured operating time. Furthermore, Figure 11 shows the consumption of glucose at a given operating time which will be used as the kinetic data for cell propagation. On the other, the surfactin production kinetic data is presented in Figure 12 given at varied initial concentrations of glucose.

Table 12. *Kinetic Data of Cell Growth of Bacillus Subtilis in 10 g/L Glucose Concentration* (Stamenković-Stojanović et al., 2020b)

Time (h)	**Concentration of *B. subtilis*** $\left(\frac{g}{L}\right)$	**Time** (h)	**Concentration of *B. subtilis*** $\left(\frac{g}{L}\right)$
0.00	0.238	13.50	4.855
1.52	0.328	15.02	5.064
3.04	0.626	16.35	5.153
4.65	1.162	18.06	5.302
6.08	1.817	19.58	5.421
7.22	2.860	21.29	5.540
7.41	3.068	22.81	5.600
8.18	3.664	25.86	5.719
9.13	3.962	28.90	5.779
9.70	4.170	34.03	5.838
10.46	4.379	38.97	5.838
12.17	4.677	47.909	5.779
$Y_{\frac{X}{S}}\left(\frac{g_{cell}}{g_{glucose}}\right)$	0.4513	$\mu_{max}\left(hr^{-1}\right)$	0.282


no glucose
5g/L glucose
X, g/L
t, h
A

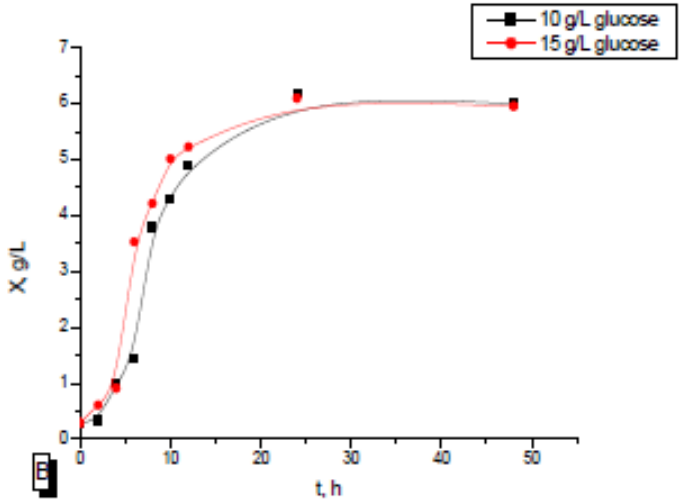

10 g/L glucose
15 g/L glucose
X, g/L
t, h
B

Figure 11. *B. subtilis Concentration (X) growth over time (t) at varying glucose concentration. (A) Glucose concentration of zero g/L (in black) and 5 g/L (in red); (B) Glucose Concentration at 10 g/L (in black) and 15 g/L (in red)* (Stamenković-Stojanović et al., 2020a)

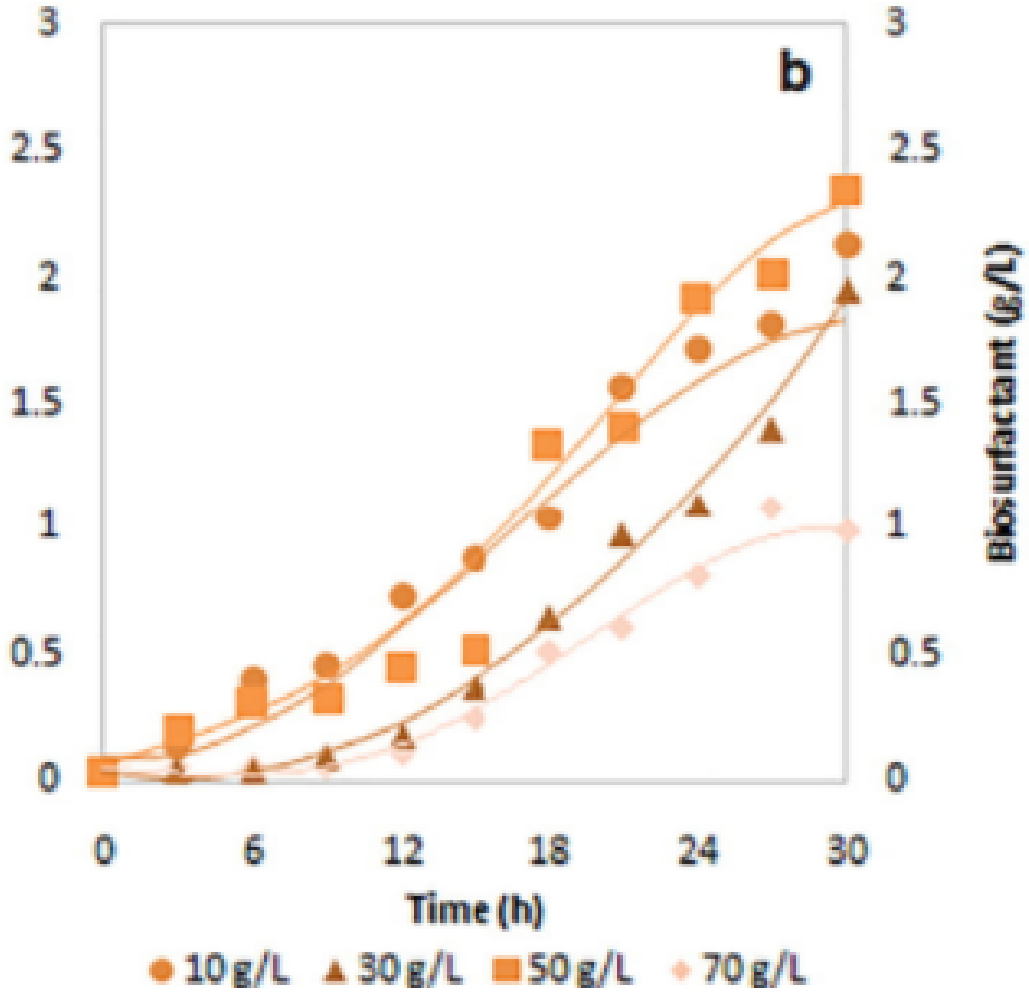


Figure 12. *Surfactin Production at varying initial glucose concentration using Bacillus subtilis culture (Heryani & Putra, 2017)*

C. Adsorption Data for Cyanide Removal via Activated Oyster Shell Ash

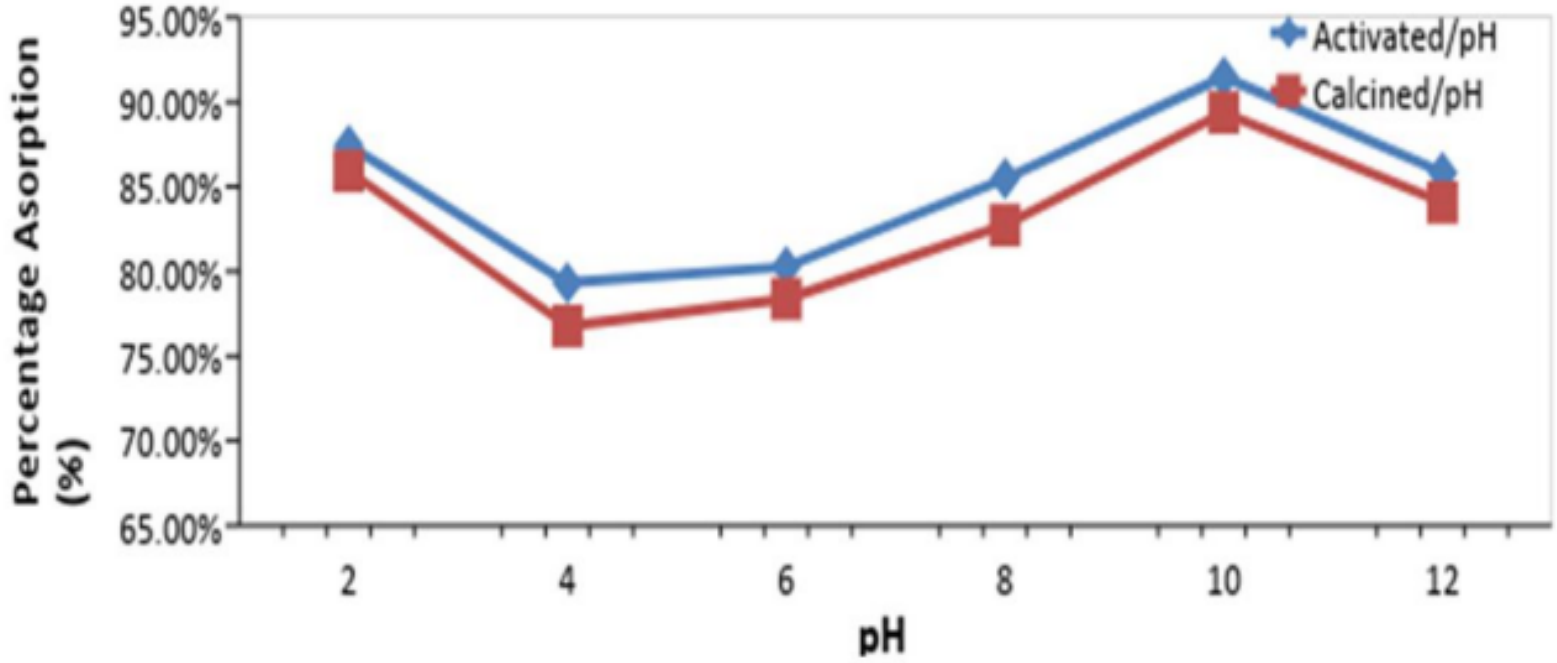


Figure 13. *Effect of pH on the adsorption of cyanide onto activated oyster shell ash (AOSA) and calcined oyster shell ash (COSA) (Akpan & Etuk, 2019)*

3.4. Basic Assumptions

In this section, the basic assumptions needed as basis of design are discussed.

3.4.1. Capacity

The lifetime of the plant for the production of surfactin from *B. subtilis* is assumed to be 15 years. Furthermore, the processing time is 7,920 h per annum at full capacity. These were as assumed by Czinkóczky & Németh (2020). Plant attainment in percentage is determined in Equation 1.

$$\%\ Attainment = \frac{Plant\ Processing\ Time}{Hours\ in\ a\ Year} \times 100\% = \frac{7{,}920\ h}{8{,}760\ h} \times 100\% = 90.41\% \qquad (1)$$

A plant attainment of 90.41% was obtained. Typically, plant attainment is between 90% to 95%.

3.4.2. Location

The location of the plant will be selected considering a set of factors: Proximity to Raw Materials, Proximity to Waste Disposal Services, Availability of Land and Infrastructure, Capital, and Risk of Calamity. This is as slightly adapted from Park et al. (2018). The description of each factor is shown in Table 13.

Table 13. *Factors for Plant Location Selection*

FACTOR	DESCRIPTION
Proximity to Raw Materials	Location should be near to suppliers of raw materials, particularly to cassava processors.
Proximity to Waste Disposal Services	The process generates processed wastewater. It is advantageous if the location is near a river or creek.
Availability of Land and Infrastructure	There should be available land in the area as well as other infrastructures that can provide the necessary utilities.
Capital	Costs for fixed capital for building, equipment, and labor for construction and installation should not be too extravagant.
Risk of Calamity	Risk of calamity should be kept at a minimum. Area should not be prone to landslides, flooding,

	and earthquakes and should not be near volcanoes.

The primary factor to be considered should be the proximity to raw materials. Thus, the location must first be near a cassava processing plant. Table 14 shows a list of cassava processors in the Philippines and their respective addresses.

Table 14. *Cassava Processors in the Philippines*

COMPANY	ADDRESS
Cassava Growers & Processors Association	Barangay Aseniero, Dapitan City, Zamboanga Del Norte
Charm's Food Products	Navotas, Barangay Ikirin, Pagbilao, Quezon
Conie Food Delicacies Center	Maharlika Highway Zone 1, Brgy. Mahon, Tanauan, Leyte
Lighthouse Cooperative	Jose Alma Arcade, 39 Luna St., Centro Arcade Tuguegarao City
Lucky 888 Food International Incorporated	126 D. Aquino St., Grace Park, Caloocan City
Marikina Food Corporation	228 Champaca Street, Parang, Marikina City 1809
Pearl Foods International, Inc.	187 Sta. Monica, San Pablo City, Laguna

Considering all the factors, a location in **Dapitan City** near Cassava Growers & Processors Association was chosen. A satellite image of the possible location in Barangay Aseniero is shown in Figure 14.

Figure 14. *Possible Plant Location in Barangay Aseniero, Dapitan City, Zamboanga del Norte*

Among the options in the selection, Cassava Growers & Processors Association is the only one that mainly specializes in cassava. Thus, a plant that utilizes its wastewater would also be very beneficial for them. There is also a river that runs through the area as shown in Figure 15.

Figure 15. *River along Barangay Aseniero in Dapitan City, Zamboanga del Norte*

Being a city, Dapitan is also well-supplied with water and electricity. However, it is not too urbanized to merit high capital costs especially in building and installment of equipment. According to the Geohazard Susceptibility Assessment of the barangays in Dapitan City, Barangay Aseniero has a low risk in landslides.

There are also no active volcanoes in Dapitan City. Earthquakes and typhoons, however, are generally frequent in the Philippines. Thus, this should be considered when building the plant and in putting up safety measures.

3.4.3. Input and Output Streams

The basis for the mass balance calculation for the entire process is the production capacity from the description on the market study. Though no known studies nor response from manufacturers from Dapitan City who can quantify the component concentrations of effluent cassava wastewater, studies outside of the Philippines are able to conduct such. Presented in Table 15 is the known composition of cassava waste water based on the study by Nitschke & Pastore (2006).

Table 15. *Composition of Cassava Wastewater* (Nitschke & Pastore, 2006*;* Imai et al., 2009)

Component	Concentration
Total carbohydrates (g/L)	35.3±1.52
Reducing sugars (g/L)	12.8±0.57
Non-reducing sugars (g/L)	22.2±0.35
Total nitrogen (g/L)	2.5±0.17
Phosphorus (mg/L)	225.9±0.34
Potassium (mg/L)	2665.1±0.45
Calcium (mg/L)	272.5±0.15
Magnesium (mg/L)	519.0±0.09
Sulfur (mg/L)	104.0±0.21
Iron (mg/L)	7.8±0.03
Zinc (mg/L)	7.3±0.08
Manganese (mg/L)	1.8±0.05
Copper (mg/L)	0.6±0.06
pH	5.9±0.02
COD* (g/L)	55.82±0.25
Cyanide (mg/L)	86.0

*COD: Chemical Oxygen Demand

Knowing that 0.1% of the total amount susfactants are made of biosurfactants, 55% of that amount is applied for cosmetic and personal care, and that 18% of the market share is made in the Asia-Pacific (specifically China), the production capacity for surfactin production is at 1,643.40 tons of crude surfactin recovered per year (Mordor Intelligence, 2020; Muthusamy et al., 2008; Vandeputte, 2012).

3.4.4. Feedstock, Chemicals, and Micoorganism

The feedstock consists of *B. subtilis* LB5a and cassava wastewater. As nitrogen source, ammonia is used. No catalysts are used in the process.

B. subtilis LB5a is a common strain of bacteria used in the production of surfactin with cassava wastewater as substrate source (Barros et al., 2008; Nitschke & Maria Pastore, 2005).

From the cassava wastewater, a total sugar concentration of 50.1 g/L is assumed to be generated. Furthermore, it is assumed that 50% of that is glucose (Nitschke & Maria Pastore, 2005).

The pure component physical properties of ammonia and water are stipulated in Table 16. Data was obtained from Perry's Chemical Engineers' Handbook by Green & Perry (2008).

Table 16. *Pure Component Physical Property Data* (Green & Perry, 2008)

Chemical	Physical Property Data						
	Formula	Formula Weight	CAS Number	Specific Gravity	Melting Point (˚C)	Boiling Point (˚C)	NFPA Rating
Ammonia	NH_3	17.03	7664-41-7	$0.817^{-79^{\circ}}$	-77.7	-33.4	3-1-0
Water	H_2O	18.02	7732-18-5	$1.00^{4^{\circ}}$ (lq); $0.915^{0^{\circ}}$ (ice)	0	100	0-0-0
Oxygen	O_2	32.00	7782-44-7	$1.14^{-188^{\circ}}$	-218.4	-183.0	0-3-0
Nitrogen	N_2	28.02	7727-37-9	$1.026^{-252.5^{\circ}}$	-209.6	-195.8	0-0-3
Glucose	$C_6H_{12}O_6$	180.16	50-99-7	-	165.5	-	0-0-0
Phosphorus	P_4	123.92	7723-14-0	$2.20^{20^{\circ}}$	590^{43atm}	ign. in air, 725	4-4-2
Potassium	K	39.10	7440-09-7	$0.86^{20^{\circ}}$	62.3	760	3-3-2
Calcium	Ca	40.08	7440-70-2	$1.55^{20^{\circ}}$	842	1484	1-3-2
Magnesium	Mg	24.32	7439-95-4	$1.74^{20^{\circ}}$	651	1110	1-0-1

Sulfur	S	32.06	7704-34-9	2.046	120	444.6	1-2-0
Iron	Fe	55.85	7439-89-6	$7.86^{20^{\circ}}$	1535	3000	0-0-0
Zinc	Zn	65.38	7440-66-6	7.140	419.4	907	1-3-1
Manganese	Mn	54.93	7439-96-5	$7.2^{20^{\circ}}$	1260	1900	3-2-1
Copper	Cu	63.57	7440-50-8	$8.92^{20^{\circ}}$	1083	2300	2-2-0

3.4.5. Product Specifications

The minimum target purity of the surfactin produced from *B. subtilis* will be based on the purity level of the surfactin produced from the preliminary mass balance which is approximately 68% w/w purity. The other characteristics of the product, as shown in Table 17.

Table 17. *Surfactin chemical product specifications after the design process*

Properties	Specifications
Appearance (Form)	Powder
Appearance (Color)	White to Faint Yellow
Elemental Analysis (%C anhydrous)	60.42 – 62.42 %
Elemental Analysis (%N anhydrous)	8.96 – 9.96 %
Purity	96 %

3.4.6. Waste Specifications

The production of surfactin from cassava wastewater using *B. subtilis* entails a significant amount of effluent which is subject to several environmental concerns. Although some components found initially in the cassava wastewater (i.e., carbohydrates, sugars, ammonia, nitrogen, and oxygen) are consumed during the surfactin production process, other components are also introduced during the purification and

separation processes. Furthermore, the additional components in the wastewater could possibly alter the pH conditions of the water which could be detrimental to the surrounding bodies of water and soil.

The location of the factory is beside the river in Dapitan, Zamboanga del Norte. According to the Water Quality Guidelines and General Effluent Standards of 2016 of the Department of Environment and Natural Resources (DENR), rivers are considered as navigable waters which refers to the waters of the Philippines, including territorial sea and inland waters suitable for water transport. Table 18 summarizes the relevant wastewater regulation level specifications in accordance with the DAO guidelines.

***Table 18**. Wastewater Parameter Regulation Levels for Navigable Waters*

Parameter	Unit	Specifications
Primary Parameters		
BOD	mg/L	15
Dissolved Oxygen (Minimum)	mg/L	2
Total Suspended Solids	mg/L	110
Temperature	^{0}C	25-32
Nitrate	mg/L	15
pH		6.0-9.0
Secondary Parameters - Inorganics		
Ammonia	mg/L	0.75
Secondary Parameters – Metals		
Iron	mg/L	7.5
Lead	mg/L	0.1
Manganese	mg/L	2.0

Zinc	mg/L	4.0
Nickel	mg/L	1.00
Copper	mg/L	0.04
Cadmium	mg/L	0.01

The wastewater component quantities should fall below the regulatory levels as listed in Table 18. Therefore, several downstream processes and treatments will be done prior to discharging the wastewater into the river.

Cell separation and decontamination processes will also be done on the wastewater to remove the *B. subtilis* from the medium. These processes will be done to avoid bacterial contamination of the surrounding waters and soils. Furthermore, the spent cells will be stored on a container tank and will be disposed appropriately.

3.4.7. Utilities

The production of surfactin will entail drying, cooling, and separation processes. These processes will involve the use of steam that will be recycled all throughout the production process to minimize steam waste. Furthermore, appropriate heat exchangers will be used to facilitate the heat exchanging process involved in the production. On the other hand, electricity for all pumps, compressors, and transporting conveyors will be provided based on the quantified power requirements.

3.5. Economic Margin

The economic margin is the financial framework utilized by design companies to evaluate and analyze the corporate performance of the designed operation based on cash flow perspective. It is a measure of the company's returns which makes it an indicator of the economic feasibility of the designed operations. Furthermore, the economic margin can be used to determine whether the company is earning above or below its cost of capital. This also provides a more comprehensive view of the company's underlying economic vitality (The Applied Finance Group, 2013).

An economic feasibility analysis of the entire plant, including the feedstock and other raw materials, as well as the plant's end-products, should be conducted. As a result, in order for the factory to operate and

sustain itself in the long run, economic profit must be achieved. The estimated economic margin assesses the profitability of the manufacturing plant.

In the design of the surfactin production using cassava wastewater and *B. subtillis* as the bacteria, there are four raw materials needed. This includes (1) Cassava wastewater, (2) Ammonium Nitrate, (3) *B. subtilis* Inoculum, and (4) Industrial Oxygen. On the other hand, the main product of the production process is the surfactin. Table 19 summarizes the consumption rates per annum and costs for each material.

Table 19. *Summary of costs and sales per material*

Raw Materials	Consumption (tons/year)	Unit Price (Php/ton)	Cost (Php/year)
Cassava Wastewater	896,620.88	28.52	25,577.331.50
Ammonium Nitrate (Commercial Grade)	32,418.76	9360.00	357,596,928.00
Bacillus subtilis Inoculum	0.87	75,600.00	65,772.00
Industrial Oxygen (99.5%)	6,577.77	416.00	2,736,352.32
Product	**Production (tons/year)**	**Selling Price (Php/ton)**	**Cost (Php/year)**
Surfactin (96% Purity)	1,643.40	2,515,225.00	4,133,520,765.00

Note: The prices are based on Alibaba and Sigma Aldrich Incorporated estimates

Based on the summarized consumption and costing details, the total costs and total sales can be determined. From these data, the economic margin can be computed using Equation 2.

$$Economic\ Margin(\%) = \frac{Total\ Sales\ (Php) - Total\ Costs\ (Php)}{Total\ Sales\ (Php)} \times 100\% \quad (2)$$

The computed economic margin for the surfactin production process is 90.66%. This computed value is based only on the feed and product streams. The computed value is positive which indicates that the proposed project is economically feasible.

REFERENCES

Abdel-Mawgoud, A. Mohammad, Aboulwafa, M. M., & Hassouna, N. A. H. (2008). Optimization of surfactin production by bacillus subtilis isolate BS5. *Applied Biochemistry and Biotechnology*, *150*(3), 305–325. https://doi.org/10.1007/S12010-008-8155-X

Abdel-Mawgoud, Ahmad Mohammad, Aboulwafa, M. M., & Hassouna, N. A.-H. (2008). Characterization of Surfactin Produced by Bacillus subtilis Isolate BS5. *Applied Biochemistry and Biotechnology 2008 150:3*, *150*(3), 289–303. https://doi.org/10.1007/S12010-008-8153-Z

Adu, S. A., Naughton, P. J., Marchant, R., & Banat, I. M. (2020). Microbial biosurfactants in cosmetic and personal skincare pharmaceutical formulations. *Pharmaceutics*, *12*(11), 1–21. https://doi.org/10.3390/pharmaceutics12111099

Andrade, C. J. de, Andrade, L. M. de, Bution, M. L., Heidi Dolder, M. A., Cavalcante Barros, F. F., & Pastore, G. M. (2016). Optimizing alternative substrate for simultaneous production of surfactin and 2,3-butanediol by Bacillus subtilis LB5a. *Biocatalysis and Agricultural Biotechnology*, *6*. https://doi.org/10.1016/j.bcab.2016.04.004

Arima, K., Kakinuma, A., & Tamura, G. (1968). Surfactin, a crystalline peptidelipid surfactant produced by Bacillus subtilis: isolation, characterization and its inhibition of fibrin clot formation. *Biochemical and Biophysical Research Communications*, *31*(3), 488–494. https://doi.org/10.1016/0006-291X(68)90503-2

Bals, B., & Dale, B. E. (2011). Economic comparison of multiple techniques for recovering leaf protein in biomass processing. *Biotechnology and Bioengineering*, *108*(3), 530–537. https://doi.org/10.1002/BIT.22973

Barros, F. F. C., Ponezi, A. N., & Pastore, G. M. (2008). Production of biosurfactant by Bacillus subtilis LB5a on a pilot scale using cassava wastewater as substrate. *Journal of Industrial Microbiology and Biotechnology*, *35*(9), 1071–1078. https://doi.org/10.1007/s10295-008-0385-y

Berk, Z. (2018). Freeze drying (lyophilization) and freeze concentration. *Food Process Engineering and Technology*, 567–581. https://doi.org/10.1016/B978-0-12-812018-7.00023-3

BYJU. (2022). *Difference between Batch and Continuous Fermentation*. https://byjus.com/biology/difference-between-batch-and-continous-fermentation/

Camylla, C. S., Adrielly, S. A. de A., Gabriela, F. D. F., Almeida, A. F. de, Janice, I. D., & Ana, K. de C. L. L. (2018). Biosurfactant production by Bacillus subtilis UFPEDA 86 using papaya (Carica papaya L.) waste as substrate: Viability studies and pH influence of the culture medium. *African Journal of Biotechnology*, *17*(30), 928–937. https://doi.org/10.5897/ajb2018.16408

Clark, D. E. (2001). Safety and the laboratory centrifuge. *Chemical Health & Safety*, *8*(6), 7–13. https://doi.org/10.1016/S1074-9098(01)00263-5

Cooper, D. G., Macdonald, C. R., Duff, S. J. B., & Kosaric, N. (1981). Enhanced Production of Surfactin from Bacillus subtilis by Continuous Product Removal and Metal Cation Additions. In *APPLIED AND ENVIRONMENTAL MICROBIOLOGY* (Vol. 42, Issue 3).

Costa, S. G. V. A. O., Nitschke, M., Lépine, F., Déziel, E., & Contiero, J. (2010). Structure, properties and applications of rhamnolipids produced by Pseudomonas aeruginosa L2-1 from cassava wastewater. *Process Biochemistry*, *45*(9), 1511–1516. https://doi.org/10.1016/J.PROCBIO.2010.05.033

Coutte, F., Lecouturier, D., Yahia, S. A., Leclère, V., Béchet, M., Jacques, P., & Dhulster, P. (2010). Production of surfactin and fengycin by Bacillus subtilis in a bubbleless membrane bioreactor. *Applied Microbiology and Biotechnology*, *87*(2), 499–507. https://doi.org/10.1007/s00253-010-2504-8

Czinkóczky, R., & Németh, Á. (2020a). Techno-economic assessment of Bacillus fermentation to produce surfactin and lichenysin. *Biochemical Engineering Journal*, *163*. https://doi.org/10.1016/J.BEJ.2020.107719

Czinkóczky, R., & Németh, Á. (2020b). The Effect of pH on Biosurfactant Production by Bacillus Subtilis DSM10. *Hungarian Journal of Industry and Chemistry*, *48*(2). https://doi.org/10.33927/HJIC-2020-25

Davis, D. A., Lynch, H. C., & Varley, J. (2001). *The application of foaming for the recovery of Surfactin from B. subtilis ATCC 21332 cultures*. www.elsevier.com/locate/enzmictec

Etoc, A., Delvigne, F., Lecomte, J., & Thonart, P. (2006). Foam control in fermentation bioprocess: from simple aeration tests to bioreactor. *App Biochem Biotech* , 392–404.

Fakruddin, M. D. (2012). Biosurfactant: Production and Application. *Journal of Petroleum & Environmental Biotechnology*, *03*(04). https://doi.org/10.4172/2157-7463.1000124

Flickinger, M. (2017). Encyclopedia of Industrial Biotechnology Bioprocess, Bioseparation, and Cell

Technology. *Multiskilling for Television Production*, *8*, 41–49. https://doi.org/10.4324/9780080512303-8

Freitas de Oliveira, D. W., Lima França, Í. W., Nogueira Félix, A. K., Lima Martins, J. J., Aparecida Giro, M. E., Melo, V. M. M., & Gonçalves, L. R. B. (2013). Kinetic study of biosurfactant production by Bacillus subtilis LAMI005 grown in clarified cashew apple juice. *Colloids and Surfaces B: Biointerfaces*, *101*. https://doi.org/10.1016/j.colsurfb.2012.06.011

Front-matter. (2016). In *Wills' Mineral Processing Technology* (pp. i–iii). Butterworth-Heinemann. https://doi.org/10.1016/b978-0-08-097053-0.00018-2

Goya, M. E., Xue, F., Sampedro-Torres-Quevedo, C., Arnaouteli, S., Riquelme-Dominguez, L., Romanowski, A., Brydon, J., Ball, K. L., Stanley-Wall, N. R., & Doitsidou, M. (2020). Probiotic Bacillus subtilis Protects against α-Synuclein Aggregation in C. elegans. *Cell Reports*, *30*(2), 367-380.e7. https://doi.org/10.1016/J.CELREP.2019.12.078

Green, D., & Perry, R. (2008). *Perry's Chemical Engineers' Handbook* (8th Editio). McGraw-Hill.

Gudiña, E. J., Fernandes, E. C., Rodrigues, A. I., Teixeira, J. A., & Rodrigues, L. R. (2015). Biosurfactant production by Bacillus subtilis using corn steep liquor as culture medium. *Frontiers in Microbiology*, *0*(FEB), 59. https://doi.org/10.3389/FMICB.2015.00059

Gudiña, E. J., Rangarajan, V., Sen, R., & Rodrigues, L. R. (2013). Potential therapeutic applications of biosurfactants. *Trends in Pharmacological Sciences*, *34*(12), 667–675. https://doi.org/10.1016/J.TIPS.2013.10.002

Gurjar, J., & Sengupta, B. (2015). Production of surfactin from rice mill polishing residue by submerged fermentation using Bacillus subtilis MTCC 2423. *Bioresource Technology*, *189*. https://doi.org/10.1016/j.biortech.2015.04.013

Ha, S., Kim, H. M., Chun, H. H., Hwang, I. M., Lee, J. H., Kim, J. C., Kim, I. S., & Park, H. W. (2018a). Effect of Oxygen Supply on Surfactin Production and Sporulation in Submerged Culture of Bacillus subtilis Y9. *Applied Sciences 2018, Vol. 8, Page 1660*, *8*(9), 1660. https://doi.org/10.3390/APP8091660

Ha, S., Kim, H. M., Chun, H. H., Hwang, I. M., Lee, J. H., Kim, J. C., Kim, I. S., & Park, H. W. (2018b). Effect of Oxygen Supply on Surfactin Production and Sporulation in Submerged Culture of Bacillus

subtilis Y9. *Applied Sciences 2018, Vol. 8, Page 1660*, *8*(9), 1660. https://doi.org/10.3390/APP8091660

Haque, E., K Kayalvizhi, K., & Hassan, S. (2020). Biocompatibility, Antioxidant and Anti-Infective Effect of Biosurfactant Produced by Marinobacter litoralis MB15. *International Journal of Pharmaceutical Investigation*, *10*(2), 173–178. https://doi.org/10.5530/ijpi.2020.2.32

Harvest Right™ | Home Freeze Dryers | Freeze Dried Food Storage Home Freeze Dryers. (n.d.). Retrieved April 3, 2022, from https://harvestright.com/product/pharmaceutical-freeze-dryer/

Henkel, M., Geissler, M., Weggenmann, F., & Hausmann, R. (2017). Production of microbial biosurfactants: Status quo of rhamnolipid and surfactin towards large-scale production. *Biotechnology Journal*, *12*(7), 1600561. https://doi.org/10.1002/BIOT.201600561

Heryani, H., & Putra, M. D. (2017). Kinetic study and modeling of biosurfactant production using Bacillus sp. *Electronic Journal of Biotechnology*, *27*, 49–54. https://doi.org/10.1016/J.EJBT.2017.03.005

Hirsch, M. (2015). *Surface Active Agents (Surfactants): Types and Applications*. Prospector. https://knowledge.ulprospector.com/3106/pc-surface-active-agents-surfactants/

Hoffmann, M., Mück, D., Grossmann, L., Greiner, L., Klausmann, P., Henkel, M., Lilge, L., Weiss, J., & Hausmann, R. (2021). Surfactin from Bacillus subtilis displays promising characteristics as O/W-emulsifier for food formulations. *Colloids and Surfaces B: Biointerfaces*, *203*. https://doi.org/10.1016/j.colsurfb.2021.111749

Hu, F., Liu, Y., & Li, S. (2019). Rational strain improvement for surfactin production: Enhancing the yield and generating novel structures. *Microbial Cell Factories*, *18*(1), 1–13. https://doi.org/10.1186/s12934-019-1089-x

Isa, M. H. M., Frazier, R. A., & Jauregi, P. (2008). A further study of the recovery and purification of surfactin from fermentation broth by membrane filtration. *Separation and Purification Technology*, *64*(2), 176–182. https://doi.org/10.1016/j.seppur.2008.09.008

Janek, T., Gudiña, E. J., Połomska, X., Biniarz, P., Jama, D., Rodrigues, L. R., Rymowicz, W., & Lazar, Z. (2021). Sustainable surfactin production by bacillus subtilis using crude glycerol from different wastes. *Molecules*, *26*(12). https://doi.org/10.3390/molecules26123488

Jimoh, A. A., & Lin, J. (2019). Biosurfactant: A new frontier for greener technology and environmental sustainability. *Ecotoxicology and Environmental Safety*, *184*. https://doi.org/10.1016/J.ECOENV.2019.109607

Joshi, S. J., Geetha, S. J., & Desai, A. J. (2015). Characterization and Application of Biosurfactant Produced by Bacillus licheniformis R2. *Applied Biochemistry and Biotechnology*, *177*(2), 346–361. https://doi.org/10.1007/s12010-015-1746-4

Knepper, T., & Berna, J. (2003). Surfactants: properties, production, and environmental aspects. *Comprehensive Analytical Chemistry*.

Kostagiolas, P. (2012). Measuring libraries' intellectual capital. *Managing Intellectual Capital in Libraries*, 87–127. https://doi.org/10.1016/b978-1-84334-678-4.50004-9

Kumar, V., Vijay, V. K., & Agarwal, U. S. (2008). Studies on Centrifugal Clarification of Sugarcane Juice - Possibilities and Limitations. *Development*, *X*, 1–11.

Lawal, N. S., Ogedengbe, K., & Oamen, E. C. (2019). *Cassava Mill Wastewater Treatment by a Combination of Physical and Nature-Based Processes : A Pilot Study | Lawal | Applied Journal of Environmental Engineering Science*. https://revues.imist.ma/index.php/AJEES/article/view/17427/10338

Lewis, L. M., Johnson, R. E., Oldroyd, M. E., Ahmed, S. S., Joseph, L., Saracovan, I., & Sinha, S. (2010). Characterizing the freeze-drying behavior of model protein formulations. *AAPS PharmSciTech*, *11*(4), 1580–1590. https://doi.org/10.1208/s12249-010-9530-9

Magar, S. T. (2021, September 28). *Batch vs Fed-Batch vs Continuous Culture- 20 Key Differences*. Microbe Notes. https://microbenotes.com/batch-vs-fed-batch-vs-continuous-culture/

Matejtschuk, P. (2007). Lyophilization of proteins. *Methods in Molecular Biology (Clifton, N.J.)*, *368*, 59–72. https://doi.org/10.1007/978-1-59745-362-2_4

Mordor Intelligence. (2020). *Bio-surfactants Market | 2021 - 26 | Industry Share, Size, Growth* . https://www.mordorintelligence.com/industry-reports/biosurfactants-market

More, A. (2021, July 13). *Global Surfactant Market Report Survey 2021-2027 With Top Countries Data Industry Research Update, Future Scope, Size Estimation, Revenue Pricing Trends, Growth*

Opportunity, Regional Outlook and Forecast. https://www.wboc.com/story/44290274/global-surfactant

Moshtagh, B., Hawboldt, K., & Zhang, B. (2019). Optimization of biosurfactant production by Bacillus Subtilis N3-1P using the brewery waste as the carbon source. *Environmental Technology (United Kingdom)*, *40*(25), 3371–3380. https://doi.org/10.1080/09593330.2018.1473502

Muthusamy, K., Gopalakrishnan, S., Ravi, K., Sivachidambaram, P., Muthusamy, K., & Gopalakrishnan, S. (2008). *Biosurfactants : Properties , commercia production and application*. *94*(6), 736–747.

Nitschke, M., & Maria Pastore, G. (2005). *Production and properties of a surfactant obtained from Bacillus subtilis grown on cassava wastewater*. https://doi.org/10.1016/j.biortech.2005.02.044

Nitschke, M., & Pastore, G. M. (2006). Production and properties of a surfactant obtained from Bacillus subtilis grown on cassava wastewater. *Bioresource Technology*, *97*(2), 336–341. https://doi.org/10.1016/j.biortech.2005.02.044

Noah, K. S., Bruhn, D. F., & Bala, G. A. (2005a). Surfactin Production from Potato Process Effluent by Bacillus subtilis in a Chemostat. *Applied Biochemistry and Biotechnology*, *122*(1–3), 0465–0474. https://doi.org/10.1385/abab:122:1-3:0465

Noah, K. S., Bruhn, D. F., & Bala, G. A. (2005b). Surfactin Production from Potato Process Effluent by Bacillus subtilis in a Chemostat. In *Twenty-Sixth Symposium on Biotechnology for Fuels and Chemicals*. Humana Press. https://doi.org/10.1007/978-1-59259-991-2_41

Palcic, I., & Lalic, B. (2009). Analytical Hierarchy Process as a tool for selecting and evaluating projects. *Article in International Journal of Simulation Modelling*, *8*, 16–26. https://doi.org/10.2507/IJSIMM08(1)2.112

Park, J. W., Oh, H. Y., Kim, D. Y., & Cho, Y. J. (2018). Plant Location Selection for Food Production by Considering the Regional and Seasonal Supply Vulnerability of Raw Materials. *Mathematical Problems in Engineering*, *2018*. https://doi.org/10.1155/2018/7494398

Porter, M. R. (1993). *Handbook of Surfactants - M.R. Porter - Google Livres*. https://books.google.com.ph/books?hl=en&lr=&id=UX3SBwAAQBAJ&oi=fnd&pg=PA1&dq=surfactants&ots=F8TVNpgy-F&sig=VKbTLgfNdbeN_-MB2xKD35kWX1g&redir_esc=y#v=onepage&q&f=false

Rahman, K. S. M., & Gakpe, E. (2008). Production, Characterisation and Applications of

Biosurfactants-Review. *Biotechnology*, *7*(2), 360–370. http://tees.openrepository.com/tees/handle/10149/93896

Randhawa, K. K. S., & Rahman, P. K. S. M. (2014). Rhamnolipid biosurfactants—past, present, and future scenario of global market. *Frontiers in Microbiology*, *5*(SEP). https://doi.org/10.3389/FMICB.2014.00454

Rodríguez-López, L., Rincón-Fontán, M., Vecino, X., Moldes, A. B., & Cruz, J. M. (2020). Biodegradability Study of the Biosurfactant Contained in a Crude Extract from Corn Steep Water. *Journal of Surfactants and Detergents*, *23*(1), 79–90. https://doi.org/10.1002/JSDE.12338

Rodríguez, A., Gea, T., Sánchez, A., & Font, X. (2020). Agro-wastes and Inert Materials as Supports for the Production of Biosurfactants by Solid-state Fermentation. *Waste and Biomass Valorization 2020 12:4*, *12*(4), 1963–1976. https://doi.org/10.1007/S12649-020-01148-5

Sahnoun, R., Mnif, I., Fetoui, H., Gdoura, R., Chaabouni, K., Makni-Ayadi, F., Kallel, C., Ellouze-Chaabouni, S., & Ghribi, D. (2014). Evaluation of Bacillus subtilis SPB1 Lipopeptide Biosurfactant Toxicity Towards Mice. *International Journal of Peptide Research and Therapeutics 2014 20:3*, *20*(3), 333–340. https://doi.org/10.1007/S10989-014-9400-5

Santos, D., Maurício, A. C., Sencadas, V., Santos, J. D., Fernandes, M. H., & Gomes, P. S. (2018). Spray Drying: An Overview. *Biomaterials - Physics and Chemistry - New Edition*. https://doi.org/10.5772/intechopen.72247

Schaechter, M. (2009). Encyclopedia of microbiology. *Encyclopedia of Microbiology*, 1–4600. https://doi.org/10.5860/choice.49-1215

Shakerifard, P., Gancel, F., Jacques, P., & Faille, C. (2009). Effect of different Bacillus subtilis lipopeptides on surface hydrophobicity and adhesion of Bacillus cereus 98/4 spores to stainless steel and Teflon. *Https://Doi.Org/10.1080/08927010902977943*, *25*(6), 533–541. https://doi.org/10.1080/08927010902977943

Show, K.-Y., Yan, Y.-G., & Lee, D.-J. (2019). Algal biomass harvesting and drying. In *Biofuels from Algae* (Second Edi). Elsevier B.V. https://doi.org/10.1016/b978-0-444-64192-2.00007-x

Stamenković-Stojanović, S., Karabegović, I., Beškoski, V., Nikolić, N., & Lazić, M. (2020a). Bacillus subtilis NCIM2063 batch cultivation: The influence of the substrate concentration and oxygen transfer rate on

the biomass yield. *Advanced Technologies*, *9*(1), 44–49. https://doi.org/10.5937/savteh2001044s

Stamenković-Stojanović, S., Karabegović, I., Beškoski, V., Nikolić, N., & Lazić, M. (2020b). Bacillus subtilis NCIM2063 batch cultivation: The influence of the substrate concentration and oxygen transfer rate on the biomass yield. *Advanced Technologies*, *9*(1), 44–49. https://doi.org/10.5937/savteh2001044s

The Applied Finance Group. (2013). *Economic Margin*. https://economicmargin.com/economic-margin/

Thompson, D. N., Fox, S. L., & Bala, G. A. (2000). Biosurfactants from potato process effluents. *Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology*, *84–86*(1–9), 917–930. https://doi.org/10.1385/ABAB:84-86:1-9:917

Twitchell, E. (1987). *Process of decomposing fats or oils into fatty acids and glycerin - Google Patents* (Patent No. 601,603). United States Patent Office. https://patents.google.com/patent/US601603A/en

Vandeputte, J. (2012). Agro-based surfactants. *Oléagineux, Corps Gras, Lipides*, *19*(2), 133–137. https://doi.org/10.1051/ocl.2012.0431

Vassaux, A., Rannou, M., Peers, S., Daboudet, T., Jacques, P., & Coutte, F. (2021). Impact of the Purification Process on the Spray-Drying Performances of the Three Families of Lipopeptide Biosurfactant Produced by Bacillus subtilis. *Frontiers in Bioengineering and Biotechnology*, *9*(December), 1–11. https://doi.org/10.3389/fbioe.2021.815337

Vecino, X., Cruz, J. M., Moldes, A. B., & Rodrigues, L. R. (2017). Biosurfactants in cosmetic formulations: trends and challenges. *Critical Reviews in Biotechnology*, *37*(7), 911–923. https://doi.org/10.1080/07388551.2016.1269053

Voulgaridou, G. P., Mantso, T., Anestopoulos, I., Klavaris, A., Katzastra, C., Kiousi, D. E., Mantela, M., Galanis, A., Gardikis, K., Banat, I. M., Gutierrez, T., Sałek, K., Euston, S., Panayiotidis, M. I., & Pappa, A. (2021). Toxicity profiling of biosurfactants produced by novel marine bacterial strains. *International Journal of Molecular Sciences*, *22*(5), 1–15. https://doi.org/10.3390/ijms22052383

Walsh, S. (2017). *Global Surfactants Market to 2022 | Industry Trends & Analysis | Smithers*. https://www.smithers.com/services/market-reports/materials/the-future-of-surfactants-to-2022

Wei, Y. H., & Chu, I. M. (1998). Enhancement of surfactin production in iron-enriched media by Bacillus subtilis ATCC 21332. *Enzyme and Microbial Technology*, *22*(8), 724–728.

https://doi.org/10.1016/S0141-0229(98)00016-7

Winterburn, J. B., & Martin, P. J. (2012). Foam mitigation and exploitation in biosurfactant production. In *Biotechnology Letters* (Vol. 34, Issue 2, pp. 187–195). https://doi.org/10.1007/s10529-011-0782-6

Yeh, M. S., Wei, Y. H., & Chang, J. S. (2006). Bioreactor design for enhanced carrier-assisted surfactin production with Bacillus subtilis. *Process Biochemistry*, *41*(8), 1799–1805. https://doi.org/10.1016/j.procbio.2006.03.027

APPENDIX I: EVALUATION AND SELECTION OF PROCESS FOR SOLIDS REMOVAL

***Table 20**. Comparison of Solids Removal Process Options*

Criteria	Centrifugal Sedimentation (Option 1) Score	Gravitational Sedimentation (Option 2) Score	Explanation
Clarifying Efficiency (41%)	3	1	Kumar et al. (2008) compared the clarifying efficiencies of centrifugal sedimentation and of gravitational sedimentation. Centrifugal sedimentation boasts of superior separation efficiency (up to 66.03%) compared to gravitational sedimentation (15.23%).
Process Time (39%)	8	1	Centrifugal sedimentation has a shortened residence time of 10 minutes as opposed to the 6-hours residence time of gravitational sedimentation (Costa et al., 2010; Lawal et al., 2019).
Ease of Operability and Technological Maturity (11%)	1	2	Gravitational sedimentation requires only gravity to operate in the settler. It is also the more mature process technologically.
Health and Safety (5%)	1	4	For these processes, no harmful waste or emissions are generated. The risk of rotor failure in centrifugation compromises health and safety to the user. However, so

			long as protective infrastructure is available (i.e. centrifuge chamber), the risk can be minimized (Clark, 2001).
Economic (4%)	6	1	Option 1 requires more operational cost due to more electricity consumed, but Option 2 requires more capital cost. Ultimately, Option 2 is less profitable for larger, industrial scale capacities (Bals & Dale, 2011).

Table 21. *Overall Composite Impact of Solids Removal Process Options*

Option	CE 0.412	PT 0.392	EoO&TM 0.106	H&S 0.050	Economic 0.039	Composite Impact
1	0.750	0.889	0.333	0.200	0.857	**0.737**
2	0.250	0.111	0.667	0.800	0.143	**0.263**

APPENDIX II: EVALUATION AND SELECTION OF PROCESS FOR SUBSTRATE PRE-TREATMENT

Table 22. *Comparison of Substrate Pre-Treatment Process Options*

Criteria	Adsorption by Activated Oyster Shell Ash (Option 1) Score	Oxidation of Cyanides (Option 2) Score	Explanation
Removal Performance (54%)	2	1	Akpan and Etuk (2019) reported a 91.48% cyanide removal using AOSA. Although Khun-anake et al. (2000) reported a 99.98% removal of cyanide using oxidation method, there is a major downside of using oxidation due to the reaction between starch and hypochlorite. Thus, adsorption will be more favorable.
Ease of Operability and Technological Maturity (24%)	1	1	For both processes, no significant difference in terms of operability and technological maturity have been observed.
Health and Safety (10%)	3	1	In terms of health and safety, adsorption method does not have much hazard as it only utilizes shell ash as adsorbents. However, for oxidation, it involves the usage of chemicals such as hypochlorite which are hazardous to the people handling the chemical especially in increased exposures.

Environment (6%)	3	1	The utilization of Shell Ash as adsorbent minimizes the shell waste. Moreover, it does not involve carcinogenic and toxic chemicals which would harm animals and humans. For oxidation, chlorine and hypochlorite are both carcinogenic and toxic.
Economic (6%)	5	1	The usage of shell ash (which is from waste) as adsorbent makes it more a cheaper option in removing cyanides. The usage of hypochlorite chemicals is very expensive and one of the downsides of oxidation process according to Khun-anake et al. (2000).

Table 23. *Overall Composite Impact of Substrate Pre-Treatment Process Options*

Option	RP 0.542	EoO&TM 0.241	H&S 0.103	Environment 0.059	Economic 0.056	Composite Impact
1	0.667	0.500	0.750	0.750	0.833	**0.649**
2	0.333	0.500	0.250	0.250	0.167	**0.351**

APPENDIX III: EVALUATION AND SELECTION OF PROCESS FOR SURFACTIN PRODUCTION

***Table 24**. Comparison of Surfactin Production Process Options*

Criteria	Foaming Fed-Batch Bioreactor (Option 1) Score	Fed-Batch Bubbleless Membrane Bioreactor (Option 2) Score	Explanation
Product Yield and Efficiency (52%)	5	1	The foaming bioreactor yields more surfactin (13.2 mg product/g substrate) than the bubbleless bioreactors (11.7-12.8 mg product/g substrate) in a study by Coutte et al. (2010). Henkel et al. (2017) compared the efficiency of different surfactin bioreactor systems through efficiency parameters. For the same *B. subtilis* strain (DSM 10^T), the foaming process resulted to higher efficiency parameters (c_{max} = 3.99 g/L and q_{sp} = 0.08 g/(g.h)) than the foam-free process (c_{max} = 0.087 g/L and q_{sp} = 0.002 g/(g.h)) and thus better efficiency. The batch process also obtained a higher yield of 0.052 g/g as opposed to 0.003 g/g.
Ease of Operability and Technological Maturity (22%)	2	1	The complexity of Option 2 may owe to its less easy operability. The foaming fermenter is more technologically mature as it has been commonly used in industrial scale applications. Bubbleless bioreactors are still in research and lab-scale development, making it relatively novel.

Health and Safety (17%)	1	1	Both processes utilize the same materials. They both do not pose any substantial risks.
Environment (7%)	1	1	For these processes, no harmful waste or emissions are generated.
Economic (3%)	6	1	`Option 1 only takes 48 hours to ferment while Option 2 takes 72 hours (Cooper et al., 1981; Coutte et al., 2010). This bloats the operational cost for Option 2. Option 2 entails a more complicated configuration as well as a membrane (Coutte et al., 2010) which may owe to greater capital and installation costs. Both utilize the same materials and therefore have the same materials cost.

Table 25. *Overall Composite Impact of Surfactin Production Process Options*

Option	PY&E 0.519	EoO&TM 0.219	H&S 0.165	Environment 0.066	Economic 0.031	Composite Impact
1	0.833	0.667	0.500	0.500	0.857	**0.721**
2	0.167	0.333	0.500	0.500	0.143	**0.279**

APPENDIX IV: EVALUATION AND SELECTION OF PROCESS FOR MOISTURE REMOVAL OF WET SURFACTIN MIXTURE

Table 26. *Comparison of Moisture Removal Process Options*

Criteria	Freeze Drying or Lyophilization (Option 1) Score	Spray Drying (Option 2) Score	Explanation
Product Quality (51%)	3	1	Option 2 greatly degrades the quality of the product due to its high operating temperature. To ensure the quality of the product, Option 1 is deemed a better method for moisture removal.
Process Time (22%)	1	2	Option 2 allows drying to occur in seconds (Show et al., 2019) while Option 1 needs at least 72 hours to achieve the desirable appearance of surfactin (Matejtschuk, 2007).
Ease of Operability and Technological Maturity (17%)	2	1	Option 1 is an older technique in drying then Option 2, hence a more mature one than the other.
Health and Safety (5%)	2	1	Between the two, Option 2 poses more harm since its process condition is at a significantly higher temperature than Option 1.
Economic (5%)	1	3	Generally, Option 2 is considered cheaper and more energy efficient than Option 1.

Table 27. *Overall Composite Impact of Moisture Removal Process Options*

Option	PQ 0.507	PT 0.222	EoO&TM 0.172	H&S 0.048	Economic 0.051	Composite Impact
1	0.750	0.333	0.667	0.667	0.250	**0.614**
2	0.250	0.667	0.333	0.333	0.750	**0.386**

APPENDIX V: PAIR-WISE COMPARISON OF PROCESS SELECTION CRITERIA

A pair-wise comparison of the criteria in selecting the process steps is performed by the Analytic Hierarchy Process. The objective is to determine the criteria weights in each selection process while also ensuring that the metric formed is reasonably consistent.

The scale of relative importance used is presented in Table 3. Intermediate values are also valid.

Table 3. *Scale of Relative Importance (Triantaphyllou and Mann, 1995)*

SCORE	DESCRIPTION
1	Equal importance
3	Moderate importance
5	Strong importance
7	Very strong importance
9	Extreme importance
2,4,6,8	Intermediate values between the two adjacent judgments

The following subsections consists of the AHP evaluation of the process options considered in this document.

Appendix V.A Solids Removal Selection Criteria

Table 28. *Scalar Pairwise Comparison of Selection Criteria for Solids Removal*

Scalar Matrix

	Clarifying Efficiency	Process Time	Ease of Operability and Technological Maturity	Health and Safety	Economy
Clarifying Efficiency	1	1	7	9	7
Process Time	1	1	5	9	8
Ease of Operability and Technological Maturity	1/7	1/5	1	3	4
Health and Safety	1/9	1/9	1/3	1	2
Economy	1/7	1/8	1/4	1/2	1
SUM	**2.40**	**2.44**	**13.58**	**22.50**	**22.00**

Normalized Matrix

	Clarifying Efficiency	Process Time	Ease of Operability and Technological Maturity	Health and Safety	Economy	Priority
Clarifying Efficiency	0.417	0.410	0.515	0.400	0.318	**0.412**
Process Time	0.417	0.410	0.368	0.400	0.364	**0.392**
Ease of Operability and Technological Maturity	0.060	0.082	0.074	0.133	0.182	**0.106**
Health and Safety	0.046	0.046	0.025	0.044	0.091	**0.050**
Economy	0.060	0.051	0.018	0.022	0.045	**0.039**
					Sum of Priorities	**1.000**

Eigenvalues

	Clarifying Efficiency	Process Time	Ease of Operability and Technological Maturity	Health and Safety	Economy	Sum	Weighted Sum
Clarifying Efficiency	1	1	7	9	7	**2.276**	**5.521**
Process Time	1	1	5	9	8	**2.103**	**5.367**
Ease of Operability and Technological Maturity	1/7	1/5	1	3	4	**0.552**	**5.5204**
Health and Safety	1/9	1/9	1/3	1	2	**0.0254**	**5.040**
Economy	1/7	1/8	1/4	1/2	1	**0.199**	**5.051**

Consistency			
λ_{max}	5.236	**Inconsistency**	0.053
n	5		
CI	0.059	**Remarks**	Consistent
RI	1.12		

Appendix V.B Substrate Pre-treatment Selection Criteria

***Table 29**. Pairwise Comparison of Selection Criteria for Substrate Pre-treatment*

Scalar Matrix

	Removal Performance	Ease of Operability and Technological Maturity	Health and Safety	Environment	Economy
Removal Performance	1	5	7	6	7
Ease of Operability and Technological Maturity	1/5	1	4	5	5
Health and Safety	1/7	1/4	1	3	2
Environment	1/6	1/5	1/2	1	1
Economy	1/7	1/5	1/2	1	1
SUM	**1.65**	**6.65**	**13.00**	**16.00**	**16.00**

Normalized Matrix

	Removal Performance	Ease of Operability and Technological Maturity	Health and Safety	Environment	Economy	Priority
Removal Performance	0.605	0.752	0.538	0.375	0.438	**0.542**
Ease of Operability and Technological Maturity	0.121	0.150	0.308	0.313	0.313	**0.241**
Health and Safety	0.086	0.038	0.077	0.188	0.125	**0.103**
Environment	0.101	0.030	0.038	0.063	0.063	**0.059**
Economy	0.086	0.030	0.038	0.063	0.063	**0.056**
			Sum of Priorities			**1.000**

Eigenvalues

	Removal Performance	Ease of Operability and Technological Maturity	Health and Safety	Environment	Economy	Sum	Weighted Sum
Removal Performance	0.542	1.204	0.719	0.353	0.392	**3.210**	**5.927**
Ease of Operability and Technological Maturity	0.108	0.241	0.411	0.294	0.280	**1.334**	**5.541**
Health and Safety	0.077	0.060	0.103	0.177	0.112	**0.529**	**5.150**
Environment	0.090	0.048	0.051	0.059	0.056	**0.305**	**5.174**
Economy	0.077	0.048	0.051	0.059	0.056	**0.292**	**5.210**

Consistency			
λ_{max}	5.400	**Inconsistency**	0.089
n	5		
CI	1.12	**Remarks**	Consistent
RI	0.100		

Appendix V.C Surfactin Production Selection Criteria

***Table 30**. Pairwise Comparison of Selection Criteria for Surfactin Production*

Scalar Matrix

	Product Yield and Efficiency	Ease of Operability and Technological Maturity	Health and Safety	Environment	Economy
Product Yield and Efficiency	1	3	6	8	9
Ease of Operability and Technological Maturity	1/3	1	1	6	8
Health and Safety	1/6	1	1	4	6
Environment	1/8	1/6	¼	1	4
Economy	1/9	1/8	1/6	¼	1
SUM	**1.74**	**5.29**	**8.42**	**19.25**	**28.00**

Normalized Matrix

	Product Yield and Efficiency	Ease of Operability and Technological Maturity	Health and Safety	Environment	Economy	Priority
Product Yield and Efficiency	0.576	0.567	0.713	0.416	0.321	**0.519**
Ease of Operability and Technological Maturity	0.192	0.189	0.119	0.312	0.286	**0.219**
Health and Safety	0.096	0.189	0.119	0.208	0.214	**0.165**
Environment	0.072	0.031	0.030	0.052	0.143	**0.066**
Economy	0.064	0.024	0.020	0.013	0.036	**0.031**
					Sum of Priorities	**1.000**

Eigenvalues

	Product Yield and Efficiency	Ease of Operability and Technological Maturity	Health and Safety	Environment	Economy	Sum	Weighted Sum
Product Yield and Efficiency	0.519	0.658	0.991	0.525	0.281	**2.974**	**5.735**
Ease of Operability and Technological Maturity	0.173	0.219	0.165	0.394	0.250	**1.201**	**5.472**
Health and Safety	0.086	0.219	0.165	0.262	0.187	**0.921**	**5.575**
Environment	0.065	0.037	0.041	0.066	0.125	**0.333**	**5.079**
Economy	0.058	0.027	0.028	0.016	0.031	**0.160**	**5.131**

Consistency			
λ_{max}	5.438	**Inconsistency**	0.098
n	5		
CI	1.12	**Remarks**	Consistent
RI	0.100		

Appendix V.D Moisture Removal of Wet Surfactin Mixture

Table 31. *Pairwise Comparison of Selection Criteria for Surfactin Recovery*

Scalar Matrix

	Process Time	Ease of Operability and Technological Maturity	Health and Safety	Environment	Economy
Process Time	1	4	5	8	6
Ease of Operability and Technological Maturity	1/4	1	3	4	5
Health and Safety	1/5	1/3	1	5	6
Environment	1/8	1/4	1/5	1	1
Economy	1/6	1/5	1/6	1	1
SUM	**1.74**	**5.78**	**9.37**	**19.00**	**19.00**

Normalized Matrix

	Process Time	Ease of Operability and Technological Maturity	Health and Safety	Environment	Economy	Priority
Process Time	0.574	0.692	0.534	0.421	0.316	**0.507**
Ease of Operability and Technological Maturity	0.144	0.173	0.320	0.211	0.263	**0.222**
Health and Safety	0.115	0.058	0.107	0.263	0.316	**0.172**
Environment	0.072	0.043	0.021	0.053	0.053	**0.048**
Economy	0.096	0.035	0.018	0.053	0.053	**0.051**
			Sum of Priorities			**1.000**

Eigenvalues

	Process Time	Ease of Operability and Technological Maturity	Health and Safety	Environment	Economy	Sum	Weighted Sum
Process Time	0.507	0.888	0.858	0.387	0.304	**2.944**	**5.804**
Ease of Operability and Technological Maturity	0.127	0.222	0.515	0.193	0.253	**1.310**	**5.901**
Health and Safety	0.101	0.074	0.172	0.242	0.304	**0.893**	**5.201**
Environment	0.063	0.056	0.034	0.048	0.051	**0.252**	**5.220**
Economy	0.085	0.044	0.029	0.048	0.051	**0.257**	**5.064**

Consistency			
λ_{max}	5.438	**Inconsistency**	0.098
n	5		
CI	1.12	**Remarks**	Consistent
RI	0.100		

APPENDIX VI: EVALUATION MATRIX FOR EQUIPMENT SELECTION

Criteria	Weight	Description
Process Efficiency and Yield	30%	Assesses how well the equipment accomplishes the specific process and how much of the desired product is produced
Main Equipment and Life Cycle	25%	Performance indicators (i.e., conversion of reactants, etc.) are sustainable, and depreciation of equipment performance is slow
Technological Maturity	20%	Considers the maturity of the technology and how much it has been applied in the industry or investigated in studies
Environmental Impact and Safety	15%	Evaluates ecological impacts (i.e., material toxicity, emissions, other wastes, etc.) and the safety of the handler in operating the equipment
Economic Value	10%	Evaluates operational, material, installation, and maintenance costs as well as the economic depreciation of the equipment

APPENDIX VII: PRELIMINARY EQUIPMENT SELECTION PROCESS

The processes stipulated in Section 2.3 and Section 3.1 shall be the basis of information gathering for the equipment options and selection phase. The required process conditions and system requirements are to be extracted from known and newly invented equipment available locally or internationally. It is of utmost concern that the equipment selected has to (a) meet required process conditions of the plant section, (b) enable a range of quantities within the equipment is safe to use, (c) have an economic value with minimal cost but of high return of investment, and (d) contributes to the upward performance efficiency of the entire process plant.

The next subsection of this paper entails the equipment selection for each process identified, its range of process conditions, and efficiency towards the production of surfactin. In connection, the same matrix used in APPENDIX III shall be used as basis of scoring for the quantified matrix of the selection process.

A. Upstream Process: Removal of Cyanide

The Chemical Mixing Machine works on the idea of converting electromagnetic field energy into various types of energy. The unit consists of a 90-136 mm diameter chamber (pipeline) inside a revolving electromagnetic field inductor. Several dozen to several hundred (0.05-5 kg) ferromagnetic cylindrical elements of 0.5-5 mm diameter and 5-60 mm length are present in the operating region, depending on the volume of the work zone.

Chemical Mixing Machine AVS-150 with ferromagnetic elements are designed to intensify various physical and chemical processes. This equipment can be multifunctional and can be used as a reactor, mixer, disintegrating machine, extractor, magnetic treatment, and materials activation. This equipment can be used for a variety of purposes, including:

- emulsions and suspensions with many components
- increasing the speed with which finely dispersed mixes are produced
- activation of materials in both dry and water dispersed forms
- resulting in enhanced resin characteristics and a shorter vulcanizing time
- for total removal of phenol, formaldehyde, heavy metals, arsenic, and cyanides from industrial waste water

- increased heat treatment speed
- from yeast cells, the synthesis of protein material
- improvement of food product microbiological stability and yeast activation in bread baking
- improvement of meat and fish raw materials and finished products
- intensification of extraction procedures, such as broth, juice, and pectin production
- creation of microbiologically safe suspensions and emulsions in food manufacturing without the use of stabilizers and enhanced product yield.

Figure 16. *AV-150 Vortex Layer for Cyanide Removal*

Table 32. *AVS-150 Vortex Layer Specifications*

Specification	**AVS-150**
Max processing rate , m^3 /h	30
Suspension production, m^3 /h	15
Operating pressure, max (MPa)	0.25
Work zone diameter, mm	136
Magnetic induction in work zone, T	0.15
Electric supply	AC network
Frequency, Hz	50
Voltage, V	380
Rotation of magnetic field, RPM	3 000
Power consumption	9.5
Dimensions, mm - unit - control panel	 1300 x 1100 x 690 1060 x 1030 x 1900
Weight, kg - unit - control panel	 500 450

B. Downstream Process: Froth/Foam Flotation

Equipment 1: Minder Water Industries Foam Fractionator

The *Minder Water Industries Foam Fractionator*, also called Protein Skimmer, is a tank that is designed with an efficient and energy-saving stealth bubble device. It is made of high-grade fiberglass and resin and is most applicable in municipal water treatment, aquaculture, and aquariums. All models of this equipment are built with a UV sanitation system to neutralize germs and bacteria.

The tank diameter ranges from 400 mm to 2,000 mm. Tank height ranges from 2,100 mm to 3,200 mm. The foam generator with needle wheel impeller claims to be 30% more effective than traditional

protein skimmers with injectors. The model flow capacities range from 10.8 m^3/hr to 226.8 m^3/hr with retention times also ranging from 67 secs to 132 secs.

The foam fractionator is equipped with a large sight glass for easy monitoring and inspection of internal contents. It is also made of corrosion-free material. The tank is protected by a 5-year warranty while the fittings and the pump are so for 1 year and 2 years respectively.

Figure 17. *Minder Water Industries Foam Fractionator (Minder Water Industries, n.d.)*

Equipment 2: Aquatic Solutions RK2 Systems RK2000PE Protein Fractionator / Protein Skimmer

The Aquatic Solutions RK2000PE Protein Fractionator is a protein skimmer with a reaction chamber made of high-density polyethylene (HDPE). The other parts of the equipment were made of non-corrosive components and the foam container is made of clear acrylic for transparency. The protein fractionator utilizes the concept of venturi injection for mass mixing of air, ozone and water which increases the gas or liquid interface. Also, there is a baffle system in the top cone of the equipment for foam structure maintenance purposes and laminar foam ejection. This foam fractionation equipment allows two minutes for a full contact time and for the separation to finish.

In this foam fractionation equipment, air or ozone are injected into the bottom portion of the reaction chamber via an ozone resistant venturi injector. The introduction of ozone enhances the fractionation process by increasing the water clarity, oxygen concentration, and the oxidation of various pathogens. The water that is to be filtered is then introduced at the top of the chamber which will exit at the bottom part of the chamber. As a result of the water travelling cross current to the air, a mass mixing effect is created, which is an essential for the effective formation of the foam. The foam then rises through the column and concentrates as it passes into the upper rise tube. The water is then drained back into the tank via gravity while the concentrated foam is ejected over the top of the riser and is then collected into a waste drain. With the equipment's automated dual wash down feature, the upper chamber is automatically washed down. This keeps the surfaces clean and prevents the foam from drying.

The equipment has an overall weight of 1,800 lbs and operating weight of 25,520 lbs. The dimensions of the equipment are 92 in. W x 168 in. H with a 114-inch depth. The reaction chamber diameter is 84 inches. The water flow rate at 2 minutes and 1.5 minutes contact time are 1,500 GPM and 2,000 GPM respectively. The equipment is built with 316ss stainless steel hardware.

Figure 18. *Aquatic Solutions RK2 Systems RK2000PE Protein Fractionator (Aquatic Solutions, 2019)*

C. Downstream Process: Lyphophilization (Freeze-Drying)

Equipment 1: Harvest Right: Pharmaceutical Freeze Dryer

The *Harvest Right: Pharmaceutical Freeze Dryer* is a patented technology that keeps the products fresh and preserves the product's potency, color, shape, and nutrition of any organic material. According to Harvest Right, this pharmaceutical freeze dryer is most suitable for freeze drying herbs, oils, medicines, remedies, pharmaceuticals, and chemical compounds.

Figure 19. *Harvest Right: Pharmaceutical Freeze Dryer (Large)* (*Harvest Right*, 2018)

Figure 19 depicts the aforementioned freeze dryer. The overall dimensions of the freeze dryer are 20.25"W x 23.75"L x 30.75"H and which are suitable for countertop or table operations. The said equipment requires a 110-volt (NEMA 5-20) outlet and a 20-A circuit. The shelf unit of the freeze dryer consists of 8 stainless steel trays with each tray having a dimension of 9" W x 20.5" L x 0.75"H) and a 10.25 square feet of tray space. It also has a 6-liter capacity for the ice compartment.

The freeze dryer comes with an Industrial Pump which is superior to the Standard Oil Pump in terms of required oil change frequency, vacuum pressure, lack of noise, power use, amperage, weight, and lifespan. This equipment is priced at $4,845.

Equipment 2: GEA RAY™ Pilot Plant Freeze Dryer

GEA's RAY ® Pilot Plant (RAY® PP) is a batch-based freeze dryer designed to process general food products such as instant coffee, fruit, vegetables, meat, seafood, and pet food, as well as very sensitive products such as lactic acid, bacteria, enzymes, and lactoferrin. RAY ® freeze drying technology claims to provide a high-quality solution by freezing products under vacuum to ensure that the inherent solvents in the product are removed as vapor.

The RAY ® PP is designed and developed for hygienic processing. This freeze drying equipment is automatically operated via a touchscreen system which monitors the product temperature, heating profile, loss of weight, and other factors. The system also provides a full documentation of the process to ensure traceability and repeatability. The full documentation profile also includes automatically generated drying profiles, which is based on maximum product temperature and/or factors such as constant weight, temperature, and vacuum difference, and determine the end point of the process.

The pilot plant freeze drying equipment is designed to operate at 0.2 mbar. However, it can tolerate pressures as low as 0.1 mbar. According to GEA, the RAY ® PP ensures short downtimes between batches, based on rapid evacuation times, effective thawing, and tray loading.

Figure 20. *GEA's RAY (R) Pilot Plant Freeze Dryer*

The RAY ® PP also allows full scalability of the pilot scale system to industrial systems. It also offers uniform drying to deliver stable products with a long shelf-life. With the equipment's online monitoring scheme, batch reporting, flexible operations, and profile generation can be done remotely. It also offers a water-based heating system.

The freeze-drying equipment cabinet is made of stainless steel. It also has a built-in condenser. The RAY ® PP also only requires water and power to function. With the equipment's high-capacity vapor trap, the freeze dryer is able to handle sublimation of 2.5 kg H_2O /m^2/h. Evacuation to 1 mbar in less than 8 minutes, and accurate vacuum regulation from 0.1-5 mbar for controlled drying is also possible with RAY ® PP, as claimed by GEA. Other specifications of the pilot plant freeze dryer are tabulated in Table 33.

Table 33. *GEA RAY TM Pilot Plant Freeze Dryer Specifications*

Specification	**RAY ® PP 1**	**RAY ® PP 2**
Drying Surface (m^2)	0.7	1.5
Load (kg/day)	20.0	38.0
Dry Matter (%)	20.0	20.0
Expected Batch Time (h)	8.0	8.0
Sublimation (kg H_2O/day)	15.3	30
Production (kg/day)	4.0	8.0

Installed Power (Kw)	12.0	25.0
Average Consumption (Kwh/h)	7.0	12.0
Water Consumption (L/h)	50.0	75.0

Equipment 3: Kemolo Freeze Dryer Lyophilizer

This type of freeze dryer is made for high-efficiency freeze drying of food materials. It necessitates a greater manufacturing capacity, cheaper equipment costs, and lower energy consumption costs. For several years, KEMOLO has focused on manufacturing this sort of freeze dryer and has extensive installation expertise both at home and abroad. The moisture in the frozen item will be evaporated to a continuous frozen vapor condenser in a sealed vacuum environment by heating the entire frozen food material. The food item is dried after 20-30 hours, and the water is transported to the freeze dryer's vapor condenser.

The freeze drying equipment is composed of several parts: the vessel, shelf plates, trays and vapor condenser, refrigeration compressor, vacuum pump, heating system, defrost system, and the control system. Each freeze dryer is made out of a vessel/chamber that contains shelf plates, trays, and vapor condensers. The vessel might be fashioned like a cylinder or like a square. Stainless steel or aluminum shelf plates and trays are available while the vapor condenser is made of stainless steel. The usual stainless steel configuration is SUS304, but SUS316 is also offered as an alternative. Welded sections are the normal configuration for shelf plates; however, entire pieces are available as an option. During in-place freezing, evacuation, and the early phases of drying, the refrigeration system freezes the shelf plates. During the drying cycle, it also freezes the vapor condenser. Larger freeze dryers employ a screw compressor, while smaller ones use a piston, and the refrigerant used is R404A, R507, and other environmentally friendly Freon. Drying chamber, condenser, vacuum pipes, valves, back pump, and holding pump make up the vacuum system. There are no leaks required, and a vacuum system is required for quick sublimation. To provide efficient capacity for evacuation, large scaled freeze dryers require backing pumps and holding pumps. A heat transfer fluid is used to cool or heat the heat-plates. During the drying cycle, this fluid can be frozen or heated to enable in-place freezing or heating of the product. An electric boiler heats the fluid directly, and an intermediate heat exchanger chills it. The heat transfer fluid is forced to circulate using a centrifugal pump. Ethanol, glycol, silicon oil, deicing fluid, alcohol, water, and other optional fluids. The vapor condenser is defrosted with water. Heat from the refrigerator compressor can be

retrieved as an optional extra to enable 60˚C hot water defrost, considerably lowering defrosting times when compared to cold water defrosting. For the control system, Siemens or Advantech PLCs that are CE and UL approved are used. All electrical components, including voltage, frequency, wire, motor circuit breakers, and overload protection, are compatible with local suppliers. The data acquisition and control of the freeze dryer is aided by a range of sensors, including pressure transducers that monitor and control the refrigeration system. At the product chamber, the vacuum pressure is measured. The heating fluid is measured and controlled using temperature sensors.

Figure 21. *Kemolo Vacuum Freeze Dryer*

Table 34*. Kemolo Vacuum Freeze Dryer*

SPECIFICATIONS	
Capacity	100 kg/batch
Trays area	Bulk or Hydraulic Stoppering
Chamber size	-70 C to 65 C
Plate temperature	Hollow Fluid Filled
Condenser temperature	12” x 24”, 316L SS, 20Ra or better
Vacuum level	-85 C
Plate size	30 L
Plate distance	20 L in 24 hours

Cooling water	Exposed Coil, 6” Vapor Port
Defrost	Hot Gas
Use Environment	3.5 HP 1st stage 2 HP 2nd stage
Power Consumption	4 Type T Thermocouples
Install Area	Corrosion Resistant
Weight	Pirani w/ Solenoid & Needle Valve
Control System	CE, UL approved Siemens or Advantech PLC
Electrical	230 V/60 Hz, 1 ph, 40 A 220 V/50 Hz, 1 ph, 40 A 380 V/50 Hz, 3 ph, 30 A

Equipment 4: MAGNUM® XL Pilot Freeze Dryer

The MAGNUM® XL Pilot Freeze Dryer is commonly used for research and development purposes, but it can also be a reliable freeze dryer for small scale or pilot scale production. This unit is designed for low-moisture content products. The manufacturer, Millrock Technology, provides an easy-to-use software (Opti-Dry® G2 software) for the unit to allow automatic freeze-drying, defrosting, and leak rate testing. Data can then be printed either numerically or graphically. Moreover, the control system (Opti-Dry® G2: PC/PLC Control) found in this specific unit is also used in industrial freeze dryers which allows for easier scaling to production. This unit also features an advanced predictive maintenance system which tracks and monitors the condition and performance of the unit during normal operation which will enable cost-saving and maximum uptime in the long run. Figure 22 shows an image of the MAGNUM® XL Pilot Freeze Dryer.

Figure 22. *MAGNUM® XL Pilot Freeze Dryer (Millrock Technology, 2022)*

As for the specifications of the said unit, it offers up to 30 sq. ft. of shelf area with a condensing capacity of 30 liters and a condensing rate of 20 liters in 24 hours. A full list of the standard features and specifications of the MAGNUM® XL Pilot Freeze Dryer is presented in Table 35.

Table 35. *MAGNUM® XL Pilot Freeze Dryer*

SPECIFICATIONS	
Shelf Area	20 to 30 sq. ft.
Shelf Assembly	Bulk or Hydraulic Stoppering
Shelf Temperature Range	-70 C to 65 C
Shelf Heat Transfer	Hollow Fluid Filled
Shelf Size/Finish	12” x 24”, 316L SS, 20Ra or better
Condenser Temperature	-85 C
Condenser Capacity	30 L
Condenser Rate	20 L in 24 hours
Condenser Style	Exposed Coil, 6” Vapor Port
Defrost	Hot Gas

Compressors (Scroll)	3.5 HP 1st stage 2 HP 2nd stage
Product Sensors	4 Type T Thermocouples
Vacuum Pump	Corrosion Resistant
Vacuum Control	Pirani w/ Solenoid & Needle Valve
Gas Backfill	Included
Control System	Opti-Dry® G2: PC/PLC Control
Trays	One per Shelf Included
Cabinet	46” w x 37” d x 88.5” h
Electrical	230 V/60 Hz, 1 ph, 40 A 220 V/50 Hz, 1 ph, 40 A 380 V/50 Hz, 3 ph, 30 A

APPENDIX VIII: CALCULATION SHEETS