FACULTY OF ENGINEERING AND BUILT ENVIRONMENT

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2ND YEAR INTEGRATED PROJECT

SEMESTER I SESSION 2020/2021

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TITLE	:	PRODUCTION OF PROBIOTIC FROM YEAST
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DECLARATION

We hereby declare the work in this project is our own except for quotations and summaries which have been duly acknowledged.

21 January 2021

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EXECUTIVE SUMMARY

Probiotics are live microorganisms that are intended to have health benefits when consumed or applied to the body. Likewise, the *Saccharomyces boulardii* (yeast cell) probiotic is one of the essential supplements in today's modern world. The *saccharomyces boulordii* probiotic able to cure a lot of diseases which involves gastrointestinal environment. The demand for this probiotic increases rapidly in the past few years. The plant capacity of our plant is 338 kg/hr which contribute about 0.3 % to the global demand. The main objective of this project is to produce *Saccharomyces boulardii* (yeast cell) in large scale. Parboiled rice effluent with 1% sucrose solution which contains glucose and rich in nitrogen and ammonia source is use as the medium for growth in batch fermenter with the presence of oxygen. Parboiled rice effluent undergoes fermentation process to produce biomass as main product and at the same time carbon dioxide and waste water is generated as waste. Carbon dioxide later on is use in photobioreactor for the growth of microalgae whereas the waste water which contains high amount of organic matter is treat by activated carbon filtration before discharge. Mass balance is performed for all the unit operation involve and energy balance is performed for fermenter and freeze dryer. In this project also, thermodynamics calculation is performed to determine the equilibrium composition of all the components in the fermenter. Moreover, we also have discuses suitable growth medium for *Saccharomyces boulardii* (yeast cell) in cell biology chapter. Finally, in the last chapter we have perform fluid mechanics calculation for rotary peristaltic pump.

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CHAPTER I

BACKGROUND OF STUDY

1.1 INTRODUCTION OF PROBIOTIC

Probiotics are defined as live organisms which confer a health benefit to the host when administered in adequate amounts, regardless of where the action takes place and the type of administration. Usually, they are recommended to help strengthen host systems (Pedro Pais 2020). *Saccharomyces boulardii* is a tropical yeast species, first isolated by French scientist Henri Boulard in 1923 from lychee and mangosteen fruit. While separate taxonomic, metabolic, and genetic properties were identified in early studies, *S. boulardii* is a strain developed by *Saccharomyces cerevisiae*, exchanging genomic relationships of > 99 percent, giving the synonym *S. boulardii var cerevisiae* (Indu Khatri 2017).

S. boulardii and *S. cerevisiae* both produce proteins, specifically pho8 and ysp3, that inhibit pathogenic bacteria and their toxins. The protein fingerprint obtained after electrophoresis of sodium dodecyl sulphate-polyacrylamide gel was similar for all isolates and the classification of *S. boulardii* to *S. cerevisiae* was therefore confirmed (Crowch 2017). *S. cerevisiae* baker's yeast does not seem to have major beneficial properties for human health (Pedro Pais 2020). In the food and nutraceutical fields, *S. boulardii* has been commonly used since it is considered to be effective in limiting diarrheal diseases. *S. boulardii* is known for its anti-inflammatory, immunomodulatory and microbiome regulatory effects and has been approved as commonly considered safe (GRAS) by the Food and Drug Administration (FDA). This yeast has been shown to prevent microbial translocation and secretion of inflammatory cytokines including IL6. Furthermore, due to its high temperature tolerance and low pH, *S. boulardii* may live in the human gastrointestinal tract. *S. boulardii* can compete for growth in the gut with

diarrhoea-causing pathogens, making it effective in treating and preventing diarrhoea (Jing-Jing Liu 2016)

The viability of probiotics is a crucial factor influencing medicinal treatment. As a feature of the strain and health effect desired, benefits and differs. Strains isolated from human or animal intestines will proliferate outside their natural environment and thus have reduced technological characteristics. In addition, probiotic micro-organisms have to show their tolerance to the acidic stomach and bile environment in order to attain high survival rates in the duodenum secreted in the gastrointestinal tract. It is difficult to spread many strains of intestinal origin and high survival is critical for both economic reasons and health effects. Furthermore, with the development of technologically unsuitable strains, more efficient technologies could lead to higher product efficacy and strain diversification. For the proliferation of probiotic bacteria, some fermentation technologies, like fed batch, continuous fermentation, membrane bioreactors and immobilised cell technology, are considered suitable. They are designed to produce higher cell yield and productivity and to minimise the downstream processing ability required for biomass harvesting. (Marimuthu Anandharaj)

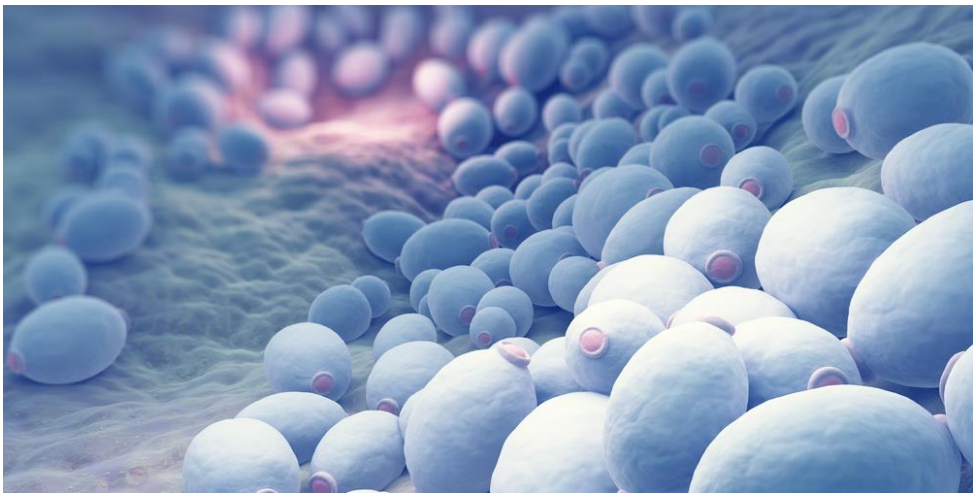


Figure 1.1 *Saccharomyces boulardii*

1.2 USAGE OF *SACCHAROMYCES BOULARDII*

There are many usages of the *Saccharomyces boulardii*, such as treating acute diarrhea in children. In children, infectious diarrhea represents a public health problem, and in the developing countries, several million children die of dehydration. This probiotic has seemed to be promising therapeutic agents. This probiotic is useful in the treatment of viral, bacterial, and protozoan induced diarrhea. *S. boulardii* works on treating diarrhea by shortening the initial phase of watery stools on the first day until the fourth day. From the expert opinion, using *S. boulardii*, the treatment time can be reduced up to 24 hours or a day (Ener Cagri 2012). The effect of diarrhea in the long term is it can cause morbidity and mortality worldwide. The *S. boulardii* is possibly useful for acne, a digestive tract infection that can lead to ulcer and travelers' diarrhea. *S. boulardii* can inactivates bacterial toxins, inhibits toxin binding to intestinal cell receptors, and reduces toxin-induced inflammation. Then, *S. boulardii* stimulates host immune systems and intestinal enzymes that enhance nutrient digestion and absorption. There are many usages of *S. boulardii*, but it does not have sufficient evidence in research, treatment and development. It can improve heart failure, Chron disease, Cystic fibrosis, high cholesterol, infection of the intestines by parasites, Lyme disease urinary tract disease, and yeast infections.

CHAPTER II

ECONOMIC ISSUES

2.1 THE DEMAND AND SUPPLY OF PROBIOTIC

Economic issue is one of the important aspect that need to be discuss. The demand for the probiotic increases drastically among Malaysians and also people all around the world from 2017 to 2019. The major reason for these drastic changes is because people start to be more aware of their internal health. Maintaining good internal environment is really important to stay healthy and also prevent ourselves from diseases. This cause the demand towards probiotic product increase in recent years and expected to grow in more upcoming years.

In 2018 the demand for the probiotic product in the world market is 17.72 kilotonnes. From the year 2019 to 2023 the demand for the probiotic product increase with Compound Annual Growth Rate (CAGR) of 6.8% and expected to reach 24.62 kilotonnes in 2023. That means we estimated in the year 2019 the demand will be 18.92 kilotonnes, 20.21 kilotonnes in the year 2020, 23.05 kilotonnes in 2021 and 23.05 kilotonnes in 2022. Thus, it is proven that the demand towards probiotic product is increasing highly every year and expected to increase more in future. (GlobeNewswire 2020)

In the other hand the supply for probiotic product in the world market is 10.91 kilotonnes in the year 2018 and increase with Compound Annual Growth Rate (CAGR) 7.14% and expected to reach 15.40 kilotonnes in the year 2023. In 2019 the supply is 11.69 kilotonnes, 12.52 kilotonnes in 2020, 13.42 kilotonnes in 2021 and 14.38 kilotonnes in 2022. (OSTER 2017)

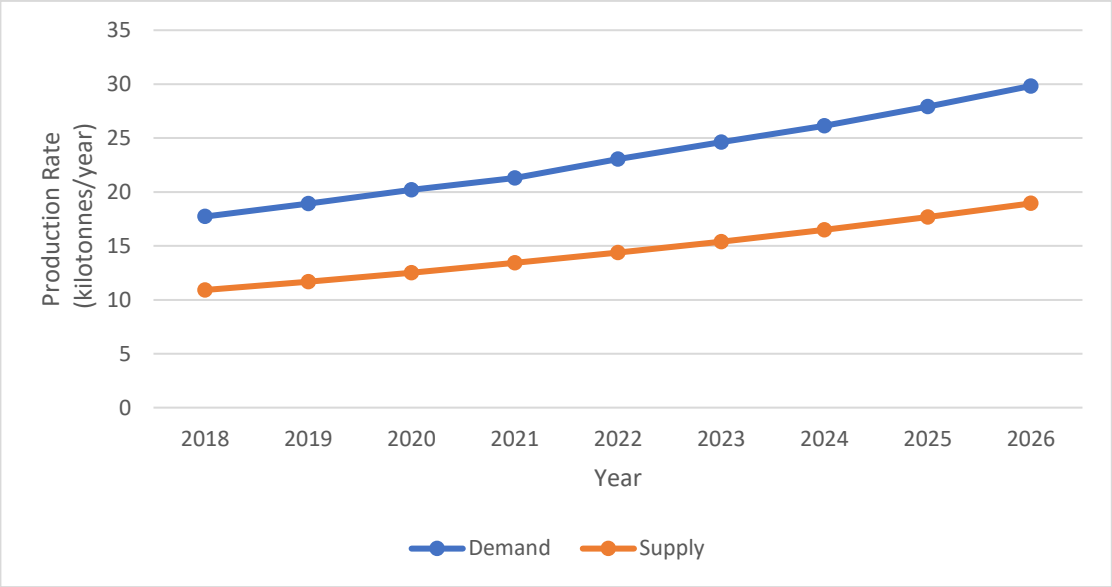


Figure 2.1 Supply and Demand for probiotic from year 2018 to 2026

(Source : Probiotic Industry Trend, 2020)

2.2 THE PRICE OF THE PROBIOTIC

Today the consumption of probiotic product by people around the world increased day by day. The demand of probiotic product in Malaysian market particularly also become higher in past few years. Thus, it is clearly proven that the demand towards probiotic product will rise in upcoming years. According to the recent market price (Amazon), the selling cost of probiotic product is $2658333.33 per ton which is equivalent to $2658.33 per kilogram. We make assumption that the market price for the probiotic product will be same all around the world which is match to the industrial standards. As for now a lot of development had been introduced for the production of probiotic which is associated with market price of probiotic products. It is targeted the market price for the probiotic product will increase in future.

Currency rate: 1 US dollar = 4.12 Malaysian Ringgit (November 11, 2020)

2.3 LIST OF COMPANIES PRODUCING PROBIOTIC

Table 2.1 List of companies producing probiotic

List of company	Country	Capacity
UF Feta Cheese Iran	Iran	117934 tonne/year
Yakult (probiotic drinks)	China	14235 tonne/year
Lonza (probiotic capsule)	Singapore	600 tonne/year
Biofarma (probiotic tablets)	Italy	525.6 tonne/year
Tianjin Goubuli Group Corp (probiotic food)	Australia	181.4 tonne/year
Tianjin Goubuli Group Corp (probiotic plant)	Australia	35 tonne/year

(Source : Journal of Food Science and Technology, 2018)

2.4 PLANT CAPACITY

From the data we obtained in Figure 2.1, we conclude that the demand of probiotic is expected to increase from 2018 due to many usages of probiotic especially in health. So, in the coming year of 2023, we have estimated the production of probiotic plant as below:

(1 tonnes = 1000 kg)

Demand = 24.62×10^3 tonnes/year

Supply = 15.40×10^3 tonnes/year

Deficient = 9.22×10^3 tonnes/year

An average value of 0.30 is taken to predict our production.

Production time per year = 341 days × 24 hours

= 8184 hours

Maintenance = 2 days/month

Plant capacity = (0.30 × deficient) ÷ production time a year

= (0.30 × 0.922 × 10^7 kg/year) ÷ [(341 days × 24)]

= 338 kg/hr

Therefore, the plant capacity is 338 kg/hr.

CHAPTER III

PRODUCTION OF PROBIOTIC

3.1 INTRODUCTION FOR PROCESS DESCRIPTION

In this chapter we will discuss about the entire process description for the production of *Saccharomyces boulordii* (yeast cell) from the beginning. There are about six operational units involve, starting from seed fermenter, main fermenter, centrifuge, filter, mixing tank and freeze dryer. The raw materials involve are parboiled rice effluent with 1% sucrose solution as the medium, betaine and with the presence of oxygen gas. The main product in this process is *Saccharomyces boulordii* (yeast cell). In addition, carbon dioxide and fermentation broth (waste water) are generated as waste from the process.

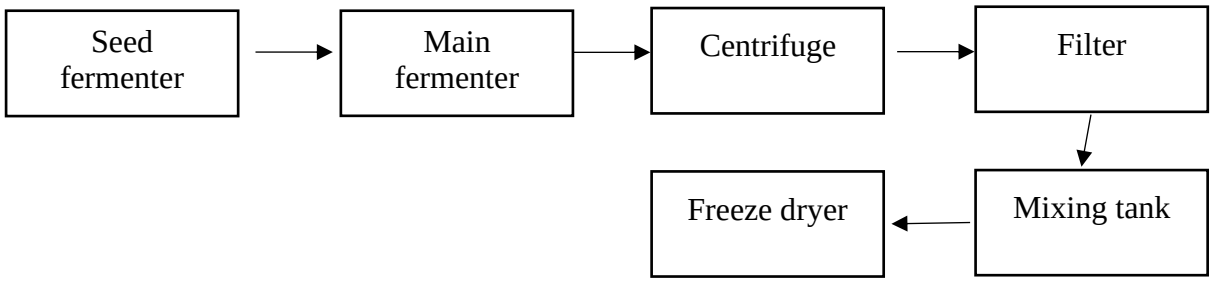


Figure 3.1 Simplified process description in block diagram

3.2 PROCESS DESCRIPTION OF SACCHAROMYCES BOULARDII

3.2.1 Seed Fermenter

The first step in the production of *Saccharomyces boulardii* probiotic starts from culturing the yeast species in a culture medium. In our process we are using parboiled rice effluent contain nitrogen and phosphorus (Queiroz and Koetz 1997) with 1%

sucrose solution as the medium for growth. The *Saccharomyces boulardii* is feed into a seed fermenter (F-101) of volume 200L containing 150L parboiled rice effluent with 1% sucrose solution. In this stage oxygen gas is supplied at T=25℃ and 1 atm and carbon dioxide gas is released from the reaction. The cell is allowed to grow.

3.2.2 Main Fermenter

After 24 hours in the seed fermenter the solution in the seed fermenter is transfer into a main fermenter of volume 2000L for fermentation process. The process is carried out to produce more *Saccharomyces boulardii* probiotic in large scale. In addition, parboiled rice and 1 % sucrose solution is feed into main reactor (F-102). Since, fermentation process is an aerobic reaction, thus oxygen gas at T=25℃ and P=1atm is supplied to the bioreactor throughout the process and carbon dioxide gas which produced from the reaction is removed from the main fermenter. In order for the process to run smoothly optimum condition is maintain at T= 28℃ P= 1 atm and at pH 7 for the cell growth. The fermenter is continuously stirred at 150 rpm. The process is carried out for 48 hours. All the ammonia is completely reacted in this stage.

3.2.3 Centrifuge

Next, after the fermentation process the solution containing *Saccharomyces boulardii* and the mixture which contain glucose and water will enter centrifugal (C-101). Centrifugation is the first separation process involve the mixture. In this stage the mixture of *Saccharomyces boulardii* and liquid solution is separated. At the end, the cell is completely separated from the liquid solution and removed as waste (fermentation broth) from centrifuge. In this stage almost 80% of water in the cell is removed together with large molecules like glucose

3.2.4 Filter

After the centrifugation process the *Saccharomyces boulardii* cell which contain water enter the filter (FT-101). In this stage, filtration take place to eliminate about 95%

remaining water from it. Filtration is one of the effective ways to purify the cell from liquid.

3.2.5 Mixture

Before the last stage the cell is feed into mixture (M-101). In this stage the cell is added with lyoprotectant which is betaine to increase the stability of the yeast. This process is important because it help to prevent the cell structure from damaging due to high stress during freezing process.

3.2.6 Freeze Dryer

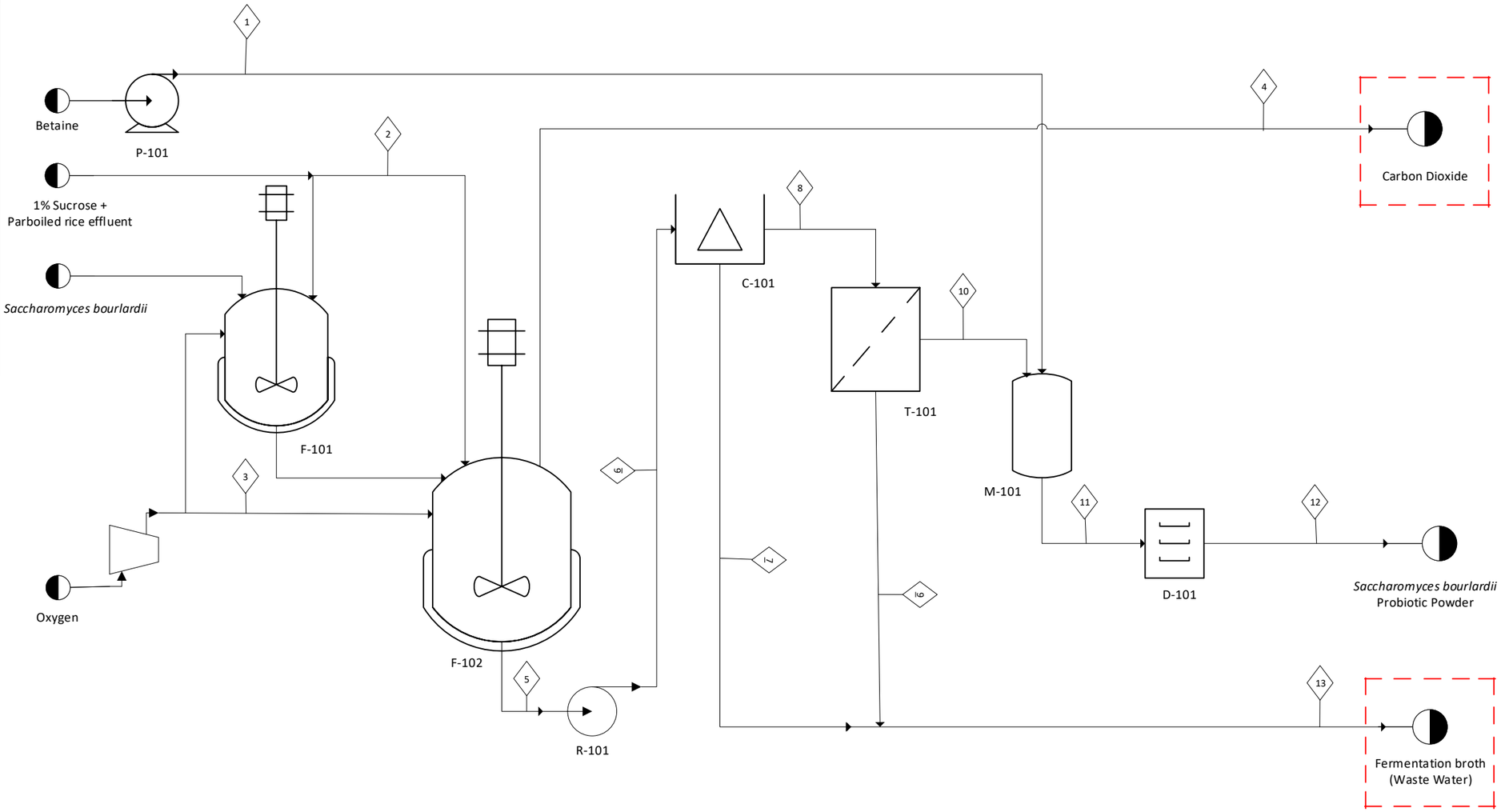
Finally, the *Saccharomyces boulardii* is extracted to become a biomass using a freeze dryer (D-101) which provides higher survival rates. Freeze drying is accomplished by three significant steps which are freezing, primary and secondary drying (Marimuthu Anandharaj 2007). This process commonly frozen at -196 °C in liquid nitrogen to increase the survival rate of yeast cultures. Then, the frozen samples are sublimated with ice under high vacuum conditions to finish the primary freezing. After the primary drying step, practically 95% of the water content in the sample is removed. Secondary drying is likewise imperative to accomplish a final water content below 4%, consequently improving survival rates and long-term storage efficiency.

3.3 PROCESS FLOW DIAGRAM

Figure 3.2 Process Flow Diagram

F-101 Seed Fermentor F-102 Main Fermentor C-101 Centrifuge T-101 Filter M-101 Mixing Tank D-101 Freeze Dryer

P-101 Centrifugal Pump R-101 Rotary Pump



Stream	1	2	3	4	5	6	7	8	9	10	11	12	13
Temperature (K)	301	301	298	301	301	301	301	301	301	301	298	77	301
Pressure (atm)	1	1	1	1	1	1.48	1	1	1	1	1	1	1
Phase	l	l	v	v	aq	aq	l	aq	l	aq	aq	s	l
Glucose (kg/h)	0	878.8	0	0	202.8	202.8	202.8	0	0	0	0	0	202.8
Oxygen (kg/h)	0	0	331.1	0	0	0	0	0	0	0	0	0	0
Ammonia (kg/h)	0	29	0	0	0	0	0	0	0	0	0	0	0
Water (kg/h)	0	605.8	0	0	878.8	878.8	703.04	175.76	166.97	8.79	8.79	8.79	870.01
Carbon Dioxide (kg/h)	0	0	0	492.7	0	0	0	0	0	0	0	0	0
Betaine (kg/h)	58.81	0	0	0	0	0	0	0	0	0	58.81	58.81	0
Saccharomyces bourladii (kg/h)	0	0	0	0	270.4	270.4	0	270.4	0	270.4	270.4	270.4	0
Total flow rate (kg/h)	58.81	1513.6	331.1	492.7	1352	1352	905.84	446.16	166.97	279.19	338	338	1072.81

UNIVERSITI KEBANGSAAN MALAYSIA
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Input Output Stream number

Date : 07 January 2021

CHAPTER IV

SAFETY ISSUES AND ENVIRONMENTAL

4.1 INTRODUCTION

Environmental concerns are the adverse effects on the biophysical environment of human activity. Environmental conservation, for the benefit of both the environment and humans, is a method of protecting the natural environment at individual, organisational or governmental levels (Cabaniss 2014). All industrial processes produce waste of some form that must be carefully treated and disposed of at varying levels in compliance with regulations set by governments. Emissions to air, water and soil, smell, noise and visual effects, and waste management are key areas for consideration. It is necessary to note that for every engineer or industry, emission considerations are both a moral and legal duty. (Wikipedia 2020)

There are a few effects on the environment due to the waste produced from the industrial production of *S. boulardii* probitic. The production of this probiotic may cause water and air pollution due to the release of great amount of carbon dioxide.

4.2 SAFETY ISSUES OF THE RAW MATERIAL AND THE METHOD OF HANDLING AND STORAGE

Industrial facilities have particular safety issues because more than just the workers on the factory floor are affected by risks and accidents. A workplace fire, lost days due to injury, or chemical hazards can impact your production quality, which can delay delivery times, distribution, relationships with sellers, and satisfaction with customers. Industrial safety works hard to prevent risks to the workplace, including chemical exposures, poor ergonomics, and physical hazards, so that without interruption to production, business can continue as normal (Resources 2018). Due to the safety issues,

the handling and storage of materials is vital. These operations provide a continuous flow of parts and assemblies through the workplace in addition to raw materials, and ensure that materials are available when needed.

4.2.1 Handling of Raw Material (Yeast)

The yeast production process can be linked to agriculture, involving preparation, seeding, cultivation and etc. The utmost care is taken in all the yeast processes to produce a product of the highest possible quality and purity. The worker regularly checks the samples and frequent cleaning and sterilization of the equipment is carried out to ensure that the correct standards are met. Sterilizing prevents the introduction of bacteria and other organisms during manufacturing (REDSTAR 2014).

During the seed fermentation, in order to avoid contamination by "wild yeast present in the air, the seed yeast is a carefully maintained laboratory culture. Yeast seeds are carefully selected according to the type of yeast to be produced and the desired specific features. All transfers are made with absolute sterility; all vessels are sterilized completely. Not only that, in large vessels, the cultivation or progress of the fermentation process is achieved. At this point, sterilizing such large vessels is impractical, but careful cleaning with steam guarantees cleanliness and quality (REDSTAR 2014)

4.2.2 Properties of Raw Materials

The properties of raw materials for *Saccharomyces boulardii* are the appearance can be fine dry granular light beige to brown powder or pellets and do not have any odour. This material is stable under recommended storage conditions. This material should avoid heat, flames, sparks, and strong oxidizing agents because they can make material unfunctional. This material is not a hazardous type.

Next, parboiled rice effluent's appearance is light brown/ beige or white/creamy, which depends on rice type. The solubility in water is moderate and have dust explosion Class 2. This material is incompatible with strong oxidizing agents.

Then, the properties of sucrose are the appearance form is crystalline, which is white. The sucrose pH is 5.5 - 7 at 25˚C which is little bit acidic and has a melting point (185 – 187)˚C. This material may form combustible dust concentration in air, and it completely soluble for water solubility at 20˚C. Sucrose is stable under recommended storage conditions and incompatible materials to strong oxidizing agents. Sucrose also has acute toxicity.

After it, the appearance form of oxygen is clear in gas and gas may explode if heated and cause or intensify fire. Oxygen doesn't have any odour and taste. The boiling point of oxygen is -187˚C, the freezing point at 218˚C, vapour pressure is 760mmHg at -183˚C and soluble in water at 25˚C. The viscosity of oxygen is 0.02075 cP at 25˚C.

Then, the appearance of carbon dioxide is clear in gas and gas may explode if heated and cause or intensify fire. Carbon dioxide doesn't have any odour, but the taste is acidic. The freezing point of carbon dioxide is -57˚C, vapour pressure is 43700mmHg at 21˚C and soluble in water at 25˚C. The viscosity of oxygen is 0.01675 cP at 0˚C. The pH for carbon dioxide is 3.7 (saturated aqueous solutions at 101.3 kPa) which is acidic. It also has toxicity.

Lastly, the properties of freeze-dried probiotic cultures make off-white to tan powder and have a faint odour. It is also slightly soluble in water and stable under recommended storage. The stability and reactivity of these materials is an open flame, spark and have static electricity.

4.2.3 Safety Issues on Raw Material and Product

Table 4.1 Safety Issues on Raw Materials and Product

Substances	First Aid Action	Protective Equipment
Saccharomyces Boulardii	▪ If the substance make contact with eyes, rinse immediately with soap and plenty of water under eyelids for at least 15 minutes. ▪ If skin contact occurs, wash off immediately with soap and plenty of water removing all contaminated clothes and shoes. Wash contaminated clothing	▪ Use tightly fitting safety goggles for the eye/face protection. ▪ For skin and body protection, use the impervious gloves and proper use glove removal technique to avoid skin contact with the product. The type of protective equipment must be selected according to the concentration and

	before reuse. ▪ Move to fresh if breathing is difficult or give oxygen. If not breathing, give the artificial respiration. ▪ Rinse mouth if taken the substance or never give anything by mouth to an unconscious person. ▪ Get medical attention.	amount of dangerous substances. ▪ Wear suitable respiratory equipment.
Parboiled rice effluent	▪ If the substance make contact with eyes, flush with water for 2-5 minutes. ▪ If skin contact occur, wash with soap and water. ▪ For inhalation part, remove from area of exposure to well ventilated area. ▪ Get medical attention.	▪ Keep dust level down by providing adequate ventilation. ▪ If dust level are high, use OSHA approved nuisance dust mask for respiratory protection. ▪ Use the safety google for eye protection. ▪ Wear body-covering clothing and closed footwear for skin protection.
Sucrose	▪ In case of skin protection, wash off with soap and plenty of water. ▪ In case inhaled, if breathed in, move person into fresh air. If not breathing, give artificial respiration. ▪ In case of eye contact, flush eyes with water as a precaution. ▪ If swallowed, never give anything by mouth to an unconscious person. Rinse mouth with water. ▪ Get medical attention.	▪ For eyes protection use equipment which tested and approved under appropriate government standards such as NIOSH and EN 166. ▪ Use proper glove and removal technique to avoid skin contact with substances. Dispose of contaminated glove after use and wash and dry hands for skin protection. ▪ Choose body protection according to the concentration and amount of dangerous substances. ▪ Wear mask type N95 or type P1. ▪ For control of environmental exposure, do not let product enter drains.
Oxygen	▪ Get medical attention if something happens.	▪ Respiratory protection may be needed for frequent or heavy exposure.
Carbon dioxide	▪ For skin contact, if frostbite or freezing occur, immediately flush with plenty of lukewarm water. ▪ For eyes contact, immediately flush eyes with plenty of water at least 15 minutes. ▪ Give artificial respiration if not breathing. ▪ Get medical attention.	▪ Wear splash resistant safety goggles if it liquid for eyes protection. ▪ Wear appropriate protective or clod insulting clothing if it liquid for skin protection. ▪ Avoid breathing vapor or mist. ▪ Wash thoroughly after handling and before eating or drinking.
Lyoprotectant (Freeze Dried	▪ For eyes exposure, rinse particulate matter from eye. ▪ For skin exposure, wash with	▪ For respiratory protection, select NIOSH/MSHA approved equipment based on actual or

Probiotic Cultures)	plenty of soap and water. ▪ If respiratory irritation or distress occurs remove victim to fresh air. ▪ Inhalation of substance may aggravate existing chronic respiratory. ▪ Seek medical attention.	potential airborne concentrations. ▪ For eye/face protection will very dependent upon work environment conditions and material handling practices like wear a minimum of safety glasses. ▪ Skin contact should be minimized through use of gloves and suitable long-sleeved clothing.

(Source : Safety Data Sheet of S. Boulardii, 2015)

4.2.4 Storage of *Saccharomyces boulardii*

We decided to covered our probiotic strain by a proprietary five-layer microencapsulation technology so our patented probiotic strains are distinct from others. The advantages of five-layer microencapsulation technology are to protect probiotics during storage. This is because the inner layer feeds the bacteria and the other layers protect against oxidation and excess moisture. The best storage conditions are in a position that is dry and cold. In the original sealed package, the shelf life is two years below 25 ° C. The shelf life can be increased by refrigerated storage (4-8 ° C) (Ingredients 2017)

4.3 ENVIRONMENTAL ISSUES

The red dotted boxes in the process flow diagram in Figure 4.1 indicate the waste that had been produced. The first waste is carbon dioxide which is in gas form. Since, fermentation process is an aerobic reaction, thus oxygen gas is supplied to the bioreactor throughout the process and carbon dioxide gas which produced from the reaction is removed from the seed fermenter and the main fermenter. Another waste is the wastewater where the centrifuge seperates the mixture of *Saccharomyces boulardii* and liquid solution. Almost 80% of water in the cell is removed together with large molecules like glucose. After that, filtration take place to eliminate about 95% remaining water from the cell.

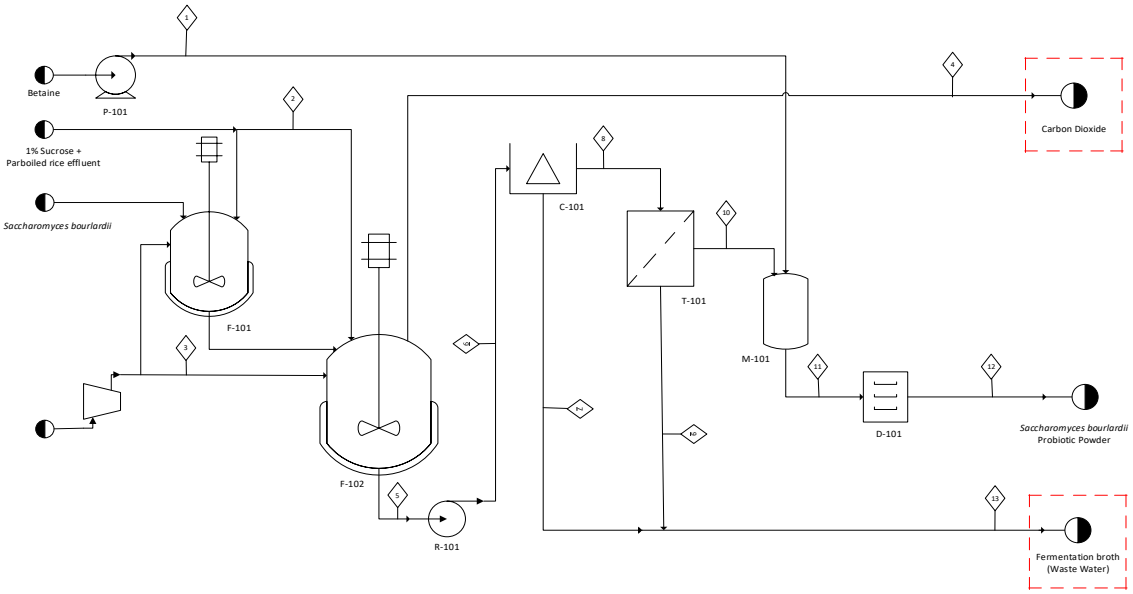


Figure 4.1 Process Flow Diagram

4.3.1 Waste Management

In the process of production of probiotic using *Saccharomyces boulardii* there are two main waste that is produced. The most common waste that is produce during the process is carbon dioxide and also fermentation broth (waste water) which rich in organic substances. Thus, a proper waste management for the waste which is produced is essential for prevent the environment from harm.

As mentioned in the previous paragraph, carbon dioxide is one of the waste that produce during the process. Since fermentation is an aerobic process thus oxygen gas is used during the process and it will produce high purity and low pressure carbon dioxide at the end of fermentation process. The amount of carbon dioxide produce during the fermentation process is large, thus it can be use in photobioreactor for the growth of algae. The carbon dioxide gas that vent from the process, is connected to a photobioreactor which contain green microalgae, Chlorella vulgaris. In the resulting continuous flow of carbon dioxide from fermenter, it makes the microalgae inside the photobioreactor to capture the carbon dioxide, in which the microalgae is placed in the membrane for exposure of light. It is make sure that the microalgae's growth is at maximum. The efficiency of microalgae in capturing and utilizing carbon dioxide is

excellent. In conventional of that the oxygen gas which produce by the microalgae during photosynthesis which later on can be used by yeast cell for growth in fermenter. Thus, by combining both of this process a symbiotic relationship can be established. It is clearly observed that the carbon dioxide emission during the process can be used for other process which can prevent it from releasing to the atmosphere.

Next, waste water also one of the important waste that produce during the process. Basically, waste water contains soluble organic matter in which the water has high level of BODs. The spent water as remaining after the process may account 90% of the initial raw organic material. Thus, the waste water produce actually can convert as a usable water in the process. The first, purified water recovery, involves the types of technologies employed for water desalination. These include evaporation of various kinds, reverse osmosis, electrodialysis, and possibly fractional crystallization. Some of these are well established technologies, but most of them are expensive and need some degree of stream pre-treatment. The high-quality water regenerated by these methods actually can be used in the process for the new batch of process.

The alternative water recycling process is the direct recycle of medium, in which the organic matter in the water is remove. Methods which can be used for removal of potential inhibitors include adsorption (such as activated carbon treatment), precipitation, ion exchange, chelating complexation, solvent extraction, membrane treatment (such as microfiltration or ultrafiltration), enzymatic hydrolysis, and thermal degradation. The choice of an optimal method will depend on the specific fermentation process. Any of the removal methods will benefit from operating with the maximum allowable inhibitor concentration. In, the recycle water, as this makes inhibitor removal easier and cheaper.

4.3.2 Enviromental Act

Environmental law is that the collection of laws, regulations, agreements and customary law that governs how humans interact with their environment. The purpose of environmental law is to guard the environment and make rules for a way people can use natural resources. Environmental laws not only aim to guard the environment from

harm, but they also determine who can use natural resources and on what terms. Laws may regulate pollution, the utilization of natural resources, forest protection, mineral harvesting and animal and fish populations. (Legal Career Path 2020)

The volume of wastewater discharged from probiotic processing plants must be treated before it is released to the sewers. The quality of effluent from treatment plants and ambient air quality standards and emission standards is regulated by the Environmental Quality Act 1974 (No.127 of 1974) states that, an act concerning to the prevention, abatement, control of pollution and enhancement of the environment. Its regulation such as the Environmental Quality (Industrial Effluent) Regulations 2009. All industries and factories in Malaysia must obey the national law of environmental acts and regulation. Any projects or production plants needs to get the authorization of Ministry of Environmental Malaysia before opening it.

CHAPTER V

MATERIAL AND ENERGY BALANCE

5.1 INTRODUCTION

One of the fundamental laws of physics states that mass can neither be produced nor destroyed. Hence, the principle of conservation of mass is used. Mass balance which is also known as material balance is the accounting of all mass in a chemical or pharmaceutical process. We will be able to identify mass flow which would have been difficult to measure by accounting the input and output of a system. The concept of mass balance can be applied to all process but with different approaches. As for multiple reaction process, the mass balance basically involves the determination of the extent of reactions for all the single reaction in a process.

In the calculations of mass balance below, our idea of doing this mass balance is that there is no flowrate at the bioreactor since our process is a semi batch process so the bioreactor is where the batch process take place. The calculation starts at the centrifuge until the end of the unit operation which is freeze dryer to obtain the product.

In the end of the calculations, the mass flow rate of *Saccharomyces boulardii* is 338 kg/h. Hence, our plant capacity is obtained.

5.2 STOICHIOMETRIC EQUATION

To explain the whole biological method, the stoichiometric equation is very important. The measures are complex for evaluating the stoichiometric equation. Stoichiometric is the quantitative relationship in a chemical reaction between the reactant and products. Stoichiometric coefficients are used in a balanced chemical equation to indicate molar component ratios in the chemical reaction.

The stoichiometric equation for fermentation of glucose to *Saccharomyces boulardii* can be expressed as follows:

$$- \alpha_1 C_6H_{12}O_6 - \alpha_2 O_2 - \alpha_3 NH_3 \rightarrow \alpha_4 H_2O + \alpha_5 CO_2 + \alpha_6 CH_{1.75}N_{0.15}O_{0.5}$$

Divide the stoichiometric equation with α_1, the equation become:

$$-C_6H_{12}O_6 - \alpha'_1 O_2 - \alpha'_2 NH_3 \rightarrow \alpha'_3 H_2O + \alpha'_4 CO_2 + \alpha'_5 CH_{1.75}N_{0.15}O_{0.5}$$

From the equation above, there are 5 unknowns need to be determined which are α'1, α'2, α'3, α'4 and α'5. There are 4 elements which are C, H, O, N present in the stoichiometric equation. Hence, 4 independent elemental equation can be formed. Degree of freedom analysis is carried out to determine whether the solution can be solved or not.

Degree of freedom = Number of unknowns – Number of equations

= 5 – 4

= 1

From the analysis above, it shows that 1 more equation is needed to obtain the unique solution. The equation can be obtained from the yield value of glucose to *Saccharomyces boulardii*.

Molecular mass of each component:

$M_{Glucose} = 6(12) + 1(12) + 6(16) = 180 g/mol$

$M_{Oxygen} = 2(16) = 32 g/mol$

$M_{saccharomyces\ boulardii} = 1(12) + 1.75(1) + 0.15(14) + 0.5(16) = 23.85 g/mol$

$M_{Carbon\ dioxide} = 1(12) + 2(16) = 44 g/mol$

$M_{Ammonia} = 1(14) + 1(3) = 17 g/mol$

$M_{Water} = 2(1) + 16 = 18\ g/mol$

Yield of yeast on glucose, $Y_{Y/G} = 0.4$ (Shuler 2002)

$$\frac{\alpha'_5 M_Y}{M_G} = 0.4$$

$$\frac{\alpha'_5 (23.85)}{180} = 0.4$$

$$\alpha'_5 = 3.019$$

4 independent elemental equation

C: $\alpha'_4 + \alpha'_5 = 6$

H: $-3\alpha'_2 + 2\alpha'_3 + 1.75\,\alpha'_5 = 12$

O: $-2\,\alpha'_1 + \alpha'_3 + 2\alpha'_4 + 0.5\alpha'_5 = 6$

N: $-\alpha'_2 + 0.15\,\alpha'_5 = 0$

To find the coefficient of each component, the Gauss Jordan method is used

$$\left(\begin{array}{ccccc|c} 0 & 0 & 0 & 1 & 1 & 6 \\ 0 & -3 & 2 & 0 & 1.75 & 12 \\ -2 & 0 & 1 & 2 & 0.5 & 6 \\ 0 & -1 & 0 & 0 & 0.15 & 0 \\ 0 & 0 & 0 & 0 & 1 & 3.019 \end{array}\right)$$

$\alpha'_1 = 2.7546$, $\alpha'_2 = 0.4529$, $\alpha'_3 = 4.0377$, $\alpha'_4 = 2.981$, $\alpha'_5 = 3.019$

Reactant: $180 + 2.7546(32) + 0.4529(17) = 275.846$

Product: 4.0377(18) + 2.981(44) + 3.019(23.85) = 275.846

From the calculation, it shows that the equation is balanced.

The final balance equation is shown as below:

$$C_6H_{12}O_6 + 2.7546O_2 + 0.4529NH_3 \rightarrow 4.0377H_2O + 2.981CO_2 + 3.019CH_{1.75}N_{0.15}O_{0.5}$$

5.3 MASS BALANCE OF EACH UNIT OPERATION

5.3.1 F-102 Main Fermenter

The fermentation of the inoculum take place in the main fermenter of 2000L which involve batch process. Where in this stage a total of 1500L of medium with 5.43×10^{-11} kg of *Saccharomyces boulardii* (yeast cell) is added into the main fermenter. The batch process is carried out for 48 hours. Since it's an aerobic reaction oxygen gas is supplied into the main fermenter and carbon dioxide is released as a waste. We also assume that all the ammonia is completely reacted in the process. As a results fermenter broth will released at outlet stream for next process.

F_2 = 1513.6 kg/h

Q_2 = 31.25 L/h

C_{W2} = 19.384 kg/L

C_{G2} = 28.125 kg/L

C_{A2} = 0.925 kg/L

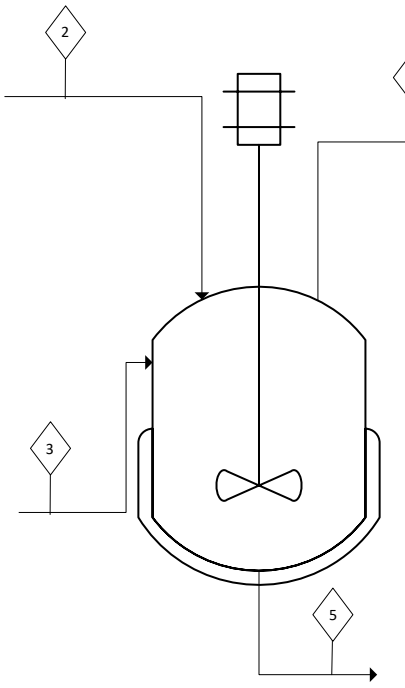
W_{W2} = 0.400205

W_{G2} = 0.5806

W_{A2} = 0.01916

F_3 = 331.1 kg/h

W_{O3} = 1



F_4 = 492.7 kg/h

W_{C4} = 1

F_5= 1352 kg/h

Q_5 = 31.25 L/h

C_{SB5}= 8.654 kg/L

C_{W5} = 28.12 kg/L

C_{G5} = 6.49 kg/L

W_{SB5} = 0.2

W_{W5} = 0.65

W_{G5} = 0.15

Figure 5.1: Main Fermenter, F-102

Table 5.1 Mass and Molar Flow Rate of Each Component in Main Fermenter F-102

		Inlet stream 2		Inlet stream 3		Outlet stream 4		Outlet stream 5	
	Molecular weight	Molar flow rate	Mass flow rate	Molar flow rate	Mass flow rate	Molar flow rate	Mass flow rate	Molar flow rate	Mass flow rate
	(g/mol)	(kmol/h)	(kg/h)	(kmol/h)	(kg/h)	(kmol/h)	(kg/h)	(kmol/h)	(kg/h)
Component									
Water	18.0	33.653	605.8	0	0	0	0	48.82	878.8
Ammonia	17	1.7	29.0	0	0	0	0	0	0
Saccharomyces bourlardii	23.85	0	0	0	0	0	0	11.34	270.4

Glucose	180	4.883	878.8	0	0	0	0	1.127	202.8
Oxygen	32	0	0	10.346	331.1	0	0	0	
Carbon Dioxide	44	0	0	0	0	11.197	492.7	0	
Total		40.236	1513.6	10.346	331.1	11.197	492.7	61.287	1352

Inlet flow rate = Outlet flow rate

1513.6 kg/h + 331.1 kg/h = 492.7 kg/h + 1352 kg/h

1844.7 kg/h = 1844.7 kg/h

5.3.2 C-101 Centrifuge

Centrifugation is the first process involve the mixture. There is a mixture of sucrose, water, glucose and *Saccharomyces bourlardii* (stream 8). In this stage, centrifugal separator is used to remove 80% of water, sucrose and glucose (stream 9).

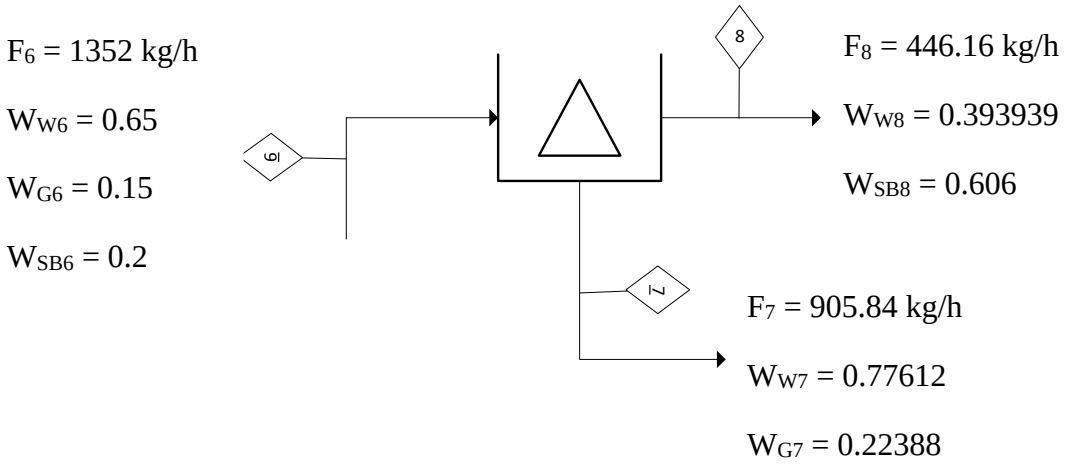


Figure 5.2: Centrifuge, C-101

Table 5.2 Mass and Molar Flow Rate of Each Component in Centrifuge C-101

		Inlet stream 6		Outlet stream 7		Outlet stream 8	
	Molecular weight (g/mol)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)
Component							
Water	18.0	48.82	878.8	39.07	703.04	9.76	175.76
Saccharomyces bourlardii	23.85	11.34	270.4	0	0	11.34	270.4
Glucose	180	1.127	202.8	1.127	202.8	0	0
Total		61.29	1352	40.19	905.84	21.1	446.16

Inlet flow rate = Outlet flow rate

1352 kg/h = 905.84 kg/h + 446.16 kg/h

1352 kg/h = 1352 kg/h

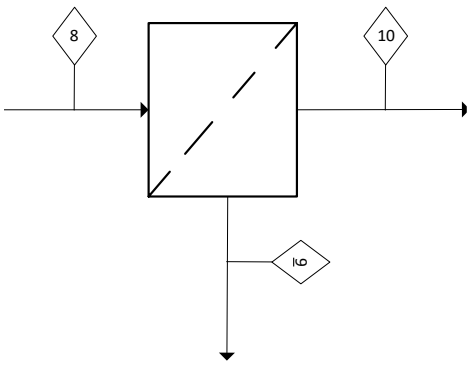
5.3.3 T-101 Filter

Filtration is one of the efficient ways to purify liquid from the cell. In this stage, almost 95% of water is removed from the *Saccharomyces bourlardii*

F_8 = 446.16 kg/h

W_{W8} = 0.393939

W_{SB8} = 0.606



F_{10} = 279.19 kg/h

W_{W10} = 0.0315

W_{SB10} = 0.9685

F_9 = 166.97 kg/h

W_{W9} = 1.000

Figure 5.3 : Filter, T-101

Table 5.3 Mass and Molar Flow Rate of Each Component in Filter T-101

		Inlet stream 8		Outlet stream 9		Outlet stream 10	
	Molecular weight (g/mol)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)
Component							
Water	18.0	9.76	175.76	9.28	166.97	0.49	8.79
Sucrose	342	0	0	0	0	0	0
Saccharomyces bourlardii	23.85	11.34	270.4	0	0	11.34	270.4
Glucose	180	0	0	0	0	0	0
Total		21.1	446.16	9.28	166.97	11.83	279.19

Inlet flow rate = Outlet flow rate

446.16 kg/h = 166.97 kg/h + 279.19 kg/h

446.16 kg/h = 446.16 kg/h

5.3.4 M-101 Mixing tank

Before the last stage the cell is feed into mixture (M-101). In this stage the cell is added with lyoprotectant which is betaine to increase the stability of the yeast

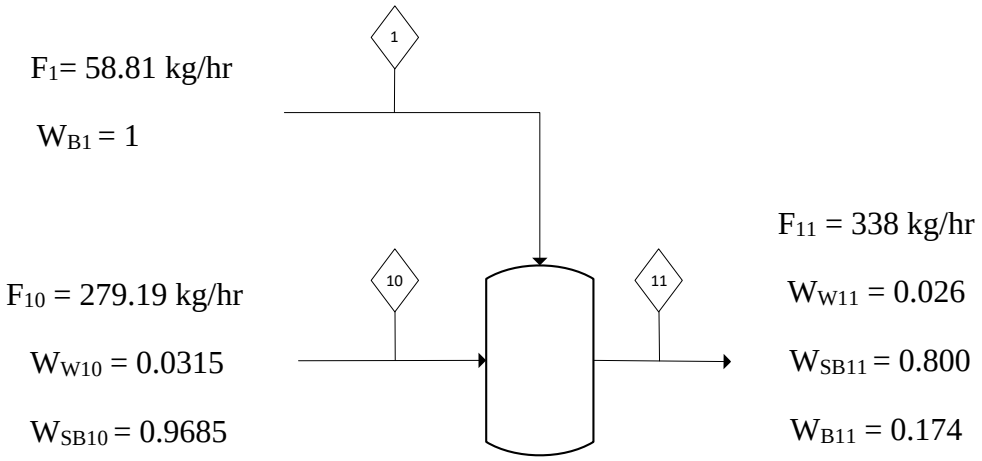


Figure 5.4 : Mixing Tank, M-101

Table 5.4 Mass and Molar Flow Rate of Each Component in Mixing Tank M-101

		Inlet stream 10		Inlet stream 1		Outlet stream 11	
	Molecular weight (g/mol)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)
Component							
Water	18.0	0.49	8.79	0	0	0.49	8.79
Sucrose	342	0	0	0	0	0	0
Saccharomyces bourlardii	23.85	11.34	270.4	0	0	11.34	270.4
Glucose	180	0	0	0	0	0	0
Betaine	117.1	0	0	0.5	58.81	0.5	58.81
Total		11.83	279.19	0.5	58.81	12.33	338

Inlet flow rate = Outlet flow rate

279.19 kg/h + 58.81 kg/h = 338 kg/h

338 kg/h = 338 kg/h

5.3.5 D101 Freeze Dryer

The stream from the freezer is freeze all the substance inside it to become powder. The water inside it will crystalize and coated the powder.

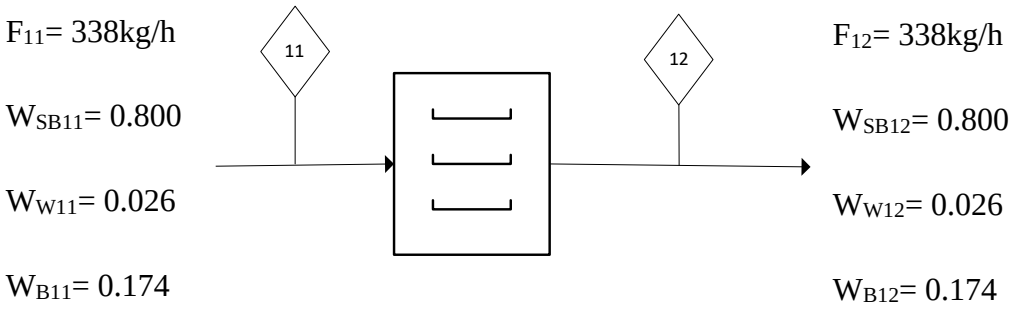


Figure 5.5 : Freeze Dryer, D-101

Table 5.5 Mass and Molar Flow Rate of Each Component in Freeze Dryer, D-101

		Inlet stream 11		Outlet stream 12	
	Molecular weight (g/mol)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)	Molar flow rate (kmol/h)	Mass flow rate (kg/h)
Component					
Water	18.0	0.49	8.79	0.49	8.79
Sucrose	342	0	0	0	0
Saccharomyces bourlardii	23.85	11.34	270.4	11.34	270.4
Glucose	180	0	0	0	0
Betaine	117.1	0.5	58.81	0.5	58.81
Total		12.33	338	12.33	338

Inlet flow rate = Outlet flow rate

338 kg/h = 338 kg/h

5.3.6 Overall Mass Balance

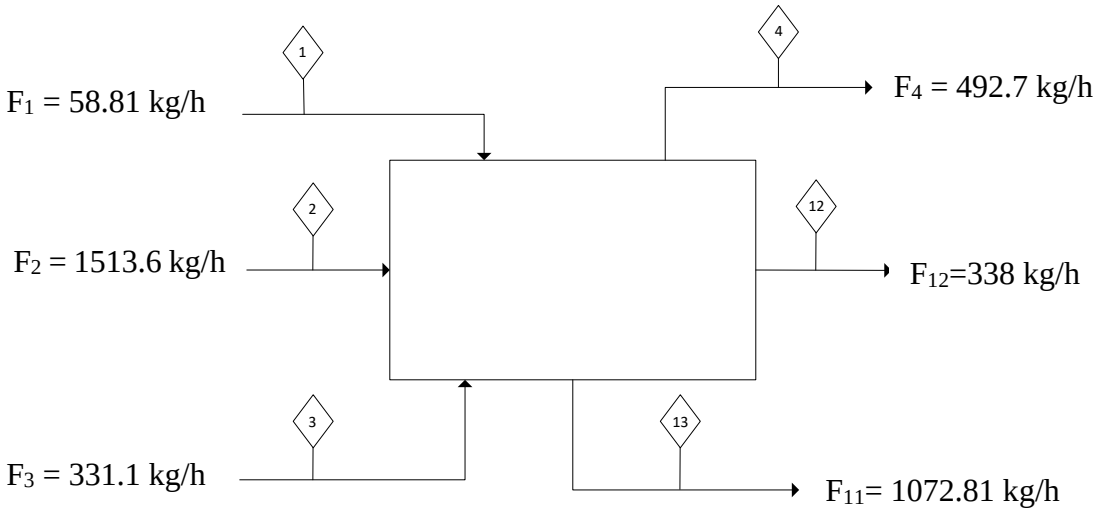


Figure 5.6 Overall Mass Balance

Total Inlet Mass flowrate = Total Outlet Mass Flowrate

$F_1 + F_2 + F_3 = F_4 + F_{11} + F_{12}$

58.81 kg/h + 1513.6 kg/h + 331.1 kg/h = 492.7 kg/h + 338 kg/h + 1072.81kg/h

1903.51 kg/h = 1903.51 kg/h

5.4 ENERGY BALANCE

5.4.1 Introduction

The arithmetical balancing of energy inputs versus outputs for the processing of a unit is energy balance. It is used to quantify the energy that the system uses or generates and to determine if the reaction is endothermic or exothermic.

5.4.2 Energy Balance Formula Data

In order to determine the total heat change, the change of enthalpy in each component is necessary. A component's molar enthalpy change is given by:

$$\Delta\hat{H} = \int_{T1}^{T2} Cp\ dT$$

Where C_P is the molar heat capacity of the component; T1 is the reference temperature (25^{o}C) and T2 is the inlet or outlet temperature. The unit of heat capacity, C_P is kJ/ kg K and temperature, T is in unit Kelvin, K.

5.5 ENERGY BALANCE OF SYSTEMS

5.5.1 F102 Main Fermenter

F_2 = 1513.6 kg/h

Q_2 = 31.25 L/h

C_{W2} = 19.384 kg/L

C_{G2} = 28.125 kg/L

C_{A2} = 0.925 kg/L

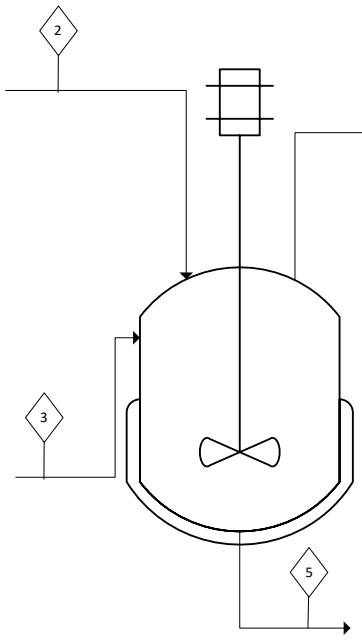
W_{W2} = 0.400205

W_{G2} = 0.5806

W_{A2} = 0.01916

F_3 = 331.1 kg/h

W_{O3} = 1



F_4 = 492.7 kg/h

W_{C4} = 1

F_5= 1352 kg/h

Q_5 = 31.25 L/h

C_{SB5}= 8.654 kg/L

C_{W5} = 28.12 kg/L

C_{G5} = 6.49 kg/L

W_{SB5} = 0.2

W_{W5} = 0.65

W_{G5} = 0.15

Figure 5.7: Energy Balance for Main Fermenter, F-102

Table 5.6 Heat of formation for each element

Component	Heat of formation, $\triangle H_f$ (kJ/mol)
Glucose	-1273.3
Ammonia	-46.0
Oxygen	0
Carbon Dioxide	-393.5
Water	-241.8
Yeast	-9.283×10^{-4}

(Source : Basic Principle and Calculations in Chemical Engineering, 2012)

$C_6H_{12}O_6 + 2.7546O_2 + 0.4529NH_3 \rightarrow 4.0377H_2O + 2.981CO_2 + 3.019CH_{1.75}N_{0.15}O_{0.5}$

$\triangle H_{rxn} = \triangle H_{fCH1.75N0.15O0.5} + \triangle H_{fH2O} + \triangle H_{fCO2} - \triangle H_{fC6H12O6} - \triangle H_{fNH3}$

$= 3.019(-9.283 \times 10^{-4}) + 4.0377(-241.8) + 2.981(-393.5) – 1(-1273.3) – 0.4529(-46)$

$= -855.209$ kJ/mol

Inlet stream enthalpy = (-1273.3)(4.883) + (-241.8)(33.653) + (-46)(1.7)

$= -14433.019 \times 10^3$ kJ/h

Outlet stream enthalpy = (-9.283×10^{-4})(11.34) + (-241.8)(48.82) + (-1273.3)(1.127) + (-393.5)(11.197)

$= -17645.715 \times 10^3$ kJ/h

Q = Outlet stream enthalpy – inlet stream enthalpy + $r\triangle H_{rxn}$

$= [-17645.715 – (-14433.019)] \times 10^3 + 2.504(-855.209)$

= -3214.837 x 10^3 kJ/h

The negative value shows the process is exothermic process. Heat is released to the surrounding.

5.5.2 D101 Freeze Dryer

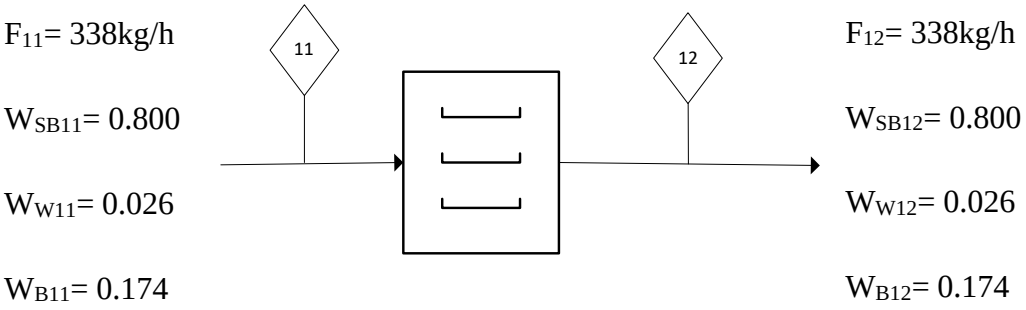


Figure 5.8 : Energy Balance for Freeze Dryer, D-101

Stream 11

Determining $\Delta\hat{H}$ for water

$\Delta\hat{H}_{water} = \int_{298}^{298} 4.18\ dT$

= 0 KJ/kg

= m (ΔH_{water})

= 0.49(0)

= 0 kJ/h

Determining $\Delta\hat{H}$ for *saccharomyces boulardii*

$\Delta\hat{H}_{saccharomyces\ boulardii} = \int_{298}^{298} 1.308\ dT$

= 0 kJ/kg

= m ($\Delta\hat{H}_{saccharomyces\ boulardii}$)

= 11.34 (0)

= 0 kJ/h

Determining $\Delta\hat{H}$ of Betaine

Table 5.7 Specific heat capacity for each element

Element	C_p ($Jmol^{-1}\ k^{-1}$)
C	8.5170
H	14.304
O	14.689
N	14.562
S	23.700

(Source : Specific Heat of all the elements in the Periodic Table, 2020)

Table 5.8 C_P for inlet Betaine

Element	Molar mass of element	Ratio of element	C_p ($Jmol^{-1}\ k^{-1}$)	$\Delta H\ (KJ/Kmol)$
C	60	0.5128	4.36750	0
H	11	0.9402	1.34486	0
O	32	0.2735	4.01744	0
N	14	0.1197	1.74307	0
Total	117	1.0000	11.4729	0

(Source : Specific Heat of all the elements in the Periodic Table, 2020)

$\Delta\hat{H}_{Betaine} = \int_{298}^{298} CPdT$

$= \int_{298}^{298} 11.4729\ dT$

= 0 kJ/h

$\sum \Delta H_{inlet} = \Delta\hat{H}_{water} + \Delta\hat{H}_{saccharomyces\ boulardii} + \Delta\hat{H}_{Betaine}$

= 0 kJ/h

Table 5.9 C_P values of species

species	C_P (KJ/kg.k)
water	4.18
Saccharomyces boulardii	1.308

(Source : Introduction to Chemical Engineering Thermodynamics, 2018)

Product enthalpy, $\Delta\hat{H} = \int_{T1}^{T2} Cp\ dT$

Temperature is from T_1 = 298K to T_2 = 77K

Stream 12

Water

$\Delta\hat{H}_{water} = \int_{298}^{77} 4.18\ dT$

= -923.78 kJ/kg

= m ($\Delta\hat{H}_{water}$)

= -932.78 (8.79)

= -8120.03 kJ/h

Saccharomyces Bourladii

$\Delta\hat{H}_{saccharomyces\ boulardii} = \int_{298}^{77} 1.308\ dT$

= -289.068 kJ/kg

= m ($\Delta\hat{H}_{saccharomyces\ boulardii}$)

= -289.068 (270.4)

= -78163.99 kJ/h

Betaine

Table 5.10 C_P for outlet Betaine

Component	Molar Mass	Ratio	C_P (kJ/kmol.K)		ΔH (kJ/kmol)
C	60	0.5128	4.3675		-965.2175
H	11	0.09402	1.34486		-297.21406
O	32	0.2735	4.01744		-887.85424
N	14	0.1197	1.74307		-385.25847
Total	77	1.00	$\sum$ Cp 11.4729	=	-2535.51

(Source : Specific Heat of all the elements in the Periodic Table, 2020)

$\Delta\hat{H}_{Betaine} = \int_{298}^{77} 11.4729$

= -2535.51 kJ/mol

ΔH = -2535.51 (Molar Flowrate)

= -2535.51(0.5)

= -1267.76 kJ/h

$\sum \Delta Hout$ = -1267.76 + (-78163.99) + (-8120.03)

= -87551.78 kJ/h

$Q = \sum \Delta\hat{H}_{product} - \sum \Delta\hat{H}_{reactant}$

= -87551.78 – 0

= -87551.78

The negative value shows the process is exothermic process. Heat is released to the surrounding.

CHAPTER VI

CHEMICAL ENGINEERING THERMODYNAMICS II

6.1 INTRODUCTION

According to the principle of Le Chatelier, if a dynamic equilibrium is disturbed by changing conditions, the equilibrium position shifts to counteract the change to reestablish an equilibrium. The equilibrium constant can describe this state of equilibrium. The equilibrium constant, K, can also be defined in a chemical reaction through the concentration of present species. The constant of equilibrium, K, is useful in determining the extent of a reaction to reach equilibrium.

If K is greater than 1, the system will favour the forward reaction of products to form. If K is less than 1, the reversed reaction will be favoured by the system to form more reactants. While, if K is equal to 1 the reaction will enter equilibrium state at which no net change between the reactants and products in the system.

6.2 EQULIBRIUM CONSTANT AND COMPOSITION

Overall equation:

$$C_6H_{12}0_6 + 2.7536O_2 + 0.4529NH_3 \longrightarrow 4.0377H_2O + 2.981CO_2 + 3.019CH_{1.75}N_{0.15}O_{0.5}$$

Table 6.1 Chemical equilibrium of each species

Species	Stoichiometric coefficient, V_i	Gibbs energy, $G°_i$ (KJ/mol)	Standard enthalpy of formation H_f^0KJ/mol)	$C°_p/R$
Glucose	-1	-917.22	-1264	26.3
Ammonia	-0.4529	-26.57	-80.893	9.718
Oxygen	-2.7546	0	0	3.535
Carbon dioxide	+2.9810	-349.39	-393.5	4.467
Water	+4.0377	-237.18	-286	9.069
Biomass	+3.019	-67	-91	1.6
Total	V = +5.8302	$\sum \Delta G° = -1046.36$	$\sum \Delta H° = -1301.90$	$\frac{\sum C°p}{R} = 14.33$

(Source : Biothermodynamics 2013)

$$\ln K = \frac{\Delta G° + \Delta H°}{RTo} + \frac{\Delta H°}{RT} + \frac{1}{T}\int_{To}^{T} \frac{\Delta C°p}{R} dT - \int_{To}^{T} \frac{\Delta C°p}{R} \frac{dT}{T}$$

$$-\ln K = \frac{-1046.36 - (-1301.9)}{8.314(293)} + \frac{(-1301.9)}{8.314(298)} + \frac{1}{T}[14.33(298 - 293)] - \left[14.33 \ln \frac{298}{293}\right]$$

$$-\ln K = 0.1049 - 0.525 + 0.240 - 0.242$$

K= 1.528

Since the value is greater than 1, the forward reactions is favors. The fermentation is feasible reaction.

Table 6.2 Stoichiometric coefficient and moles of component

Species	nio	vi
Glucose	1	-1
Ammonium	0.5	-0.4529
Oxygen	2.8	-2.7546
Carbon Dioxide	-	+2.981
Water	-	+4.0377
Biomass	-	+3.019
	$\sum n_{io}$ =4.3	$\sum v_i$ = +5.8302

The relationship of K, equilibrium constant and extent of reaction are as follows:

$$\prod_i (x_{1i})^{v_i} = k_i$$

The component in the fermenter is assumed as ideal solution where

$$\left(\frac{1-\varepsilon}{4.3+5.8302\varepsilon}\right)^{-1}\left(\frac{0.5-0.4529\varepsilon}{4.3+5.8302\varepsilon}\right)^{-0.4529}\left(\frac{2.8-2.7546\varepsilon}{4.3+5.8302\varepsilon}\right)^{-2.7546}\left(\frac{4.0377\varepsilon}{4.3+5.8302\varepsilon}\right)^{4.0377}$$

$$\left(\frac{2.981\varepsilon}{4.3+5.8302\varepsilon}\right)^{2.981}\left(\frac{3.019\varepsilon}{4.3+5.8302\varepsilon}\right)^{3.019} = 1.528$$

Since the relationship shows high complexity and non-linearity in finding the solutions for extent of reactions, we have decided to use trial and error in finding the solution for extent of reaction in the fermentation using proper guesses and assumptions. From the assumption calculated, the value of extent of reactions is 0.5.

$$X_{Glucose} = \frac{1-0.5}{4.3+5.8302(0.5)} = 0.0693$$

$$X_{Ammonia} = \frac{0.5-0.4529(0.5)}{4.3+5.8302(0.5)} = 0.0379$$

$$X_{Oxygen} = \frac{2.8-2.7546(0.5)}{4.3+5.8302(0.5)} = 0.1971$$

$$X_{Carbon\ dioxide} = \frac{2.981(0.5)}{4.3+5.8302(0.5)} = 0.2798$$

$$X_{Water} = \frac{4.0377(0.5)}{4.3+5.8302(0.5)} = 0.2064$$

$$X_{Biomass} = \frac{3.019(0.5)}{4.3+5.8302(0.5)} = 0.2092$$

$$\sum x_i = 0.997 \approx 1$$

Table 6.3 shows the comparison of molar composition of the components based on the equilibrium constant, K calculation and mass balance calculation. The percentage error is then calculated for respective product components.

Table 6.3 Comparison of molar composition of the components between thermodynamics calculation and material balance calculation

Component	Data from mass balance			Data from thermodynamics calculation	Percentage error (%)
	Kg/hr	Kmol/hr	Composition		
Glucose	202.8	1.127	0.01568	0.0693	341.96
Carbon dioxide	492.7	11.197	0.147	0.2798	90.34
Water	878.8	48.82	0.6793	0.2064	69.62
Biomass	270.4	11.34	0.1577	0.2092	32.66

$$Percentage\ error = \left|\frac{Molar\ composition\ on\ material\ balance - \ molar\ compositionbased\ on\ k}{Molar\ composition\ based\ on\ material\ balance}\right| \times 100\%$$

The percentage error of the comparison falls around the range of 32.66 % to 341.96 %. There is a difference between the molar composition based on equilibrium constant K and material balance it is because for molar composition calculation based on equilibrium constant, K we assume that all the components are in liquid phase at ideal solution.

6.3 HEAT OF REACTION

$\Delta H_{Reaction} = \sum \Delta H_{Product} - \sum \Delta H_{Reactant}$

= [4.0377(-286) + 2.981(-393.5) + 3.019(-91)] – [1(-1264) + 2.7536(0) +

0.4529(-80.893)]

= -2602.53 – (- 1300.64)

= -1301.89 KJ/mol

The heat of reaction in the fermenter is -1301.89 KJ/mol, thus the reaction in the fermenter is exothermic. In order to increase the product formation, the temperature in the fermenter need to decrease, as a result, equilibrium constant, K will increase

CHAPTER VII

CELL BIOLOGY FOR ENGINEERS

7.1 THE CHEMICAL AND BIOCHEMICAL PROPERTIES

Table 7.1 shows the chemical and biochemical properties of *S. boulardii*

Table 7.1 Chemical and Biochemical Properties of *S. Boulardii*

CHEMICAL	BIOCHEMICAL
S. boulardii had better heat tolerance and acid tolerance growing well at 37˚C and pH 2.0 to survive in a gastric environment.	*S. boulardii* can exist in two different forms: haploid or diploid. Haploid and diploid cells can reproduce asexually in a process called budding, where the daughter cell protrudes off a parent cell.
The thermal death temperature is 55-56˚C.	*S. boulardii* is a non-pathogenic and biotherapeutic features.
S. boulardii is well ethanol-tolerant, and the highest concentration of ethanol that can be tolerated is 20%.	*S. boulardii* good fermentative power, no H_2S production, killer activity, flocculation ability, and production of flavoring compounds.
Has a density of 1095.2 kg/m3.	*S. boulardii* does not produce spores.
Molecular weight of *S. boulardii* is 23.85 g/mol.	*S. boulardii* is resistant to antibiotics, and it can be prescribed to patients receiving antibiotics.
Yield of product (g/g) is 0.5.	*S. boulardii* has only one, DNA polymerase IV (pol IV). Pol IV has intrinsic 5′-2-deoxyribose-5-phosphate lyase activity. Pol IV has low processivity and can fill short gaps in DNA.

(Source: Journal of Biological Chemistry, 2010)

Table 7.2 Biochemical Characteristics of *s. boulardii* strain

TEST		RESULT
Nitrogen utilization	Nitrate	Negative
	Peptone	Positive
	Ammonium sulfate	Positive
Carbon utilization	Glucose	Positive
	Fructose	Positive
	Sucrose	Positive
	Lactose	Negative
	Starch	Positive
Acid production		Positive
Ester production		Positive
Urea hydrolysis		Negative
Gelatin Liquefaction Test		Negative
H_2S Test		Brownish, Black

(Source: Frontiers in Nutrition, 2020)

Table 1.2 shows the the test of *Saccharomyces boulardii* with nitrogen utilization, carbon utilization and others test. The result show that not all nitrogen utilization can be used as nitrogen source such as nitrate and not all carbon source can be used as carbon source such as lactose. In our production, sucrose has been chosen as carbon source and ammonia has been used as nitrogen source.

7.2 SELECTED PRODUCER CELL

There are few cells that can be involved in the production of probiotics from yeast. The cell that can be a candidate for this production is *Saccharomyces boulardii, Saccharomyces cerevisiae,* and *Debaryomyces hansenii*. Table 7.3 shows the taxonomy classification among 3 types of cells, and table 7.3 shows the differences between those strains.

Table 7.3 The Taxonomic Classification of 3 Types of Cells

Strains	Classification
Saccharomyces boulardii *Saccharomyces cerevisiae*	Domain: Eukarya Kingdom: Fungi Subkingdom: Dikarya Phylum: Ascomycota Subphylum: Saccharomycotina Class: Saccharomycetes Order: Saccharomycetales Family: *Saccharomycetaceae* Genus: *Saccharomyces*
Debaryomyces hansenii	Kingdom: Fungi Domain: Eukarya Kingdom: Fungi Subkingdom: Dikarya Phylum: Ascomycota Subphylum: Saccharomycotina Class: Saccharomycetes Order: Saccharomycetales Family: *Debaryomycetaceae* Genus: *Debaryomyces*

(Source: National Center for Biotechnology Information)

Morphological and physiological of S*accharomyces boulardii* almost have the same characteristic as *Saccharomyces cerevisiae* but different strain. Then, the present study's result strongly indicates a close relatedness of *S. boulardii* to *S. cerevisiae* and thereby supports the recognition of *S. boulardii* as a member of *S. cerevisiae* and not as a separate species (Lene Jespersen, 2003). *S. boulardii* and *S. cerevisiae* are genetically very similar, each containing 16 chromosomes with greater than 99% relatedness by

average nucleotide identity (Khatri et al., 2017). Some of the important differences include those in the genes expressing some flocculation proteins, which contribute to a different adhesion profile of *S. boulardii* when compared to *S. cerevisiae* (Edwards-Ingram et al., 2007). A major genetic difference between *S. boulardii* and other *S. cerevisiae* is chromosome IX trisomy in *S. boulardii*, though its impact on the probiotic attributes of *S. boulardii* has not been definitively demonstrated (Edwards-Ingram et al., 2007).

Table 7.4 shows the differences between three types of strains.

Table 7.4 The Difference Between Three Types of Strains

Strains	***S. boulardii***	***S. cerevisiae***	***D. hansenii***
Shape	Ellipsoid/ oval/ spherical	Ellipsoid/ovoid	Lenticular
Optimum temperature (°C) to growth	37	30	32
Resistance to temperature	High	Low	Medium
Resistance to acidic	High (viability up to 75% at pH 2)	Medium (viability up to 30% at pH 2)	Medium (pH around 3-10)
Yield of Product (g/g)	0.5	0.3	0.1

(Source : Can. J. Microbial. Vol. 50, 2004)

As shown from the table above, three types of strains compared: *Saccharomyces boulardii*, *Saccharomyces cerevisiae*, and *Debaryomyces hansenii*. *Saccharomyces boulardii* is chosen as the type of yeast to produce probiotics as it is the most suitable cell and most common type of yeast that applies in the healthcare industry. Our basis is yield of product so *Saccharomyces boulardii* have higher yield of product which is 0.5 compare to others cells. Another the reason is *S. boulardii* grew faster than *S. cerevisiae* at both temperatures, which are 30˚C and 37˚C. This characteristic is an essential advantage for *S. boulardii* concerning its utilization as a probiotic. Then, the

extremely low pH in the gastric environment (usually pH ~2.0) is severe stress and lethal to most microorganisms.

For this reason, the yeasts are exposed to a simulated gastric environment (Charteris et al. 1998) for 60 min. Viability levels under these conditions were indistinguishable for the first 10 min, but after 15 min, *S. boulardii* appeared to be more resistant, maintaining its cell viability at about 75%. The viability of *S. cerevisiae* fell to about 30% after 60 min. This characteristic is important because it will be applied in human health, so the biotherapeutic agents must survive passage throughout the upper gastrointestinal tract since viability is necessary for probiotics at their sites of action. During their passage in the digestive tract, they are submitted to very different stress conditions such as exposure to low gastric pH, bile salts, organic acids, and digestive enzymes and competition with intestinal microbiota and its secondary metabolism products (H2S, bacteriocins, and organic acids) (Holzapfel et al. 1998). In conclusion, *S. boulardii* has more benefits and functions when it comes to probiotics.

7.3 CULTIVATION/PROPAGATION OF CELLS

7.3.1 The Source of The Cell

Saccharomyces boulardii is a cell that will use to produce probiotics from yeast in this study. The company that generously supplied it is Van Wankum Ingredients Co., Ltd. In Maarsen, The Netherlands. The same company purchased sucrose. This company is selected due to its high reputation and reasonable prices, and at the same time, it produces various products. Then, parboiled rice effluent is rich in organic matter and nutrients such as nitrogen and phosphorus (Queiroz and Koetz, 1997). It requires treatment before disposal so that it can get from the parboiled rice industries.

Saccharomyces boulardii yeast is a consolidated probiotic for use in man and animals. The first step to cultivate the producer cell is the microorganism and selection of culture media. Many culture media can be used to cultivate *Saccharomyces boulardii*, so the culture media that has been selected is parboiled rice effluent + 1% sucrose. The media was sterilized for 15min at 121˚C. The culture media were inoculated with the *S.*

boulardii culture and incubated in a feed fermenter at 150 rpm and 28˚C for 48 h. The cell viability was analysed by serial dilutions and counting of the colony units ($CFU.mL^{-1}$).

The second step is the determination of Chemical Oxygen Demand (COD), nitrogen, and phosphorus in media selection. The samples were acidified to pH < 2.0 with sulfuric acid and stored at 4˚C. COD was determined by closed reflux, nitrogen by the Total Kjeldahl nitrogen method (TKN), and total phosphorus by the ascorbic acid method and previous digestion with sulfuric acid-nitric acid.

The last step is yeast culture in the bioreactor. The pre-inoculum and inoculum of *S. boulardii* were performed at 250 rpm, 1 vvm, and 28˚C for 48h in a bioreactor (New Brunswick Scientific, NJ, USA) containing parboiled rice effluent + 1% sucrose with 10% of inoculum (volume). The media were stabilized in the fermentation tank for 45min at 121˚C. During the culturing process, the culture was evaluated cell viability, biomass, COD, nitrogen, phosphorus, and sucrose consumption.

In conclusion, the growth of yeast *S. boulardii* in parboiled rice effluent supplemented with sucrose was evaluated to select the culture medium that most effectively generates biomass and provides removal of COD, nitrogen, and phosphorus from this effluent.

7.3.2 Physical and Chemical Factors that Influence Growth

There is a particular condition that will influence the growth of the *Saccharomyces boulardii*. As mentioned, the tables 7.5 and 7.6 below show the physical and chemical factors that influence the growth of *Saccharomyces boulardii.*

Table 7.5 The Physical Factors Which Influence The Growth of *Saccharomyces boulardii*

Physical factor	Explanation
Temperature	-*S. boulardii* are the best yeast to adapted to growth at high temperatures. -The *Saccharomyces* genus, with the highest optimum (32.3°C) and maximum (45.4°C) growth temperatures.
pH value/ acidity	-Yeasts can grow in a pH range of 4 to 4.5. -They can grow at lower pH than most bacteria but do not grow well under alkaline conditions.

(Source : Act For Libraries, 2017)

Table 7.6 The Chemical Factors Which Influence The Growth of *Saccharomyces boulardii*

Chemical factor	Explanation
Nitrogen concentration	- Fermentation was strongly dependent on nitrogen availability. The product formation by *s. boulardii* under anaerobic conditions is affected by the nitrogen source
Sugar concentration	-Yeast can delay its growth at high concentrations of glucose and fructose
Water activity	- In terms of water requirements, yeasts are intermediate between bacteria and molds. - Normal yeasts require a minimum water activity of 0.85 or relative humidity of 88%.
Minerals	- Minerals such as magnesium, potassium, and several other trace elements are essential for yeast growth and should encourage growth when provided externally.
Phosphorus	- When considering its part as an element in the nucleic acids and phospholipids, phosphorus is essential for effective yeast growth.
Growth factors	- Yeasts require certain vitamins, purines, pyrimidines, amino acids, and fatty acids for catalyzing the biosynthesis, although they do not act as energy sources for yeasts.

(Source : Act For Libraries, 2017)

7.4 TYPE OF CARBON SOURCE

The main carbon source for the production of probiotics from yeast is sucrose. Sucrose is a carbon source option that can be employed in growing media without significantly increasing the production cost. The addition of a carbon source in this effluent increased cell viability in *S. boulardii* cultures. Enrichment with additional carbon source allowed exponential phase extension up to 24 h and 15 h in cultures with sucrose. Supplementation with 1% sucrose caused a lower COD increase, so it exhibited the highest COD reduction. Supplemented culture exhibits a significantly higher nitrogen reduction after 24 h of cultivation in parboiled rice effluent + 1% sucrose. Nitrogen uptake due to increased metabolism and cell multiplication is confirmed by higher cell viability in these cultures. The effluents supplemented with 1% sucrose exhibits the best results in cell viability and COD, nitrogen, and phosphorus removal rates.

7.5 NUTRIENTS COMPOSITION IN GROWTH MEDIUM

Nutritional yeast is produced by culturing a yeast in a nutrient medium for several days. The primary ingredient in the growth medium is glucose. Nutritional values for nutritional yeast vary from one manufacturer to another. Table 7.7 shows the suitable nutritional composition of the media.

Table 7.7 Suitable Nutritional Composition

Component/Element	Composition (g/g)
Ash	1.37
Nitrogen	0.480
Phosphorus	0.349
Magnesium	0.157
Calcium	0.018
Iron	0.00216
Zink	0.0000143
Manganese	0.000011
Copper	0.00000239
Molybdenum	0.000000775
Chromium	0.000000088

(Source: Journal Science Food, 1979)

Table 7.8 Function of component

Component/Element	Function
Nitrogen	- Used in building cellular material
	- Support a healthy yeast population
Phosphorus	- Vital for yeast cells to grow healthily
Magnesium, Calcium, Manganese, Molybdenum, Iron	- Act as key cofactors in enzymatic reactions and participate in the metabolism of yeast cells.
	- Increase enzyme formation.
Zink	- Zinc deficiency can cause growth inhibition.
	- Zinc may directly regulate DNA synthesis.
	- Zinc also influences hormonal regulation of cell division.
	- Zinc specifically acts on cartilage growth it is involve in multiple enzymatic reactions.
Copper	- Influence the growth and bioaccumulation properties of adapted and growing cells.

(Source: The Journal of Nutrition, 2000)

CHAPTER VIII

FLUID MECHANICS

8.1 FRICTION LOSS

Friction loss occurs in pipe due to the effect of the fluid's viscosity near the surface of the pipe and the movement of the fluid molecules against each other and against the wall of pipe. From the process flow diagram, a pump (R-101) between fermenter (F-102) and centrifugal separator (C-101) is chosen to be analyzed. The pump that we used is rotary peristaltic pump since it can pump fluids or slurries with a high degree of fluid content. The temperature of the substances that flow in the pipe is 28°C. Figure 8.1 shows the dimension of pipeline calculated

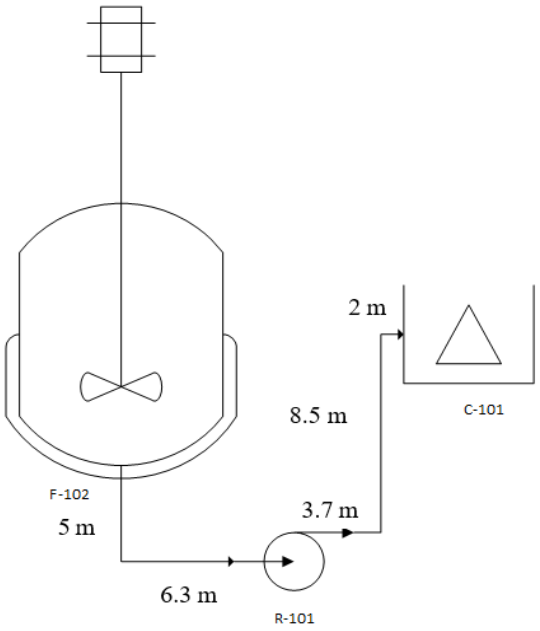


Figure 8.1 Process Flow Diagram from Fermenter (F-101) to Centrifugal separator (C-101)

8.1.1 Friction loss in pipe

Density of *Saccharomyces bourladii*, $\rho = 1095.2$ kg/m^3

Viscosity of *Saccharomyces bourladii*, $\mu = 0.18$ Pa.s

Mass flow rate, $\dot{m} = 0.376$ kg/s

Inlet pipe used is commercial steel, schedule 40, 4-inch nominal diameter.

Outlet pipe used is commercial steel, schedule 40, 2-inch nominal diameter.

Based on (Appendix A.5)

Inlet diameter, D_1 = 0.1023 m

Outlet diameter, D_2 = 0.0525 m

Area of inlet pipe, $A_1 = \pi D_1^2/4$

$= \pi (0.1023)^2/4$

$= 8.219 \times 10^{-3}$ m^2

Area of outlet pipe, $A_2 = \pi D_2^2/4$

$= \pi (0.0525)^2/4$

$= 2.165 \times 10^{-3}$ m^2

Velocity of the flow at inlet stream, $v_1 = \frac{\dot{m}}{A\rho}$

$= \frac{0.376}{8.219 \times 10^{-3}(1095.2)}$

$= 0.0418$ m/s

Velocity of the flow at outlet stream, $v_2 = \frac{\dot{m}}{A\rho}$

$= \frac{0.376}{2.165 \times 10^{-3}(1095.2)}$

$= 0.1586$ m/s

Reynolds Number at inlet, $N_{Re} = \frac{\rho v_1 D}{\mu}$

$$= \frac{(1095.2)\ (0.0418)(0.1023)}{0.18}$$

$$= 26.02$$

The Reynolds number, $N_{Re} = 26.02 < 2100$, hence the flow is laminar flow.

Reynolds Number at outlet, $N_{Re} = \frac{\rho v_2 D}{\mu}$

$$= \frac{(1095.2)\ (0.1586)(0.1023)}{0.18}$$

$$= 50.66$$

The Reynolds number, $N_{Re} = 50.66 < 2l00$, hence the flow is laminar flow.

Table 8.1 Summary of calculated value

Stream properties	**Pump inlet stream**	**Pump outlet stream**
Density of *Saccharomyces bourladii*, ρ (kg/m^3)	1095.2	1095.2
Viscosity, μ (Pa.s)	0.18	0.18
Mass flowrate, ṁ (kg/s)	0.376	0.376
Nominal pipe (inches)	4	2
Inside Diameter, ID (m)	0.1023	0.0525
Cross-sectional Area, (m^2)	8.219×10^{-3}	2.165×10^{-3}
Velocity, v (m/s)	0.0418	0.1586
Reynolds Number, N_{Re}	26.02	50.66
Type of flow	Laminar	Laminar

Total friction loss considering all bends, expansions, contractions along pipe is given by:

The total friction loss, $\sum F$ include:

i. Contraction loss at the exit fermenter.

For contraction loss from A_1 to A_2 cross-sectional area, $\frac{A_2}{A_1} = 0$ since A_1 of the fermenter is very large compared to A_2.

$K_C = 0.55\ (1 - \frac{A_2}{A_1})$ = 0.55 (1-0) = 0.55

For laminar flow, α = ½ = 0.5

$h_C = K_C\ \frac{V^2}{2a}$ = $(0.55)\frac{(0.0418)^2}{2(0.5)}$ = 9.61×10^{-4} J/kg

ii. Friction loss in 4-inch pipe.

Fanning factor, $f = \frac{16}{Re}$ for Re < 2100 (laminar flow)

$f = \frac{16}{Re} = \frac{16}{26.02}$ = 0.6149

Δ L = 5 + 6.3 = 11.3 m

$F_f = 4f\ \frac{\Delta L}{D}\frac{V_1^2}{2}$

F_f = 4 (0.6149) $\frac{11.3}{0.1023}\frac{0.0418^2}{2}$

= 0.2374 J/kg

iii. Friction loss in 2-inch pipe.

Fanning factor, $f = \frac{16}{Re}$ for Re < 2100 (laminar flow)

$f = \frac{16}{Re} = \frac{16}{50.66}$ = 0.3158

Δ L = 3.7 + 8.5 + 2.0 = 14.2 m

$$F_f = 4f \frac{\Delta L}{D} \frac{V_1^2}{2}$$

$$F_f = 4\ (0.3158) \frac{14.2}{0.0525} \frac{0.1586^2}{2}$$

$$= 4.334 \text{ J/kg}$$

iv. Friction in 3 elbows, 90°

From table 2.10-2 (Geankoplis, 4th edition), $K_f = 17$

$$h_f = K_f \frac{V_1^2}{2}$$

$$= 17 \frac{(0.0418)^2}{2}$$

$$= 0.0149 \text{ J/kg}$$

$$h_f = 2K_f \frac{V_2^2}{2}$$

$$= 2\ (17) \frac{(0.1586)^2}{2}$$

$$= 0.4276 \text{ J/kg}$$

Total friction in 3 elbows = 0.0149 + 0.4276

$$= 0.4425 \text{ J/kg}$$

v. Expansion loss at the centrifuge

$$K_{ex} = (1 - \frac{A_1}{A_2}) = (1\text{-}0) = 1$$

For laminar flow, $\alpha = ½ = 0.5$

$$h_{ex} = K_{ex} \frac{V^2}{2}$$

$$= (1) \frac{(0.1586^2)}{2(0.5)}$$

$$= 0.0252 \text{ J/kg}$$

Therefore, the total friction loss, $\sum F$:

$$\sum F = h_C + F_f + h_f + h_{ex}$$

$= 9.61 \times 10^{-4} + 0.2374 + 4.334 + 0.4425 + 0.0252$

$= 5.04$ J/kg

8.1.2 Mechanical Energy Balance

Substituting value of $\sum F$ into mechanical energy balance equation 2.7-28 (Geankoplis, 4th edition).

$$\frac{1}{2}(V_{2av}^2 - V_{1av}^2) + g\,(Z_2 - Z_1) + \frac{P_2 - P_1}{\rho} + \sum F + W_S = 0$$

$$\frac{0.1586^2 - 0.0418^2}{2(0.5)} + 9.81(8.5 - 5.0) + \frac{150000 - 101000}{1095.2} + 5.04 + W_s = 0$$

$$W_s = -84.14 \text{ J/kg}$$

8.2 PERFORMANCE RATING OF PUMP

8.2.1 Peristaltic Pump

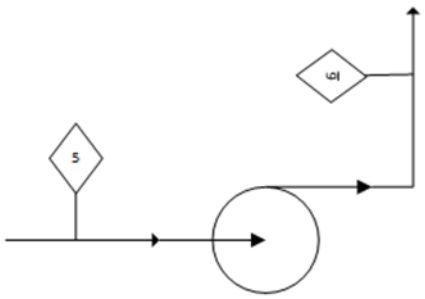


Figure 8.2 Rotary peristaltic pump

A rotary peristaltic pump is a type of positive displacement pump used for pumping a variety of fluids. A rotary peristaltic pump transport fluid through a flexible duct using traveling contraction waves. When the viscosity increases the efficiency will

increase because of the frictional losses in the pump. Therefore, there are a few assumptions before doing the calculation:

1. The reference point is the pump position to the fermenter and centrifugal separator.

2. Z1 is the reference height of fermenter from reference point.

3. Z2 is the reference height of centrifugal separator from reference point.

8.2.2 Suction head calculation

The total friction loss, $\sum F$ include:

i. Contraction loss at the exit fermenter.

For contraction loss from A_1 to A_2 cross-sectional area, $\frac{A_1}{A_2} = 0$ since A_1 of the fermenter is very large compared to A_2.

$K_C = 0.55\ (1 - \frac{A_2}{A_1})$ = 0.55 (1-0) = 0.55

For laminar flow, α = ½ = 0.5

$h_C = K_C\ \frac{V^2}{2a}$ = $(0.55)\frac{(0.0418)^2}{2(0.5)}$ = 9.61×10^{-4} J/kg

ii. Friction loss in 4-inch pipe.

Fanning factor, $f = \frac{16}{Re}$ for Re < 2100 (laminar flow)

$f = \frac{16}{Re} = \frac{16}{26.02}$ = 0.6149

Δ L = 5 + 6.3 = 11.3 m

$F_f = 4f\ \frac{\Delta L}{D}\frac{V_1^2}{2}$

F_f = 4 (0.6149) $\frac{11.3}{0.1023}\frac{0.0418^2}{2}$

= 0.2374 J/kg

iii. Friction in 1 elbow, 90°

From table 2.10-2 (Geankoplis, 4th edition), $K_f = 17$

$$h_f = K_f \frac{V_1^2}{2}$$

$$= 17 \frac{(0.0418)^2}{2}$$

$$= 0.0149 \text{ J/kg}$$

Therefore, the total friction loss, $\sum F_1$:

$$\sum F_1 = h_C + F_f + h_f$$

$$= 9.61 \times 10^{-4} + 0.2374 + 0.0149$$

$$= 0.2533 \text{ J/kg}$$

Suction head, $H_1 = \frac{V_1^2}{2} + gz_1 + \frac{P_1}{\rho} + \sum F_1$

$$H_1 = \frac{0.0418^2}{2} + 9.81\ (5) + \frac{101000}{1095.2} + 0.2533$$

$$= 141.525 \text{ J/kg}$$

8.2.3 Discharge head calculation

The total friction loss, $\sum F$ include:

i. Friction loss in 2-inch pipe.

Fanning factor, $f = \frac{16}{Re}$ for Re < 2100 (laminar flow)

$f = \frac{16}{Re} = \frac{16}{50.66} = 0.3158$

Δ L = 3.7 + 8.5 + 2.0 = 14.2 m

$$F_f = 4f \frac{\Delta L}{D} \frac{V_1^2}{2}$$

$$F_f = 4\ (0.3158) \frac{14.2}{0.0525} \frac{0.1586^2}{2}$$

$$= 4.334 \text{ J/kg}$$

ii. Friction in 2 elbows, 90°

From table 2.10-2 (Geankoplis, 4th edition), $K_f = 17$

$$h_f = 2K_f \frac{V_2^2}{2}$$

$$= 2\ (17) \frac{(0.1586)^2}{2}$$

$$= 0.4276 \text{ J/kg}$$

iii. Expansion loss at the centrifuge

$$K_{ex} = (1 - \frac{A_1}{A_2}) = (1\text{-}0) = 1$$

For laminar flow, $\alpha = ½ = 0.5$

$$h_{ex} = K_{ex} \frac{V^2}{2}$$

$$= (1) \frac{(0.1586^2)}{2(0.5)}$$

$$= 0.0252 \text{ J/kg}$$

Therefore, the total friction loss, $\sum F_2$:

$$\sum F_2 = F_f + h_f + h_{ex}$$

$$= 4.334 + 0.4276 + 0.0252$$

$$= 4.7868 \text{ J/kg}$$

Discharge head, $H_2 = \frac{V_2^2}{2} + gz_2 + \frac{P_2}{\rho} + \sum F_2$

$$H_2 = \frac{0.1586^2}{2} + 9.81\ (8.5) + \frac{150000}{1095.2} + 4.7868$$

= 225.146 J/kg

8.2.4 Pump fluid power calculation

Pump head, $\Delta H = H_2 - H_1$

ΔH = 225.146 – 141.525

= 83.624 J/kg

Pump head in unit length, $\frac{\Delta H}{g} = \frac{83.624}{9.81} = 8.52\ m$

Volumetric flow rate that enters the pump are calculated using formula:

$$Q = A.v$$

Volumetric flow rate = (8.219×10^{-3} m^2) (0.0418 m/s) (60min)

= 0.0206 m^3/min

Based on the Geankoplis, 4th edition, pump efficiencies of centrifugal pump at rated capacities are as follow:

Table 8.2 Rotary peristaltic pump rated capacities

Efficiency	Volumetric Flow Rate (m^3/min)
50	0.075
62	0.19
68	0.38
75	0.76
82	1.89
85	3.80

Since the volumetric flow rate = 0.0206 m^3/min.

The efficiency of pump calculated from the extrapolation as follow:

0.5	0.075
X	0.0206
0.62	0.19

$$x = 0.5 + \frac{0.0206 - 0.075}{0.19 - 0.075}(0.62 - 0.5) = 0.4432$$

Hence, the pump efficiency is 44.32% for 0.0206 m^3/min.

Pump power, $-W_S = -W_P \eta$

$$-W_P = \frac{-84.14}{0.4432}$$

W_P = 189.85 J/kg

$\dot{m}W_P = 0.376\ (189.85)$

= 71.38 W

8.2.5 Net Positive Suction Head Actual $(NPSH)_A$:

The required net positive suction head ($NPSH_{required}$), define as the minimum NPSH necessary to avoid cavitation in the pump. The available $NPSH_A$ of the system should always exceed the required $NPSH_{(required)}$ to avoid vaporization and cavitation of the pump.

Assumption:

1. The height Z_1 is 5.0 m.
2. All temperature at 28° C.

Vapor pressure of *Saccharomyces bourladii*, $P_{vp} = 101000\ Pa$

Total friction loss, $\sum F = 0.2533\ J/kg$

Based on equation 3.3-6 (Geankolis, 4th edition):

$$g\ (NPSH)_A = \frac{P_1 - P_{vp}}{\rho} + gZ_1 - \frac{V^2}{2} - \sum F$$

$$9.81\ (NPSH)_A = \frac{101000-101000}{1095.2} + 9.81(5.0) - \frac{0.0418^2}{2} - 0.2533$$

$(NPSH)_A = 4.97$ m

8.2.6 Pump efficiency curve

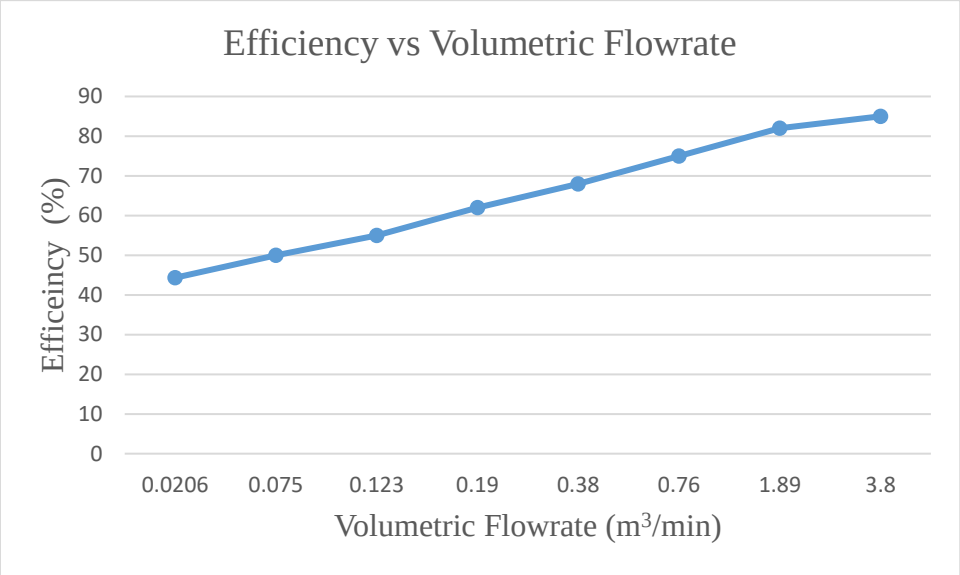


Figure 8.3 Pump Efficiency Curve

Figure 8.3 shows the curve of the pump efficiency against the volumetric flow rate. It shows that when the volumetric flow rate increase, the efficiency of the pump also increases.

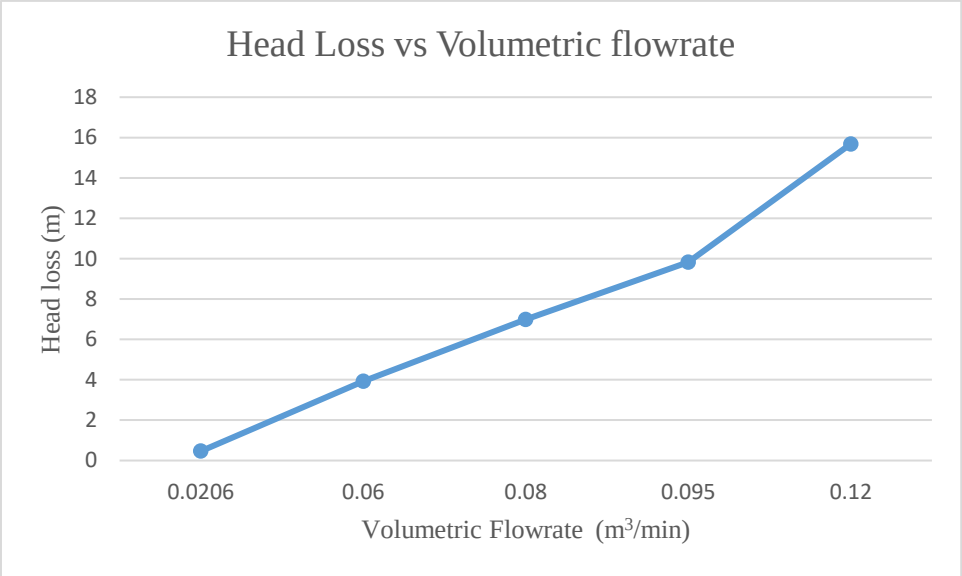


Figure 8.4 Pump Head Loss Curve

Figure 8.4 shows the head loss against the volumetric flow rate. From the graph plotted, it shows that when the volumetric flow rate increase, the head loss also increases.

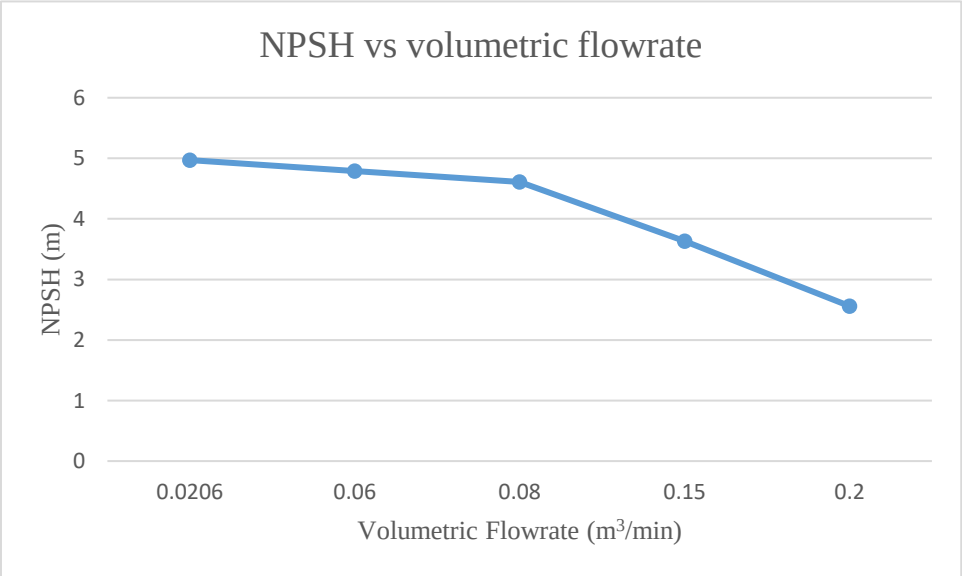


Figure 8.5 NPSH Curve

Figure 8.5 shows the NPSH against volumetric flow rate. Based on the graph, it shows that when the volumetric flow rate increase, the NPSH decrease.

Fluidization is the process of making a powder behave like a liquid by entraining the powder in a flowing gas. Fluidization is widely used in energy, chemical, pharmaceutical and others industries. In this process, the fluidization process does not involve because the freeze dryer (D-101) was used to provides higher survival rates of biomass. Freeze drying is accomplished by three significant steps which are freezing, primary and secondary drying (Marimuthu Anandharaj, 2007). This process commonly frozen at -196 °C in liquid nitrogen to increase the survival rate of yeast cultures. Then, the frozen samples are sublimated with ice under high vacuum conditions to finish the primary freezing. After the primary drying step, practically 95% of the water content in the sample is removed. Secondary drying is likewise imperative to accomplish a final water content below 4%, consequently improving survival rates and long-term storage efficiency.

CONCLUSION

To conclude, *Saccharomyces boular*dii probiotic is very essential product in the world because of its uniqueness. The *Saccharomyces boulardii* probiotic able to cure a lot of disease which involves gastrointestinal environment. The main objective of this project which is to produce biomass of the saccharomyces boulardii in large scale is achieved.

For the economic aspect of production of *Saccharomyces boulardii* probiotic, the world consumption of *Saccharomyces boulardii* probiotic is increasing year to year. The market growth of *Saccharomyces boulardii* probiotic is expected to grow with CAGR of 6.8% over 2019 to 2023. The plant capacity of *Saccharomyces boulardii* probiotic in our process is 338 kg/hour which contribute about 0.3% of global demand.

Later, in the following chapter we have discuss about the safety issue on material and method to handle our *Saccharomyces boulardii* cell. We have also discussed about environmental issue where we discuss about the waste generated in our process which are carbon dioxide and fermentation broth (waste water) and techniques on how the waste is managed in our process. We have also included environmental acts.

Based on the project, we have performed mass and energy balance, we have performed mass balance for entire plant and energy balance for fermenter and freeze dryer. From referring to overall mass balance inlet mass flow rate is equal to outlet mass flowrate which is 1876.6 kg/hour. The energy balance performed in the fermenter shows that the reaction is -9.0049×10^6 KJ/hour, thus the reaction is exothermic. Whereas the energy balance perform in the freeze dryer is -87551.78 KJ/hour. The negative value shows the process is exothermic. Heat is released to the surrounding.

Next in following chapter 6, for the thermodynamics part we have performed thermodynamics equilibrium composition to determine molar composition and the value we obtained we had compared with material balance calculation. The value of equilibrium constant K, for this process is 1.528. In chapter 7, we have discussed chemical and biochemical properties of *Saccharomyces boulardii* (yeast cell), producer

cell and cell cultivation technique are studied. Lastly, in chapter 8, we have studied fluid mechanics on friction loss in pipe, mechanical energy balance, pump head, pump efficiency and pump power.

In conclusion, *Saccharomyces boulardii* probiotic is very essential in modern medicine. By this project we have applied all the knowledge that we learned in this semester to complete this project.

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APPENDIX A CELL GROWTH

The formula to calculate the amount of cell is derive by using differential equation.

It is given that the growth rate is proportional to the number of organisms present in that population. If each member of that population has the same mass, then the growth rate is proportional to the mass concentration of members (x = cell mass concentration).

$$\frac{dx}{dt} \propto x$$

Thus, the specific growth rate (h^{-1}), $\mu = \frac{1}{x}\frac{dx}{dt}$

By using separable differential equation method,

$$\mu \, dt = \frac{1}{x}\frac{dx}{dt}$$

$$\int_{t_0}^{t} \mu \, dt = \int_{x_0}^{x} \frac{1}{x}\frac{dx}{dt}$$

$$[\mu t]_{t_0}^{t} = [\ln x]_{x_0}^{x}$$

$$\ln\frac{x}{x_0} = \mu_{net}(t - t_0)$$

Where x= cell mass concentration (kg/hr)

t = time (hr)

<u>Calculation to determine the amount of yeast cell that have to feed into fermenter</u>

Cell flowrate at output stream = 270.4 kg/hr

We assume that the overall process take place for 8 hours.

Amount of cell produce = 270.4 kg/hr × 8 hr

= 2163.2 kg

In main fermenter contains about 2163.2 kg of *saccharomyces boulardii* cell,

By using,

$$\ln \frac{x}{x_0} = \mu_{net}(t - t_0)$$

Let the rate of cell growth is μ_{net} = 0.5 /hr

Main fermenter contains about 1500L of solution.

So, the concentration of cell = 2163.2 kg ÷ $1500\ L$

$$x = 1.44 \text{ kg/L}$$

Hence the value we calculate for initial amount of cell in main fermenter,

The reaction take place about 48 hours.

$$\ln \frac{x}{x_0} = \mu_{net}(t - t_0)$$

$$\ln \frac{(1.44)}{x_0} = 0.5(48\text{-}0)$$

$$x_0 = 5.43 \times 10^{-11} \text{ kg/L}$$

$$Amount\ of\ cell\ in\ main\ \ fermenter\ initially = 8.15 \times 10^{-8} \text{ kg}$$

Now x = 8.15 × 10^{-8} kg for seed fermenter,

The concentration of cell in seed fermenter = 8.15 × 10^{-8} kg ÷ 150 L

$$x = 5.44 \times 10^{-10} \text{ kg/L}$$

Hence the amount of cell inserted in seed fermenter,

The reaction take place for about 24 hours in seed fermenter.

$$\ln \frac{x}{x_0} = \mu_{net}(t_0 - t)$$

$$\ln \frac{5.44 \times 10 - 10}{x_0} = 0.5(24\text{-}0)$$

$$x_0 = 3.34 \times 10^{-15} \text{ kg/L}$$

So about 5.01× 10^{-13} kg of yeast cell is feed into the seed fermenter. We assume that there is no dead cell during the process.

APPENDIX B MASS BALANCE

Main Fermenter, F-102

C_{SB5} = (11.34 kmol/h x 48h x 23.85 g/mol) / 1500L

= 8.654 kg/L

C_{W5} = (48.82 kmol/h x 48h x 18 g/mol) / 1500 L

= 28.12 kg/L

C_{G5} = (1.127 kmol/h x 48h x 180 g/mol) / 1500L

= 6.492 kg/L

$Q_5C_{SB5} = F_6W_{SB6}$

$Q_5(8.654) = 270.4$

$Q_5 = 31.25$ L/h

$Q_5C_{W5} = F_6W_{W6}$

$Q_5(28.12) = 878.8$

$Q_5 = 31.25$ L/h

$Q_5C_{G6} = F_6W_{G6}$

$Q_5(6.49) = 202.8$

$Q_5 = 31.25$ L/h

saccharomyces boulardii balance

$N_{SB2} = N_{SB5} - \alpha rv$

$0 = 31.25(8.654x1000)/23.85 – (3.019)\ r\ (1500)$

$r = 2.504$ mol/L.h

Water balance

$31.25\ C_{W2}/18 = 31.25928.12x1000)/18 – (4.0377)(2.504)(1500)$

$C_{W2} = 19.384$ kg/L

Ammonia balance

$31.25\ C_{A2}/17 = 0 – (-0.4529)(2.504)(1500)$

$C_{A2} = 0.925$ kg/L

Glucose balance

$31.25\ C_{G2}/180 = 31.25(6.489x1000)/180 – (-1)(2.504)(1500)$

$C_{G2} = 28.123$ kg/L

Oxygen balance

$N_{O3} = 0 – (-2.7546)(2.504)(1500)$

$= 10346.278$ mol/h (32g/mol)

$F_3 = 331.08$ kg/h

Carbon Dioxide balance

$0 = N_{C4} – (2.981)(2.504)(1500)$

$N_{C4} = 11196.636$ mol/h (44 g/mol)

$F_4 = 492.7$ kg/h

$F_2 + F_3 = F_4 + F_5$

$F_2 = 1352 + 492.7 – 331.1$

$= 1513.6$ kg/h

The composition for inlet stream 6

$Q_2C_{W2} = F_2W_{W2}$

$W_{W2} = 31.25(19.384)/1513.6 = 0.4$

$Q_2C_{G2} = F_2W_{G2}$

$W_{G2} = 31.25(28.123)/1513.6 = 0.581$

$Q_2C_{A2} = F_2W_{A2}$

$W_{A2} = 31.25(0.925)/1513.6 = 0.019$

Centrifuge, C-101

Overall mass balance

$F_6 = F_7 + F_8$

$1352 = F_7 + F_8$

Water balance

$F_6W_{W6} = F_7W_{W7} + F_8W_8$

$(1352)\ (0.65) = (F_7)\ (0.77612) + (F_8)\ (0.393939)$

$878.8 = 0.77612(1352– F_8) + 0.393939F_8$

$0.38218F_8 = 170.51424$

$F_8 = 446.16$ kg/h

$F_7 = 905.84$ kg/h

Water Mass Flow Rate

F_{6W}= F_6W_{W6} = (1352) (0.65) = 878.8 kg/h

F_{7W} = F_7W_{W7} = (905.84) (0.77612) = 703.4 kg/h

F_{8W} = F_8W_{W8} = (446.16) (0.393939) = 175.76 kg/h

saccharomyces bourlardii Mass Flow Rate

F_{6SB} = F_6W_{SB6} = (1352) (0.2) = 270.4 kg/h

F_{7SB} = F_7W_{SB7} = (446.16) (0.606) = 270.4 kg/h

Glucose Mass Flow Rate

F_{6G} = F_6W_{G6} = (1352) (0.15) = 202.8 kg/h

F_{7G} = F_7W_{G7} = (905.84) (0.22388) = 202.8 kg/h

Filter, F-101

Overall mass balance

$F_8 = F_9 + F_{10}$

$446.16 = F_9 + F_{10}$

Water balance

$F_8W_{w8} = F_9W_{w9} + F_{10}W_{w10}$

446.16(0.393939) = F11(1) + F12(0.0315)

175.76 = (446.16-F12) + 0.0315F12

$0.9685F_{10}$ = 270.4

F_{10} = 279.19

F_9 = 446.16 – 279.19

= 166.97

Water Mass Flow Rate

$F_{8W} = F_8W_{W8} = (446.16)\ (0.393939) = 175.76$ kg/h

$F_{9W} = F_9W_{W9} = (166.97)\ (1.0) = 166.97$ kg/h

$F_{10W} = F_{10}W_{W10} = (279.19)\ (0.0315) = 8.79$ kg/h

saccharomyces bourlardii Mass Flow Rate

$F_{SB8} = F_8W_{SB8} = (446.16)\ (0.606) = 270.4$ kg/h

$F_{SB10} = F_{10}W_{SB10} = (279.19)\ (0.9685) = 270.4$ kg/h

Mixing tank, M-101

Overall mass balance

$F_1 + F_{10} = F_{11}$

$F_1 + 279.18 = 338$

$F_1 = 58.81$ kg/hr

Water balance

$F_{10}W_{W10} = F_{11}W_{W11}$

(279.18) (0.0315) = F13(0.026)

$F_{11} = 338$ kg/hr

Betaine balance

$F_1W_{B1} = F_{11}W_{B11}$

$(58.811)\ (1) = (338)\ (W_{B13})$

$W_{B11} = 0.174$

saccharomyces boulardii balance

$W_{B11} + W_{SB11} + W_{W11} = 1$

$0.174 + W_{SB11} + 0.026 = 1$

$W_{SB11} = 0.800$

$F_{10}W_{SB10} = F13W_{SB11}$

(279.18) (0.9685) = (338) (0.8)

270.38 kg/hr = 270.38 kg/hr

Water Mass Flow Rate

$F_{10}W_{W10}$ = (279.18) (0.0315) = 8.79 kg/hr

$F_{11}W_{W11}$ = (338) (0.026) = 8.79 kg/hr

Betaine Mass Flow Rate

F_1W_{B1} = (58.81) (1) = 58.81 kg/hr

$F_{11}W_{B11}$ = (338) (0.184) = 58.81 kg/hr

saccharomyces boulardii Mass Flow Rate

$F_{10}W_{SB10}$ = (279.18) (0.9685) = 270.38 kg/hr

$F_{11}W_{SB11}$ = (338) (0.8) = 270.38 kg/hr

APPENDIX C FLUID MECHANICS

For graph head loss against volumetric flow rate:

Friction factor for Laminar flow: $f_1 = \frac{64}{Re}$

$$= \frac{64}{26.02} = 2.4596$$

$$f_2 = \frac{64}{Re}$$

$$= \frac{64}{50.66} = 1.263$$

Head loss, h_{Lmajor}

$$h_{L\,major} = h_{Lin} - h_{Lout}$$

$$h_{Lmajor} = f_1 \frac{L_1}{D_1} \frac{V_1^2}{2g} + f_2 \frac{L_2}{D_2} \frac{V_2^2}{2g}$$

$$h_{Lmajor} = (2.4596) \frac{11.3}{0.1023} \frac{0.0418^2}{2(9.81)} + 1.263 \frac{14.2}{0.0525} \frac{0.1586^2}{2(9.81)}$$

$$h_{Lmajor} = 0.4622\ m$$

SAFETY DATA SHEET

Preparation Date 30-Sep-2011 **Revision Date** 20-Jan-2015 **Revision Number** 3

1. IDENTIFICATION OF THE SUBSTANCE/PREPARATION AND OF THE COMPANY/UNDERTAKING

Product Identifier

Product Name **SACCHAROMYCES BOULARDII**

Other means of identification

Product Code 4691300
CAS Number Not available
Formula Unspecified
Synonyms Saccharomyces Boulardii

Recommended use of the chemical and restrictions on use

Recommended Use Nutritionals
Uses advised against Repeated contact with food. Avoid prolonged contact with eyes, skin, and clothing.

Details of the supplier of the safety data sheet

Supplier Address
ACETO CORPORATION
4 Tri Harbor Court
Port Washington, NY 11050-4661
Phone: (516) 627-6000
Fax: (516) 627-6093
Email: aceto@aceto.com

Emergency Telephone Number(s)

ChemTrec: 1-800-424-9300 Outside of the United States: 1-703-527-3887

2. HAZARDS IDENTIFICATION

Classification

Not a dangerous substance or mixture according to the Globally Harmonized System (GHS)

Label elements

Emergency Overview

The product contains no substances which at their given concentration, are considered to be hazardous to health

Appearance	Physical State	Odor
Fine dry Granular Light Beige to Brown Powder or Pellets	Solid	Not available

Hazards not otherwise classified (HNOC)

None known

Other Information

100 % of the mixture consists of ingredient(s) of unknown toxicity

3. COMPOSITION/INFORMATION ON INGREDIENTS

Synonyms Saccharomyces Boulardii

Formula Unspecified

Chemical Name	CAS-No	Weight %	North American Hazard Indicator
SACCHAROMYCES BOULARDII	N/A	100	False

4. FIRST AID MEASURES

First Aid Measures

General Advice Show this safety data sheet to the doctor in attendance.

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Consult a physician.

Skin Contact Wash off immediately with soap and plenty of water removing all contaminated clothes and shoes. Wash contaminated clothing before reuse. Consult a physician.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. If not breathing, give artificial respiration. Consult a physician.

Ingestion Rinse mouth. Never give anything by mouth to an unconscious person. Consult a physician.

Protection of First-aiders Use personal protective equipment. Avoid contact with skin, eyes and clothing.

Most important symptoms and effects, both acute and delayed

Main Symptoms No information available.

Indication of any immediate medical attention and special treatment needed

Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Suitable Extinguishing Media Water spray. Carbon dioxide (CO2). Dry powder. Foam.

Unsuitable Extinguishing Media No information available.

Hazardous Combustion Products Thermal decomposition or combustion may produce hazardous gases and/or materials.

Explosion Data

Sensitivity to mechanical impact Not available.
Sensitivity to static discharge Not available.

Specific Hazards Arising from the Chemical
Keep product and empty container away from heat and sources of ignition.

Protective Equipment and Precautions for Firefighters
As in any fire, wear self-contained breathing apparatus pressure-demand, MSHA/NIOSH (approved or equivalent) and full protective gear.

6. ACCIDENTAL RELEASE MEASURES

Personal precautions, protective equipment and emergency procedures

Personal Precautions Evacuate personnel to safe areas. Ensure adequate ventilation. Use personal protective equipment. Avoid contact with skin, eyes and clothing. In case of insufficient ventilation, wear suitable respiratory equipment.

Environmental Precautions Local authorities should be advised if significant spillages cannot be contained. Prevent further leakage or spillage if safe to do so. Do not allow material to contaminate ground water system. Prevent product from entering drains. Do not flush into surface water or sanitary sewer system. Should not be released into the environment.

Methods and material for containment and cleaning up

Methods for Cleaning up Evacuate personnel to safe areas. Sweep up and shovel into suitable containers for disposal. Avoid dust formation. Clean contaminated surface thoroughly.

7. HANDLING AND STORAGE

Precautions for Safe Handling

Handling Use only in an area equipped with a safety shower. Ensure that eyewash stations and safety showers are close to the workstation location. Ensure adequate ventilation. Do not breathe vapours/dust. Avoid contact with skin, eyes and clothing. Avoid repeated exposure.

Conditions for safe storage, including any incompatibilities

Storage Keep containers tightly closed in a dry, cool and well-ventilated place. Keep in properly labelled containers. Keep at temperatures between 2 - 8°C.

Incompatible Materials Strong oxidizing agents.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Exposure Guidelines This product does not contain any hazardous materials with occupational exposure limits established by the region specific regulatory bodies.

Chemical Name	ACGIH TLV	OSHA PEL	NIOSH IDLH
SACCHAROMYCES BOULARDII N/A	-	-	-

Appropriate engineering controls

Engineering Controls Ensure adequate ventilation, especially in confined areas.

Individual protection measures, such as personal protective equipment

Eye/face Protection Tightly fitting safety goggles.

Skin and body protection Impervious gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching the glove's outer surface) to avoid skin contact with this product. The type of protective equipment must be selected according to the concentration and amount of dangerous substance at the specific workplace.

Respiratory Protection In case of insufficient ventilation, wear suitable respiratory equipment.

General Hygiene Considerations Handle in accordance with good industrial hygiene and safety practice.

9. PHYSICAL AND CHEMICAL PROPERTIES

Information on basic physical and chemical properties

Appearance Fine dry Granular Light Beige to Brown Powder or Pellets
Physical State Solid
Odor Not available
Odor Threshold Not available

Property	Values	Remarks • Method
pH	No data available	
Melting/freezing point	No data available	Literary Reference
Boiling Point/Range	No information available	
Flash Point	No data available	No information available
Evaporation Rate	Not available	
Flammability Limits in Air		
upper flammability limit	No information available	
lower flammability limit	Not available	
Vapor Pressure	No information available	
Vapor Density	Not available	
Specific Gravity	No information available	
Water Solubility		
Solubility in other solvents	No information available	
Partition coefficient: n-octanol/water	Not available	
Autoignition temp (°C)	No data available	Estimated
Decomposition temperature	No data available	
Viscosity, kinematic	No information available	
Viscosity, dynamic	Not available	
Explosive Properties	No information available	
Oxidizing Properties	No information available	

Other Information

Softening Point No information available
Molecular Weight No information available

VOC Content No information available
Density No information available
Bulk Density No information available

10. STABILITY AND REACTIVITY

Reactivity Not applicable.
Chemical Stability Stable under recommended storage conditions

Conditions to Avoid Heat, flames and sparks.

Incompatible Materials Strong oxidizing agents.

Hazardous Decomposition Products No information available.

Possibility of Hazardous Reactions None under normal processing

11. TOXICOLOGICAL INFORMATION

Product Information To the best of our knowledge, the complete toxicological properties have not been thoroughly investigated.

Chemical Name	LD50 Oral	LD50 Dermal	LC50 Inhalation
SACCHAROMYCES BOULARDII N/A	-	-	-

The following values are calculated based on chapter 3.1 of the GHS document .

Information on likely routes of exposure

Eyes Contact with eyes may cause irritation. Avoid contact with eyes.

Skin May be harmful by skin contact. Substance may cause slight skin irritation. Avoid contact with skin.

Inhalation May be harmful by inhalation. May cause irritation of the mucous membranes. May cause irritation of respiratory tract. Avoid breathing dust.

Ingestion May be harmful if swallowed. Do not ingest.

Information on toxicological effects

Symptoms No information available.

Delayed and immediate effects as well as chronic effects from short and long-term exposure

Carcinogenicity There are no known carcinogenic chemicals in this product.

Eye damage/irritation No information available.

Skin Corrosion/Irritation No information available.

Sensitization No information available.

Reproductive Effects	No information available.
Mutagenic Effects	No information available.
Developmental Effects	No information available.
STOT - single exposure	No information available.
STOT - Repeated Exposure	No information available.
Chronic Toxicity	Avoid repeated exposure.
Aspiration Hazard	No information available.

Numerical measures of toxicity - Product Information

Acute Toxicity	100 % of the mixture consists of ingredient(s) of unknown toxicity

12. ECOLOGICAL INFORMATION

Ecotoxicity

There is no known ecological information for this product.

100 % of the mixture consists of components(s) of unknown hazards to the aquatic environment

Persistence / Degradability	No information available.
Bioaccumulation / Accumulation	No information available.
Mobility in Soil	No information available.

13. DISPOSAL CONSIDERATIONS

Waste treatment methods

Waste Disposal Method	Contact waste disposal services. Dispose of in accordance with local regulations.
Contaminated Packaging	Dispose of in accordance with local regulations. Empty containers should be taken for local recycling, recovery or waste disposal.

14. TRANSPORT INFORMATION

DOT	Not regulated
TDG	Not regulated
MEX	Not regulated

ICAO Not regulated

IATA Not regulated

IMDG/IMO Not regulated

RID Not regulated

ADR Not regulated

ADN Not regulated

15. REGULATORY INFORMATION

International Inventories

U.S.A. (TSCA)	Does not Comply
Canada (DSL)	Does not Comply
EU (EINECS)	Does not Comply
EU (ELINCS)	Does not Comply
Japan (ENCS)	Does not Comply
China	Does not Comply
Korea (KECL)	Does not Comply
Philippines (PICCS)	Does not Comply
Australia (AICS)	Does not Comply

Chemical Name	U.S.A. (TSCA)	Canada (DSL)	EU (EINECS)	EU (ELINCS)
SACCHAROMYCES BOULARDII	-	-	-	-

Chemical Name	Japan (ENCS)	China	Korea (KECL)	Philippines (PICCS)	Australia (AICS)
SACCHAROMYCES BOULARDII	-	-	-	-	-

Federal Regulations

SARA 313

Section 313 of Title III of the Superfund Amendments and Reauthorization Act of 1986 (SARA). This product does not contain any chemicals which are subject to the reporting requirements of the Act and Title 40 of the Code of Federal Regulations, Part 372.

SARA 311/312 Hazardous Categorization

Acute Health Hazard	No
Chronic Health Hazard	No
Fire Hazard	No
Sudden Release of Pressure Hazard	No
Reactive Hazard	No

CWA (Clean Water Act)
This product does not contain any substances regulated as pollutants pursuant to the Clean Water Act (40 CFR 122.21 and 40 CFR 122.42)

CERCLA
This material, as supplied, does not contain any substances regulated as hazardous substances under the Comprehensive Environmental Response Compensation and Liability Act (CERCLA) (40 CFR 302) or the Superfund Amendments and Reauthorization Act (SARA) (40 CFR 355). There may be specific reporting requirements at the local, regional, or state level pertaining to releases of this material

State Regulations

California Proposition 65
This product does not contain any Proposition 65 chemicals.

State Right-to-Know
This product does not contain any State Right-to-Know chemicals.

U.S. EPA Label Information

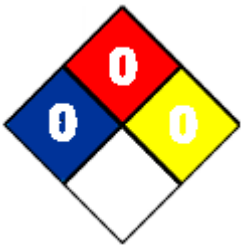
EPA Pesticide Registration Number Not applicable

Canada

WHMIS Statement
This product has been classified in accordance with the hazard criteria of the Controlled Products Regulations (CPR) and the MSDS contains all the information required by the CPR.

16. OTHER INFORMATION

NFPA
Health 0 **Flammability** 0 **Instability** 0 **Physical Hazard** 0



Preparation Date 30-Sep-2011
Revision Date 20-Jan-2015
Revision Summary
Not available

Disclaimer

The information provided on this MSDS is correct to the best of our knowledge, information and belief at the date of its publication. The information given is designed only as a guide for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered as a warranty or quality specification. The information relates only to the specific material designated and may not be valid for such material used in combination with any other material or in any process, unless specified in the text.

End of MSDS

SAFETY DATA SHEET

1. IDENTIFICATION OF PRODUCT AND COMPANY

Product Name:	Parboiled White Rice
Manufacturer:	Gulf Pacific Rice Co., Inc.
	12010 Taylor Road, Houston, Texas 77041
	Telephone Number: 713-464-0606 Fax: 713-466-8377 Website: www.gulfpac.com

2. COMPOSITION AND INGREDIENTS

Parboiled white rice obtained from milling of parboiled brown rice.

3. HAZARD IDENTIFICATION

Eyes:	May cause slight irritation upon contact due to mechanical action.
Skin:	May cause slight irritation upon contact due to mechanical action.
Inhalation:	May aggrevate preexisting respiratory problems.
Ingestion	Non anticipated from incidental ingestion.

4. FIRST AID MEASURES

Eyes:	Flush with water for 2 - 5 minutes.
Skin:	Wash with soap and water.
Inhalation:	Remove from area of exposure to well ventilated area.

5. FIRE FIGHTING MEASURES

Flash point:	Not applicable.
Fire Hazard:	None in open containers. Material will combust if ignited

6. ACCIDENTAL RELEASE MEASURES

Personal precaution:	Avoid direct contact with skin and eyes. Do not inhale dust.
Spill/Cleanup:	Sweep up and repackage or dispose according to state or federal laws.

7. HANDLING AND STORAGE

Handling:	Minimize dust generation and accumulation.
Storage:	Store in a cool dry place.

8. EXPOSURE CONTROLS AND PERSONAL PROTECTION

Ventilaion:	Keep dust levels down by providing adequate ventilation.
Respiratory protection:	If dust levels are high, use OSHA approved nuisance dust mask.
Eye protection:	Safety goggles.
Skin protection:	Wear body-covering clothing and closed footwear.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	White/Creamy
Odor:	Characteristic of parboiled rice
Taste:	Characteristic of parboiled rice
Vapour density:	Not applicable
Vapour pressure	Not applicable
Volatile by vol. (%):	Not applicable
Flash point:	Not applicable
Auto ignition temp (^{o}C):	Not applicable
Boling point:	Not applicable

10. STABILITY AND REACTIVITY

Stability:	Stable
Conditions to avoid:	Ignition sources
Hazardous decomposition products:	None

11. OTHER INFORMATION

Disclaimer: This information relates only to the specified material designated and may not be valid for such material used in combination with any other material. Such information is to the best of Gulf Pacific's knowledge accurate and reliable at the time of publication.

SAFETY DATA SHEET

1. IDENTIFICATION OF PRODUCT AND COMPANY

Product Name:	Parboiled Brown Rice
Manufacturer:	Gulf Rice Milling Inc.
Address:	12010 Taylor Road, Houston, Texas 77041
Emergency Contact:	713-464-0606
Recommended Use:	Food Ingredient

2. HAZARD IDENTIFICATION

Eyes:	May cause slight irritation upon contact due to mechanical action.
Skin:	May cause slight irritation upon contact due to mechanical action.
Inhalation:	May aggrevate preexisting respiratory problems.
Ingestion	Non anticipated from incidental ingestion.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Parboiled Brown Rice:	100%
Hazardous Components:	None

4. FIRST AID MEASURES

Eyes:	Flush with water for 2 - 5 minutes.
Skin:	Wash with soap and water.
Inhalation:	Remove from area of exposure to well ventilated area.
Ingestion:	No known hazard.

5. FIRE FIGHTING MEASURES

Extinguishing Media:	Water spray, Foam, Carbon Dioxide, Dry Powder.
Unsuitable Extinguishing Media:	Not applicable
Special Fire Fighting Procedures:	In case of fire, self-contained breathing apparattus and full protective clothing must be worn.
Unusual Fire and Explosion Hazard:	Not applicable
Hazardous Combustion Products:	Carbon oxides

6. ACCIDENTAL RELEASE MEASURES

Personal precaution:	Avoid direct contact with skin and eyes. Do not inhale dust.
Spill/Cleanup:	Sweep up and dispose according to state or federal laws.

7. HANDLING AND STORAGE

Safe handling;	Not applicable
Storage:	Store in a cool dry place.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Ventilaion:	Keep dust levels down by providing adequate ventilation.
Respiratory protection:	If dust levels are high, use OSHA approved nuisance dust mask.
Eye protection:	Not required but safety goggles may be worn.
Skin protection:	Wear body-covering clothing and closed footwear.
Work/Hygiene practice:	Observe good personal hygiene.

9. PHYSICAL AND CHEMICAL PROPERTIES

Appearance:	Light Brown/Beige
Odor:	Characteristic of parboiled brown rice
Odor Threshold:	No data available
pH:	Not applicable
Melting Point:	Not applicable
Freezing Point:	Not applicable
Boiling Point:	Not applicable
Flash Point:	No data available
Evaporation Rate:	Not applicable
Flammability Limit - Upper (%):	No data available
Flammability Limit - Lower (%):	No data available
Vapour Pressure	Not applicable
Vapour Density:	Not applicable
Specific Graivity:	> 1
Solubility in Water:	Moderate
Solubility(Other):	No data available
Partition Coefficient: n-octanol/water:	No data available
Autoignition Temperature:	No data available
Decomposition Temperature:	No data available
Viscosity:	Not applicable
Explosive Properties:	Dust explosion Class II

10. STABILITY AND REACTIVITY

Stability:	Stable
Conditions to avoid:	Ignition sources
Incompatible Materials:	Strong oxidizing agents
Hazardous decomposition products:	No data available

11. TOXICOLOGICAL INFORMATION

No information available.

12. ECOLOGICAL INFORMATION

Biodegradable

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods:	Material is not considered hazardous under federal regulations. Dispose in accordance with local authority requirements.

14. TRANSPORATION INFORMATION

Department of Transportaion:	Not regulated.
Product Shipping Name:	Not applicable
Transport Hazard:	Not applicable
Packaging Group Number:	Not applicable
IBC Code:	Not applicable
Identification Number:	Not applicable

15. REGULATORY INFORMATION

None.

16. OTHER INFORMATION

Disclaimer:	This information relates only to the specified material designated and may not be valid for such material used in combination with any other material. Such information is to the best of Gulf Rice Milling's knowledge accurate and reliable at the time of publication.

Safety Data Sheet
Oxygen

Section 1: Product and Company Identification

Tech Air
50 Mill Plain Rd.
Danbury, CT 06811
203-792-1834 | http://techair.com
Email: Safety@techair.com

EMERGENCY PHONE: P.E.R.S #800-633-8253

International: 1-801-629-0667

Product Code: Oxygen

Section 2: Hazards Identification

Danger

Hazard Classification:
Gases Under Pressure
Oxidizing Gas (Category 1)

Hazard Statements:
Contains gas under pressure; may explode if heated
May cause or intensify fire; oxidizer

Precautionary Statements
Prevention:
Keep reduction valves/valves and fittings free from oil and grease.
Keep and store away from clothing and combustible materials.

Response:
In case of fire: Stop leak if safe to do so.

Storage:
Protect from sunlight.
Store in well-ventilated place.

Section 3: Composition/Information on Ingredients

CAS #
7782-44-7

Chemical Substance	Chemical Family	Trade Names
OXYGEN, COMPRESSED GAS	Inorganic gases	OXYGEN; DIOXYGEN; MOLECULAR OXYGEN; OXYGEN MOLECULE; PURE OXYGEN; UN 1072; O2

Section 4: First Aid Measures

Skin Contact	Eye Contact	Ingestion	Inhalation	Note to Physicians
None expected	None expected	Not likely route of exposure	If adverse effects occur, remove to uncontaminated area. Give artificial respiration if not breathing. Get immediate medical attention.	None

Section 5: Fire Fighting Measures

Suitable Extinguishing Media	Products of Combustion	Protection of Firefighters
Non-flammable. Use extinguishing agent appropriate for the material which is burning. Use water in large quantities for fires involving oxygen.	Oxides of burning material	▪ Respiratory protection may be needed for frequent or heavy exposure. ▪ None

Section 6: Accidental Release Measures

Personal Precautions	Environmental Precautions	Methods for Containment
Keep unnecessary people away, isolate hazard area and deny entry. Ventilate closed spaces before entering.	Avoid contact with combustible materials.	Stop leak if possible without personal risk.

Methods for Cleanup	Other Information
Stop leak and ventilate	None

Section 7: Handling and Storage

Handling	Storage
Store and handle in accordance with all current regulations and standards. Subject to storage regulations: U.S. OSHA 29 CFR 1910.101.	Keep separated from incompatible substances.

Section 8: Exposure Controls/Personal Protection

Exposure Guidelines
OXYGEN, COMPRESSED GAS: No occupational exposure limits established.

Engineering Controls

Handle only in fully enclosed systems.

Eye Protection	Skin Protection	Respiratory Protection

Eye Protection	Skin Protection	Respiratory Protection
Eye protection not required, but recommended.	Protective clothing is not required.	Respiratory protection may be needed for frequent or heavy exposure.

General Hygiene considerations

- Avoid breathing vapor or mist
- Avoid contact with eyes and skin
- Wash thoroughly after handling and before eating or drinking

Section 9: Physical and Chemical Properties

Physical State	Appearance	Color	Change in Appearance	Physical Form	Odor	Taste
Gas	Clear	Colorless	N/A	Gas	Odorless	Tasteless

Flash Point	Flammability	Partition Coefficient	Autoignition Temperature	Upper Explosive Limits	Lower Explosive Limits
Not flammable	Not available	Not available	Nonflammable	Nonflammable	Nonflammable

Boiling Point	Freezing Point	Vapor Pressure	Vapor Density	Specific Gravity	Water Solubility	pH	Odor Threshold	Evaporation Rate	Viscosity
-297 F (-183 C)	-360 F (-218 C)	760 mmHg @ -183 C	1.1 (Air=1)	Not applicable	3.2% @ 25 C	Not applicable	Not available	Not applicable	0.02075 cP @ 25 C

Molecular Weight	Molecular Formula	Density	Weight per Gallon	Volatility by Volume	Volatility	Solvent Solubility
31.9988	O2	1.309 g/L @ 25 C	Not available	Not applicable	Not applicable	Soluble: Alcohol

Section 10: Stability and Reactivity

Stability	Conditions to Avoid	Incompatible Materials
Stable at normal temperatures and pressure.	Stable at normal temperatures and pressure.	Combustible materials, halo carbons, metals, bases, reducing agents, amines, metal salts, oxidizing materials, alkaline earth and alkali metals

Hazardous Decomposition Products	Possibility of Hazardous Reactions
Miscellaneous decomposition products	Will not polymerize.

Section 11: Toxicology Information

Acute Effects

Oral LD50	Dermal LD50	Inhalation
Not established	Not established	Irritation, changes in body temperature, nausea, difficulty breathing, irregular heartbeat, dizziness, disorientation, hallucinations, mood swings, pain in extremities, tremors, lung congestion, convulsions

Eye Irritation	Skin Irritation	Sensitization
No information on significant adverse effects	No information on significant adverse effects	No significant target effects reported.

Chronic Effects

Carcinogenicity	Mutagenicity	Reproductive Effects	Developmental Effects
Not known.	Available.	Available.	No data

Section 12: Ecological Information

Fate and Transport

Eco toxicity	Persistence / Degradability	Bioaccumulation / Accumulation	Mobility in Environment

Fish toxicity: Not available Invertibrate toxicity: Not available Algal toxicity: Not available Phyto toxicity: Not available Other toxicity: Not available	Not available	Low bioaccumulation	Not available

Section 13: Disposal Considerations

Dispose in accordance with all applicable regulations. Subject to disposal regulations: U.S. EPA 40 CFR 262. Hazardous Waste Number(s): D001.

Section 14: Transportation Information

U.S. DOT 49 CFR 172.101

Proper Shipping Name	ID Number	Hazard Class or Division	Packing Group	Labeling Requirements	Passenger Aircraft or Railcar Quantity Limitations	Cargo Aircraft Only Quantity Limitations	Additional Shipping Description
Oxygen, compressed	UN1072	2.2	Not available	2.2; 5.1	75 kg or L	150 kg	N/A

Canadian Transportation of Dangerous Goods

Shipping Name	UN Number	Class	Packing Group / Risk Group
Oxygen, compressed	UN1072	2.2; 5.1	Not applicable

Section 15: Regulatory Information

U.S. Regulations

CERCLA Sections	SARA 355.30	SARA 355.40
Not regulated.	Not regulated.	Not regulated.

SARA 370.21

Acute	Chronic	Fire	Reactive	Sudden Release
No	No	Yes	No	Yes

SARA 372.65

Not regulated.

OSHA Process Safety

Not regulated.

State Regulations

CA Proposition 65
Not regulated.

Canadian Regulations

WHMIS Classification
A,C

National Inventory Status

US Inventory (TSCA)	TSCA 12b Export Notification	Canada Inventory (DSL/NDSL)
Listed on inventory.	Not listed.	Not determined.

Section 16: Other Information

NFPA Rating
HEALTH=0 FIRE=0 REACTIVITY=0

0 = minimal hazard, 1 = slight hazard, 2 = moderate hazard, 3 = severe hazard, 4 = extreme hazard

Safety Data Sheet
Carbon Dioxide

Section 1: Product and Company Identification

Tech Air
50 Mill Plain Rd.
Danbury, CT 06811
203-792-1834 | http://techair.com
Email: Safety@techair.com

EMERGENCY PHONE: P.E.R.S #800-633-8253

International: 1-801-629-0667

Product Code: Carbon Dioxide

Section 2: Hazards Identification

Warning

Hazard Classification:
Gases Under Pressure

Hazard Statements:
Contains gas under pressure; may explode if heated

Precautionary Statements

Storage:
Protect from sunlight.
Store in well-ventilated place.

Section 3: Composition/Information on Ingredients

CAS #
124-38-9

Chemical Substance	Chemical Family	Trade Names
CARBON DIOXIDE, GAS	Inorganic gases	CARBONIC ACID GAS; CARBONIC ANHYDRIDE; CARBON DIOXIDE; CARBON OXIDE; UN 1013; CO2

Section 4: First Aid Measures

Skin Contact	Eye Contact	Ingestion	Inhalation	Note to Physicians
If frostbite or freezing occur, immediately flush with plenty of lukewarm water (105-115 F; 41-46 C). DO NOT USE HOT WATER. If warm water is not available, gently wrap affected parts in blankets. Get immediate medical attention.	Contact with liquid: Immediately flush eyes with plenty of water for at least 15 minutes. Then get immediate medical attention.	Do not induce vomiting.	If adverse effects occur, remove to uncontaminated area. Give artificial respiration if not breathing. If breathing is difficult, oxygen should be administered by qualified personnel. Get immediate medical attention.	For inhalation, consider oxygen.

Section 5: Fire Fighting Measures

Suitable Extinguishing Media	Products of Combustion	Protection of Firefighters
Non-flammable	Non-flammable	▪ Any appropriate escape-type, self-contained breathing apparatus. ▪ Non-flammable

Section 6: Accidental Release Measures

Personal Precautions	Environmental Precautions	Methods for Containment
Keep unnecessary people away, isolate hazard area and deny entry. Ventilate closed spaces before entering. Do not touch spilled material.	Subject to California Safe Drinking Water and Toxic Enforcement Act of 1986 (Proposition 65). Keep out of water supplies and sewers.	Stop leak if possible without personal risk.

Methods for Cleanup	Other Information
Stop leak, evacuate, remove source of ignition.	None

Section 7: Handling and Storage

Handling	Storage
Subject to storage regulations: U.S. OSHA 29 CFR 1910.101. Keep separated from incompatible substances.	Store and handle in accordance with all current regulations and standards

Section 8: Exposure Controls/Personal Protection

Exposure Guidelines
CARBON DIOXIDE, GAS: CARBON DIOXIDE: 5000 ppm (9000 mg/m3) OSHA TWA 10000 ppm (18000 mg/m3) OSHA TWA (vacated by 58 FR 35338, June 30, 1993) 30000 ppm (54000 mg/m3) OSHA STEL (vacated by 58 FR 35338, June 30, 1993) 5000 ppm ACGIH TWA 30000 ppm ACGIH STEL 5000 ppm (9000 mg/m3) NIOSH recommended TWA 10 hour(s) 30000 ppm (54000 mg/m3) NIOSH recommended STEL

Engineering Controls

Handle only in fully enclosed systems.

Eye Protection	Skin Protection	Respiratory Protection

Eye Protection	Skin Protection	Respiratory Protection
For the gas: Eye protection not required, but recommended. For the liquid: Wear splash resistant safety goggles. Contact lenses should not be worn. Provide an emergency eye wash fountain and quick drench shower in the immediate work area.	For the gas: Protective clothing is not required. For the liquid: Wear appropriate protective, cold insulating clothing.	Any appropriate escape-type, self-contained breathing apparatus.

General Hygiene considerations

- Avoid breathing vapor or mist
- Avoid contact with eyes and skin
- Wash thoroughly after handling and before eating or drinking

Section 9: Physical and Chemical Properties

Physical State	Appearance	Color	Change in Appearance	Physical Form	Odor	Taste
Gas	Colorless	Colorless	N/A	Gas	Odorless	Acid taste

Flash Point	Flammability	Partition Coefficient	Autoignition Temperature	Upper Explosive Limits	Lower Explosive Limits
Not flammable	Not available	N/A	Nonflammable	Nonflammable	Nonflammable

Boiling Point	Freezing Point	Vapor Pressure	Vapor Density	Specific Gravity	Water Solubility	pH	Odor Threshold	Evaporation Rate	Viscosity
Not available	-71 F (-57 C) @ 4000 mmHg	43700 mmHg @ 21 C	1.5 (Air=1)	1.522 @ 21 C	Soluble	3.7 (saturated aqueous solution) @ 101.3 kPa (carbonic acid)	Not available	Not applicable	0.01657 cP @ 0 C

Molecular Weight	Molecular Formula	Density	Weight per Gallon	Volatility by Volume	Volatility	Solvent Solubility
44.01	C-O2	0.114	Not available	Not applicable	Not applicable	Soluble: Alcohol, acetone, hydrocarbons, organic solvents

Section 10: Stability and Reactivity

Stability	Conditions to Avoid	Incompatible Materials
Stable at normal temperatures and pressure.	Stable at normal temperatures and pressure.	Combustible materials, oxidizing materials, metal salts, reducing agents, metal carbide, metals, bases

Hazardous Decomposition Products	Possibility of Hazardous Reactions
Carbon monoxide	Will not polymerize.

Section 11: Toxicology Information

Acute Effects

Oral LD50	Dermal LD50	Inhalation
Not established	Not established	Ringing in the ears, nausea, irregular heartbeat, headache, drowsiness, dizziness, tingling sensation, visual disturbances, suffocation, convulsions, coma

Eye Irritation	Skin Irritation	Sensitization
Irritation, frostbite, blurred vision	Liquid: blisters, frostbite	Difficulty breathing

Chronic Effects

Carcinogenicity	Mutagenicity	Reproductive Effects	Developmental Effects
Not available	Not established	Available.	No data

Section 12: Ecological Information

Fate and Transport

Eco toxicity	Persistence / Degradability	Bioaccumulation / Accumulation	Mobility in Environment
Fish toxicity: 150000 ug/L 48 day(s) (Mortality) Brown trout (Salmo trutta) Invertibrate toxicity: Not available Algal toxicity: Not available Phyto toxicity: Not available Other toxicity: Not available	Relatively non-persistent in the environment. Moderately volatile from water.	Accumulates very little in the bodies of living organisms.	Leaches through the soil

Section 13: Disposal Considerations

Dispose in accordance with all applicable regulations.

Section 14: Transportation Information

U.S. DOT 49 CFR 172.101

Proper Shipping Name	ID Number	Hazard Class or Division	Packing Group	Labeling Requirements	Passenger Aircraft or Railcar Quantity Limitations	Cargo Aircraft Only Quantity Limitations	Additional Shipping Description
Carbon dioxide	UN1013	2.2	Not applicable	2.2	75 kg or L	150kg	None

Canadian Transportation of Dangerous Goods

Shipping Name	UN Number	Class	Packing Group / Risk Group
Carbon dioxide	UN1013	2.2	Not applicable

Section 15: Regulatory Information

U.S. Regulations

CERCLA Sections	SARA 355.30	SARA 355.40
Not regulated.	Not regulated.	Not regulated.

SARA 370.21

Acute	Chronic	Fire	Reactive	Sudden Release
Yes	No	No	No	Yes

SARA 372.65

Not regulated.

OSHA Process Safety

Not regulated.

State Regulations

CA Proposition 65
Not regulated.

Canadian Regulations

WHMIS Classification
A

National Inventory Status

US Inventory (TSCA)	TSCA 12b Export Notification	Canada Inventory (DSL/NDSL)
Listed on inventory.	Not listed.	Listed on inventory.

Section 16: Other Information

NFPA Rating
HEALTH=2 FIRE=0 REACTIVITY=0

0 = minimal hazard, 1 = slight hazard, 2 = moderate hazard, 3 = severe hazard, 4 = extreme hazard

SAFETY DATA SHEET

Version 3.9
Revision Date 08/13/2014
Print Date 02/23/2018

1. PRODUCT AND COMPANY IDENTIFICATION

1.1 Product identifiers

Product name : Sucrose

Product Number : 18219
Brand : Sigma-Aldrich

CAS-No. : 57-50-1

1.2 Relevant identified uses of the substance or mixture and uses advised against

Identified uses : Laboratory chemicals, Manufacture of substances

1.3 Details of the supplier of the safety data sheet

Company : Sigma-Aldrich
3050 Spruce Street
SAINT LOUIS MO 63103
USA

Telephone : +1 800-325-5832
Fax : +1 800-325-5052

1.4 Emergency telephone number

Emergency Phone # : +1-703-527-3887 (CHEMTREC)

2. HAZARDS IDENTIFICATION

2.1 Classification of the substance or mixture

GHS Classification in accordance with 29 CFR 1910 (OSHA HCS)
Combustible dust,

For the full text of the H-Statements mentioned in this Section, see Section 16.

2.2 GHS Label elements, including precautionary statements

Pictogram none

Signal word Warning

Hazard statement(s)
May form combustible dust concentrations in air

Precautionary statement(s) none

2.3 Hazards not otherwise classified (HNOC) or not covered by GHS

Combustible dust

3. COMPOSITION/INFORMATION ON INGREDIENTS

3.1 Substances

Synonyms : α-D-Glucopyranosyl β-D-fructofuranoside
α-D-Glc-(1→2)-β-D-Fru
D(+)-Saccharose
Sugar
β-D-Fructofuranosyl-α-D-glucopyranoside
Saccharum

Formula	:	$C_{12}H_{22}O_{11}$
Molecular weight	:	342.30 g/mol
CAS-No.	:	57-50-1
EC-No.	:	200-334-9

Hazardous components

Component	Classification	Concentration
Sucrose		
		90 - 100 %

For the full text of the H-Statements mentioned in this Section, see Section 16.

4. FIRST AID MEASURES

4.1 Description of first aid measures

If inhaled
If breathed in, move person into fresh air. If not breathing, give artificial respiration.

In case of skin contact
Wash off with soap and plenty of water.

In case of eye contact
Flush eyes with water as a precaution.

If swallowed
Never give anything by mouth to an unconscious person. Rinse mouth with water.

4.2 Most important symptoms and effects, both acute and delayed
The most important known symptoms and effects are described in the labelling (see section 2.2) and/or in section 11

4.3 Indication of any immediate medical attention and special treatment needed
No data available

5. FIREFIGHTING MEASURES

5.1 Extinguishing media

Suitable extinguishing media
Use water spray, alcohol-resistant foam, dry chemical or carbon dioxide.

5.2 Special hazards arising from the substance or mixture
No data available

5.3 Advice for firefighters
Wear self-contained breathing apparatus for firefighting if necessary.

5.4 Further information
No data available

6. ACCIDENTAL RELEASE MEASURES

6.1 Personal precautions, protective equipment and emergency procedures
Avoid dust formation. Avoid breathing vapours, mist or gas.
For personal protection see section 8.

6.2 Environmental precautions
Do not let product enter drains.

6.3 Methods and materials for containment and cleaning up
Sweep up and shovel. Keep in suitable, closed containers for disposal.

6.4 Reference to other sections
For disposal see section 13.

7. HANDLING AND STORAGE

7.1 Precautions for safe handling

Further processing of solid materials may result in the formation of combustible dusts. The potential for combustible dust formation should be taken into consideration before additional processing occurs.
Provide appropriate exhaust ventilation at places where dust is formed.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Keep container tightly closed in a dry and well-ventilated place.

7.3 Specific end use(s)

Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

8.1 Control parameters

Components with workplace control parameters

Component	CAS-No.	Value	Control parameters	Basis
Sucrose	57-50-1	TWA	10 mg/m3	USA. ACGIH Threshold Limit Values (TLV)
	Remarks	Dental erosion Not classifiable as a human carcinogen		
		TWA	15 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		TWA	5 mg/m3	USA. Occupational Exposure Limits (OSHA) - Table Z-1 Limits for Air Contaminants
		TWA	5 mg/m3	USA. NIOSH Recommended Exposure Limits
		TWA	10 mg/m3	USA. NIOSH Recommended Exposure Limits

8.2 Exposure controls

Appropriate engineering controls
General industrial hygiene practice.

Personal protective equipment

Eye/face protection
Use equipment for eye protection tested and approved under appropriate government standards such as NIOSH (US) or EN 166(EU).

Skin protection
Handle with gloves. Gloves must be inspected prior to use. Use proper glove removal technique (without touching glove's outer surface) to avoid skin contact with this product. Dispose of contaminated gloves after use in accordance with applicable laws and good laboratory practices. Wash and dry hands.

Full contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:Dermatril® (KCL 740 / Aldrich Z677272, Size M)

data source: KCL GmbH, D-36124 Eichenzell, phone +49 (0)6659 87300, e-mail sales@kcl.de, test method: EN374
If used in solution, or mixed with other substances, and under conditions which differ from EN 374, contact the supplier of the CE approved gloves. This recommendation is advisory only and must be evaluated by an industrial hygienist and safety officer familiar with the specific situation of anticipated use by our customers. It should not be construed as offering an approval for any specific use scenario.

Body Protection
Choose body protection in relation to its type, to the concentration and amount of dangerous substances, and to the specific work-place., The type of protective equipment must be selected according to the concentration and amount of the dangerous substance at the specific workplace.

Respiratory protection
Respiratory protection is not required. Where protection from nuisance levels of dusts are desired, use type N95 (US) or type P1 (EN 143) dust masks. Use respirators and components tested and approved under appropriate government standards such as NIOSH (US) or CEN (EU).

Control of environmental exposure
Do not let product enter drains.

9. PHYSICAL AND CHEMICAL PROPERTIES

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: crystalline Colour: white
b)	Odour	No data available
c)	Odour Threshold	No data available
d)	pH	5.5 - 7 at 342 g/l at 25 °C (77 °F)
e)	Melting point/freezing point	Melting point/range: 185 - 187 °C (365 - 369 °F)
f)	Initial boiling point and boiling range	No data available
g)	Flash point	No data available
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	May form combustible dust concentrations in air
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapour pressure	No data available
l)	Vapour density	No data available
m)	Relative density	No data available
n)	Water solubility	342 g/l at 20 °C (68 °F) - completely soluble
o)	Partition coefficient: n-octanol/water	No data available
p)	Auto-ignition temperature	No data available
q)	Decomposition temperature	No data available
r)	Viscosity	No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	No data available

9.2 Other safety information
No data available

10. STABILITY AND REACTIVITY

10.1 Reactivity
No data available

10.2 Chemical stability
Stable under recommended storage conditions.

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
No data available

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
Other decomposition products - No data available
In the event of fire: see section 5

11. TOXICOLOGICAL INFORMATION

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - 29,700 mg/kg
Remarks: Behavioral:Somnolence (general depressed activity). Cyanosis Diarrhoea

Inhalation: No data available

Dermal: No data available

No data available

Skin corrosion/irritation
No data available

Serious eye damage/eye irritation
No data available

Respiratory or skin sensitisation
No data available

Germ cell mutagenicity
No data available

Carcinogenicity

IARC: No component of this product present at levels greater than or equal to 0.1% is identified as probable, possible or confirmed human carcinogen by IARC.

NTP: No component of this product present at levels greater than or equal to 0.1% is identified as a known or anticipated carcinogen by NTP.

OSHA: No component of this product present at levels greater than or equal to 0.1% is identified as a carcinogen or potential carcinogen by OSHA.

Reproductive toxicity
No data available

No data available

Specific target organ toxicity - single exposure
No data available

Specific target organ toxicity - repeated exposure
No data available

Aspiration hazard
No data available

Additional Information
RTECS: WN6500000

To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

12. ECOLOGICAL INFORMATION

12.1 Toxicity
No data available

12.2 Persistence and degradability
No data available

12.3 Bioaccumulative potential
No data available

12.4 Mobility in soil
No data available

12.5 Results of PBT and vPvB assessment
PBT/vPvB assessment not available as chemical safety assessment not required/not conducted

12.6 Other adverse effects

No data available

13. DISPOSAL CONSIDERATIONS

13.1 Waste treatment methods

Product
Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

14. TRANSPORT INFORMATION

DOT (US)
Not dangerous goods

IMDG
Not dangerous goods

IATA
Not dangerous goods

15. REGULATORY INFORMATION

SARA 302 Components
No chemicals in this material are subject to the reporting requirements of SARA Title III, Section 302.

SARA 313 Components
This material does not contain any chemical components with known CAS numbers that exceed the threshold (De Minimis) reporting levels established by SARA Title III, Section 313.

SARA 311/312 Hazards
No SARA Hazards

Massachusetts Right To Know Components

	CAS-No.	Revision Date
Sucrose	57-50-1	1991-07-01

Pennsylvania Right To Know Components

	CAS-No.	Revision Date
Sucrose	57-50-1	1991-07-01

New Jersey Right To Know Components

	CAS-No.	Revision Date
Sucrose	57-50-1	1991-07-01

California Prop. 65 Components
This product does not contain any chemicals known to State of California to cause cancer, birth defects, or any other reproductive harm.

16. OTHER INFORMATION

Full text of H-Statements referred to under sections 2 and 3.

May form combustible dust concentrations in air

HMIS Rating

Health hazard:	0
Chronic Health Hazard:	
Flammability:	0
Physical Hazard	0

NFPA Rating

Health hazard:	0
Fire Hazard:	0
Reactivity Hazard:	0

Further information

The above information is believed to be correct but does not purport to be all inclusive and shall be used only as a guide. The information in this document is based on the present state of our knowledge and is applicable to the product with regard to appropriate safety precautions. It does not represent any guarantee of the properties of the product. Sigma-Aldrich Corporation and its Affiliates shall not be held liable for any damage resulting from handling or from contact with the above product. See www.sigma-aldrich.com and/or the reverse side of invoice or packing slip for additional terms and conditions of sale.

Preparation Information
Sigma-Aldrich Corporation
Product Safety – Americas Region
1-800-521-8956

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