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The National University of Malaysia

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COURSE CODES:	KKKR3583, KKKR3733, KKKR3723, KKKR3713
LECTURERS:	KKKR3583: PROF. MADYA TS. EN. NOORHISHAM TAN KOFLI TS. DR. AHMAD RAZI OTHMAN KKKR3733: PROF. MADYA IR. DR. HASSIMI ABU HASAN PROF. MADYA TS. EN. NOORHISHAM TAN KOFLI PROF. MADYA TS. DR. ROSIAH ROHANI TS. DR. DARMAN NORDIN KKKR3723: PROF. MADYA IR. DR. SHUHAIDA HARUN PROF. MADYA IR. DR. TEOW YEIT HAAN KKKR3713: PROF. MADYA DR. NORLIZA ABD. RAHMAN DR. JARINAH MOHD ALI
GROUP:	KK1
SUBMISSION DATE:	08th JULY 2022
MEMBERS:	MUHAMMAD ARIF DANIAL BIN HASSAN (A173470) AZRUL ZULHILMI BIN AHMAD ROSLI (A173752) NUQMAN HAZIQ BIN MOHD AYUB KHAN (A174188) AHMAD NAWWAR IRSYAD BIN MOHAMED (A174340)

DECLARATION

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

09 July 2022

MUHAMMAD ARIF
DANIAL BIN HASSAN
A173470

AZRUL ZUHILMI BIN
AHMAD ROSLI
A173752

NUIQMAN HAZIQ BIN
MOHD AYUB KHAN
A174188

AHMAD NAWWAR
IRSYAD BIN MOHAMED
A174340

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EXECUTIVE SUMMARY

Our project is focused on the production of Plasmid DNA from the cultivation of *Escherichia coli* (*e. coli* K12). *E. coli* is a gram-negative bacterium and plasmid DNA is an intermediate chemical to be used in the production of antibiotic and others. By 2027, the market expected to growth to 2344.29 million by 2027, registering CAGR of 23.38% during 2022-2027. Plant capacity in this project is 8.94 kg/hr with 2.5% from the deficient. The process in this plant is start with fermentation which use to cultivate the *e. coli*. Then, biomass will undergo centrifugation and remove unwanted component. Biomass which is *e. coli* will undergo alkaline lysis to produce plasmid DNA., genetic DNA, protein, cell debris and others. Next, precipitation process will happen to precipitate unwanted component from the mixture. Then, the process will go through filtration and microfiltration. After it, mixture will go through ultrafiltration and chromatography as purification step to purify the plasmid DNA to achieved 96% purity. Tris-EDTA will be using as buffer in Anion Exchange Chromatography. In this project, the waste product are solid waste which contains gDNA, RNA and others, liquid waste which contains Tris-EDTA buffer, SDS, and others, and carbon dioxide. In this production, glucose was used as carbon source, and ammonia as nitrogen source. Mass balance for unit operations such as bioreactor, mixer, chromatography, microfiltration, ultrafiltration, and filter are calculated, and the overall mass are balance. The value of entalphy obtained from the energy balance in bioreactor is -48737.49 kJ/hr, hence is an exothermic reaction. For biochemistry part, the enzyme used is galactose permease (galP) with commission number EC 2.7.1.204. Galactose permease will be used to create the new strain to make the production of acetate low. Furthermore, the main fermentor and CSTR are the pressure vessels designed under respective internal and external pressure according to ASME Code procedure. Then, at utility part, heat exchanger be designed is E-101 and E-102. For separation part, centrifuge is selected as the unit operation. 97.77 % recovery of solid shows that the high efficiency of the centrifuge separation process of solid from mixture. In dynamic and process control, the control configuration (Main fermenter, R-102, Centrifuge, C-101, CSTR, R-103 and Chromatography, CR-101) has been specified before the designing a process control system.

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LIST OF NOMENCLACTURE

O_2	Oxygen
NH_3	Ammonia
H_2O	Water
$C_6H_{12}O_6$	Glucose
$C_2H_4O_2$	Acetic Acid
CO_2	Carbon Dioxide

CHAPTER I

INTRODUCTION

1.1 INTRODUCTION OF PLASMID DNA

Biopharmaceuticals are recombinant therapeutic proteins produced by biotechnological techniques and the source of the products were from biological sources which is microorganisms, animal fluids and many more (Jozala et al., 2016). The word plasmid was first discovered by Joshua Lederberg in 1952 to describe 'any extrachrosomal hereditary element' (*Plasmids Are Independent DNA Loops Used to Clone DNA*, 2018). Plasmids are tiny circular extrachromosomal double-stranded DNA units that may be found in a variety of bacterial species (Reames, 2021). Figure 1.1 below shows the structure of Plasmid DNA in *Escherichia coli.*

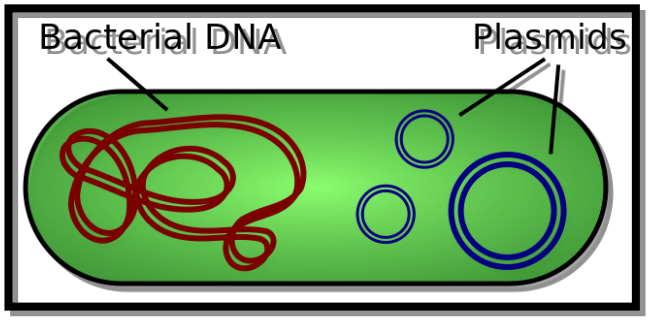


Figure 1.1 Plasmid DNA inside *E. coli*

Source: Rafael at el., 2021

In this project, the Plasmid DNA for pharmaceuticals applications were extract from bacteria *Escherichia coli* (*E. coli*). Bacteria *E. coli* was chosen because it is commonly used in the production of recombinant proteins and it production of recombinant proteins requires a high copy number plasmid in order to boost gene dosage (Cunningham et al., 2009).

Plasmid DNA plays a significant part in the pharmaceutical industry, and it is currently being evaluated as a viable vector alternative for gene therapies and vaccinations since it is safer, non-toxic and allows for larger inserts (Cunningham et al., 2009). For the non-viral approaches, plasmid DNA has emerged as a viable gene delivery vector due to its ease of genetic manipulation (Voß, 2007).

1.2 PURITY AND PROPERTIES OF PLASMID DNA

Purity of plasmid DNA was used in our production is 96%. Purity has decided based on our unit process and market value. General properties polyurethane is shown in Table 1.1.

Table 1.1 Properties of plasmid DNA

Properties	**Plasmid DNA**
Physical state	Liquid
Specific gravity	1
Appearance	Colorless liquid
Boiling point	$100°C$
pH	6.4

Source: Biotage, 2018

CHAPTER II

ECONOMIC ANALYSIS

2.1 SUPPLY AND DEMAND

The global plasmid market demand at 19.13 kilo tonnes in 2017 and is expected to grow at a compound annual growth rate (CAGR) of 23.8% from 2021 to 2030. The expected value of plasmid DNA demand in 2030 is 306.96 kilo tonnes. According to Precedence Research, the plasmid DNA manufacturing market size was valued at US$ 306.4 million in 2020. The plasmid DNA manufacturing market has witnessed an upsurge in the global biopharmaceutical industry. The growing adoption of plasmid DNA for developing vaccines and drugs for various diseases in the biopharmaceutical industry is significantly propelling the demand for the plasmid DNA. Furthermore, the growing adoption of gene therapy for the treatment of various infectious and genetic disorder has exponentially contributed towards the growth of the market in the recent years (plasmid DNA Market, 2021).

Plasmid DNA is also extensively used in applying gene therapy to diagnose diseases in patients, fuelling the demand for the gene therapy. Moreover, the growing prevalence of cancer is propelling the market growth. According to The International Agency for Research on Cancer (IARC), in 2020, the global cancer cases reached to 19.3 million and around 10 million deaths due to cancer had been recorded. Breast cancer is the dominant type of cancer with 24.5% of the total cancer cases. Hence, the growing adoption of gene therapy in the treatment of cancer is propelling the growth of the global plasmid DNA manufacturing market (plasmid DNA Market, 2021). Figure 2.1 show the demand and supply of plasmid DNA from 2017 until 2030.

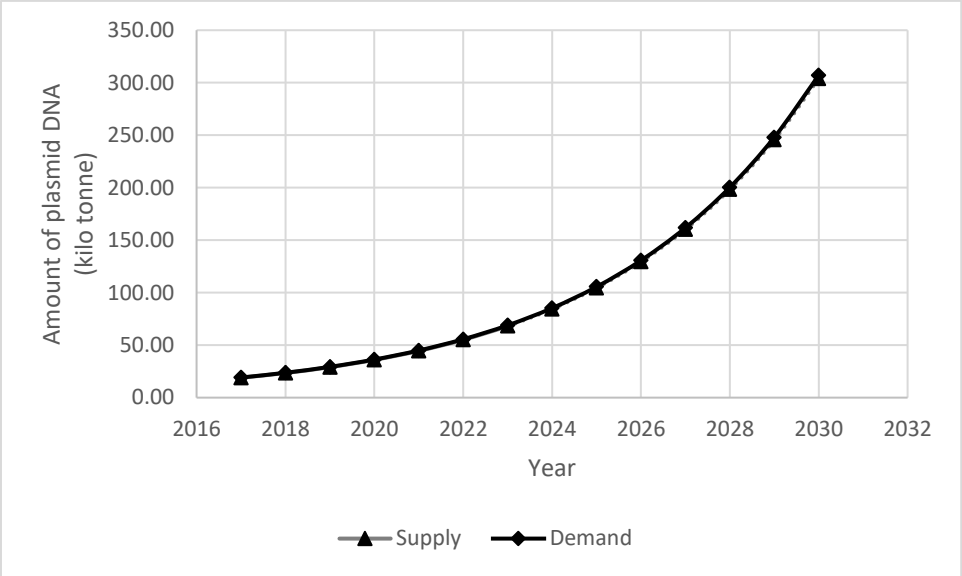


Figure 2.1 Graph of supply and demand of plasmid DNA from 2017 to 2030

Source: Coherent Market Insight, 2022

2.2 PLANT CAPACITY

Based on the Figure 1.1, the demand for plasmid DNA at 2030 is expected to be at 306.96 kilo tonnes per year, while the supply for that year is expected at 304 kilo tonnes per year. For our plasmid DNA production on the year 2030, we decided to fulfill for 2.5% of the deficiency which means we produced about 74 tonnes per year and equals to 8.94 kg per hour. The chemical plant will operate for 345 days with 20 days left for maintenance purposes.

Plant Capacity for year 2030:

Demand = 306 960 tonnes/ year

Supply = 304 000 tonnes/ year

Deficient = Demand – Supply
= 306 960 – 304 000
= 2 960 tonnes/ year

Production time = 345 days × 24 hours
= 8 280 hours

Our production fulfils 2.5% from the deficient.

Plant Capacity = 0.025 × 2 960
= 74 tonnes/year
= 74 000 kg/year
= 74 000 ÷ 8 280
= 8.94 kg/ hour

2.3 LIST OF COMPANY

Nowadays there are so many companies that produce the Plasmid DNA (pDNA) around the world especially north America and Europe. Because of development in pharmaceutical industry and research its promising market potential, so that the company keep produce the pDNA because of it benefits towards humanity. Table 2.1 shows top or major companies operating in the global Plasmid DNA manufacturing market.

Table 2.1 List of the major company produce pDNA

Company	Location	Capacity, kg
Lonza	Pennsylvania, USA, Basel, Switzerland	2000
Advanced BioScience Laboratories. Inc	Maryland, U.S. A	4500
Novasep Holding S.A. S	Leverkusen, Germany	3540
Cobra Biologics	Northern Sweden	1000
BioReliance	Glasgow, U.K	1000

Source: DataIntelo, 2020

2.4 PRICE OF PLASMID DNA

Plasmid DNA has been affected by recent logistical challenges, which have limited supply and as a result, driven up prices. Key factors that are driving the market and

price growth include government restrictions, lockdowns and factories being shut-down because of Covid-19 (Mordor Intelligence, 2021). Then, the market price for plasmid DNA is $36,000 per kilogram (Biocat Expert, 2022). Assumption has been considered that the market price for plasmid DNA will be same all around the world which is match to the industrial standards. Figure 2.2 below shows the price of plasmid DNA in year 2017.

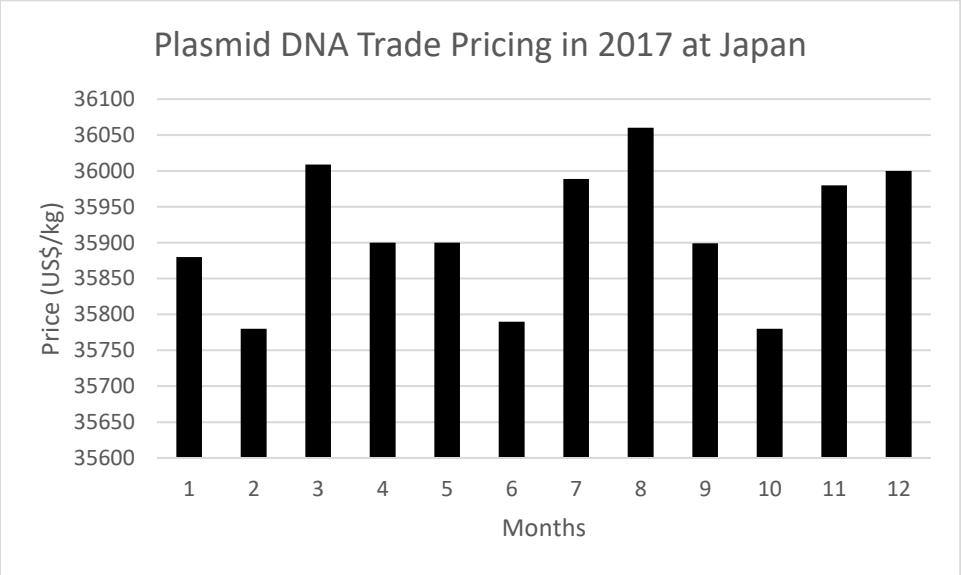


Figure 2.2 Price of Plasmid DNA in 2017 at Japan

Source: Prudour, 2019

Currency rate: 1 US dollar = 4.23 Malaysian Ringgit (April 14, 2022)

2.5 FUTURE MARKET POTENTIAL

The global plasmid DNA manufacturing market is estimated to grow within the estimated period, the Market size in 2021 is USD 635.1 million and expected to reach USD 2733.8 million with exhibit a 23.3% CAGR by 2028 (Medgadget, 2022). Nowadays many companies produce to fulfil the demand.

The key factor or the main issue for increasing the demand and production of plasmid DNA is rising number of gene therapy-based discovery in pharmaceutical industry. The keys trends lead to production of these plasmid DNA or gene therapy. The application of the Plasmid DNA also the main factor for market potential because

of its advantages in Pharmaceutical for diseases like infectious disease, Genetic disorder, and cancer. Besides that, it also applies in Gene therapy, DNA Vaccines, immunotherapy, gene cloning and Nanoparticles (Stratview Research, 2021). So, it has been produced in the large number because of its advantages in pharmaceutical use.

Other than that, among the region North America will dominate the worldwide pDNA manufacturing Market by 2024 because of the increasing research and development for pDNA application. It expects significant growth in market that specialize in expanding the facilities. The COVID-19 outbreak is positively impacting the pDNA manufacturing market, much research is using the gene and plasmid DNA for study the virus. So that, the market expected to growth to 2344.29 million by 2027, registering CAGR of 23.38% during 2022-2027 (and, 2022). In North America, Asia-Pacific and Europe are promising future market because of increasing technology in Pharmaceutical and biotechnology.

CHAPTER III

ENVIRONMENTAL AND SAFETY ISSUES

3.1 INTRODUCTION

The environmental and safety aspects of biochemical process production are critical characteristics that must be clarified. This chapter will focus on the environmental aspects and project's safety. This chapter will go over the physical properties of all compounds as well as the recommended storage methods. Then, it will be discussed about the waste generated by this plant and how it should be handled legally.

3.2 ENVIRONMENTAL ACT

To keep the ecosystem and environment in great shape, Malaysia has regulations in place to prevent pollution, preserve the environment, and ensure environmental quality. The Ministry of Energy, Science, Technology, and Climate Change oversees the Department of Environment that responsible for pollution prevention and control, as well as industrial waste management in Malaysia. Department of Environment has done so by establishing rules and regulations, as well as encouraging industry to invest more in greener technologies which can minimize hazardous waste. The following are some of the environmental laws in Malaysia that are mentioned in the Environmental Quality Act 1974:

1. Environmental Quality (Licensing) Regulations 1977

2. Environmental Quality (Industrial Effluents) Regulations 2009

3. Environmental Quality (Clean Air) Regulations 2014.

All the acts mentioned can improve environmental quality, limit pollution from all sources, and prevent the establishment or operation of any industrial facility on environmental grounds.

3.3 WASTE GENERATION

In the production of Plasmid DNA (pDNA) from *E. coli*, there will be a few by-products that known as waste will be generated. The waste product generated may be harmful to the environment, so the waste product needs to be treated first before it can be released to the surrounding. Figure 3.1 shows the waste generated by the plant.

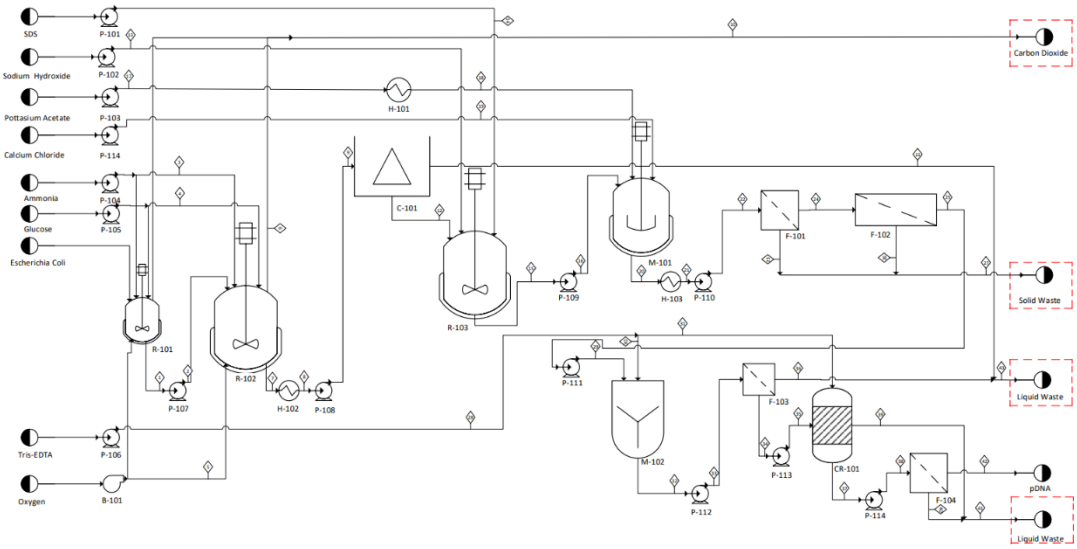


Figure 3.1 Waste generated by plant

The waste product that will be generated in the production of pDNA are liquid waste, solid waste and carbon dioxide gas. Table 3.1 below shows the waste generation in the production process.

Table 3.1 Waste generation from Extraction plasmid DNA from *E. coli*

Waste	Waste generation
Solid waste	pDNA is internal product of the cell, so that after cell disruption another component of the cell need to be separated. To separate the pDNA and other component of the cell, precipitation using potassium acetate and calcium chloride are used to precipitated gDNA, protein and RNA. These solid are separated from the mixture using filter (F-101).
Liquid waste	Liquid waste in these processes containing chemical and buffer such as

	potassium acetate, calcium chloride that used in precipitation process, Tris-EDTA buffer to maintain the pH to avoid degradation of pDNA water and acetate acid that produce during fermentation. These wastes were generated during purification processes which is ultrafiltration (F-103) and chromatography (CR-101).
Carbon dioxide	During fermentation in Fermenter (R-102) carbon dioxide has been produce during aerobic condition of growth the *E. coli.* Because of the small amount of CO_2 produced, it will directly be released to environment because it will not give negative impact to environment.

3.4 WASTE MANAGEMENT

The waste that produced during production of Plasmid DNA need to be treated after the production took place. Table 3.2 below shows the waste that produce during the processes and the treatment for waste generated during the processes.

Table 3.2 Treatment for waste from Extraction of Plasmid DNA

Waste	Treatment
Solid waste	The small amount of solid waste which is the cell component of the *E. coli* will be directly dispose because no negative impact and no toxic to environment and other organism.
Liquid waste	From this production, the liquid waste can be treated by using same method of wastewater treatment which is contain 3 steps. First step is primary treatment which is used to remove solid contain in the wastewater. The sedimentation of solid contain in the wastewater is the first step and this is done after the wastewater has been filtered to remove bigger solid contain (Bloganica, 2017). Second step, Secondary treatment is meant to significantly degrade the biological component of the wastewater through aerobic biological processes (Arvia Technology, 2018). The process is done in three ways, which is biofiltration, aeration and oxidation ponds. Lastly, the tertiary treatment is the last step before the water can be discharge. It is basically to increase the quality of the water to achieve the domestic and industrial standards (Arvia Technology, 2018).
Carbon dioxide	Carbon dioxide from fermentation is directly to be released to environment because it will not give negative impact to environment.

3.5 SAFETY ISSUES

Table 3.3 shows few of safety issues such as first aid measures and protective equipment on raw materials and product that we must considered. This is to ensure the risks in workplace can be minimized so that employers and workers can be safe at their workplace and activities at the workplace can be done at ease.

Table 3.3 Safety Issues on raw materials and products

Components	First Aid Measures	Protective Equipment	References
E. Coli	▪ Eye contact: Immediately flush eyes with water for at least 15 minutes ▪ Skin contact: Wash skin with soap and water.	▪ Wear gloves if skin contact possible. Wear protective clothing such as lab coats, uniforms, disposable overalls and etc	Material Safety Data Sheet
Ammonia	▪ Inhalation: Move exposed individual to fresh air. Loosen clothing as necessary ▪ Skin contact: Wash skin with soap and water.	▪ Use suitable respiratory protective device when high concentration involved. The glove material has to be impermeable and resistant to substance.	Material Safety Data Sheet
Sodium Hydroxide	▪ Skin contact: Wipe off dry product from skin. Remove clothing before washing. ▪ Eye contact: Rinse immediately with plenty of water for 15 minutes.	▪ Should wear good resistance protective clothing such as rubber, neoprene and nitrile rubber. Wear a face shield and in case of dust production, wear protective googles.	Material Safety Data Sheet
Calcium Chloride	▪ Inhalation: Provide fresh air to exposed individual ▪ Skin contact: Rinse skin with water	▪ Respiratory protection is necessary if there is dust formation. Wear proper protective clothing to avoid skin contact.	Material Safety Data Sheet
Potassium Acetate	▪ Eye contact: Rinse immediately with plenty of water for 15 minutes. ▪ Skin contact: wash off immediately with plenty of water for at least 15 minutes	▪ Wear suitable protective eyeglasses or chemical safety googles. Wear approriate protective gloves and clothing to avoid skin contact.	Material Safety Data Sheet
Glucose	▪ Skin contact: wash off immediately with plenty of water for at least 15 minutes ▪ Inhalation: Remove to fresh air	▪ Wear appropriate protective eyeglasses and chemical safety googles. Wear suitable protective gloves and clothing to prevent skin exposure.	Material Safety Data Sheet
Oxygen	▪ Inhalation: Remove to fresh air ▪ Eye contact: Immediately rinse with plenty of water for 15 minutes	▪ Safety eyewear with an approved standard shoul be used to avoid eye exposure. Chemical resistant gloves should be worn at all times when handling chemical.	Material Safety Data Sheet
Plasmid DNA	▪ Inhalation: Move to fresh air ▪ Skin contact: Wash off immediately with plenty of water for at least 15 minutes	▪ Wear appropriate protective gloves and clothing to avoid skin exposure. Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149.	Material Safety Data Sheet
SDS	▪ Skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower. ▪ Eye contact: rinse out with plenty of water. Immediately call-in ophthalmologist. Remove contact lenses.	▪ Change contaminated clothing as soon as possible. ▪ Apply sunscreen to your skin as a preventative measure. ▪ After working with the material, wash your hands and face. ▪ Use eye protection that has been tested and approved according to regulatory regulations. ▪ When dust is created,	Material Safety Data Sheet

		respiratory protection is essential.	

3.6 MATERIAL HANDLING AND STORAGE

The most crucial step in handling raw materials and products in the manufacturing of plasmid DNA is to use comprehensive personal protection equipment such as gloves, safety glasses, and protective clothes to avoid contact with the skin and eyes, as shown in Table 3.4. Ensure that all materials, whether raw or finished, are stored in a firmly closed container.

Table 3.4 Method of Handling and Storage of the raw materials and products

Components	Handling Procedure	Storage Requirement	References
E. Coli	▪ Use suitable personal protection equipment when handling. ▪ Use with caution and plenty of ventilation. ▪ Avoid coming into touch with your eyes, skin, or clothing.	▪ Store under refrigeration in closed container. The suitable temperature: 2 - 7°C ▪ This material can be denatured or inactivated by a variety of organic solvents, salts or heavy metals	Material Safety Data Sheet
Ammonia	▪ Hands should be washed after handling. ▪ When handling chemical materials, use adequate hygiene procedures. ▪ When working with chemical chemicals, do not eat, drink, smoke, or use personal care items.	▪ Avoid storing anything near sources of high heat, ignition, or an open flame. ▪ Keep it away from food. ▪ Keep out of the reach of oxidising agents. ▪ Store in well-sealed containers in a cold, dry environment.	Material Safety Data Sheet
Sodium Hydroxide	▪ Avoid allowing the material to come into touch with water. ▪ Carry out operations outside, with local exhaust/ventilation, or with respiratory protection. ▪ Remove any contaminated clothing as soon as possible. ▪ Remove contaminated garments and wash it.	▪ Store in a cool, dry place. ▪ Keep the container out of direct sunlight and in a well-ventilated area. ▪ Keep everything locked up. ▪ Keep at room temperature. ▪ Only use the container that came with it.	Material Safety Data Sheet
Calcium Chloride	▪ Dust should be avoided at all costs. ▪ Removal of dust accumulations. ▪ Hands should be washed before and after work breaks. ▪ Keep a safe distance from food, water, and animal feed.	▪ Store in a cool, dry location. Keep the container tightly shut. ▪ Needs for ventilation: Make use of both local and overall ventilation. ▪ 15–25 °C is the recommended storage temperature.	Material Safety Data Sheet
Potassium	▪ Wear PPE (personal	▪ Containers should be kept	Material Safety

Acetate	protective equipment) and face shields. ▪ Make sure you have enough ventilation. ▪ Avoid coming into touch with your skin, eyes, or clothes.	tightly closed in a dry, cold, and well-ventilated location. ▪ Incompatible Materials: Oxidizing agents have a strong oxidation process.	Data Sheet
Glucose	▪ Wear PPE (personal protective equipment) and face shields. ▪ Make sure you have enough ventilation. ▪ Avoid coming into touch with your skin, eyes, or clothes. ▪ Moisture should be avoided.	▪ Containers should be kept tightly closed in a dry, cold, and well-ventilated location. ▪ Incompatible Materials: Oxidizing agents have a strong oxidation process.	Material Safety Data Sheet
Oxygen	▪ Make sure you're wearing the right safety gear. ▪ Gas is held under pressure in this container. ▪ Take care not to inhale any gas. ▪ Container should not be punctured or incinerated. ▪ After each usage and when the container is empty, close the valve.	▪ Keep it away from incompatible materials and out of direct sunlight ▪ Temperatures in the cylinder should not exceed 52 ° C. (125 °F). ▪ Separate from flammable items and reducing agents. ▪ Keep grease and oil at a distance.	Material Safety Data Sheet
Plasmid DNA	▪ Make sure you have enough ventilation. ▪ Personal protection equipment should be worn. ▪ Avoid coming into touch with your skin, eyes, or clothing.	▪ In the long run. Keep the container well closed in a dry, cold location. ▪ Maintain a temperature of 4°C.	Material Safety Data Sheet
SDS	▪ Work in fume hood. Do not inhale any of the chemical or mixture. ▪ Keep a safe distance from open flames, hot surfaces, and ignition sources. Prevent static discharge by taking precautions. ▪ Change contaminated clothing as soon as possible. ▪ Apply sunscreen to your skin as a preventative measure. ▪ After working with the material, wash your hands and face.	▪ Closed tightly. Keep away from heat and ignition sources. ▪ 4.1B: Flammable solid hazardous materials, according to TRGS 510.	Material Safety Data Sheet

CHAPTER IV

PROCESS DESCRIPTION

4.1 INTRODUCTION

Escherichia coli (E. coli) is commonly employed in batch or fed batch plasmid DNA (pDNA) manufacturing using complicated or specified media. It is feasible to obtain very high cell densities of bacteria using fed-batch (*E. coli*). The bacteria are pelleted and resuspended in a resuspension buffer (Tris EDTA), which aids in DNA denaturement and contains EDTA, which binds to divalent cations, causing membrane destabilization and blocking DNases (enzymes degrade DNA). RNases are also used to digest the RNA that has been released. Centrifugation is used to harvest the E. coli cells, which are then broken to release the intracellular pDNA content (Voß, 2007). The density of the cells was accomplished in this experiment using aerobic fermentation, which used glucose as the carbon source, aqueous ammonia as the nitrogen source, and air as the oxygen source and other nutrient such as Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, ammonium sulfate and magnesium sulfate (Voß, 2007). The process to extract pDNA is complex especially for separation or purification step to achieved high purity product.

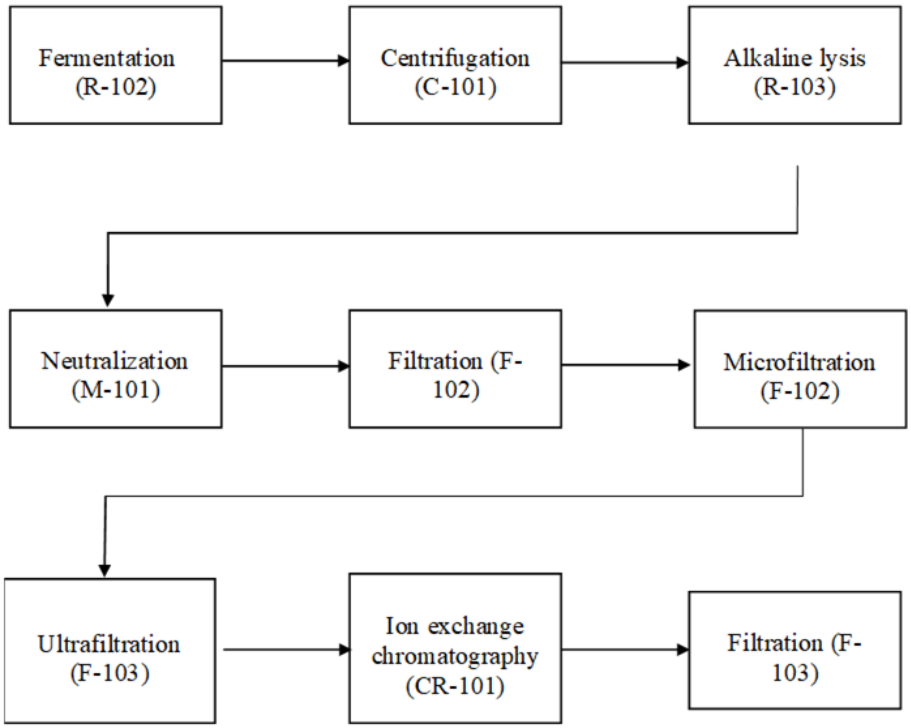


Figure 4.1 Simplified process diagram in block diagram

4.1.1 Fermentation (R-102)

E. coli is cultured in a fermenter with three stages of seeds fermenter at the start of the procedure. The first step is to grow the E. coli colonies in a flask with a small capacity (5 ml) (de la Cruz et al., 2020). Two seed fermenters are used before the main fermenter is used. Glucose is used as a carbon source, air is used as an oxygen source, and ammonia solution is used as a nitrogen source in the fermentation medium. By adding aqueous ammonia to the fermentation process, it is carried out aerobically at 37°C and pH 7. In addition, the medium is added to the main fermenter to achieve the appropriate cell density on an industrial scale (Borja et al., 2012). An antibiotic is supplied to each fermenter in addition to batch medium to ensure plasmid maintenance throughout the process. This fermentation takes roughly 12 hours.in the shake flask, 30 h in seed fermenter and 40 h in main fermenter (Demetri Petrides at al. 2021). Stoichiometric equation shows in … (4.1).

$$C_6H_{12}O_6 + 0.8801O_2 + 0.1038NH_3 \longrightarrow 0.4325CH_{1.77}O_{0.49}N_{0.24} + 0.8877CO_2 + 2.3399C_2H_4O_2 + 1.093H_2O \quad \ldots (4.1)$$

4.1.2 Centrifugation (C-101)

For primary recovery, the first step is the harvest of *E. coli* cell by centrifugation. The fermented broth undergoes centrifugation to separate biomass and the liquid waste which is acetate acid, water, and unreacted reactant from fermentation to ensure 97% of biomass is recovered in the bottom stream. Disc type centrifuge were used in this process. This takes the place of the gravitational force that is normally necessary for settling. As a result, sedimentation can be accomplished in less time. The broth is fed into centrifuge at 30 ℃ and 1 bar and operates at 14000 rpm. This process operates for 1 hour with efficiency 90%.

4.1.3 Cell disruption through Alkaline lysis

Alkaline lysis is utilized instead of mechanical methods in cell disruption since plasmid and genomic DNA are sensitive to shear stress and mechanical methods are not preferred. The cell suspension is gently mixed with a strong alkali, sodium hydroxide 0.2 M, and a detergent, sodium dodecyl sulphate (SDS) 1 percent w/w, to complete the procedure (Eon-Duval et al., 2003). This is done in CSTR for large-scale alkaline lysis to preserve the purity of plasmid DNA while avoiding fragmentation of gDNA. It's critical to mix thoroughly during lysis to avoid pH extremes that could cause pDNA denaturation. This alkaline lysis takes 10 minutes in CSTR at room temperature (25°C). The SDS detergent dissolves the membrane's phospholipids and proteins, causing cell lysis and release the cell contents. The high cell concentration of sodium hydroxide denatures the genomic and pDNA as well as cellular protein. The cellular DNA becomes linearized, and the strands are separated whereas plasmid DNA is circular and remains topologically constrained (Demetri Petrides at al. 2021). In this condition, almost all proteins are denatured, supercoiled pDNA remains in structure and not denatured. Equation …(4.2) is the stochiometric equation for plasmid DNA production.

$$100\ \text{Biomass} \longrightarrow 30\ \text{cell debris} + 3\ \text{genomic DNA} + 4\ \text{plasmid DNA} + 40\ \text{Proteins} + 23\ \text{RNA} \quad \ldots(4.2)$$

4.1.4 Neutralization and Calcium Chloride precipitation (M-101)

The precipitation methods are employed to precipitate the gDNA, proteins, and big RNA molecules neutralizing process is performed to remove other cell components. A 3 M potassium acetate (pH 5.5), prechilled at 4°C, is added to the mixture from alkaline lysis using a mixing approach with mixer, with the process taking 10 minutes at 4°C. To counteract the severe alkaline conditions, potassium acetate is added, resulting in a high salt concentration and a precipitate (white) containing SDS and proteins. Furthermore, this procedure enables for DNA renaturation. Denatured protein and chromosomal DNA do not return to their original form as a result, making these molecules soluble. Precipitate captures large chromosomal DNA, and the small plasmid DNA remains in solution (Carsten, 2007). Reaction below:

gDNA = gDNA(s)	90% conversion
Proteins = Proteins(s)	80% conversion
RNA = RNA(s)	20% conversion

Following potassium acetate precipitation, a solution of calcium chloride (5 M) is added to the mixture via another stream to precipitate most of the residual RNA, gDNA, and protein, leaving just pDNA. This procedure takes place in the same CSTR as Neutralization, with conversion rates of 98 percent, 96 percent, and 85 percent, respectively. To remove the solid, the pDNA mixture generated from alkaline lysis, neutralization, and calcium chloride precipitation will be separated.

4.1.5 Filtration

In this part, the mixture from the previous process is sent to a two-step filtration to remove its high loads of solid to achieve high yield of pDNA.

i. Filtration

The mixture will be sent to filtration process to remove the solid precipitate such as RNA, Protein, gDNA and chromosomal DNA. The soluble plasmid DNA can be purified from the solution with this process.

ii. Microfiltration

The last step of filtration is using microfilter. It has had ability to separates molecules of difference sizes which is pore size 0.02 to 10 μm. So that the remaining impurities can be removed to achieve the high concentration of pDNA. Microfilter can remove 90 to 100% undesired product.

4.1.6 Purification

i. Ultrafiltration (UF) (F-103)

The principal contaminant in solution, RNA, did not precipitate after the primary recovery process. Ultrafiltration was used to extract significant amounts of RNA. The purifying process begins with this phase. The pDNA is filtered via a UF membrane with a molecular weight cutoff of 100 kDa and a filtration area of around 1 m2/g (Demetri Petrides at al. 2021). The plasmid molecules are retained by this membrane, while smaller RNA molecules, proteins, and other contaminants are allowed to flow through.

ii. Chromatography (AEC) (CR-101)

Anion Exchange Chromatography is used to continue the purifying process. Anion exchange chromatography is a technique for sorting molecules based on their charge. During operation, a binding capacity of 10 g/L was specified, and the column retained 90 percent of the pDNA and 5% of the RNA. At 30 °C and 1 bar, the pDNA solution from UF was fed into AEC. AEC has an efficiency of around 82 percent and runs for 1 hour. The buffer is used to wash the column (Tris-EDTA with Nacl 0.5 m). The column is eluted with a buffer gradient that starts at 0.5 M Nacl and

increases to 1.0 M Nacl, releasing 100% of the bound pDNA in the process (Demetri Petrides at al. 2021).

4.1.7 Filtration (F-104)

The final product which is pDNA in buffer elution is then filter using a single-use normal filter

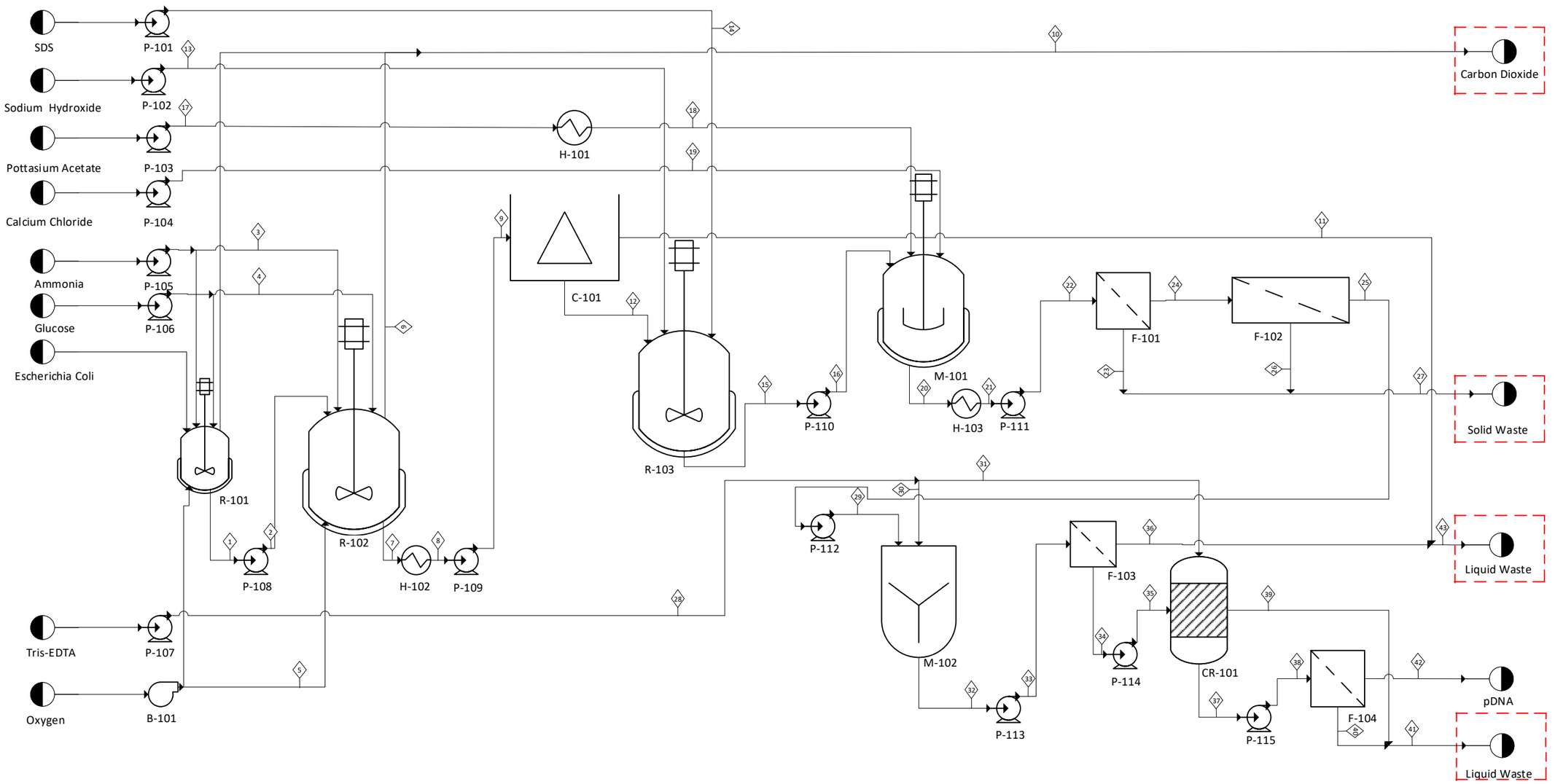
4.2 RAW MATERIAL SELECTION

Our raw materials in this plant are *Escherichia coli* (*E. coli),* and glucose. *E. coli* is a favoured host for gene cloning because of the remarkable efficiency with which DNA molecules are introduced into cells. It had a quick growth and capacity to express proteins at very high levels, E. coli is a desirable host for protein synthesis (Gheorghe M Borja et al, 2012).

4.3 PROCESS FLOW DIAGRAM

Process flow diagram will be shown in the following page.

P-101 Pump 1	P-105 Pump 5	R-101 Reactor 1	P-109 Pump 9	P-111 Pump 11	M-101 Mixer 1	P-113 Pump 13	H-102 STHE 2
P-102 Pump 2	P-106 Pump 6	P-107 Pump 7	H-101 STHE 1	C-101 Centrifuge 1	F-102 Filter 2	F-103 Filter 3	P-114 Pump 14
P-103 Pump 3	B-101 Blower 1	R-102 Reactor 2	R-103 Reactor 3	F-101 Filter 1	P-115 Pump 15	CR-101 Chromatography	H-103 STHE 3
P-104 Pump 4	P-108 Pump 8	P-110 Pump 10	P-112 Pump 12	M-102 Mixer 2	F-104 Filter 4	C-102 Centrifuge 2	



Stream	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
Temperature ©	25	25	25	25	25	37	37	30	30	37	30	30	25	25	25	25	25	4	25	4	30	30	30	30	30	30	30	25	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30
Pressure (bar)	1.0	2.0	2.0	2.0	2.0	1.2	1.0	1.0	2.0	1.2	1.0	1.0	2.0	2.0	1.0	2.0	2.0	2.0	2.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	2.0	2.0	2.0	2.0	1.0	2.0	1.0	2.0	1.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0
Phase	L	L	L	L	G	G	L	L	L	G	L	S	L	L	L	L	L	L	L	S/L	S/L	S/L	S	L	L	S	S	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L	L
SDS(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	0	70.61	70.61	70.61	0	0	0	70.61	70.61	70.61	70.61	0	0	0	70.61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sodium Hydroxide(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	70.61	0	70.61	70.61	0	0	0	70.61	70.61	70.61	0	70.61	7.01	63.6	63.6	0	7.01	0	0	7.01	7.01	7.01	7.01	3.98	1.21	1.21	5.8	1.14	6.94	0.12	3.98
Potassium Acetate(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	70.67	70.67	0	70.67	70.67	70.67	63.6	7.07	0.71	6.36	69.98	0	0.71	0	0	0.71	0.71	0.71	0.71	0.51	0	0	0.71	0	0.71	0	0.51
Calcium Chloride(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	70.67	70.67	70.67	63.6	7.07	0.71	6.36	69.98	0	0.71	0	0	0.71	0.71	0.71	0.71	0.51	0	0	0.71	0	0.71	0	17.73
Ammonia(kg/h)	0	0	96.29	0	0	0	87.28	87.28	87.28	0	87.28	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	87.28
Glucose(kg/h)	0	0	0	950.0	0	0	161.51	161.51	161.51	0	159.89	1.61	0	0	1.61	1.61	0	0	0	1.61	1.61	1.61	0	1.61	0.16	1.45	1.45	0	0.16	0	0	0.13	0.13	0.16	0.16	0.12	0.03	0.03	0.13	0	0.13	0.03	87.39
Escherichia Coli(kg/h)	47.50	47.50	0	0	0	0	145.77	145.77	145.77	0	4.37	141.4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.37
Tris-EDTA(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.77	0	2.88	2.88	2.88	2.88	2.88	2.88	2.88	5.77	5.77	0	5.77	5.77	0	2.88
Oxygen(kg/h)	0	0	0	0	142.51	18.96	0	0	0	18.96	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Carbon Dioxide(kg/h)	0	0	0	0	0	172.34	0	0	0	172.34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Acetic Acid(kg/h)	0	0	0	0	0	0	512.07	512.07	512.07	0	506.95	5.12	0	0	5.12	5.12	0	0	0	5.12	5.12	5.12	0	5.12	0.51	4.61	4.61	0	0.51	0	0	0.51	0.51	0.51	0.51	0.37	0	0	0.51	0	0.51	0	507.32
Water(kg/h)	0	0	0	0	0	0	138.15	138.15	138.15	0	137.03	1.38	0	0	1.38	1.38	0	0	0	1.38	1.38	1.38	0	1.38	0.14	1.24	1.24	0	0.14	0	0	0.14	0.14	0.14	0.14	0	0.14	0.14	0	0	0	0.14	137.03
Proteins(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	53.52	53.52	0	0	0	53.53	53.53	53.53	53.1	0.43	0.04	0.39	53.48	0	0.04	0	0	0.04	0.04	0.03	0.03	0.04	0	0	0	0	0	0.03	0.04
Cell debris(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	41.05	41.05	0	0	0	41.05	41.05	41.05	41.05	0	0	0	41.05	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
gDNA(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5.69	5.69	0	0	0	5.69	5.69	5.69	5.64	0.05	0.05	0	5.64	0	0.05	0	0	0.05	0.05	0	0	0.05	0	0	0	0	0	0	0	0.05
RNA(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	32.47	32.47	0	0	0	32.47	32.47	32.47	28.61	3.86	0.35	3.51	32.12	0	0.35	0	0	0.35	0.35	0.04	0.04	0.31	0.04	0.04	0	0	0	0.04	0.31
pDNA(kg/h)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8.66	8.66	0	0	0	8.66	8.66	8.66	0	0	0	0	0	0	8.66	0	0	8.66	8.66	8.66	8.66	0	8.66	8.66	0	0	0	8.66	0
Total Flowrate(kg/h)	47.50	47.50	96.29	950.0	142.51	191.30	1045.03	1045.03	1045.03	191.3	895.52	149.52	70.61	70.61	290.74	290.74	70.67	70.67	70.67	432.08	423.08	432.08	326.21	105.87	18.34	87.53	413.77	5.77	18.34	2.88	2.88	21.23	21.23	20.82	20.82	8.77	15.85	15.85	7.85	6.91	14.77	8.94	848.9

UNIVERSITI KEBANGSAAN MALAYSIA
The National University of Malaysia

Title: Production of Plasmid DNA From Escherichia coli

Lecturers:

KKKR3583
Prof. Madya Ts. En. Noorhisham Tan Kofli
Ts. Dr. Ahmad Razi Othman

KKKR3733
Prof. Madya Ts. En. Noorhisham Tan Kofli
Ts. Dr. Darman Nordin
Prof. Madya Ir. Dr. Hassimi Abu Hassan
Prof. Madya Ts. Dr. Rosiah Rohani

KKKR3723
Prof. Madya Ir. Dr. Shuhaida Harun
Prof. Madya Ir. Dr. Teow Yeit Haan

KKKR3713
Prof. Madya Dr. Norliza Abd. Rahman
Dr. Jarinah Mohd Ali

Group: KK1

Group Members:

1. Muhammad Arif Danial Bin Hassan (A173470)

2. Azrul Zulhilmi Bin Ahmad Rosli (A173752)

3. Nuqman Haziq Bin Mohd Ayub Khan (A174188)

4. Ahmad Nawwar Irsyad Bin Moham (A174340)

Input

OutputStream number

Date : 19th May 2022

4.4 EQUIPMENT SELECTION

4.4.1 Type of Reactor

In this production, *E. coli* growth in large stainless-steel bioreactors (Fermenter). All the necessary components for biological process extraction are provided by a fermenter or bioreactor. It has several gadgets that aid in the preservation of the internal environment, which leads to the manufacturing of the product. Heat can be produced during fermentation via biological reactions and agitation. By withdrawing or giving heat, a temperature control aids in maintaining a comfortable temperature. The impeller in the fermenter is utilised for bulk fluid and gas mixing, air dispersion, heat transfer, oxygen transfer, and solid panicle suspension, as well as maintaining the vessel's condition. Other than that, fermenter is design with pH control which contain pH sensor and a port to maintain the pH (Somak Banerjee, 2021).

4.4.2 Bioreactor design equation and sizing

The general balance equation,

$$\left[\begin{array}{c}Molar\ flowrate\\ of\ A\ into\\ the\ system\\ \left(\frac{moles}{time}\right)\end{array}\right]-\left[\begin{array}{c}Molar\ flowrate\\ of\ A\ out\ of\\ the\ system\\ \left(\frac{moles}{time}\right)\end{array}\right]+\left[\begin{array}{c}Molar\ generation\\ of\ A\ by\ chemical\\ reaction\ within\\ the\ system\\ \left(\frac{moles}{time}\right)\end{array}\right]=\left[\begin{array}{c}Molar\ \ accumulation\\ of\ A\ within\\ the\ system\\ \left(\frac{moles}{time}\right)\end{array}\right]$$

In + Out + Generation = Accumulation

$$F_{AO} - F_A + G_A = \frac{dN_A}{dt}$$

$$G_A = r_A \times V$$

$$G_A = \sum_{i=1}^{M} \Delta G_{Ai} = \sum_{i=1}^{M} r_{Ai} \Delta V_i$$

$$G_A = \int_0^V r_A dV$$

$$F_{AO} - F_A + \int_0^V r_A dV = \frac{dN_A}{dt}$$

For batch reactor (fermenter) (R-102)

Assumptions:

1. No molar flowrate involve.

$$F_{AO} = F_A = 0$$

2. There are no spatial variations in the rate of reaction which is perfectly mix

$$\int_0^V r_A dV = r_A V$$

Thus,

$$r_A V = \frac{dN_A}{dt}$$

The conversion of X is a function of the time the reactants spend in the reactor.

In terms of conversion,

$$X = \frac{mole\ of\ A\ reacted}{mole\ of\ A\ fed}$$

$$\begin{bmatrix} Moles\ of\ A \\ that\ has\ been\ consumed \end{bmatrix} = \begin{bmatrix} Moles\ of \\ A\ fed \end{bmatrix} \begin{bmatrix} \frac{mole\ of\ A\ reacted}{mole\ of\ A\ fed} \end{bmatrix}$$

$$= [N_{AO}][X]$$

At time, t:

$$\begin{bmatrix} Moles\ of\ A \\ in\ reactor\ at\ time, t \end{bmatrix} = \begin{bmatrix} Moles\ of\ A\ initially \\ fed\ to\ reactor\ at\ time, t = 0 \end{bmatrix} - \begin{bmatrix} mole\ of\ A\ that\ has \\ been\ consumed \end{bmatrix}$$

$$N_A = N_{AO} - (N_{AO} \times X)$$

Rearrange,

$$N_A = N_{AO}(1 - X)$$

Substituting with equation 1,

$$N_{AO}\frac{dX}{dt} = -r_A V$$

$$dt = N_{AO}\frac{dX}{-r_A V}$$

$$t = N_{AO}\int_0^X \frac{dX}{-r_A V}$$

Wheres,

t = time

rA = the rate of formation of species per unit volume

V = the volume of the reactor

N_{AO}= Moles of A fed

X= Conversion

4.5 PHYSICAL AND CHEMICAL PROPERTIES

4.5.1 Properties of culture Medium

The medium used in this production in Glucose for CO_2 source, ammonia as Nitrogen source and air as Oxygen source. Other nutrient such as Potassium dihydrogen phosphate, dipotassium hydrogen phosphate, ammonium sulfate. This medium will supply nutrient to *E. coli* to gives high yield production. The properties of medium shown in Table 4.1.

Table 4.1 Physical and chemical properties of Medium

Properties	Glucose	Ammonia solution	Potassium dihydrogen phosphate	Dipotassium hydrogen phosphate	Ammonia sulfate
Molecular formula	$C_6H_{12}O_6$	$NH_3(H_2O)$	KH_2PO_4	K_2HPO_4	$(NH_4)_2SO_4$
Appearance	Crystaline	Colourless	White powder	deliquescent	Crystal
State	Solid	Liquid	Solid	Solid	Solid
Boiling point	527.1 °C ± 50°C	37.7 °C	252.6 °C	158 °C	330°C
Melting point	146 °C	-57.5 °C	400 °C	> 465 °C	235 to 280 °C
pH	5.9	11-12	8	9.2(H_2O)	5.5
Solubility in water	Soluble in water	Miscible	22.6 g/100 mL (20 °C)	Soluble in water	Soluble in water

Source: Admin, 2019

4.5.2 Properties of the Product

Purity of plasmid DNA was used in our production is 80%. Purity has decided based on our unit process and market value. General properties plasmid DNA is shown in Table 4.2.

Table 4.2 Properties of main Product

Properties	Plasmid DNA
State	Liquid
Appearance	Colourless
Specific gravity	1
Boiling point	100°C
pH	6.4

Source: Biotage, 2018

4.5.3 Properties of by product

Table 4.3 Physical and chemical properties of by products

Properties	Carbon dioxide	Acetate acid
Formula	CO2	$C_2H_4O_2$
State	Gas	liquid
Appearance	Colourless	Colourless
Melting point	-56.6°C	16.6°C
Boiling point	-78.5°C	118°C
pH	3.7	2.87

Source: Admin, 2018

CHAPTER V

MATERIAL AND ENERGY BALANCE

5.1 INTRODUCTION

Mass balance is known as a material balance and frequently used in chemical engineer. The basic of the law of conservation of mass, which states that mass can neither be created nor destroyed. A close system's mass will remain constant; hence the output value must be the same as the input value. It's critical to calculate mass balance to ensure that no mass is lost throughout the procedure (Himmelblau,1967). Statements based on the law of conservation of mass such as $\sum mass_{input} = \sum mass_{output}$ are examples of material balance. Energy balance is used to decide whether heat is released or absorbed in a chemical reaction.

The plant capacity for Plasmid DNA (pDNA) production by *Escherichia coli* (*E. coli*) per year, as calculated in Chapter II, is 309.93 kg/hr. Glucose, ammonia aques and oxygen was used as feed material. However, a seed train consisting of three growth phases is used to provide enough inoculum for plasmid fermentation. The first stage is carried out in shakes flasks in batch mode for 12 hours. The second stage is done in fed-batch mode, with two 14-liter fermenters staggered for 30 hours. In two 140 L staggered fermenters, the third phase is carried out in fed-batch mode for 40 hours. (Da & Petrides, 2021).

Plasmid DNA as main product while acetic acid, water, and carbon dioxide as by-products of the reaction. Then, the fermentation broth will undergo several downstream processes to produce high purity and yield of pDNA. The downstream process that involved are centrifugation, microfiltration, ultrafiltration (UF)/ dialfiltration (DF), ion- exchange chromatography (AEC), and sterile filtration. The process involved in each unit operation is explained in mass balance operation below.

5.2 STOICHIOMETRY EQUATION

The coefficient for the biochemical reaction for production of plasmid DNA is shown in …(5.1).

$$C_6H_{12}O_6 + \alpha_1 O_2 + \alpha_2 NH_3 \longrightarrow \alpha_3 CH_{1.77}O_{0.49}N_{0.24} + \alpha_4 CO_2 + \alpha_5 C_2H_4O_2 + \alpha_6 H_2O \quad \ldots(5.1)$$

Table 5.1 Molecular weight of each component

Components	**Molecular weight (g/mol)**
Glucose, $C_6H_{12}0_6$	180.156
Ammonia, NH_3	17.031
Oxygen, O_2	32
Escherichia coli, $CH_{1.77}O_{0.49}N_{0.24}$	24.97
Carbon dioxide, CO_2	44
Acetic acid, $C_2H_4O_2$	50
Water, H_2O	18.015

Degree of freedom analysis:

Number of unknowns = 6 (α_1, α_2, α_3, α_4, α_5, α_6)

Material balance for each component:

Carbon, C: $-6 + \alpha 3 + \alpha 4 + 2\alpha 5 = 0$ ---(1)

Hydrogen, H: $-12 - 3\alpha_2 + 1.77\alpha_3 + 4\alpha_5 + 2\alpha_6 = 0$ ---(2)

Oxygen, O: $-6 - 2\alpha_1 + 0.49\alpha_3 + 2\alpha_4 + 2\alpha_5 + \alpha 6 = 0$ ---(3)

Nitrogen, N: $-\alpha_2 + 0.24\alpha_3 = 0$ ---(4)

Degree of reduction:

<u>Substrate</u>

Glucose, $C_6H_{12}0_6$: [6(4) +12(1) + 6(-2)]/6 = 4

Oxygen, O_2: 4

Ammonia, NH_3: 0

Product:

Escherichia coli, $CH_{1.77}O_{0.49}N_{0.24}$: [1(4) + 1.77(1) +0.49(-2) + 0.24(-3)]/1 = 4.07

Acetic acid, $C_2H_4O_2$: [2(4) + 67(1) + 4(1) + 2(2)]/2= 4

Water, H_2O: 0

Carbon dioxide, CO_2: 0

Electron balance equation: -

$$Y_s - 2\alpha_1 + Y_b\,\alpha_3 + Y_p\,\alpha_4 = 0 \quad \text{---(5)}$$

So, α_1: 0.8801

Yield of Cell Biomass mass basis g/g:

$$Y_{x/s} = \alpha_3\, MW_b / MW_s$$

$$0.06 = \alpha_3(24.97) / 180 \quad \text{--- (6)}$$

$$\alpha_3 = 0.4326$$

Number of independent equations: 4+ 2 = 6

Degree of freedom = Number of unknown - Number of independent

= 6 – 6

= 0

N: $0.24\alpha_3 = \alpha_2$

$\alpha_2 = 0.1038$

H: $-11.5459 = -4\,\alpha_5 + -2\alpha_6$

$-11.5459 = -2(2\alpha_5 + \alpha_6)$

$5.7729 = 2\alpha_5 + \alpha_6$

O: $-7.548275 + 2\alpha_4 + 5.7729 = 0$

$\alpha_4 = 0.8877$

C: $-6 + \alpha 3 + \alpha 4 + 2\alpha_5 = 0$

$2\alpha_5 = 4.6798$

$\alpha_5 = 3.3399$

So,

$5.7729 = 2\alpha_5 + \alpha_6$

$\alpha_6 = 3.3399$

The stoichiometry was obtained as below:

$$C_6H_{12}O_6 + 0.8801O_2 + 0.1038NH_3 \longrightarrow 0.4325CH_{1.77}O_{0.49}N_{0.24} + 0.8877CO_2 + 2.3399C_2H_4O_2 + 1.093H_2O \quad \ldots(5.2)$$

Reactant:

$180 + 0.8801(32) + 0.1038(17.031) = 209.93$

Product:

$0.4325\ (24.97) + 0.8877(44) + 2.3399(50) + 1.093(18.015) = 209.93$

Total:

$209.93 = 209.03$

$209 \approx 209$

The biochemical equation is balanced.

5.3 MATERIAL BALANCE

Mass balance is an application of conservation of mass to the analysis of physical system. As the law of conservation state that, matter cannot disappear or created spontaneously (Himmeblau et al., 1967). This analysis is mean to keep track of any material as it is moving or forming in the environment. Mass balance also shows the accumulation in the system as the input is higher that the output.

5.3.1 Seed Fermenter (R-101)

Seed fermenter used for the culturing of bacterial *Escherichia coli* (*E. coli*). Glucose, ammonia, oxygen, and *E. coli* to the seed fermenter. After cultivation, *E. coli* will be transferred to the main fermenter F-102. Seed Fermenter serve the purpose of providing sufficient nutrient *E. coli* to inoculate in the main fermenter. The main production of Plasmid DNA (pDNA) will be taking place in the main fermenter.

5.3.2 Main Fermenter (R-102)

E. coli and medium such as glucose and ammonia will be transferred from seed fermenter to main fermenter. Oxygen will directly be supplied from air to the fermenter *E. coli* will continuedly grow and reproduce. Figure 5.1 below shows the main fermenter (F-102) which use to cultivate the *E. coli*. Table 5.2 shows the molar flowrate and mass flowrate of each material across the main fermenter.

Reaction:

$$C_6H_{12}O_6 + 0.8801O_2 + 0.1038NH_3 \longrightarrow 0.4325CH_{1.77}O_{0.49}N_{0.24} + 0.8877CO_2 + 2.3399C_2H_4O_2 + 1.093H_2O \quad \ldots(5.3)$$

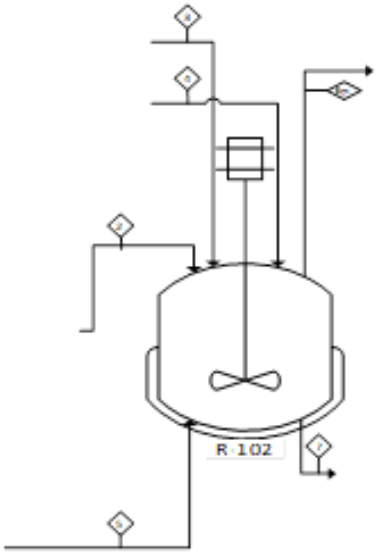


Figure 5.1 Fermenter (R-102)

Degree of Freedom Analysis

Number of unknown: 6

Number of independent component equation balance: 5

Reaction (r) and equation conversion of glucose: 1

Number of degrees of freedom: 6-6 = 0

Derivative general mole balance

$$F_{AO} - F_A + \int_0^V r_A . v \, dv - \int_0^V r_A . v \, dv = \frac{dC_A}{dt} \quad \ldots(5.4)$$

Mass in – Mass out + Generation – Consumption = Accumulation

Assumption:

i. The process is unsteady-state so there is no mass in and mass out, $F_{AO} - F_A = 0$
ii. Generation, $\int_0^V r_A . v \, dv = 0$
iii. The liquid density is constant.
iv. The rate of cell growth r is given by the Monod equation.
v. The feed stream is sterile and thus contains no cells

By using assumption, the mass balance equation we get stated as below:

$$\frac{dC_A}{dt} = \int_0^V r_A . v \, dv$$

$$\frac{dC_A}{dt} -= r_A . v$$

$$\frac{dC_A}{dt} -= -r_A$$

Theory yield coefficient, $\frac{\alpha_A MW_A}{\alpha_B MW_B}$

Where,

α refers to stochiometric coefficient for biomass

β refers stochiometric coefficient for substrate

So,

$Y_{X/O}$ = 0.383 g biomass/ g oxygen

$Y_{X/N}$ = 6.120 g biomass/ g ammonia

$Y_{X/S}$ = 0.060 g biomass / g substrate Retrieved from Da & Petrides, 2021

$Y_{W/S}$ = 0.109 g water/ g substrate

$Y_{A/S}$ = 0.6494 g acid acetic/ g substrate

$Y_{C/S}$= 0.217 g carbon dioxide / g substrate

While the parameters: X = Biomass (*Escherichia coli*), $CH_{1.77}O_{0.49}N_{0.24}$, O = Oxygen, O_2, N = Ammonia, NH_3, W= Water, H_2O, S = Substrate (Glucose), $C_6H_{12}0_6$, A = Acetic acid, $C_2H_4O_2$ and C = Carbon dioxide, CO_2.

Monod equation:

$$\mu = \frac{\mu_{max} . S}{k_s + S}$$

Where,

μ_{max} = maximum specific growth rate (h^{-1})

$$\mu_{max} = \frac{1}{time, t}$$

S = substrate concentration(g/L)

K_s = half − saturation constant (g/L)

So,

Time.t = 40 hour

Substrate concentration; 20 g/L

k_s = 01019 g/l

x = 50 g/l Retrieved from Da & Petrides, 2021

$$\mu = \frac{\frac{1}{40} . 20}{0.019 + 20}$$

$$\mu = 0.0249\ h^{-1}$$

Rate law:

$$r_x = \mu . x$$

$$r_s = \frac{r_x}{Y_{x/s}}$$

So, reaction rate for production of biomass, r_x :

$$r_x = 0.0249(50)$$

$$r_x = 1.245\ gL^{-1}h^{-1}$$

and, reaction rate of substrate consumption, r_s :

$$r_s = \frac{1.245}{0.060}$$

$$r_s = 20.75\ gL^{-1}h^{-1}$$

and, reaction rate for other substrate and products:

Substrate

$r_o = 3.251\ gL^{-1}h^{-1}$

$r_N = 0.2034\ gL^{-1}h^{-1}$

By-Product

$r_w = 2.262\ gL^{-1}h^{-1}$

$r_A = 13.475\ gL^{-1}h^{-1}$

$r_c = 4.5028\ gL^{-1}h^{-1}$

Material balance for each component

a. *Escherichia coli,* $CH_{1.77}O_{0.49}N_{0.24}$

$$\frac{dC_A}{dt} = \mu . C_x$$

$C_x = C_{xo}$, at t = c

$$C_x = C_{xo} e^{\mu t}$$

Let C_{xo} = 50 g/L, (Da & Petrides, 2021)

$$C_x = 50e^{(0.0249)(40)}$$

$$C_x = 135.37\ \text{g/l}$$

b. Acetic acid, $C_2H_4O_2$

$$\frac{dC_A}{dt} = r_A$$

$C_A - C_{AO} = r_A t$

$C_A = \ 13.475(40)$

$C_A = 539$ g/l

c. Glucose, $C_6H_{12}0_6$

Let $C_{So} = 1000$ g/L, (Da & Petrides, 2021)

$\frac{dC_S}{dt} = -r_S$

$C_s - C_{SO} = -r_S t$

$C_S = \ -20.75(40) + 1000$

$C_S = 170$ g/l (unreact)

d. Ammonia, NH_3

Let $C_{NO} = 100$ g/L, (Da & Petrides, 2021)

$\frac{dC_N}{dt} = -r_N$

$C_N - C_{NO} = -r_N t$

$C_N = \ -0.2034(40) + 100$

$C_N = 91.864$ g/l (unreact)

e. Oxygen, O_2

Let $C_{OO} = 150$ g/L, (Da & Petrides, 2021)

$$\frac{dC_O}{dt} = -r_O$$

$$C_O - C_{OO} = -r_O t$$

$$C_O = -3.251(40) + 150$$

C_O = 19.96 g/l (unreact)

f. Carbon dioxide, CO_2

$$\frac{dC_C}{dt} = r_C$$

$$C_C - C_{CO} = r_C t$$

$$C_C = 4.5021(40)$$

C_C = 180.11 g/l (produce)

Formula: Mass (kg) = Concentration (g/L) x (1/1000 kg) x 1000L

Table 5.2 Summarized stream table of Fermenter, R-102

Component	**Molecular Weight (g/mole)**	**Inlet Mass flowrate, (kg/batch)**				**Outlet Mass flowrate, kg/batch**	
		Stream 3	**Stream 2**	**Stream 4**	**Stream 5**	**Stream 6**	**Stream 7**
E coli	24.97		47.50				145.77
Acetic acid	50						512.07
Glucose	180.156			950.00			161.51
Ammnonia	17.031	96.29					87.28
Oxygen	32				142.51	18.96	
Carbon dioxide	44					172.34	
Water	18.015						138.15
Total		96.29	47.50	950.00	142.51	191.30	1045.03

Check for balance:

$\sum F_{in} = F_{3in} + F_{2in} + F_{4in} + F_{5in}$ = 96.29 kg/batch + 47.50 kg/batch + 950 kg/batch + 142.51 kg/batch

$\sum F_{in}$ = 1236.30 kg/hr

$\sum F_{out} = F_{6out} + F_{7out}$ = 191.30 kg/batch + 1045.03 kg/batch

$\sum F_{out}$ = 1236.33 kg/hr

1236.30 ≈ 1236.33

$\sum F_{in} = \sum F_{out}$

Balance.

Comparison with SuperPro

COMPONENT	INITIAL	INPUT	OUTPUT	FINAL	IN-OUT
Acetic-Acid	0.000	0.000	570.095	0.000	- 570.095
Ammonia	0.000	96.290	89.036	0.082	7.172
Carb. Dioxide	0.000	0.000	158.358	0.146	- 158.504
Escherichia Col	0.000	47.500	91.537	0.000	- 44.037
Glucose	0.000	950.000	219.073	0.000	730.927
Nitrogen	1.034	0.000	1.033	0.001	0.000
Oxygen	0.314	142.510	28.539	0.026	114.259
Water	0.000	0.000	79.887	0.000	- 79.887
TOTAL	1.348	1,236.300	1,237.559	0.255	0.165

Figure 5.2 Mass in and out from SuperPro

Figure 5.2 shows the mass in and out from the SuperPro simulation. Table 5.3 shows the percentage error between manual calculation and SuperPro calculation at stream out from fermentation.

Table 5.3 Percentage error between SuperPro and manual calculation

Component	SuperPro Calculation (kg/hr)	Manual Calculation (kg/hr)	Percentage Error
E coli	91.537	145.77	37%
Acetic acid	570.095	512.07	10%
Glucose	219.30	161.51	26%
Ammonia	89.036	87.28	2%
Water	79.887	138.15	40%
Carbon dioxide	158.358	172.34	8%

Oxygen	28.539	18.96	33%

5.3.3 Centrifuge, (C-101)

The main purpose of centrifuge is to separate particles suspended in solution according to its size, density, viscosity of medium and rotor speed. In our plant, biomass, water, acetic acid and the rest of glucose and ammonia from stream 11 that are in the form of fermentative broth is fed into centrifuge to separate the biomass. The efficiency of centrifuge is 90% with 14,000 rpm and this centrifugation process occurs for 1 hour at 1 bar (Da & Petrides, 2021). Figure 5.3 shows the outlet and inlet centrifuge.

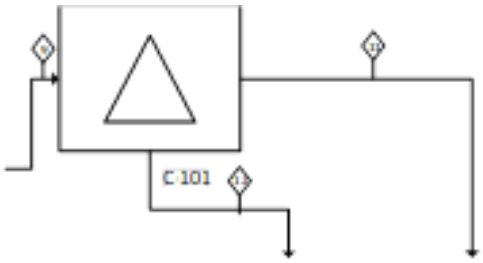


Figure 5.3 Inlet and outlet Centrifuge (C-101)

Table 5.4 Summarized stream table of Centrifuge, C-101

Components	**Inlet, kg/hr**	**Outlet. kg/hr**	
	Stream 9	**Stream 11**	**Stream 12**
E. coli	145.77	4.37	141.40
Acetic acid	512.07	506.94	5.12
Glucose	161.51	159.89	1.61
Ammonia	87.28	87.28	
Water	138.15	137.03	1.38
Total	1045.03	895.52	149.52

Table 5.4 shows the summarized stream table of centrifuge (C-101). The material balance is balance.

5.3.4 Reactor, (R-103)

The main purpose of this reactor is disrupting the cell using alkaline lysis using sodium hydroxide (NaOH) and a detergent like sodium dodecyl sulfate (SDS). Amount of NaOH and SDA should be same as the reactant. It will lead to production of cell debris, proteins, genomic deoxyribonucleic acid (gDNA), plasmid deoxyribonucleic acid (pDNA)and ribonucleic acid (RNA). At here, pH control is required to make sure plasmid DNA (pDNA) don't denature. At stream 16, SDS will enter the reactor at room temperature (25˚C). At stream 15, NaOH will enter the reactor at room temperature (25˚C). and at stream 17, product from the disruption cell will go out along with others substrate. Yield of Plasmid DNA 0.060 g/g. Figure 5.4 shows the inlet in and out of the reactor.

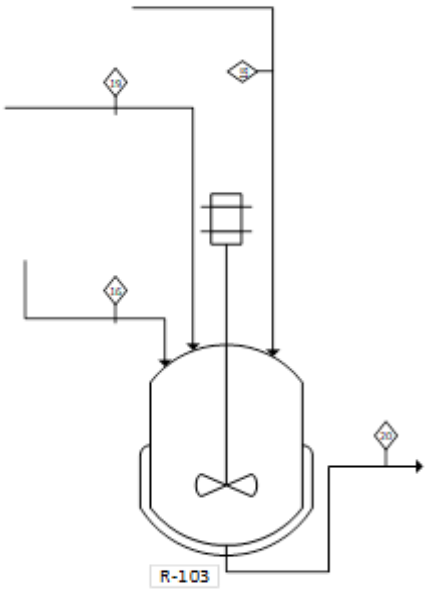


Figure 5.4 Inlet stream out and in at Reactor (R-103)

Table 5.5 Summarized stream table of Reactor, R-103

Components	**Inlet, kg/hr**			**Outlet, kg/hr**
	Stream 12	**Stream 13**	**Stream 14**	**Stream 15**
E. coli	141.40			
Acetic acid	5.12			5.12
Glucose	1.61			1.61

SDS			70.61	70.61
NaoH		70.61		70.61
Water	1.38			1.38
pDNA				8.66
gDNA				5.69
RNA				32.47
Proteins				53.53
Cell debris				41.05
Total	149.52	70.61	70.61	290.74

Table 5.5 shows the summarized of material balance at reactor (R-103). According to Da & Petrides, 2021, volume of SDS and NaOH is equivalent to total biomass in. Then for the cell disruption, it follows the stoichiometry given by Da & Petrides, 2021. The stoichiometry equation …(5.5).

$$100\ \text{Biomass} \longrightarrow 30\ \text{cell debris} + 3\ \text{genomic DNA} + 4\ \text{plasmid DNA} + 40\ \text{Proteins} + 23\ \text{RNA} \quad \ldots(5.5)$$

So, the material balance is balance.

5.3.5 Mixer, (M-101)

The main purpose of this mixer to do the neutralization and precipitation. Neutralization to make sure pDNA at the right pH environment by adding an acidic buffer which is potassium acetate ($C_2H_3O_2K$) at pH 5.5. Then, it will lead to precipitation of gDNA and others substance. $C_2H_3O_2K$ will enter the mixer through stream 20 at 4˚C and Calcium chloride (CaCl) will enter through stream 21 at room temperature (25˚C). Amount of CaCl and $C_2H_3O_2K$ should be same as reactant. The mixture will go out from mixer at stream 32. Figure 5.5 shows the material in and out from the mixer (M-101).

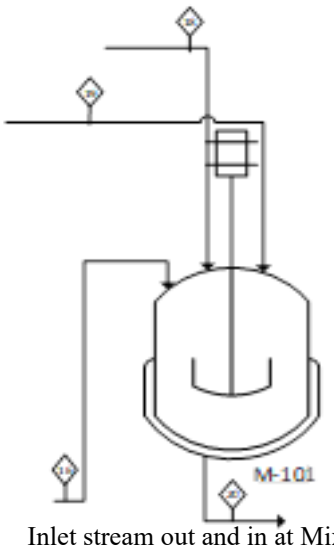


Figure 5.5 Inlet stream out and in at Mixer (M-101)

Table 5.6 Summarized stream table of Mixer, M-101

Components	**Inlet, kg/hr**			**Outlet, kg/hr**
	Stream 16	**Stream 18**	**Stream 19**	**Stream 20**
Acetic acid	5.12			5.12
Glucose	1.61			1.61
SDS	70.61			70.61
NaoH	70.61			70.61
Water	1.38			1.38
pDNA	8.66			8.66
gDNA	5.69			5.69
RNA	32.47			32.47
Proteins	53.53			53.53
Cell debris	41.05			41.05
Potassium acetate		70.67		70.67
CaCl			70.67	70.67
Total	290.74	70.67	70.67	432.08

Table 5.6 show the material balance at mixer (M-101). The flowrate of $C_2H_3O_2K$ enter the mixer is equivalent to stream in from disruption cell and CaCl based on their molarity (Da & Petrides, 2021). Material balance is balanced.

5.3.6 Filtration, (F-102)

The main purpose of filtration is to separate solids and liquids after precipitation and neutralization. At here, assume all the SDS is precipitate and cell debris is all in solid. Then, for RNA, gDNA, protein will precipitate at 20%, 90% and 80%. Then, it will go further precipitation for gDNA, protein, RNA will filtrate at 98%, 96% and 85% (Da & Petrides, 2021). $C_2H_3O_2K$ and CaCl will be filtrate at 90% (Da & Petrides, 2021). Figure 5.6 show the outlet and inlet centrifuge.

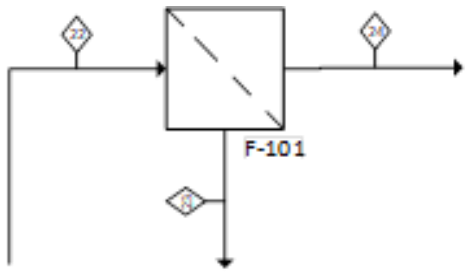


Figure 5.6 Inlet stream out and in at Filtration (F-101)

Table 5.7 Summarized stream table of Filtration, F-101

Components	**Inlet, kg/hr**	**Outlet, kg/hr**	
	Stream 22	**Stream 23**	**Stream 24**
Acetic acid	5.12		5.12
Glucose	1.61		1.61
SDS	70.61	70.61	
NaoH	70.61		70.61
Water	1.38		1.38
pDNA	8.66		8.66
gDNA	5.69	5.64	0.05
RNA	32.47	28.61	3.86
Proteins	53.53	53.10	0.43
Cell debris	41.05	41.05	
Potassium acetate	70.67	63.60	7.07
CaCl	70.67	63.60	7.07
Total	432.08	326.21	105.87

Table 5.7 show the material balance at Filter (F-101). Material balance is balanced.

5.3.7 Microfiltration, (F-102)

The main purpose of microfiltration is to further filtrate the remaining unneeded substances. At here, assume all the unneeded substances will be 90% remove from the mixture. Figure 5.7 show the outlet and inlet microfiltration.

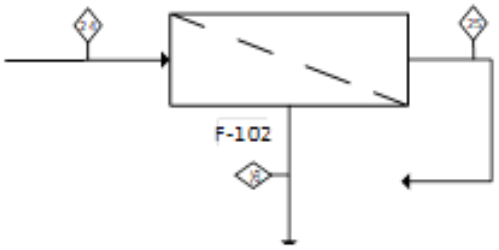


Figure 5.7 Inlet stream out and in at Microfiltration (F-102)

Table 5.8 Summarized stream table of Microfiltration, F-102

Components	**Inlet, kg/hr**	**Outlet, kg/hr**	
	Stream 24	**Stream 25**	**Stream 26**
Acetic acid	5.12	0.51	4.61
Glucose	1.61	0.16	1.45
NaoH	70.61	7.01	63.60
Water	1.38	0.14	1.24
pDNA	8.66	8.66	
gDNA	0.05	0.05	
RNA	3.86	0.35	3.51
Proteins	0.43	0.04	0.39
Potassium acetate	7.07	0.71	6.36
CaCl	7.07	0.71	6.36
Total	105.87	18.34	87.53

Table 5.8 show the material balance at microfiltration (F-101). Material balance is balanced.

5.3.8 Ultrafiltration at (M-102)

Ultrafiltration is step for purification to remove remaining unneeded substances. Tris-EDTA will be use as buffer to remove the impurities (Da & Petrides, 2021). At here,

Tris-EDTA will enter the mixer through stream 30 at room temperature (25˚C). Figure 5.8 show the outlet and inlet ultrafiltration.

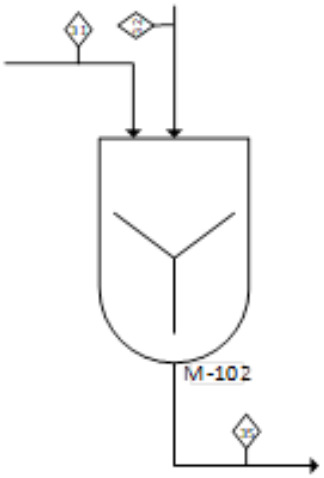


Figure 5.8 Inlet stream out and in at Mixer (M-102)

Table 5.9 Summarized stream table of Mixer, M-102

Components	**Inlet, kg/hr**		**Outlet, kg/hr**
	Stream 30	**Stream 29**	**Stream 32**
Acetic acid	0.51		0.51
Glucose	0.16		0.16
NaoH	7.01		7.01
Water	0.14		0.14
pDNA	8.66		8.66
gDNA	0.05		0.05
RNA	0.35		0.35
Proteins	0.04		0.04
Potassium acetate	0.71		0.71
CaCl	0.71		0.71
Tris-EDTA		2.88	2.88
Total	18.34	2.88	21.23

Table 5.9 show the material balance at mixer (M-102). Material balance is balanced.

5.3.9 Ultrafiltration at (F-103)

At here, 90% of RNA, protein and gDNA will be remove from the mixture. Figure 5.9 show the outlet and inlet ultrafiltration at F-103.

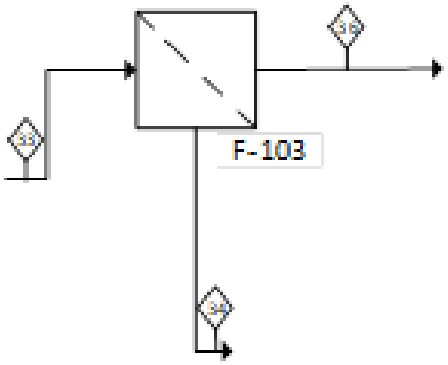


Figure 5.9 Inlet stream out and in at Ultrafiltration at (F-103)

Table 5.10 Summarized stream table of Ultrafiltration, F-103

Components	**Inlet, kg/hr**	**Outlet, kg/hr**	
	Stream 33	**Stream 34**	**Stream 36**
Acetic acid	0.51	0.51	
Glucose	0.16	0.16	
NaoH	7.01	7.01	
Water	0.14	0.14	
pDNA	8.66	8.66	
gDNA	0.05		0.05
RNA	0.35	0.04	0.31
Proteins	0.04		0.04
Potassium acetate	0.71	0.71	
CaCl	0.71	0.71	
Tris-EDTA	2.88	2.88	
Total	21.23	20.81	0.41

Table 5.10 show the material balance at ultrafiltration at F-103. Material balance is balanced.

5.3.10 Chromatography, (CR-101)

Chromatography is using Anion Exchange Chromatography (AEC). At here, Tris-EDTA will be use as buffer to remove the impurities Then, 90% of the pDNA together with 5% of RNA are retained by the column. The pDNA solution from UF/DF fed into AEC at 30 °C and 1 bar (Da & Petrides, 2021). The efficiency of AEC is about

82% and operates for 1 hours. Assume Tris-EDTA flowrate is 100kg/hr. Figure 5.10 show the outlet and inlet chromatography.

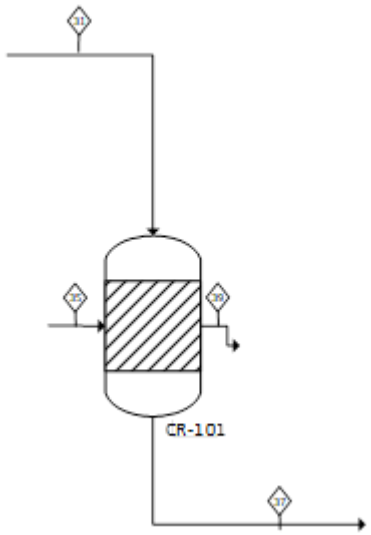


Figure 5.10 Inlet stream out and in at Chromatography (CR-101)

Table 5.11 Summarized stream table of Chromatography, CR-101

Components	**Inlet, kg/hr**		**Outlet, kg/hr**	
	Stream 35	**Stream 31**	**Stream 37**	**Stream 39**
Acetic acid	0.51			0.51
Glucose	0.16		0.03	0.13
NaoH	7.01		1.21	
Water	0.14		0.14	
pDNA	8.66		8.66	
RNA	0.04		0.04	
Potassium acetate	0.71			0.71
CaCl	0.71			0.71
Tris-EDTA	2.88	2.88	5.77	
Total	20.81	2.88	15.85	7.85

Table 5.11 show the material balance at chromatography (CR-101).

5.3.11 Filtration, (F-104)

The final product will be filtered to remove the buffer at some unneeded impurities. All the Tris-EDTA is removed from the mixture. Figure 5.11 show the outlet and inlet filter.

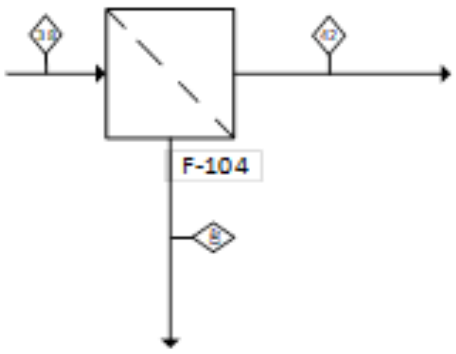


Figure 5.11 Inlet stream out and in at Filter (F-104)

Table 5.12 Summarized stream table of Filter, F-104

Components	**Inlet, kg/hr**	**Outlet, kg/hr**	
	Stream 38	**Stream 40**	**Stream 42**
Glucose	0.03		0.03
NaoH	1.21	1.14	0.07
Water	0.14		0.14
pDNA	8.66		8.66
RNA	0.04		0.04
Tris-EDTA	5.77	5.77	
Total	15.85	6.91	8.94

Table 5.12 show the material balance at filter (F-104). Then, the final product is obtained. Material balance is balanced.

5.3.12 Overall Material Balance

In our plant to produce plasmid DNA, the inlet streams are stream 16, stream 15, stream 21, stream 20, stream 4, stream 6, stream 2, stream 30, and stream 7 while the outlet streams are stream 11, stream 29, stream 36, stream 38, and stream 12. Figure 5.12 show the outlet and inlet overall mass balance.

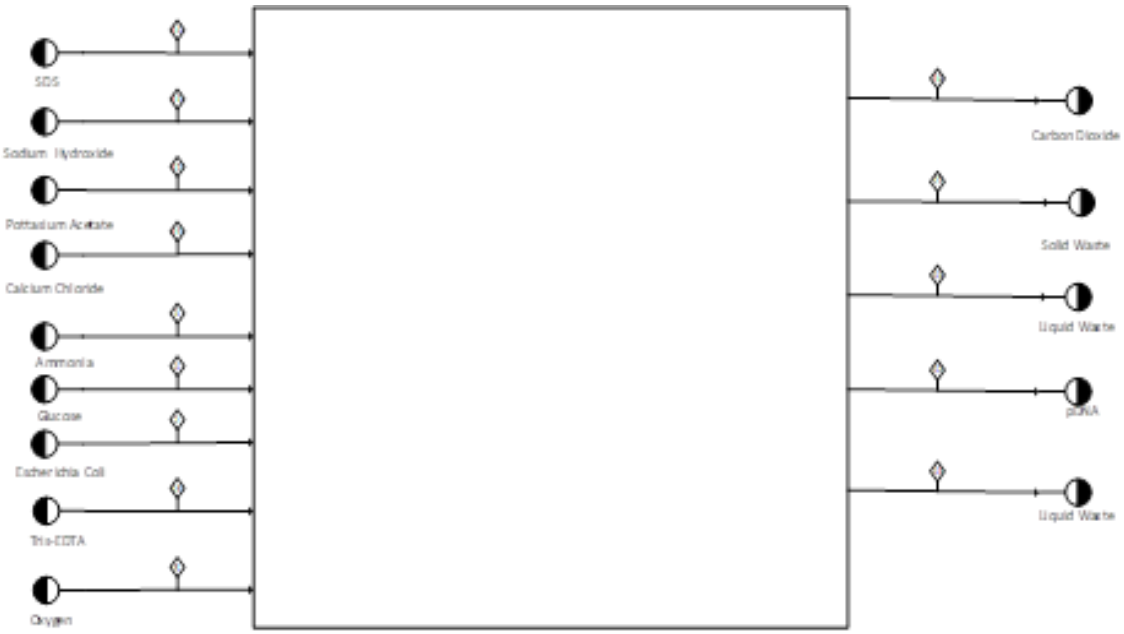


Figure 5.12 Inlet stream out and in at overall plant operation

Check for balance:

$$\sum F_{in} = F_{14in} + F_{13in} + F_{17in} + F_{19in} + F_{3in} + F_{4in} + F_{1in} + F_{28in} + F_{5in}$$

= 70.61 kg/hr + 70.61 kg/hr + 70.67 kg/hr + 70.67 kg/hr + 96.29 kg/hr + 950.03 kg/hr + 47.50 kg/hr + 5.77 kg/hr + 142.51 kg/hr

$$\sum F_{in} = 1524.66 \text{ kg/hr}$$

$$\sum F_{out} = F_{10out} + F_{27out} + F_{43out} + F_{42out} + F_{41out} =$$

= 191.30 kg/hr + 413.74 kg/hr + 895.92 kg/hr + 8.94 kg/hr + 14.77 kg/hr

$$\sum F_{out} = 1524.67 \text{ kg/hr}$$

$1524.66 \approx 1524.67$

$$\sum F_{in} = \sum F_{out}$$

Balance.

5.4 ENERGY BALANCE

The quantity of energy that flows into or out of each process unit is determined by a system's energy balance. During a reaction, it determines how much heat is received or emitted. When heat is emitted during a reaction, it is called exothermic; when heat is absorbed, it is called endothermic.

The assumptions used in the calculations are as follows:

1. The flow in the unit processes are in steady state.
2. The reference temperature is fixed at 25℃ or 298 K
3. There is no potential energy, kinetic energy and work done by the system.

The enthalpy change of components is calculated using the formula below:

$$\Delta H = \int_{Tref}^{T} C_p dT$$

Table 5.13 The Specific heat capacity for each component

Component	C_p (J/mol.K)
Ammonia	80.8
Biomass	38.2
Glucose	219.19
Oxygen	29.19
Carbon Dioxide	37.35
Acetic acid	63.4
Water	75.32

Table 5.14 The Standard Heat of Formation for each component

Component	ΔH_f (kJ/mol)
Ammonia	-46.0
Biomass	-88.95
Glucose	-1273.3
Oxygen	0
Carbon Dioxide	-393.52
Acetic acid	-484.1
Water	-285.82

Reference temperature=298.15K

Rate of heat transfer can be calculated by using the formula below:

Reference temperature=298.15K

Rate of heat transfer can be calculated by using the formula below:

ΔH_{rxn} *can be calculated by using the formula* $\Delta H_{rxn} = \Delta H_{product} - \Delta H_{reactant}$

5.4.1 Main fermenter (R-102)

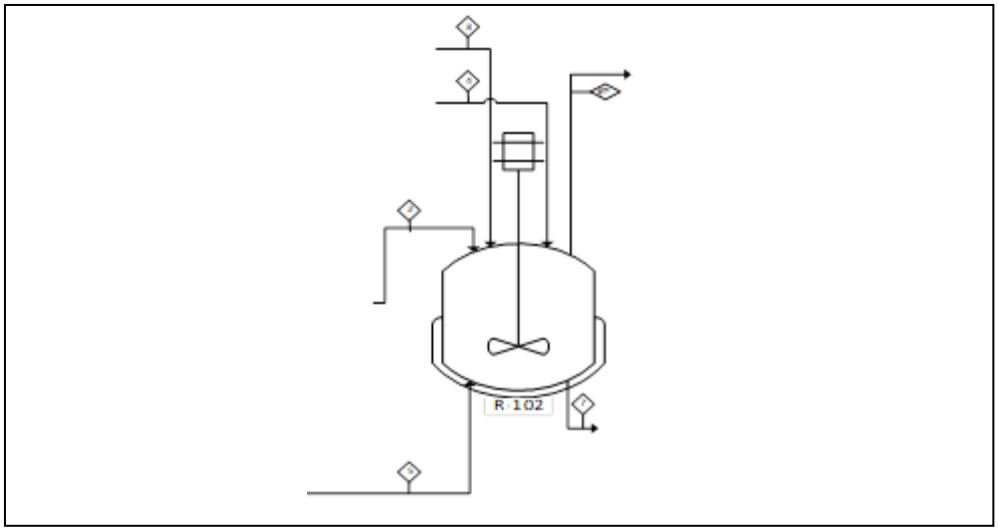


Figure 5.13 Main Fermenter R-102

Table 5.13 the standard heat of formation for each component

Table 5.15 The molar flow rate in for each component

Component	Molar Flowrate In (kmol/hr)			
	Stream inlet 3	Stream inlet 2	Stream inlet 4	Stream inlet 5
Ammonia	5.65	0	0	0
E. coli	0	1.90	0	0
Glucose	0	0	5.27	0
Oxygen	0	0	0	4.45

Table 5.16 The molar flowrate out for each component

Component	Molar Flowrate Out (kmol/hr)	
	Stream outlet 6	Stream outlet 7
Ammonia	0	5.12
E. coli	0	5.84
Glucose	0	0.90
Oxygen	0.59	0
Carbon dioxide	3.92	0
Acetic Acid	0	10.24
Water	0	7.67

Table 5.17 The heat change and heat of reaction for each component

Components	Enthalpy change, ΔH (J/mol)	Heat of reaction, Q (kJ/hr)
Ammonia	969.6	5478.24
E. coli	458.4	870.96
Glucose	145.32	765.84
Oxygen	350.28	1558.75
Total		8673.79

Table 5.18 The heat change and heat of reaction for each component

Components	Enthalpy change, ΔH (J/mol)	Heat of reaction, Q (kJ/hr)
Ammonia	969.6	4964.35
E. coli	458.4	2677.06
Glucose	145.32	130.79
Oxygen	350.28	206.67
Carbon Dioxide	448.2	1756.94
Acetic acid	760.8	7790.59
Water	903.84	6932.45
Total		24458.85

Rate of reaction in the fermenter R-102, r = 20.74

Heat of reaction for the reaction, ΔH_{rxn} = -1832.95 -1278.07

=-3111.02

Overall heat change, $Q = \sum \Delta H_{Product} N_{Product} - \sum \Delta H_{Reactant} N_{Reactant} + r\Delta Hrxn$

$$Q = 24458.85 - 8673.79 + (20.74)(-3111.02)$$

$$Q = -48737.49 kJ/hr$$

The negative sign indicates that the reaction occurred in the fermenter R-102 is exothermic reaction.

CHAPTER VI

BIOCHEMISTRY AND BIOCHEMICAL ENGINEERING

6.1 INTRODUCTION

Plasmid DNA is a circular double-stranded DNA present in the cytoplasm of a bacteria that replicate independently from the bacterial chromosome. Plasmid DNA can be found in some species are unicellular, meaning they only have one cell, while others are multicellular, meaning they have multiple cell types. Prokaryotes are single-celled organisms, whereas eukaryotes are multicellular organisms. Prokaryotes include bacteria and viruses (Tushar Chauhan, 2019). Figure 6.1 shows the general structure of bacteria contains plasmid DNA and chromosomal DNA.

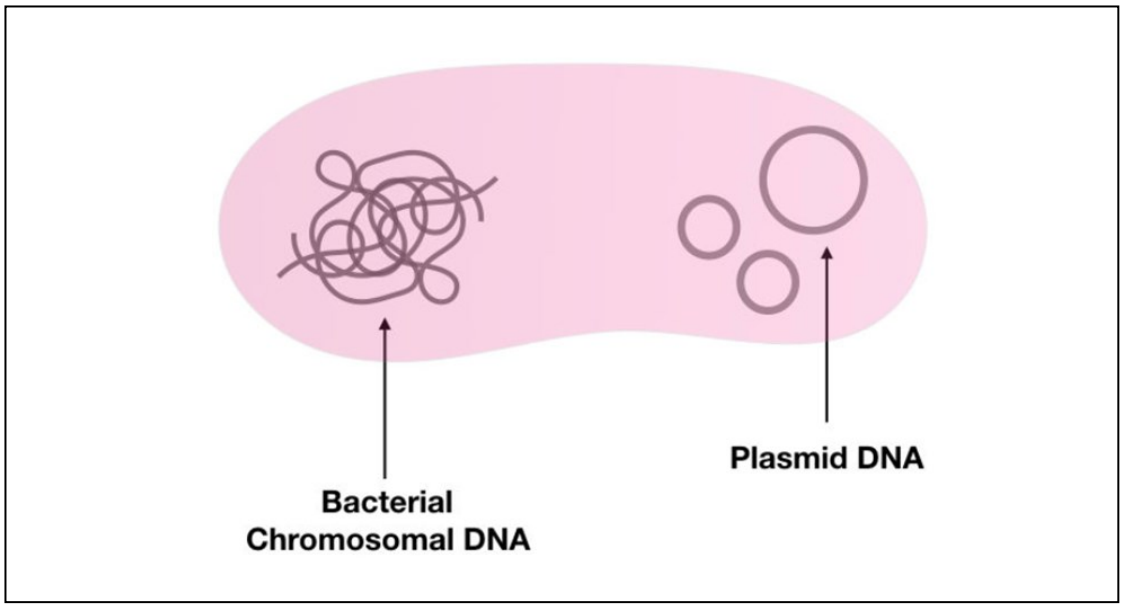


Figure 6.1 Structure of plasmid DNA and chromosomal DNA

Source: Tushar Chauhan, 2019

Properties of plasmid DNA is a circular molecule made up of double-stranded DNA and has a replication origin. It has an antibiotic resistance gene for bacterial survival, which aids in the development of resistance to several natural antibiotics. Horizontal gene transfer by conjugation is one of the most essential features of plasmid DNA (Tushar Chauhan, 2019). Plasmids are self-replicating elements that each bacterium inherits during cell division. The plasmid DNA aids in the organism's survival by kills other host cells by generating poisonous proteins, and it defends its own cells by acquiring tolerance to the invading organism or killing it. In some severe conditions, the plasmid also aids in the replication of bacterial DNA. There are 5 types of plasmids depends on their function which are F-plasmid, Col-plasmid, R-plasmid, V-plasmid, and D-plasmid (Tushar Chauhan, 2019).

This plant is using industrial *E. coli* K12 because it a typical host identity for the plasmid DNA production (Rinal et al., 2014). *E. coli* was used as host cell because it has a lot of common gene mutations that helps the production of plasmid DNA. First, gene endA which called as Endonuclease I mutation. endA is a nonspecific cleavage of dsDNA that use to improves plasmid yield. Then, recA, recA1, recA13 is essential for recombination and general DNA repair so it will reduces plasmid recombination and increases plasmid stability (Ferenc, 2014).

6.2 MAIN ENZYME USE IN PRODUCTION

The production of plasmid deoxyribonucleic acid (pDNA) is obtained by cultivating the bacteria *Escherichia coli* (*E. coli*) in the suitable medium for the bacteria to grow. Full genomic sequencing revealed *E. coli* K12 is a 5.11174 Mb long, circular double-stranded extrachromosomal with 4,727 protein coding genes (*NCBI*, 2022). The enzyme used in this process is galactose permease. Galactose permease, or galP, is a membrane protein discovered in Escherichia coli that transports monosaccharides, especially hexoses, for use by *E. coli* in glycolysis and other metabolic and catabolic pathways.

The galactose permease from the galP gene of the Escherichia coli genome called as galP/H+ symporter. Galactose is a carbon source that can be used instead of

glucose. galP expression is almost certainly regulated by the cAMP/CRP catabolite repression regulator (Moller et., 2022). HTH-type transcriptional regulator GalR (galR) and Protein GalS (galS) are the two proteins that block transcription from the gal regulon. The main structural sequences of GalR and GalS are quite similar, and the operator binding sites are identical. GalR and GalS, which are repressors are inhibited in the presence of D-galactose (El Qaidi et al., 2009). GalR and GalS will bind the promoter operator site when galP is not necessary like when glucose is available, it will be limiting transcription and preventing cAMP-CRP activation (Samsey et al., 2007).

Then, GalS binds only when GalR is present to indicate both proteins are required for repression. CRP is modulated at the promoter by cAMP. The cAMP-CRP complex activates the gal regulon, which leads to GalP upregulation. In the presence of glucose, GalP is also suppressed because the cell prefers glucose to galactose (Samsey et al., 2007).

6.3 CHARACTERISTICS OF ENZYME

Function of galactose permease is involving the uptake of galactose or glucose into the cell across the boundary membrane (McDonald et al., 2001). Table 6.1 shows names and taxonomy of galactose permease.

Table 6.1 Names and taxonomy of galactose permease

Names and Nomenclatures	Properties
Protein name	Galactose-proton symporter
Alternative name	Galactose transporter
Enzyme commission number	EC 2.7.1.204
Gene name	galP
Organisms	Escherichia coli (strain K12)

Source: UniProt, 2022

Structure of galP is belongs to the MFS family of proteins and is a homologue of the human GLUT1 transporter. The C- and N-termini of all MFS members are positioned on the cytoplasmic side of the membrane, and the membrane spans 12 alpha(α)-helices (Zheng et al., 2010). Helixes 6 and 7 are connected by a lengthy hydrophilic cytoplasmic loop, which divides the 12 helices into two pseudo-

symmetric halves of 6 helices. These two halves join to form a hole for substrate transport; galactose, glucose, and H+ are the most common substrates in galP. galP monomers have a pore diameter of about 10Å, which is like the pore diameters found in other MFS members, which range from 10-15Å (Zheng et al., 2010). galP has been discovered as an oligomer made up of a homotrimer of galP monomers with p3 rotational symmetry. galP is the first MFS member to be discovered as a trimer and to be physiologically active in its trimeric form; the galP oligomer is assumed to be generated for stability. Figure 6.2 shows the 3D structure of galactose permease (Zheng et al., 2010).

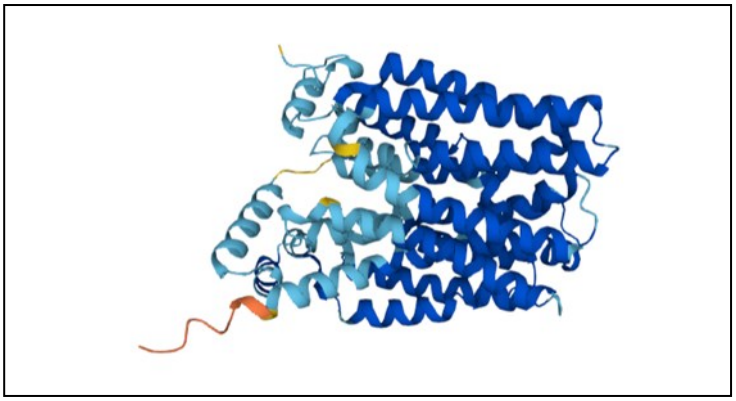


Figure 6.2 3D-structure of galactose permease

Source: UniProt, 2022

6.4 ENHANCEMENT OF PLASMID DNA PRODUCTION

The plasmid DNA usually will be cultivate using Escherichia coli as a host cell. There are many types of strain in the *E. coli*. Galactose permease can be used to make a new strain from parent strain W3110 (Wunderlich et al., 2014). Two engineered *E. coli* strains that be derive from W3110 is VH33 and VH34 and it will be compared during plasmid DNA (pDNA) production. There are several factors can influence the production of pDNA which are carbon source, C/N ration, production of acetate, plasmid size, temperature, pH and others (Lara & Ramírez, 2011).

Strain VH34 was modified from strain VH33 to reduce overflow metabolism. Production or accumulation of acetate also became lower so *E. coli* can continue cultivate or devide without being inhibit by acetate. The phosphotransferase system

(PTS) for glucose transport was inactivated in VH33, so it will be substituted by the galactose permease (GalP) and overexpressed in the chromosome. The pyruvate kinase A gene (pykA) has been deleted in strain VH34 (Knabben et al., 2010). Figure 6.3 shows the simplified metabolic map of modifications made to the engineering *E. coli* strain.

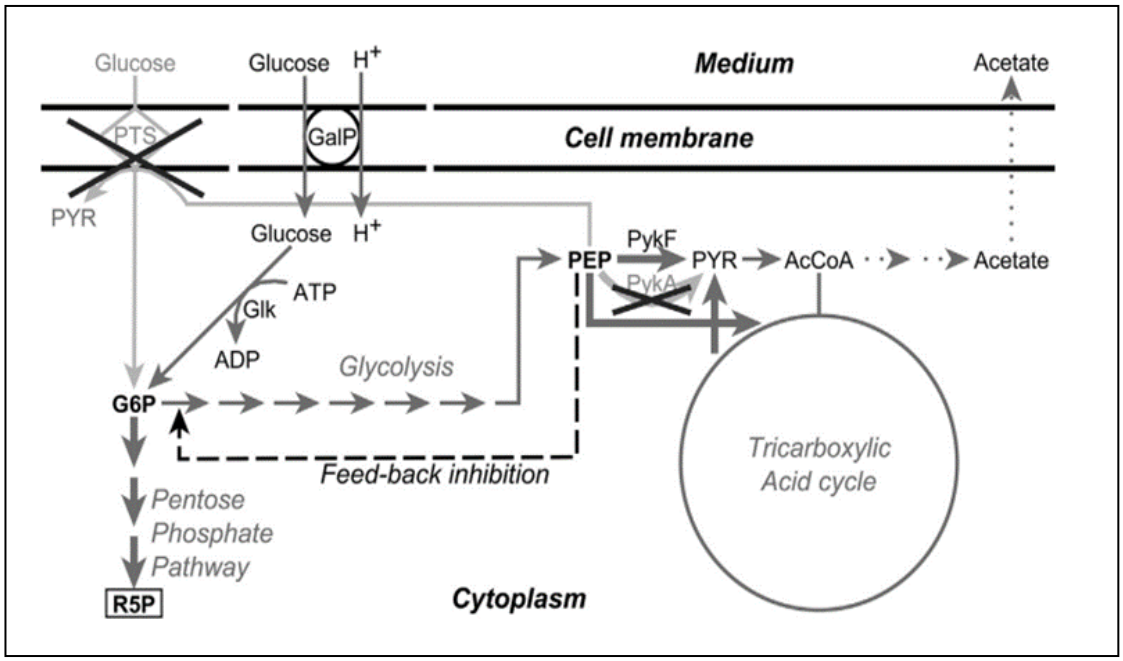


Figure 6.3 Simplified metabolic map of modifications made to the engineering *E. coli* strain.

Source: Wunderlich et al., 2014

At initial growth phase, temperature at 30-37˚C was used and followed by temperature up-shift at 42-45˚C. Thermal induction has been used to successfully raise plasmid copy number from 20-50 to 400-600 (Silva et al., 2011). Next, during batch cultivations with starting glucose concentrations of up to 130 g/L, the modified strain VH33 demonstrated a clear reduction in acetate production and allowing for high cell densities. In batch and fed-batch modes, strain VH33 has also been studied to produce pDNA. When cultured in batch mode with 100 g/L glucose as the beginning concentration, VH33 was found to yield twice as much pDNA as the parent strain W3110. Then, VH34 produced 70% more pDNA in batch mode using complex media than VH33 (Soto et al., 2011).

Then, overflow metabolism at strains VH33 and VH34 is produced more pDNA and accumulated less acetate than W3110 at dilution rate 0.025 to 0.25h^{-1}. Figure 6.4 shows the yield of pDNA for strain W3110, VH33 and VH34 at dilution rate 0.025 to 0.25h^{-1} (Wunderlich et al., 2014).

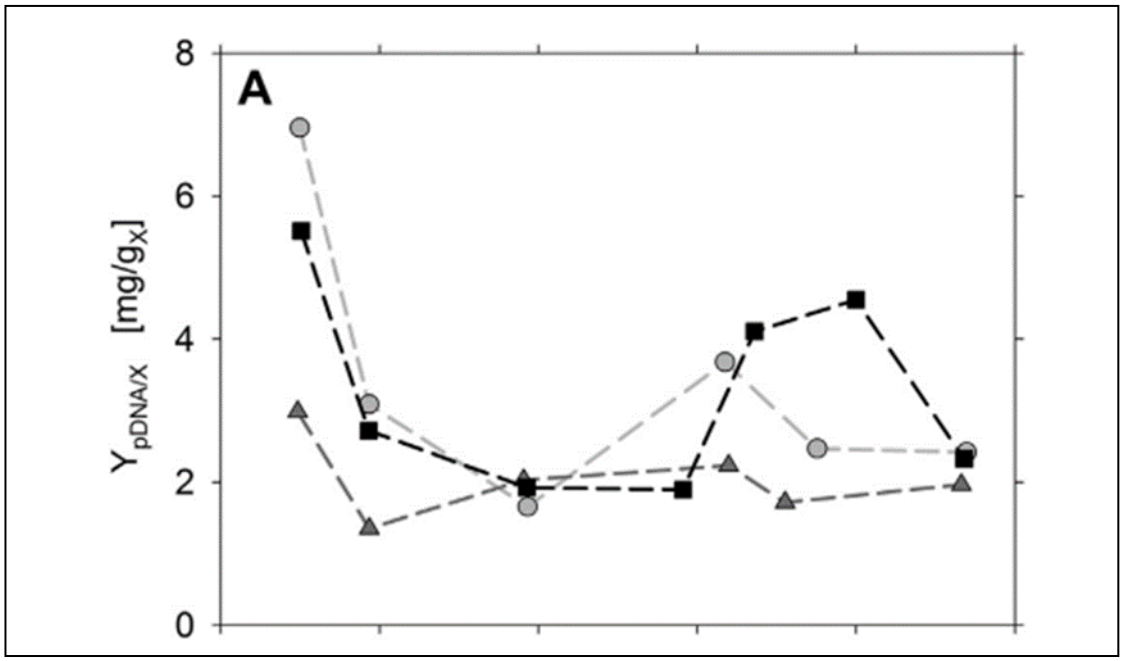


Figure 6.4 Yield of pDNA for strain W3110, VH33, and VH34
Circle is strain W3110, triangles is strain VH33, square is strain VH34.
Source: Wunderlich et al., 2014

In conclusion, engineering *E. coli* for strain VH34 is have better production of pDNA compared to strain W3110 and VH33.

CHAPTER VII

SEPARATION PROCESS II

7.1 INTRODUCTION

In this process, there are 5 different separation unit used in this process to increases the purity of the pDNA which disc type centrifuge, filter, microfilter, ultrafiltration and chromatography (AEC). Disk stack centrifuge were used to separate biomass and the liquid waste which is acetate acid, water, and unreacted reactant from fermentation to ensure 97% of biomass is recovered in the bottom stream. Centrifuges use a quick rotation to create an accelerated gravitational force that separates the particles (Salim et al, 2013). This takes the place of the gravitational force that is normally necessary for settling. As a result, by substituting the sedimentation tank/vessel with a centrifuge, sedimentation can be accomplished in less time. The disc type centrifuge was chosen because of the good solid discharge, which is high efficiency and recovery of bowl cooling possible solid (Harisson et al, 2003).

7.2 MASS BALANCE OF DISC STACK CENTRIFUGE

The mass balance for all component at centrifuge shown in Table 7.1.

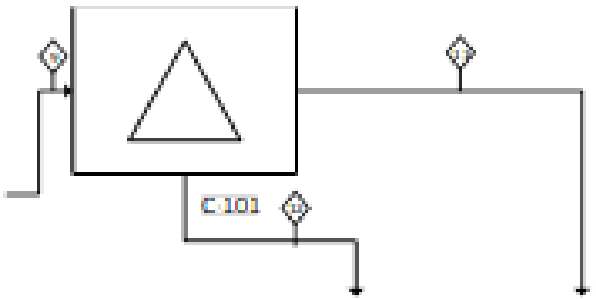


Figure 7.1 Centrifugation process

Table 7.1 Summarized stream table of centrifuge, C-101

Components	**Inlet, kg/hr**	**Outlet. kg/hr**	
	Stream 9	**Stream 11**	**Stream 14**
E. coli	145.771	4.371	141.400
Acetic acid	512.070	506.949	5.121
Glucose	161.506	159.891	1.615
Ammonia	87.277	87.277	0.00
Water	138.152	137.030	1.383
Total	1044.775	895.518	149.517

7.3 DESIGN AND CALCULATION OF DISK STACK BOWL CENTRIFUGE

The centrifugal stack disc may apply a force between 4000 and 14000 times that of gravity, shortening separation time. The separation process can be improved by dispersing the flow to multiple parallel thin channels between conical dishes when using this centrifuge. The centrifuge was design based on the performance and specification show in Table 7.2 below.

Table 7.2 Specification and performance characteristics of centrifugal stack disc bowl

Bowl D, (mm)	**Speed (rpm)**	**Maximum acceleration**	**Throughput/Liquid(L/min)**
254	10000	14200	40-150
406	6250	8850	100-570
686	4200	6760	150-1500
762	3300	4630	150-1500

(Source: Council, 2022)

The operating pressure, temperature and phase of the feed and outlet stream of centrifuge show in Table 7.3 below. The bowl usually air-driven and operated at low pressure or in an atmosphere of nitrogen reduce generation of frictional heat.

Table 7.3 Properties of stream in and out at centrifuge

	Inlet	**Outlet**	
Stream	**9**	**11**	**12**
Temperature (℃)	25	30	30
Pressure (bar)	2	1	1
Phase	Slurry	Liquid	Solid

Table 7.4 below shows the properties of particles.

Table 7.4 Properties of particles

Properties	**Values**
Diameter of particles, d (μm)	1
Density of particles (kg/m^3)	1032.02
Size of particles, $\boldsymbol{\alpha}$ (μm)	2

(Source: Council, 2022)

Table 7.5 below shows the properties of medium in the fluid.

Table 7.5 Properties of medium in the fluid

Properties	**Values**
Viscosity of medium (Pa·s)	0.25
Density (kg/m3)	997

(Source: Goudar et al., 1999)

7.3.1 Heat Duty

Total mass flowrate in (kg/h) = 1044.775 kg/h

Specific heat capacity of fluid in stream. Cp (kJ/kg.K) = 321.68 kJ/kg.K

Temperature different, ΔT (K) = T_{out} - T_{in}

Heat duty has been calculate using formula, Q $= mCp\Delta T$

$$Q = mCp\Delta T$$

$$= (1044.775)\ (321.68)\ (303.15\text{-}298.15)$$

$$= 1680375.9 \text{ kJ/h}$$

=466.77 kW

7.3.2 Design Calculation

A disc centrifuge is a system of fast rotating concentric inverted cones positioned close together to reduce the time it takes to catch dense particles or liquids while permitting continuous feed and discharge liquids through forced flow. The feed suspension enters on the axis of rotation and is driven to the bottom of the revolving bowl in this centrifuge. The suspension is pushed upward by pressure. If the nozzle is open, dense particles are expelled; otherwise, they gather on the bowl's outer wall.

The following assumptions simplify the analysis:

1. Typically, v0, the flow velocity, is much greater than v_{ω}.
2. v0 = *Q*/*A*, where *A* decreases as particles move toward the centre.
3. The fluid velocity v_0 is a function of *y* and goes to zero at the surface of the disks.

The equation of motion in the *x* direction is,

$$\frac{dx}{dt} = v_o - v_\omega sin\theta \qquad \text{Eqn (7.1)}$$

$$\frac{dy}{dt} = v_\omega Cos\theta = \frac{v_g \omega^2 r}{g} Cos\theta \qquad \text{Eqn (7.2)}$$

Eqn (7.1) is simplified by the assumption that v0 is much greater than vω sin θ. The average value of v_0, denoted v_0, is given by the flow rate *Q* divided by the cross-sectional area *A* perpendicular to the flow for *n* disks: The local value of v0 can be found by multiplying v0 by a function *f* (*y*) that gives the velocity variation between the disks:

$$v_o = \frac{Q}{2\pi nrl} f(y) \qquad \text{Eqn (7.3)}$$

Substitute Eqn (7.3) into Eqn (7.1), noted that $\frac{dx}{dt} = v_o$,

$$\frac{dx}{dt} = \frac{Q}{2\pi nrl} f(y) \qquad \text{Eqn (7.4)}$$

Multiply Eqn (7.2) with Eqn (7.4), dy/dx = dy/dt x dt/dx and get,

$$\frac{dy}{dx} = \frac{v_g \omega^2 2\pi n l}{Qgf(y)} r^2 cos\theta \qquad \text{Eqn (7.5)}$$

Substitute., $r^2 = (R_o - xsin\theta)^2$ into Eqn (7.5) and get,

$$\frac{dy}{dx} = \frac{v_g \omega^2 2\pi n l}{Qgf(y)} (R_o - xsin\theta)^2 cos\theta \qquad \text{Eqn (7.6)}$$

Integrate, $\frac{1}{f(y)}$ $respect\ to\ y$,

$$\frac{1}{l}\int_0^l f(y)\, dy = 1 \qquad \text{Eqn (7.7)}$$

Substitute $x = \frac{(R_o - R_1)}{sin\theta}$, y = l, into Eqn (7.6) and then rearrange,

$$Q = v_g \frac{2\pi n \omega^2}{3g} ({R_0}^3 - {R_1}^3)\cot\theta \qquad \text{Eqn (7.8)}$$

$$Q = v_g \Sigma \qquad \text{Eqn (7.9)}$$

Wheres;

Q = Feed volumetric flowrate

n = number of discs

ω = angular rotation

θ = angle for disc fit from the vertical

r_o = outer radius of discs

r_1 = inner radius of discs

v_g = terminal velocity of particle

Σ = factor/area

7.3.3 Design Calculation

This centrifuge operates in continuous or batch mode, depending on whether the nozzle is open or closed. As a result, an appropriate relationship between v0, the flow velocity, and v, the particle sedimentation velocity, must be found. Table 7.6 below shows the properties of disk stack centrifuge.

Table 7.6 Design parameter of centrifuge

Properties	Values
Number of discs, n	50
Rotational speed (rpm)	10000
angle for disc fit from the vertical, θ	40
outer radius of discs, r_o (m)	0.05
inner radius of discs, r_1 (m)	0.02
Height (m)	1.0

(Source: Yang, M. et al. 2019)

Formula of feed flowrate in disk stack centrifuge,

$$Q = v_g \Sigma$$

Rotation speed (rpm) = 10000 rpm

$$\omega = \frac{2\pi(10000)}{60} = 1047.2 \text{ rad/s}$$

Sedimentation velocity under gravity, Vg:

$$V_g = \frac{d^2}{18\mu}(\rho_s - \rho_0)\omega^2 r$$

$$V_g = \frac{(0.5 \times 10^{-6})^2}{18(0.25)}(1032.02 - 997)(1047.2)^2(0.25)$$

$$V_g = 5.334 \times 10^{-7} \text{ m/s}$$

Sigma Factor, $\sum$, represent area $(\text{length})^2$ or size of the centrifuge The angle chosen typically between 40° to 55° and the number of discs used is between 50 to 150. (Perry, 1973).

$$\sum = \frac{2\pi n({r_0}^3 - {r_1}^3)\omega^2}{3g}\cot\theta$$

$$\sum = \frac{2\pi(50)(0.05^3 - 0.02^3)(1047.2)^2}{3(9.81)}cot(40)$$

$$\sum = 1.632 \times 10^3 m^2$$

Volumetric flowrate, Q:

$$Q = V_g \sum$$

$$Q = (5.334 \times 10^{-7}\ \mathrm{m/s}) \times (1.632 \times 10^3 m^2)$$

$$Q = 8.7 \times 10^{-4}\ \frac{m^3}{s}$$

$$Q = 52.2\ L/min$$

Residence time, t:

$$ln(\frac{r_0}{r_1}) = \frac{2\alpha^2(\rho_s - \rho_0)\omega^2 t}{9\mu}$$

$$lln(\frac{0.05}{0.02}) = \frac{2(2 \times 10^{-6})^2(1032.02 - 997)(1047.2)^2 t}{9(0.0250)}$$

$$t = 673\ s$$

$$t = 11.22\ min$$

Volume of fluid, v:

$$t = \frac{V}{Q}$$

$$V = 8.7 \times 10^{-4}\ m^3/s \times 673\ s$$

$$V = 0.58\ m^3$$

Relative centrifugal force (RCF), G force:

$$G = \frac{\omega^2 r}{g}$$

$$G = \frac{(1047.2)^2(0.025)}{(9.81)}$$

$$G = 27415.7$$

Percentage solid recovery,

Mass flowrate at inlet = $\frac{145.771\ \text{kg/h}}{3600}$ = 0.0405 kg/s

Mass of solid supposed to collect, $m_1 = 0.0404 kg/s \times 936 = 37.81 kg$

Mass flowrate at outlet (stream 12) = $\frac{141.400\ 1\ \text{kg/h}}{3600}$ = 0.01393 kg/s

Mass of solid collected, $m_2 = 0.01393 kg/s \times 673 = 26.38\ kg$

Percentage biomass recovery (%) = $\frac{m_2}{m_1} \times\ 100\%$ (Matt, 2018)

$$\%\ =\ \frac{26.38\ kg}{27.26\ kg} \times 100\%$$

$$\% = 96.77\%$$

7.3.4 Summary of Design Calculation

By assuming the parameter in Table 7.6 such as outer radius and inner radius, the rotation has been set 10000 RPM the calculation of has been done by determine the value of Vg, Sigma (∑), volumetric flowrate, Q, retention time, Volume, relative centrifugal force, G, and percentage recovery. 97.77 % recovery of solid shows that the high efficiency of the centrifuge separation process of solid from mixture. All these calculations have been done using that assuming value the volumetric flowrate

of centrifuge that has been calculated is in range in Table 7.2 which is the specification of centrifuge. Table 7.7 below show the overall design calculation.

Table 7.7 Overall design calculation

Specification	**Values**
Volumetric flowrate, Q (L/min)	52.2
Rotational speed (rpm)	10000
Outer radius of discs, ro (m)	0.05
Inner radius of discs, r1 (m)	0.02
Height (m)	1.0
G force	27946.7
Residence time (min)	11.22
Recovery (%)	96.77
Heat duty (kW)	466.77
Volume (m3)	0.58
Sedimentation velocity under gravity, Vg (m/s)	5.334×10^{-7}
Sigma Factor, $\sum (m2)$	1.632×10^{3}

CHAPTER VIII

UTILITY AND PRESSURE VESSEL

8.1 INTRODUCTION TO UTILITY DESIGN

In our production, we use one type of utility unit, shell and tube heat exchanger. In this part, the calculation for utility design is for shell and tube heat exchanger unit H-101.

8.2 DESIGN OF HEAT EXCHANGER H-101

In the production, the heat exchanger H-101 is used to lower the temperature for potassium acetate from 25°C to 4°C. Figure 8.1 below shows the temperature profile for counter current flow.

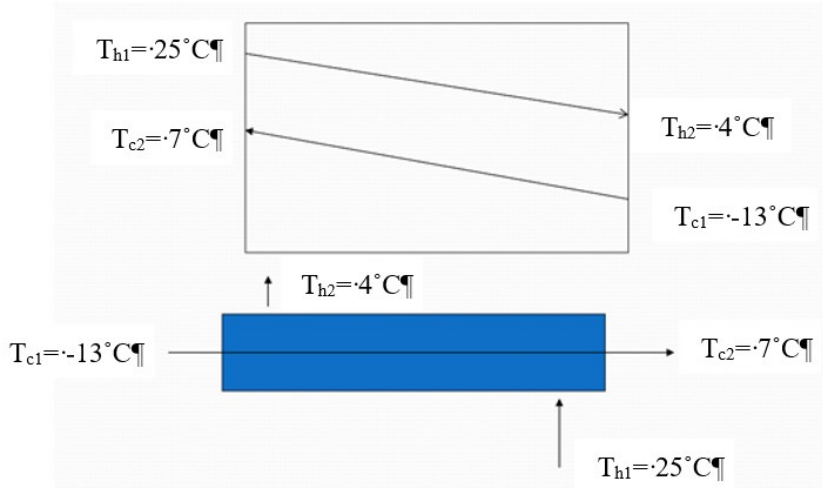


Figure 8.1 Temperature profile

Source: BYJU'S Exam Prep, 2017

The type of flow for temperature profile is in counter current flow. Counter current flow is more efficient compared to co-current flow due to higher efficiency in creating more uniform temperature difference.

8.2.1 Physical Properties for Fluids

Table shows the physical properties of service and process fluid.

Table 8.1 Physical Properties of Service and Process Fluid

Physical Properties	Brine (Tube)	Potassium Acetate (Shell)
Density (kg/m^3)	1230	1600
Specific heat capacity (kJ/kg.K)	3.11	1.114
Viscosity (kg/m.s)	0.0036	0.0055
Thermal Conductivity (W/m. °C)	0.600	0.500
Inlet Temperature (°C)	-13	25
Outlet Temperature (°C)	7	4
Flowrate (kg/h)	26.58	70.67

8.2.2 Summary of Design

Table 8.2 and Table 8.3shows the specification of tube side and shell side of heat exchanger H-101.

Table 8.2 Tube Side Specification

Parameter	Value
Tube pitch	Square pitch
Outer diameter, d_o (m)	0.0195
Inner diameter, d_i (m)	0.0174
Length, L (m)	1.83
Area of one tube, A_t (m^2)	0.1095
Number of tubes, N_t	4
Linear velocity, u_t (m/s)	5.4819 x 10^{-5}
Heat transfer coefficient, h_i (W/m. °C)	47.52028
Pressure drop, ΔPt (pa)	8 x 10^{-5}

Table 8.3 Shell Side Specification

Parameter	Value
Bundle diameter, D_b (mm)	103.186
Shell diameter, D_s (mm)	115.186
Cross section area, A_s (m^2)	7.958 x 10^{-4}
Baffle spacing, I_B (mm)	34.56
Equivalent diameter, d_e (m)	18.803 x 10^{-3}
Heat transfer coefficient, h_s (W/m. °C)	0.02847
Pressure drop, ΔP_s (pa)	137.857

8.3 DESIGN CALCULATION

Perform mass balance:

$$q_h = q_c$$

$$(m_h)(C_{ph})(\Delta T_h) = (m_c)(C_{pc})(\Delta T_c)$$

$$(70.67)(1.114)(21) = (m_c)(3.11)(20)$$

$$m_c = 26.58\ kg/h$$

Log mean temperature difference:

$$LMTD, \Delta T_{LM} = \frac{(T_{h1} - T_{c2}) - (T_{h2} - T_{c1})}{ln\frac{(T_{h1} - T_{c2})}{(T_{h1} - T_{c1})}}$$

$$LMTD, \Delta T_{LM} = \frac{(25 - 7) - (4 - ((-13))}{ln\frac{(25 - 7)}{(4 - ((-13))}}$$

$$LMTD, \Delta T_{LM} = 17.5\ °C$$

True temperature differences:

$$\Delta T_M = F_T \times \Delta T_{LM}$$

$$R = \frac{T_{h1} - T_{h2}}{T_{c2} - T_{c1}}$$

$$R = 1.05$$

$$S = \frac{T_{c2} - T_{c1}}{T_{h1} - T_{c1}}$$

$$S = 0.53$$

Based on temperature correction factor chart (in TEMA)

When R = 1.05 and S = 0.53 :

$F_T = 0.77$

$\Delta T_M = 13.5\ ℃$

Heat duty:

$$q_h = 459.24\ W$$

Area of one tube:

$A_t = \pi LD$

L = 2.83 m

D = 19.05 mm

$$A_t = 0.1095\ m^2$$

Area:

$$A = \frac{Q}{U\Delta T_{LM}}$$

U = 500 W/m^2 ℃ (Assumed)

$$A = 0.262\ m^2$$

Number of tubes:

$$N_t = \frac{A}{A_t}$$

$$N_t \approx 4\ tubes$$

Bundle diameter:

$$D_b = d_o(\frac{N_t}{K_1})^{\frac{1}{n_1}}$$

Number passes = 2

Square pitch

So, $K_1 = 0.156$ and $n_1 = 2.291$

$$D_b = 19.05(\frac{10}{0.156})^{\frac{1}{2.291}}$$

$$D_b = 103.186\ mm$$

Shell diameter:

$$D_s = D_b + C$$

Fix tube heat exchanger. So C, clearance = 12 mm

$$D_s = 115.186\ mm$$

Tube side: Brine

Heat transfer coefficient:

$$h_i = 4200(1.35 + 0.02t)(\frac{{u_t}^{0.8}}{{d_i}^{0.2}})$$

Linear velocity:

$$u_t = \frac{w}{\rho A_t}$$

$$u_t = \frac{(26.58)(\frac{1}{3600})}{(1230)(0.1095)}$$

$U_t = 5.4819 \times 10^{-5}$ m/s

Inner diameter:

$d_i = 17.399$ mm

Average temperature:

t = -3 °C

$$h_i = 4200(1.35 + 0.02(-3))(\frac{5.4819\ x\ 10^{-5^{0.8}}}{17.399\ x\ 10^{-3^{0.2}}})$$

$h_i = 47.52028$ W/m^2 °C

Heat transfer factor:

$$j_h = \frac{h_1 d_i}{k}\left(\frac{\mu C_p}{k}\right)^{-\frac{1}{3}}$$

$$\mu = 0.0036 \text{ Ns/m}^2$$

$$j_h = \frac{(47.52)(17.399)}{0.600}\left(\frac{0.0036(3.11\ x\ 10^3)}{0.600}\right)^{-\frac{1}{3}}$$

$$j_h = 519.532$$

Pressure drop:

$$\Delta P_t = N_p(8j_f\left(\frac{L}{d_i}\right) + 2.5)(\frac{\rho {u_t}^2}{2})$$

Reynold number:

$$Re = \frac{\rho u_t d_i}{\mu}$$

$$Re = \frac{(1230)(5.819\ x\ 10^{-5})(17.399)}{0.0036}$$

$$Re = 325.8802$$

Friction factor, j_f:

Based on Reynold number, $j_f = 2.5 \text{ x } 10^{-2}$

$$\Delta P_t = 2(8(2.5\ x\ 10^{-2})\left(\frac{1.83}{17.399\ x\ 10^{-3}}\right) + 2.5)(\frac{(1230)(5.4819\ x\ 10^{-5})^2}{2})$$

$$\Delta P_t = 8\ x\ 10^{-5}\ pa$$

<u>Shell side: Potassium Acetate</u>

Baffle spacing:

$$I_B = 0.3D_s$$

$$I_B = 34.56\ mm$$

Tube pitch:

$$p_t = 1.25d_o$$

$$p_t = 23.81mm$$

Cross sectional area:

$$A_s = \frac{(p_t - d_o)D_s I_B}{p_t}$$

$$A_s = 7.958\ x\ 10^{-4}\ \text{m}^2$$

Mass flux:

$$G_s = \frac{W_s}{A_s}$$

$$G_s = 24.67\ \text{kg/m}^2\ \text{s}$$

Fluid linear velocity:

$$u_s = \frac{G_s}{\rho}$$

$$u_s = 0.0154\ \text{m/s}$$

Hydraulic diameter:

$$d_e = \frac{1.27}{d_o}({p_t}^2 - 0.785{d_o}^2)$$

$$d_e = 18.803\ mm$$

Reynold number:

$$Re = \frac{G_s d_e}{\mu}$$

$$\mu = 0.0055 \text{ Ns/m}^2$$

$$Re = 84.34$$

Prandtl number:

$$p_r = \frac{C_p \mu}{k_f}$$

$$p_r = 0.0123$$

Heat transfer factor:

Based on baffle cut = 25%, square pitch and Re = 84.34

So, heat tranfor factor, $j_f = 5.5 \times 10^{-2}$

Heat transfer coefficient:

$$N_u = \frac{h_s d_e}{k_f} = j_h Re {p_r}^{1/3}$$

$$\frac{h_s d_e}{k_f} = 0.02847 \text{ W/m}^2 \text{ °C}$$

Friction factor:

Based on baffle cut = 25%, square pitch and Re = 84.34

So, friction factor, $j_f = 2.8 \times 10^{-1}$

Pressure drop:

$$\Delta P_s = 8 j_f (\frac{D_s}{d_e})(\frac{L}{I_B})(\frac{\rho {u_s}^2}{2})$$

$$\Delta P_s = 137.857$$

8.4 PROCESS DESIGN OF MAIN FERMENTER R-102

The main fermenter, R-102 unit are operating in batch operation where the temperature, pH strictly control. The temperature is control and maintain at 37 ºC to make sure *E. coli* in optimum temperature.

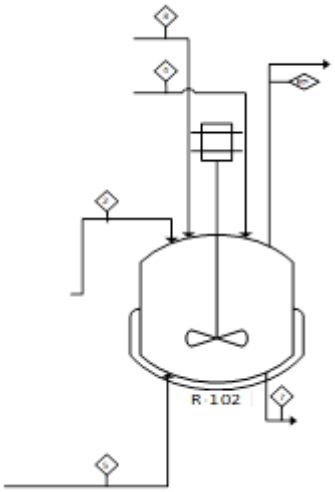


Figure 8.2 Fermenter (R-102)

Fermenter is used in the production of plasmid DNA from *Escherichia coli*. Stainless steel grade 316 has high amounts of chromium and nickel and also silicon, manganese and carbon with the majority of the compositionbeing iron. This type of stainless steel which contains 18% chromium, 10% nickel and 2.5% molybdenum are used (Jagani et al., 2010). Plus, stainless steel grade 316 is the most used type of fermenter for industrial operation. The higher molybdenum content increase corrosion resistance and increase strength at high temperature. It's also perfect for marine equipment, chemical and pharmaceutical manufacturing equipment (Bergsen Metal, 2021). It is a more susceptible to weld decay and they are particularly effective in acidic environments. Since stainless steel has high wear resistance, smooth surface, it is ease for cleaning process to avoid any adhere and growth of germs.

8.4.1 Design specification of fermenter, R-102

The vessels for microbiological work should have a ratio about 2.5-3:1 (Jagani et al., 2010). In our design, height to diameter ratio of 2.5:1 has been used to design the fermenter R-102. Material used is stainless steel grade 316 and corrosion allowance is set at 2mm. vertical vessel with a ratio of 2:1 ellipsoidal head as the top head and bottom. Table 8.4 below shows the operating conditions of fermenter R-102, T Table 8.5 shows the dimension of fermenter R-102 and Table 8.6 shows the inlet component of fermenter R-102.

Table 8.4 Operating conditions of fermenter R-102

Condition	Operating selection
Operating pressure	1 bar
Temperature	37°C
Material selection	Stainless steel grade 316
Corrosion allowance	2 mm
Joint efficiency	0.85

Table 8.5 Dimension of fermenter R-102.

Part	Type
Top head	Ellipsoidal 2:1
Shell vessel	Cylindrical
Bottom head	Ellipsoidal 2:1

Table 8.6 Inlet component of fermenter R-102

Components	Flow rate (kg/h)	Density(kg/m^3)
Ammonia	96.295	880.0
Eschericia coli	47.50	1105.0
Glucose	950.0	1560
Oxygen	142.51	1.429
Total	1236.305	804.884

$$\sum\rho = \frac{96.25}{1236.305}(880) + \frac{47.50}{1236.305}(1105.0) + \frac{950.0}{1236.305}(1560) + \frac{142.51}{1236.305}(1.429)$$

$$\sum\rho = 804.884\frac{kg}{m^3}$$

Volumetric flow rate is calculated using this equation,

$$V = \frac{\mathrm{m}}{\rho}$$

$$V = 0.651 \frac{m^3}{h}$$

Volume required for fermenter for operation hour about 40 hours,

Volume fermenter = 0.0651 x 40

Volume fermenter = 26.042 m^3

Table 8.7 below shows the dimension of fermenter R-102

Table 8.7 Dimension of fermenter R-102

Dimension	Values(ft)	Value(in)
Vessel height, H	37.58	450.96
Vessel inside diameter, D_i	15.03	180.36
Cylindrical shell height, h_c	30.06	360.72
Ellipsoidal top head height, h_e	3.76	45.12
Ellipsoidal bottom height, h_e	3.76	45.12

Diameter inside, D_i

$$V = \frac{\pi}{4} {D_i}^2 (2.5 D_i)$$

$$27 = \frac{\pi}{4} {D_i}^2 (2.5 D_i)$$

$${D_i}^3 = 13.75$$

$$D_i = 15.03\ ft = 180.6''$$

Height vessel, H

$$H = 2.5(15.03)$$

$$H = 37.58\ ft$$

Ellipsoid height head and bottom, h_e

$$h_e = \frac{D_i}{4}$$

$$h_e = 3.76\ ft$$

Cylindrical shell height, h_c

$$h_c = H - 2h_e$$

$$h_c = 30.06\ ft$$

8.4.2 Design pressure, hydrostatic pressure, minimum wall thickness, MAWP of fermenter R-102

The calculation involved will be shown after the table below. Table 8.8 shows the design pressure, P_d, hydrostatic pressure, P_H, minimum wall thickness, t_{min} and MAWP of fermenter R-102.

Table 8.8 Design pressure, hydrostatic pressure, minimum wall thickness and MAWP of fermenter R-102.

Part	P_D (psi)	P_H (psi)	t_{min} (mm)	$MAWP_p$ (part) (psi)	$MAWP_v$ (vessel) (psi)
Ellipsoidal head	16.1318	1.628	2.1844	16.1318	14.5038
Cylindrical Shell (Circumferential)	29.1478	14.644	3.937	29.189	14.545
Cylindrical Shell (Longitudinal)	29.1478	14.644	1.9558	29.041	14.397
Ellipsoidal bottom	30.7758	16.272	4.1402	30.7758	14.5038

The MAWP for vessel is 14.397 psi. The least amount among all the MAWP vessel calculated.

Hydrostatic pressure, P_H

$P_H = 0.433H$

$P_{H\ (head)}$ = 1.628 psi

$P_{H\ (shell)}$ = 14.644 psi

$P_{H\ (bottom)}$ = 16.272 psi

Design pressure, P_D

$P_D = P_o + P_H$

$P_{D\ (head)}$ = 16.1318 psi

$P_{D\ (shell)}$ = 29.1478 psi

$P_{D\ (bottom)}$ = 30.7758 psi

Minimum Wall Thickness, t_{min}

Ellipsoidal head and bottom

With $h_e = D/4$, so

$$t = \frac{PD}{2SE - 0.2P}$$

Top Head

$$t = \frac{(16.1318)(180.36'')}{2(20000)(0.85) - 0.2(16.1318)}$$

$$t = 0.086\ in = 2.1844\ mm$$

Bottom head

$$t = \frac{(30.7758)(180.36'')}{2(20000)(0.85) - 0.2(30.7758)}$$

$$t = 0.163\ in = 4.1402\ mm$$

Cylindrical Shell

Circumferential stress:

With t<R/2, so

$$t = \frac{PR}{SE - 0.6P}$$

$$t = \frac{(29.1478)(90.18)}{(20000)(85) - 0.6(29.1478)}$$

$$t = 0.155\ in = 3.937\ mm$$

Longitudinal stress:

With t<R/2, so

$$t = \frac{PR}{2SE + 0.4P}$$

$$t = \frac{(29.1478)(90.18)}{2(20000)(85) + 0.4(29.1478)}$$

$$t = 0.077\ in = 1.9558\ mm$$

MAWP part

Ellipsoid Head and Bottom

$$P = \frac{2SEt}{D + 0.2t}$$

Top Head

$$P = \frac{2(20000)(0.85)(0.086)}{180.36 + 0.2(0.086)}$$

$$P = 16.1318\ psi$$

Bottom Head

$$P = \frac{2(20000)(0.85)(0.163)}{180.36 + 0.2(0.163)}$$

$$P = 30.7758\ psi$$

Cylindrical Shell

Circumferential stress:

$$P = \frac{SEt}{R + 0.6t}$$

$$P = \frac{(20000)(0.85)(0.155)}{90.18 + 0.6(0.155)}$$

$$P = 29.189\ psi$$

Longitudinal stress:

$$P = \frac{2SEt}{R - 0.4t}$$

$$P = \frac{2(20000)(0.85)(0.077)}{90.18 - 0.4(0.077)}$$

$$P = 29.041\ psi$$

MAWP Vessel

$MAWP_v = MAWP_p - P_H$

Ellipsoidal head:

$MAWP_v = 14.5038$ psi

Cylindrical shell

Circumferential stress:

$MAWP_v = 14.545$ psi

Longitudinal stress:

$MAWP_v = 14.397$ psi

Ellipsoidal bottom:

$MAWP_v = 14.5038$ psi

8.4.3 Thickness uniformity and nominal thickness correction of fermenter R-102

From the values of t_{min} calculated, a thickness of 0.163 in was chosen to be the uniform wall thickness for the whole vessel. Among a nominal thickness of 0.25, 0.3125 and 0.375 in, a nominal thickness of 0.25 in was chosen. By using nominal thickness, the new minimum wall thickness can be calculated by using equation:

$$t_{\min(new)} = t_{nominal} - CA$$

$$t_{\min(new)} = 0.25'' - 0.079''$$

$$t_{\min(new)} = 0.171\ in$$

Hence the new MAWP part and vessel can be calculated using the new t_{min}. Table 8.9 shows the new t_{min}, and MAWP.

Table 8.9 New t_{min}, and MAWP

Part	T_{min} (in)	$MAWP_p$ (psi)	$MAWP_v$ (psi)
Ellipsoidal head	0.171	32.229	30.601
Cylindrical shell (circumferential)	0.171	32.199	17.555
Cylindrical shell (longitudinal)	0.171	64.52	49.876
Ellipsoidal bottom	0.171	32.229	15.957

Therefore, new MAWP for vessel is 15.957 psi with minimum wall thickness of 0.171 inches.

With

$$t = 0.171\ inch$$

New MAWP part

Ellipsoid Head and Bottom

$$P = \frac{2SEt}{D + 0.2t}$$

Top Head

$$P = \frac{2(20000)(0.85)(0.171)}{180.36 + 0.2(0.171)}$$

$$P = 32.229\ psi$$

Bottom Head

$$P = \frac{2(20000)(0.85)(0.171)}{180.36 + 0.2(0.171)}$$

$$P = 32.229\ psi$$

Cylindrical Shell

Circumferential stress:

$$P = \frac{SEt}{R + 0.6t}$$

$$P = \frac{(20000)(0.85)(0.171)}{90.18 + 0.6(0.171)}$$

$$P = 32.199\ psi$$

Longitudinal stress:

$$P = \frac{2SEt}{R - 0.4t}$$

$$P = \frac{2(20000)(0.85)(0.171)}{90.18 - 0.4(0.171)}$$

$$P = 64.52\ psi$$

New MAWP Vessel

$MAWP_v = MAWP_p - P_H$

Ellipsoidal head:

$$MAWP_v = 30.601 \text{ psi}$$

Cylindrical shell

Circumferential stress:

$$MAWP_v = 17.555 \text{ psi}$$

Longitudinal stress:

$$MAWP_v = 49.876 \text{ psi}$$

Ellipsoidal bottom:

$$MAWP_v = 15.957 \text{ psi}$$

8.4.4 Combine loading analysis for fermenter R-102

Analysis of Primary Stress

Table 8.10 below shows the parameter used in analysis of primary stress for fermenter R-102.

Table 8.10 Parameter used in analysis of primary stress for fermenter R-102

Parameter	Value(N/mm^2)
Longitudinal stress (σ_L)	52.74
Circumferential stress (σ_h)	105.42
Direct stress (σ_w)	6.1757×10^{-4}
Bending stress (σ_b)	neglected

Longitudinal stress, σ_L

$$\sigma_L = \frac{PD_i}{4t}$$

$$\sigma_L = \frac{(0.22)(4581.144)}{4(4.3434)}$$

$$\sigma_L = 52.74 \frac{N}{mm^2}$$

Circumferential stress, σ_h

$$\sigma_h = \frac{PD_i}{2t}$$

$$\sigma_h = \frac{(0.22)(4581.144)}{2(4.3434)}$$

$$\sigma_h = 105.42$$

Direct stress, σ_w

$$\sigma_w = \frac{w}{\pi(D_i + t)t}$$

$$w = 240 C_v D_m (H_v + 0.8 D_m) t$$

C_v = a factor account for the weight of nozzles, manway internal supports, which can be taken as 1.08 for vessels with only few internal fittings.

H_v = tangent between tangent line, m

$$H_v = \frac{1}{3}(e.head\ H) + (shell\ H) + \frac{1}{3}(e.bottom\ H)$$

$$H_v = \frac{1}{3}(1.146) + (9.162) + \frac{1}{3}(1.146)$$

$$H_v = 9.926\ m$$

D_m = mean diameter of the vessel, m

$$D_m = (D_i + t)$$

$$D_m = (4.581 + 0.0043434)$$

$$D_m = 4.58534\ m$$

$$w = 240(1.08)(4.585)\big(9.926 + 0.8(4.585)\big)(0.00434)$$

$$w = 70.12\ N$$

$$\sigma_w = \frac{70.12}{\pi(4.581 + 0.00434)0.00434}$$

$$\sigma_w = 1121.58\frac{N}{m^2}$$

To calculate the principal stress for circumferential stress and longitudinal stress, equation below were used:

$$\sigma_1 = \frac{1}{2}\left[\sigma_h + \sigma_z + \sqrt{(\sigma_h - \sigma_z)^2 + 4T^2}\right]$$

$$\sigma_2 = \frac{1}{2}\left[\sigma_h + \sigma_z - \sqrt{(\sigma_h - \sigma_z)^2 + 4T^2}\right]$$

$$\sigma_3 = 0.5P\ (neglected\ for\ thin\ vessel)$$

With torsional shear stresses = 0

$$\sigma_1 = \sigma_h$$

$$\sigma_2 = \sigma_z$$

Total longitudinal stresses for upwind and downwind can be calculated as follow:

Upwind, σ_z

$$\sigma_z = \sigma_L + \sigma_w + \sigma_b = 52.74112 \frac{N}{mm^2}$$

Downwind, σ_z

$$\sigma_z = \sigma_L + \sigma_w + \sigma_b = 52.74112 \frac{N}{mm^2}$$

$$\Delta\sigma_{max} = \sigma_h - \sigma_{z(upwind)} = 52.259 \frac{N}{mm^2}$$

$$\Delta\sigma_{max} = \sigma_h - \sigma_{z(downwind)} = 52.259 \frac{N}{mm^2}$$

Hence, maximum allowable stress intensity ($\Delta\sigma_{max}$) is equal to 52.259 N/mm^2

The thickness of the vessel's wall is sufficient to ensure that the maximum stress intensity at any point does not exceed the design stress for the construction material because the maximum allowable stress intensity calculated were less than the material maximum allowable stress which is 137.89515 N/mm^2.

Analysis of elastic stability

From the analysis of the combine loading, the material chosen met both the maximum stress intensity and the elastic stability requirements:

$$\Delta\sigma_{max} \leq S \text{ and } \Delta\sigma_{compressive} \leq \sigma_c$$

σ_c = critical buckling stress

$$\sigma_c = 0.6E\left(\frac{t}{D_i}\right)$$

$$\sigma_c = 0.6(200000)\left(\frac{4.3434}{4581.144}\right)$$

$$\sigma_c = 113.77\frac{N}{mm^2}$$

$$\Delta\sigma_{\text{compressive}} = \sigma_b + \sigma_w$$

$$\Delta\sigma_{\text{compressive}} = 617.37\ x\ 10^{-6}\frac{N}{mm^2}$$

In conclusion, the design is safe.

8.4.5 Vessel Support Analysis for Fermenter, R-102

Cylindrical skirt is chosen as the vessel support for our fermenter, R-102 and CSTR, R-103. It is suitable for tall vessels rather than other support. The skirt support is weld at the base of the shell. Material used to fabricate support must be high in strength, which is stainless steel (SA-240) with nominal composition 18Cr-2Mo. As the material fulfil minimum content to avoid corrosion, which is 12% chromium and contain molybdenum that can improve corrosion resistance.

8.4.6 Skirt Thickness of Fermenter, R-102

The skirt thickness must be sufficient to withstand the dead weight load and bending moment imposed on it by the vessel. Cylindrical skirt is suitable for high vessel with subject to wind loading. The specification for skirt thickness of fermenter is shown in Table 8.11 below

Table 8.11 Specification in skirt thickness for fermenter, R-102

Parameters	Value
Height vessel between tangent line, m	9.926
Diameter of the vessel, m	4.58
Skirt support height, mm	3

Operating pressure, bar	1
Typical liquid loading, kN/m^{-2}	1.2
Skirt thickness, inch	0.25

Table 8.12 The resultant stresses in the skirt design of fermenter, R-102

Parameters	Values
Bending stress in the skirt, σ_{bs}, N/mm^2	0.409
Dead weight stress in the skirt, σ_{ws}, N/mm^2	0.00791
Maximum σ_s tensile, N/mm^2	0.4011
σ_s (compressive), N/mm^2	0.41691

Assume the skirt diameter (D_s) is equal to inner diameter of vessel (D_i) and the skirt thickness (t_s) is equal to the nominal thickness.

Bending stress in the skirt, σ_{bs}

$$\sigma_{bs} = \frac{4m_s}{\pi(D_s + t_s)t_s D_s}$$

$M_s = 42818960$ Nmm

$$\sigma_{bs} = 0.409 \frac{N}{mm^2}$$

Dead weight stress of vessel, σ_{ws}

$$\sigma_{ws} = \frac{W}{\pi(D_s + t_s)t_s}$$

$$W = C_v \pi \rho_m D_m g(H_v + 0.8D_m) + 10^{-3}$$

$$W = 723.97\ N$$

$$\sigma_{ws} = 0.00791 \frac{N}{mm^2}$$

Therefore, the resultant stresses:

Maximum σ_s (tensile) = $\sigma_{bs} - \sigma_{ws}$

Maximum σ_s (tensile) = 0.4011 N/mm^2

σ_s (compressible) = $\sigma_{bs} + \sigma_{ws}$

σ_s (compressible) = 0.41691 N/mm^2

Rhe skirt thickness under worst condition of wind and dead weight loading should not exceed the following design criteria,

σ_s (tensile) < $f_s J sin\theta_s$

σ_s (compressible) < $0.125E(t_s/D_s)sin\theta_s$

Assumptions:

1) Straight cylindrical skirt
2) Maximum allowable stress for skirt material, f_s = 137.9 N/mm^2
3) Weld joint factor, J = 0.85
4) Base angle, θ_s = 90^o
5) Young's modulus, E = 20000

Therefore,

σ_s (tensile) < $f_s J sin\theta_s$

0.4011 < 117.215 N/mm^2

σ_s (compressible) < $0.125E(t_s/D_s)sin\theta_s$

0.4169 < 3.465 N/mm^2

Therefore, the skirt thickness was inacceptable range and with addition of CA, 2 mm.

Base ring and anchor bolt design

The loads carried the by the skirt will transmit to the foundation slab by the skirt base ring.

Assumptions made to determine base ring thickness:

1) Bolt circle diameter, D_b = 2 m
2) Circumference of bolt circle = 2000πmm
3) Maximum allowable bolt stress, f_b = 125 N/mm^2
4) Number of bolts, $N_b \geq 8$
5) Bolt spacing > 600 mm

Table 8.13 shows the base ring and anchor bolt design specification of fermenter, R-102.

Table 8.13 The base ring and anchor bolt design specification of fermenter, R-102

Parameters	Value
Bolt spacing, mm	785.4
Bending moment at the base, M_s, Nmm	9329
f_b, N/m	616.6
L_b, mm	0.18
Base ring thickness, t_b, mm	2.36

The anchor bolt is assumed to share the overturning load equally and the bolt area required:

Bolt spacing = circumference of bolt circle/number of bolts

Bolt spacing = 785.4 mm > 600 mm

Thickness skirt = 25 mm

$$A_b = \frac{1}{N_b f_b}\left(\frac{4M_s}{D_b} - W\right)$$

Wind loading, w = 0.00128 N/mm^2

The bending moment at the skirt base,

$$M_s = \frac{wx^2}{2}$$

$$M_s = 9329\ mm$$

$$A_b = 18\ mm^2$$

Use M24 bolts (BS44190:1967) root area = 353 mm^2

Total compressive load on the base ring per unit length, f_b

$$f_b = \frac{4M_s}{\pi {D_s}^2} + \frac{W}{\pi D_s}$$

$$f_b = 616.6\frac{N}{m}$$

Bearing pressure = 3.5 N/mm^2, so the minimum width of the base rings, L_b

$$L_b = \frac{f_b}{f_c} x \frac{1}{10^3}$$

$$L_b = 0.18\, mm$$

The required thickness base ring found by treating the rings as cantilever beam:

$$t_b = L_r \sqrt{\frac{3f_c'}{f_r}}$$

Lr = the distance from the edge of the skirt to the outer edge of the ring, mm = 76 mm

F'c= actual bearing pressure, N/mm^2 = 0.045 N/mm^2

Fr = allowable design stress in the ring of material, N/mm^2 = 140 N/mm^2

$$t_b = 2.36\, mm$$

8.5 PROCESS DESIGN OF CSTR (R-103)

CSTR is a unit operation used for alkaline lysis or cell disruption. The feed contains biomass recovery from centrifuge. The process occurs in ambient temperature 25℃. SDS and sodium hydroxide added into CSTR with different stream to disrupt the cell to release the cell component. Figure 8.3 shows CSTR (R103).

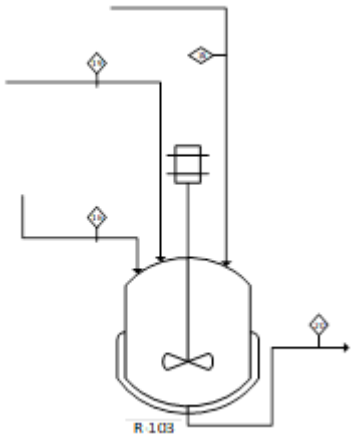


Figure 8.3 CSTR (R-103)

CSTR is used for cell lysis in the production of plasmid DNA from *Escherichia coli*. Stainless steel grade 316 has high amounts of chromium and nickel and silicon, manganese, and carbon with most of the compositionbeing iron. This type of stainless steel which contains 18% chromium, 10% nickel and 2.5% molybdenum are used (Jagani et al., 2010). Plus, stainless steel grade 316 is the most used type of CSTR for industrial operation. The higher molybdenum content increase corrosion resistance and increase strength at high temperature. It is a more susceptible to weld decay and they are particularly effective in acidic environments. Since stainless steel has high wear resistance (*Processdesign*, 2015), smooth surface, it is ease for cleaning process to avoid any adhere and growth of germs.

8.5.1 Design specification of CSTR (R-103)

The vessels for microbiological work should have a ratio about 2.5-3:1 (Jagani et al., 2010). In our design, height to diameter ratio of 2.5:1 has been used to design the CSTR (R-103). Material used is stainless steel grade 316 and corrosion allowance is set at 2mm. Vertical vessel with a ratio of 2:1 ellipsoidal head as the top head and bottom. Table 8.14 below shows the operating conditions of the CSTR (R-103), Table 8.15 shows the dimension of the CSTR (R-103), and Table 8.16 shows the inlet component of the CSTR (R-103).

Table 8.14 Operating conditions of CSTR (R-103)

Condition	Operating selection
Operating pressure	1 bar
Temperature	25°C
Material selection	Stainless steel grade 316
Corrosion allowance	2 mm
Joint efficiency	0.85

Table 8.15 Dimension of CSTR (R-103)

Part	Type
Top head	Ellipsoidal 2:1
Shell vessel	Cylindrical
Bottom head	Ellipsoidal 2:1

Table 8.16 Inlet component of CSTR (R-103)

Components	Flow rate (kg/h)	Density(kg/m^3)
SDS	70.61	1010
Sodium Hydroxide	70.61	2100
E. coli	141.4	1105
Glucose	1.61	1560
Acetic Acid	5.12	3250
Water	1.38	997.77
Total	290.73	1363.370215

$$\sum\rho = \frac{70.61}{290.73}(1010) + \frac{70.61}{290.73}(2100) + \frac{141.4}{290.73}(1105) + \frac{1.61}{290.73}(1.61) + \frac{5.12}{290.73}(3250) + \frac{1.38}{290.73}(997.77)$$

$$\sum\rho = 1363.370215\ \frac{kg}{m^3}$$

Volumetric flow rate is calculated using this equation,

$$V = \frac{\mathrm{m}}{\rho}$$

$$V = 0.213244\,\frac{m^3}{h}$$

Volume required for fermenter for operation hour about 10 minutes,

Volume CSTR = 8.956232 m^3

Table 8.17 below shows the dimension of CSTR (R-103)

Table 8.17 Dimension of CSTR (R-103)

Dimension	Values(ft)	Value(in)
Vessel height, H	12.475	149.69
Vessel inside diameter, D_i	4.990	59.88
Cylindrical shell height, h_c	9.980	119.75
Ellipsoidal top head height, h_e	1.247	14.97
Ellipsoidal bottom height, h_e	1.247	14.97

Diameter inside, D_i

$$V = \frac{\pi}{4} {D_i}^2 (2.5 D_i)$$

$$8.956232 = \frac{\pi}{4} {D_i}^2 (2.5 D_i)$$

$$D_i = 4.990 ft = 59.88''$$

Height vessel, H

$$H = 2.5(4.990)$$

$$H = 12.475 ft$$

Ellipsoid height head and bottom, h_e

$$h_e = \frac{D_i}{4}$$

$$h_e = 1.247 ft$$

Cylindrical shell height, h_c

$$h_c = H - 2h_e$$

$$h_c = 9.980\ ft$$

8.5.2 Design Pressure, Minimum wall thickness, Maximum Allowable External Working Pressure

The operational pressure, P_o for the CSTR R-103 is 1 bar while the pressure act on the CSTR is 2 bar from the jacket around the CSTR. Table 8.18 below shows the minimum wall thickness, t_{min} and maximum allowable external working pressure, P_a

Table 8.18 Minimum wall thickness, t_{min} and maximum allowable external working pressure, P_a

Part	T_{min} (mm)	P_a (psi)
Ellipsoidal head	15	20.0072
Cylindrical shell	1.27	18.1594
Ellipsoidal bottom	15	20.0072

Minimum Wall Thickness, t_{min}

Ellipsoidal head and bottom

Let t assume = 15 mm

$\frac{D_o}{2h_o}$ *to determine* K_o

$$\frac{D_o}{2h_o} = \frac{1523.455}{2\left(\left(\frac{2}{3}\right)(380.2102) + 3041.682\right)}$$

$$\frac{D_o}{2h_o} = 2.99$$

By referring Table UG-33.1 from ASME Code to determine K_o value, we need to do the interpolation.

K_o = 1.355

$R_o = K_o D_o$

$R_o = 2065.0434$

Factor A:

$$A = \frac{0.125}{\frac{R_o}{t}}$$

$$A = 0.0009079$$

From figure HA, Subpart 3, Section II, Part D of ASME Code, we can determine factor B,

B = 2500

$$P_a = \frac{B}{\frac{R_o}{t}}$$

$$P_a = 18.159\ psi$$

Since the value for P_a is near to the $P_{D,}$ we can conclude that the t assumed were acceptable. Hence when P_a at 18.159 psi, the t_{min} is at 15 mm.

Cylindrical Shell

Let t assume = 1.27 mm

L/D_o ratio and D_o/t,

$$\frac{L}{D_o} = \frac{3295.316}{1523.455}$$

$$\frac{L}{D_o} = 2.163$$

$$\frac{D_o}{t} = \frac{1523.455}{1.27}$$

$$\frac{D_o}{t} = 1199.571$$

We can determine factor A from Figure G, Subpart 3, Section II, Part D because ratio of L/D_o in the range of $0.05< L/D_o<50$

Factor A = 0.00045

We can determine factor B from Figure HA, Subpart 3, Section II, Part D.

Factor B = 18000

$$P_a = \frac{4B}{3(\frac{D_o}{t})}$$

$$P_a = 20.0075\ psi$$

Since the value for P_a is near to the $P_{D,}$ we can conclude that the t assumed were acceptable. Hence when P_a at 20.0075 psi, the t_{min} is at 1.27 mm.

8.5.3 Thickness uniformity

From the values of t_{min} calculated, a thickness of 15 mm was chosen to be the uniform wall thickness for the whole vessel.

8.5.4 Combine loading analysis for CSTR R-103

Analysis of Primary Stress

Table 8.19 below shows the parameter used in analysis of primary stress for CSTR R-103.

Table 8.19 Parameter used in analysis of primary stress for CSTR R-103

Parameter	Value(N/mm²)
Longitudinal stress (σ_L)	66.626
Circumferential stress (σ_h)	82.617
Direct stress (σ_w)	0.3723
Bending stress (σ_b)	neglected

Longitudinal stress, σ_L

$$\sigma_L = \frac{PD_i}{4t}$$

$$\sigma_L = \frac{(0..13798)(1520.841)}{4(1.27)}$$

$$\sigma_L = 66.626 \frac{N}{mm^2}$$

Circumferential stress, σ_h

$$\sigma_h = \frac{PD_i}{2t}$$

$$\sigma_h = \frac{(0.13798)(1520.841)}{2(1.27)}$$

$$\sigma_h = 82.617 \frac{N}{mm^2}$$

Direct stress, σ_w

$$\sigma_w = \frac{w}{\pi(D_i + t)t}$$

$$w = 240 C_v D_m (H_v + 0.8 D_m) t$$

C_v = a factor account for the weight of nozzles, manway internal supports, which can be taken as 1.08 for vessels with only few internal fittings.

H_v = tangent between tangent line, m

$$H_v = \frac{1}{3}(e.head\ H) + (shell\ H) + \frac{1}{3}(e.bottom\ H)$$

$$H_v = \frac{1}{3}(0.38021) + (3.04168) + \frac{1}{3}(0.38021)$$

$$H_v = 3.295155\ m$$

D_m = mean diameter of the vessel, m

$$D_m = (D_i + t)$$

$$D_m = (1.520841 + 0.00127)$$

$$D_m = 1.5221\ m$$

$$w = 240(1.08)(1.5221)\big(3.2952 + 0.8(1.5221)\big)(0.00127)$$

$$w = 2.2612\ N$$

$$\sigma_w = \frac{2.2612}{\pi(1.5208 + 0.00127)0.00127}$$

$$\sigma_w = 0.3723\frac{N}{m^2}$$

To calculate the principal stress for circumferential stress and longitudinal stress, equation below were used:

$$\sigma_1 = \frac{1}{2}\left[\sigma_h + \sigma_z + \sqrt{(\sigma_h - \sigma_z)^2 + 4T^2}\right]$$

$$\sigma_2 = \frac{1}{2}\left[\sigma_h + \sigma_z - \sqrt{(\sigma_h - \sigma_z)^2 + 4T^2}\right]$$

$$\sigma_3 = 0.5P\ (neglected\ for\ thin\ vessel)$$

With torsional shear stresses = 0

$$\sigma_1 = \sigma_h$$

$$\sigma_2 = \sigma_z$$

Total longitudinal stresses for upwind and downwind can be calculated as follow:

Upwind, σ_z

$$\sigma_z = \sigma_L + \sigma_w + \sigma_b = 66.998 \frac{N}{mm^2}$$

Downwind, σ_z

$$\sigma_z = \sigma_L + \sigma_w + \sigma_b = 66.998 \frac{N}{mm^2}$$

$$\Delta\sigma_{max} = \sigma_h - \sigma_{z(upwind)} = 15.61816 \frac{N}{mm^2}$$

$$\Delta\sigma_{max} = \sigma_h - \sigma_{z(downwind)} = 15.61816 \frac{N}{mm^2}$$

Hence, maximum allowable stress intensity ($\Delta\sigma_{max}$) is equal to 15.61816 N/mm^2

The thickness of the vessel's wall is sufficient to ensure that the maximum stress intensity at any point does not exceed the design stress for the construction material because the maximum allowable stress intensity calculated were less than the material maximum allowable stress which is 137.89515 N/mm^2.

Analysis of elastic stability

From the analysis of the combine loading, the material chosen met both the maximum stress intensity and the elastic stability requirements:

$$\Delta\sigma_{max} \leq S \text{ and } \Delta\sigma_{compressive} \leq \sigma_c$$

σ_c = critical buckling stress

$$\sigma_c = 2\ x\ 10^4 \left(\frac{t}{D_o}\right)$$

$$\sigma_c = 2\ x\ 10^4 \left(\frac{1.27}{1523.455}\right)$$

$$\sigma_c = 16.6726 \frac{N}{mm^2}$$

$$\Delta\sigma_{compressive} = \sigma_b + \sigma_w$$

$$\Delta\sigma_{compressive} = 0.3723 \frac{N}{mm^2}$$

In conclusion, the design is safe.

8.5.5 Vessel Support Analysis for CSTR, R-103

Cylindrical skirt is chosen as the vessel support for our fermenter, R-102 and CSTR, R-103. It is suitable for tall vessels rather than other support. The skirt support is weld at the base of the shell. Material used to fabricate support must be high in strength,

which is stainless steel (SA-240) with nominal composition 18Cr-2Mo. As the material fulfil minimum content to avoid corrosion, which is 12% chromium and contain molybdenum that can improve corrosion resistance.

8.5.6 Skirt Thickness of CSTR, R-103

The skirt thickness must be sufficient to withstand the dead weight load and bending moment imposed on it by the vessel. Cylindrical skirt is suitable for high vessel with subject to wind loading. The specification for skirt thickness of fermenter is shown in Table 8.20 below.

Table 8.20 Specification in skirt thickness for CSTR, R-103

Parameters	Value
Height vessel between tangent line, m	3.295
Diameter of the vessel, m	1.52
Skirt support height, mm	3
Operating pressure, bar	1
Typical liquid loading, kN/m^{-2}	1.2
Skirt thickness, inch	0.25

Table 8.21 The resultant stresses in the skirt design of CSTR, R-103

Parameters	Values
Bending stress in the skirt, σ_{bs}, N/mm^2	0.409
Dead weight stress in the skirt, σ_{ws}, N/mm^2	0.00791
Maximum σ_s tensile, N/mm^2	0.4011
σ_s (compressive), N/mm^2	0.41691

Assume the skirt diameter (D_s) is equal to inner diameter of vessel (D_i) and the skirt thickness (t_s) is equal to the nominal thickness.

Bending stress in the skirt, σ_{bs}

$$\sigma_{bs} = \frac{4m_s}{\pi(D_s + t_s)t_s D_s}$$

M_s = 42818960 Nmm

$$\sigma_{bs} = 0.409 \frac{N}{mm^2}$$

Dead weight stress of vessel, σ_{ws}

$$\sigma_{ws} = \frac{W}{\pi(D_s + t_s)t_s}$$

$$W = C_v \pi \rho_m D_m g(H_v + 0.8D_m) + 10^{-3}$$

$$W = 723.97\ N$$

$$\sigma_{ws} = 0.00791 \frac{N}{mm^2}$$

Therefore, the resultant stresses:

Maximum σ_s (tensile) = $\sigma_{bs} - \sigma_{ws}$

Maximum σ_s (tensile) = 0.4011 N/mm^2

σ_s (compressible) = $\sigma_{bs} + \sigma_{ws}$

σ_s (compressible) = 0.41691 N/mm^2

The skirt thickness under worst condition of wind and dead weight loading should not exceed the following design criteria,

σ_s (tensile) < $f_s J sin\theta_s$

σ_s (compressible) < $0.125E(t_s/D_s)sin\theta_s$

Assumptions:

1. Straight cylindrical skirt

2. Maximum allowable stress for skirt material, f_s = 137.9 N/mm^2

3. Weld joint factor, J = 0.85

4. Base angle, θ_s = 90°

5. Young's modulus, E = 20000

Therefore,

σ_s (tensile) < $f_s J sin\theta_s$

0.4011 < 117.215 N/mm^2

σ_s (compressible) < $0.125E(t_s/D_s)sin\theta_s$

0.4169 < 3.465 N/mm^2

Therefore, the skirt thickness was in acceptable range and with addition of CA, 2 mm.

Base ring and anchor bolt design

The loads carried the by the skirt will transmit to the foundation slab by the skirt base ring.

Assumptions made to determine base ring thickness:

1. Bolt circle diameter, D_b = 2 m

2. Circumference of bolt circle = 2000πmm

3. Maximum allowable bolt stress, f_b = 125 N/mm^2

4. Number of bolts, $N_b \geq 8$

5. Bolt spacing > 600 mm

Table 8.22 shows the base ring and anchor bolt design specification of CSTR, R-103.

Table 8.22 The base ring and anchor bolt design specification of CSTR, R-103

Parameters	Value
Bolt spacing, mm	785.4
Bending moment at the base, M_s, Nmm	9329
f_b, N/m	616.6
L_b, mm	0.18
Base ring thickness, t_b, mm	2.36

The anchor bolt is assumed to share the overturning load equally and the bolt area required:

Bolt spacing = circumference of bolt circle/number of bolts

Bolt spacing = 785.4 mm > 600 mm

Thickness skirt = 25 mm

$$A_b = \frac{1}{N_b f_b}\left(\frac{4M_s}{D_b} - W\right)$$

Wind loading, w = 0.00128 N/mm^2

The bending moment at the skirt base,

$$M_s = \frac{wx^2}{2}$$

$$M_s = 9329\ mm$$

$$A_b = 18\ mm^2$$

Use M24 bolts (BS44190:1967) root area = 353 mm^2

Total compressive load on the base ring per unit length, f_b

$$f_b = \frac{4M_s}{\pi {D_s}^2} + \frac{W}{\pi D_s}$$

$$f_b = 616.6 \frac{N}{m}$$

Bearing pressure = 3.5 N/mm^2, so the minimum width of the base rings, L_b

$$L_b = \frac{f_b}{f_c} x \frac{1}{10^3}$$

$$L_b = 0.18\ mm$$

The required thickness base ring found by treating the rings as cantilever beam:

$$t_b = L_r \sqrt{\frac{3f_c'}{f_r}}$$

Lr = the distance from the edge of the skirt to the outer edge of the ring, mm = 76 mm

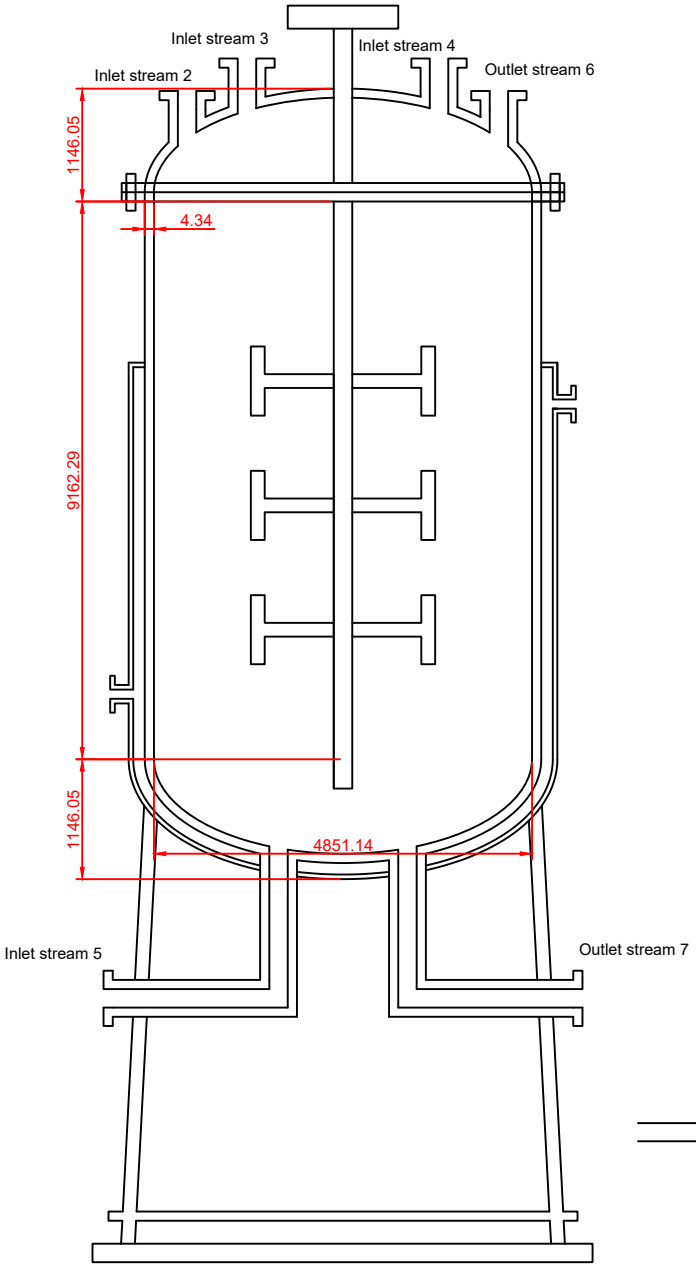
F'c= actual bearing pressure, N/mm^2 = 0.045 N/mm^2

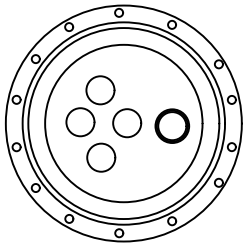
Fr = allowable design stress in the ring of material, N/mm^2 = 140 N/mm^2

$$t_b = 2.36\ mm$$

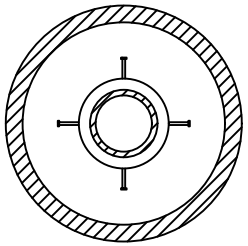
8.6 MECHANICAL DRAWING

Mechanical drawing for Fermenter, F-102 and CSTR, CR-103 will be shown in the following page.

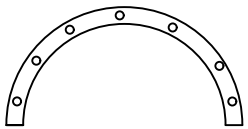




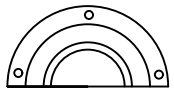
Top view of R-102



Cross sectional of R-102

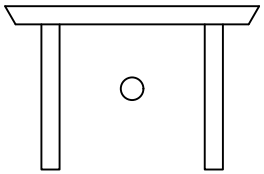


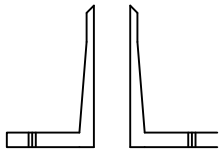
Cross sectional are of manhole

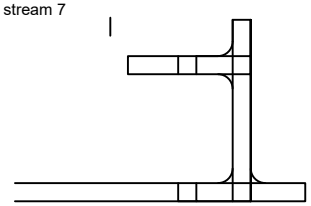


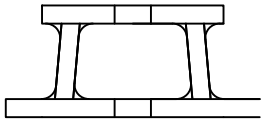
weld flenge

Anchor bolt chair









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FAKULTI KEJURUTERAAN DAN ALAM BINA
JABATAN KEJURUTERAAN KIMIA DAN PROSES

PRODUCTION OF PLASMID DNA FROM *E.COLI*

FERMENTER, R-102 DESIGN

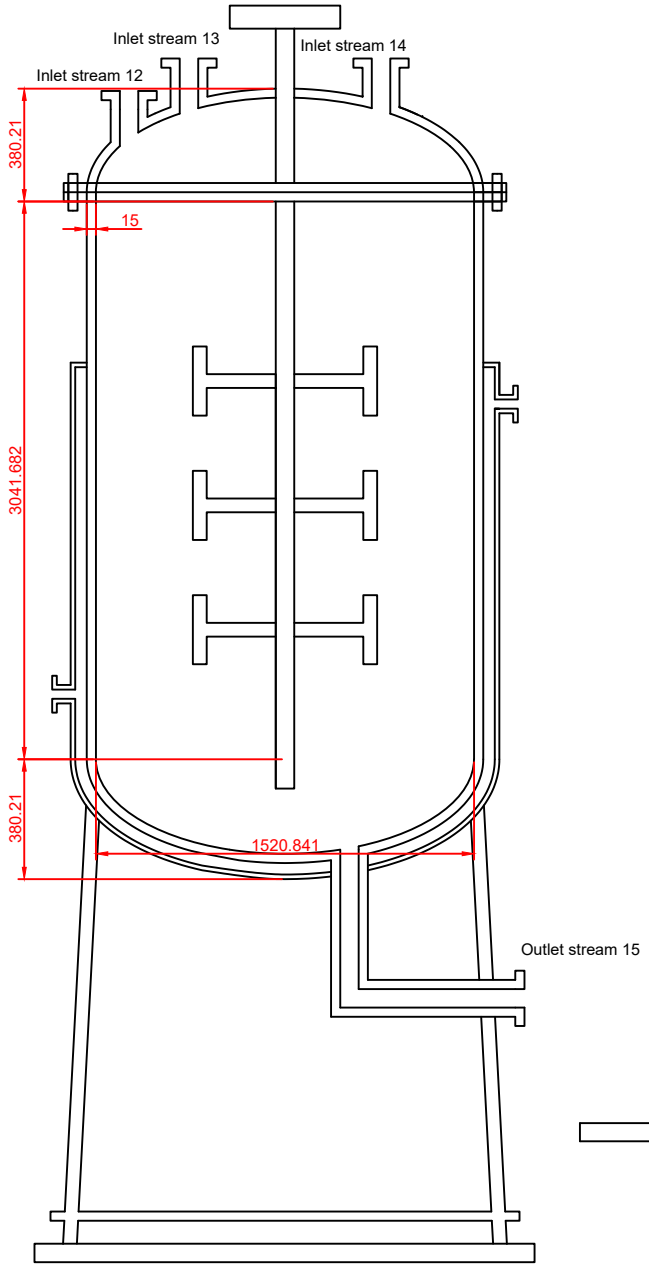
Group KK1:
1) Muhammad Arif Danial bin Hassan (A173470)
2) Azrul Zulhilmi bin Ahmad Rosli (A173752)
3) Nuqman Haziq bin Mohd Ayub Khan (A174118)
4) Ahmad Nawwar Irsyad bin Mohamed (A174340)

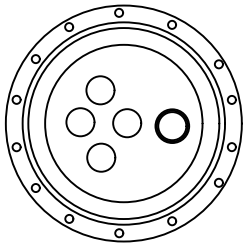
Submission Date: 8/7/2022

Design Specification

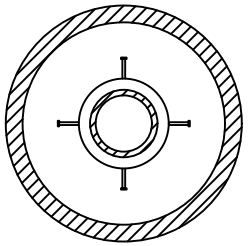
Operating Pressure: 1 bar
Operating Temperature: 37C
Material: Stainless Steel Grade 316
Thickness: 0.171 in
Corrosion Allowance: 2 mm
Head: Ellipsoidal 2:1
Shell: Cylindrical
Bottom: Ellipsoidal 2:1
Design Bolt: M24 (BS4410:1967)
Design Flanges: Well neck flangesclass 150

Unit dimension in mm

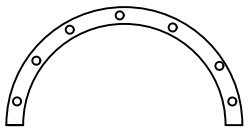




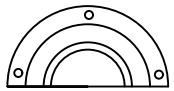
Top view of R-103



Cross sectional of R-103

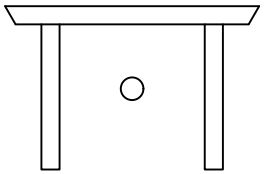


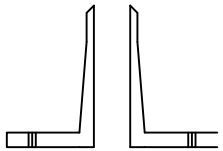
Cross sectional are of manhole

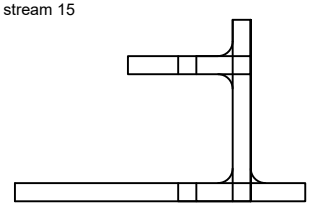


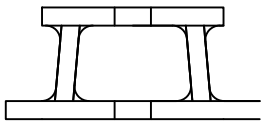
weld flenge

Anchor bolt chair









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FAKULTI KEJURUTERAAN DAN ALAM BINA
JABATAN KEJURUTERAAN KIMIA DAN PROSES

PRODUCTION OF PLASMID DNA FROM *E.COLI*

CSTR, R-103 DESIGN

Group KK1:
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3) Nuqman Haziq bin Mohd Ayub Khan (A174118)
4) Ahmad Nawwar Irsyad bin Mohamed (A174340)

Submission Date: 8/7/2022

Design Specification

Operating Pressure: 1 bar
Operating Temperature: 25C
Material: Stainless Steel Grade 316
Thickness: 15 mm
Corrosion Allowance: 2 mm
Head: Ellipsoidal 2:1
Shell: Cylindrical
Bottom: Ellipsoidal 2:1
Design Bolt: M24 (BS4410:1967)
Design Flanges: Well neck flanges class 150

Unit dimension in mm

CHAPTER IX

DYNAMIC AND PROCESS CONTROL

9.1 INTRODUCTION

Dynamic and process control is one of the important subject which has proven that it is an effective technique for developing and designing control system which is easy, efficient and safe to operate chemical plants. It is critical to have a strong background in dynamic process modelling and control in order to construct a chemical and biochemical process model that meets the requirements for control system design and design of feedback controllers for chemical and biochemical processes (Dennis, 2011).

9.2 VALVES SELECTION

The materials employed in relation to the carried fluid, as well as the valve's mechanical design, determine a valve's suitability for a certain application. Gate valves, globe valves, ball valves, butterfly valves, check valves, pressure valves, and diaphragm valves are examples of industrial valves. These valves serve a variety of purposes and operate on a separate concept. The Table 9.1 below shows the valves that have been selected for our project.

Table 9.1 Valve selection according to the type of controller

Type of Controller	Type of Valve	Reason
Temperature	Globe valve	▪ Good flow control ▪ The opening-closing time is shorter ▪ Can be used as stop-check valves
Flowrate	Globe valve	▪ Good flow control ▪ The opening-closing time is shorter ▪ Is used primarily for throttling
Level	Ball valve	▪ Contain a compact and low-maintenance design that requires no lubrication

pH	Globe valve	▪ Provide leak-proof service ▪ The opening-closing is short ▪ Good flow control ▪ The opening-closing time is shorter ▪ Can be used as stop-check valves
DO	Globe valve	▪ Good flow control ▪ The opening-closing time is shorter ▪ Is used primarily for throttling

Source: Different Types of Valves used in Piping, 2015

9.3 CONTROL CONFIGURATION FOR EACH MAIN UNIT OPERATION

9.3.1 Control Configuration of Main Fermenter (R-102)

Streams 2, 3, 4, and 5 consist of cultured cells of *E. coli*, ammonia, glucose and sterile oxygen are fed into main fermenter to undergo fermentation process for 40 hours at 37°C. At the end of the reaction, more *E. Coli* cells is produced as the main product while water, carbon dioxide and acetic acid are produced as the by-products. Equation for batch fermenter R-102 can refer to Appendix A of dynamic and process control.

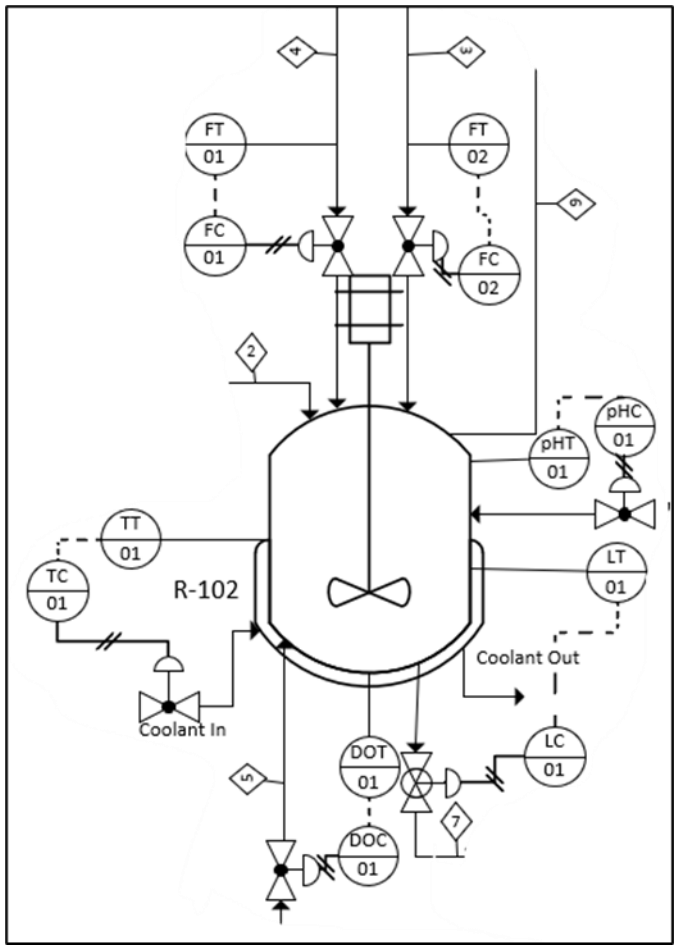


Figure 9.1 Control Configuration of Main Fermenter (R-102)

Table 9.2 Control Configuration of Main Fermenter (R-102)

Type of Controller	Objective	Control Variable	Manipulated Variable	Control Action	Type of Valve
Flowrate	To maintain the required flowrate of ammonia and glucose at Stream 3 and 4	Flowrate of ammonia and glucose at 96.29 and 950 kg/hr respectively.	Flowrate of stream 3 and 4	When the feed flowrate is low, increase the valve opening to obtain the required flowrate. When the flowrate is high, decrease the valve opening to obtain the optimum flowrate.	Globe valve
Temperature	To control the temperature of Main Fermenter	Temperature of the main fermenter at 37°C	Inlet flowrate of cooling water	When the temperature is higher than 37°C, increase the valve opening to let cooling water in. When the temperature is lower than the setpoint, decrease the valve opening to increase the temperature.	Globe valve
Level	To maintain the level of fluid and avoid overflow in the main fermenter	Level of fluid in the main fermenter	Outlet flowrate of the main fermenter at stream 7	When the level of fluid reached the optimum level of the fermenter, the valve will be opened to let the fluid flow out. After the process is done, the valve will be closed	Ball valve
pH	To maintain the pH level	Main fermenter pH level is at	Inlet flowrate of buffer	If the pH is higher than desired, adjust it using an acid	Globe valve

inside main fermenter	6.5	solution enters main fermenter	solution. If the pH is lower than desired, adjust it using an alkaline solution.

Source: Different Types of Valves used in Piping, 2015

9.3.2 Control Configuration of Centrifuge (C-101)

The first step is the harvest of E. coli cell by centrifugation. The main purpose of centrifuge is to separate particles suspended in solution according to its size, density, viscosity of medium and rotor speed. In our plant, biomass, water, acetic acid and the rest of glucose and ammonia from stream 11 that are in the form of fermentative broth is fed into centrifuge to separate the biomass and ensure 97% of biomass is recovered in the bottom stream.

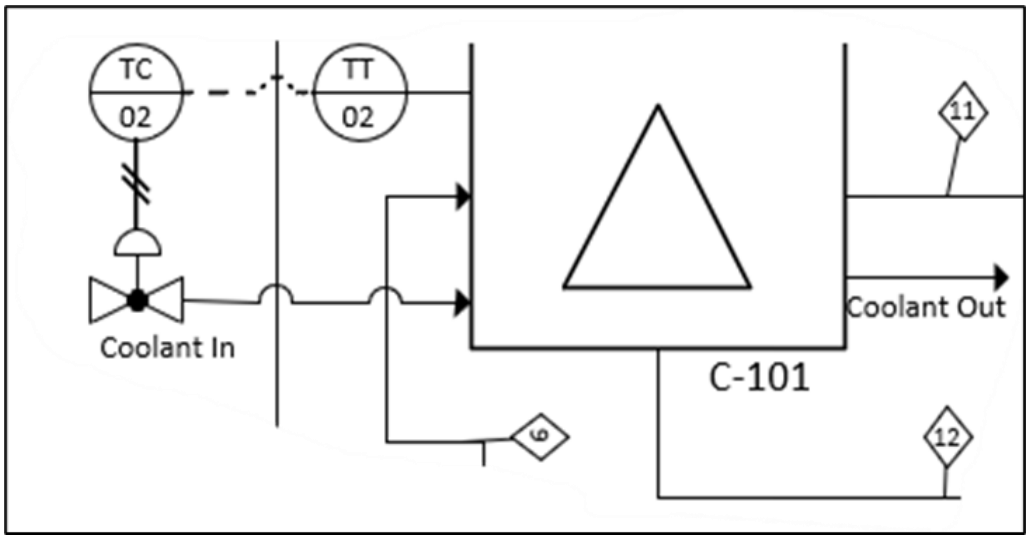


Figure 9.2 Control Configuration of Centrifuge (C-101)

Table 9.3 Control Configuration of Centrifuge (C-101)

Type of Controller	Objective	Control Variable	Manipulated Variable	Control Action	Type of Valve
Temperature	To control the temperature of centrifuge	Temperature of the centrifuge at 30°C	Inlet flowrate of cooling water	When the temperature is higher than 30°C, increase the valve opening to let cooling water in. When the temperature is lower than the setpoint, decrease the valve opening to increase the temperature.	Globe valve

Source: Different Types of Valves used in Piping, 2015

9.3.3 Control Configuration of CSTR (R-103)

In CSTR R-103 cell disruption occur. Alkaline lysis is used for cell disruption other than mechanical method because plasmid and genomic DNA are sensitive to shear stress. This process is done by gently mixing the cell suspension with strong alkali sodium hydroxide 0.2 M and a detergent which is sodium dodecyl sulfate (SDS) 1% w/w. this is done using in CSTR for alkaline lysis for large scale to preserve the quality of plasmid DNA as well as to avoid fragmentation of gDNA. Proper mixing during lysis is crucial to avoids pH extremes that might lead to pDNA denaturation. This alkaline lysis occurs in CSTR at ambient temperature in 10 minutes. The SDS detergent solubilizes the phospholipids and protein of the membrane resulting in cell lysis and the release of the cell contents. The high cell concentration of sodium hydroxide denatures the genomic and pDNA as well as cellular protein.

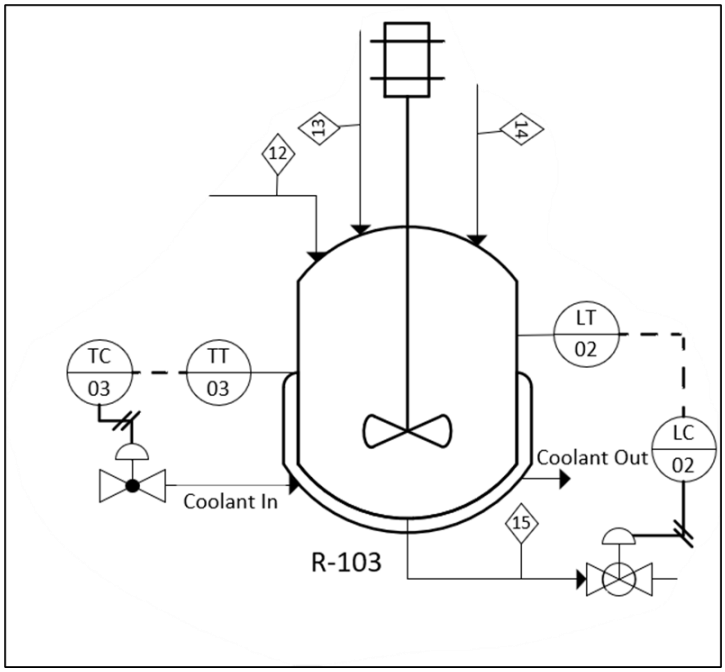


Figure 9.3 Control Configuration of CSTR (R-103)

Table 9.4 Control Configuration of CSTR (R-103)

Type of Controller	Objective	Control Variable	Manipulated Variable	Control Action	Type of Valve
Temperature	To control the temperature of CSTR	Temperature of the CSTR at 25°C	Inlet flowrate of cooling water	When the temperature is higher than 25°C, increase the valve opening to let cooling water in. When the temperature is lower	Globe valve

				than the setpoint, decrease the valve opening to increase the temperature.	
Level	To maintain the level of fluid and avoid overflow in the CSTR	Level of fluid in the CSTR	Outlet flowrate of CSTR at stream 15	When the level of fluid reached the optimum level of the CSTR, the valve will be opened to let the fluid flow out. After the process is done, the valve will be closed	Ball valve

Source: Different Types of Valves used in Piping, 2015

9.3.4 Control Configuration of Chromatography (CR-101)

For Chromatography (CR-101), we are using Anion Exchange Chromatography (AEC). Tris-EDTA being used as a buffer to remove impurities in our pDNA. Then, 90% of the pDNA together with 5% of RNA are retained by the column.

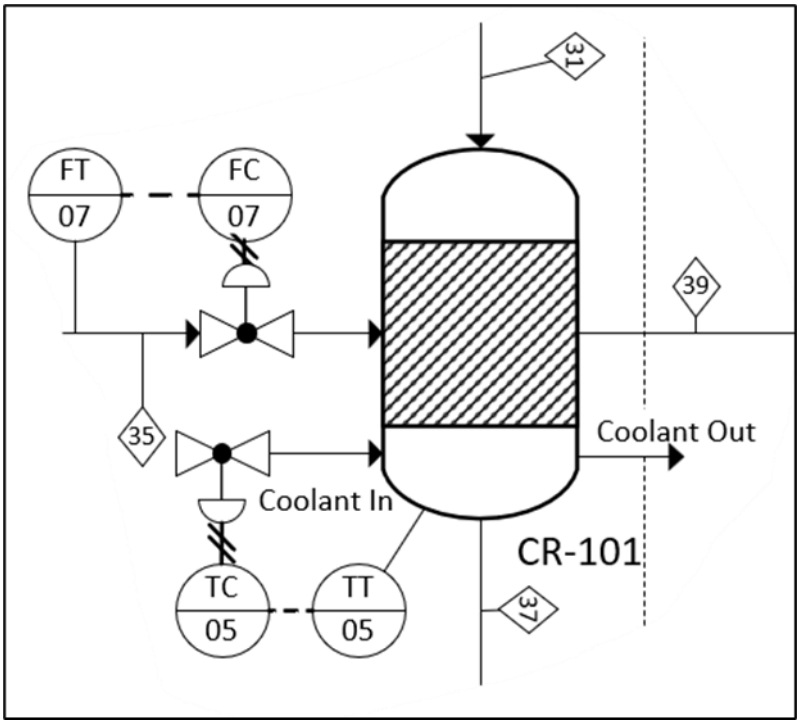


Figure 9.4 Control Configuration of Chromatography (CR-101)

Table 9.5 Control Configuration of Chromatography (CR-101)

Type of Controller	Objective	Control Variable	Manipulated Variable	Control Action	Type of Valve
Flowrate	To maintain the required flowrate of pDNA in stream 35	Flowrate of pDNA at 8.66 kg/hr	Flowrate of stream 35	When the feed flowrate is low, increase the valve opening to obtain the required flowrate. When the flowrate is high, decrease the valve opening to obtain the optimum flowrate.	Globe valve

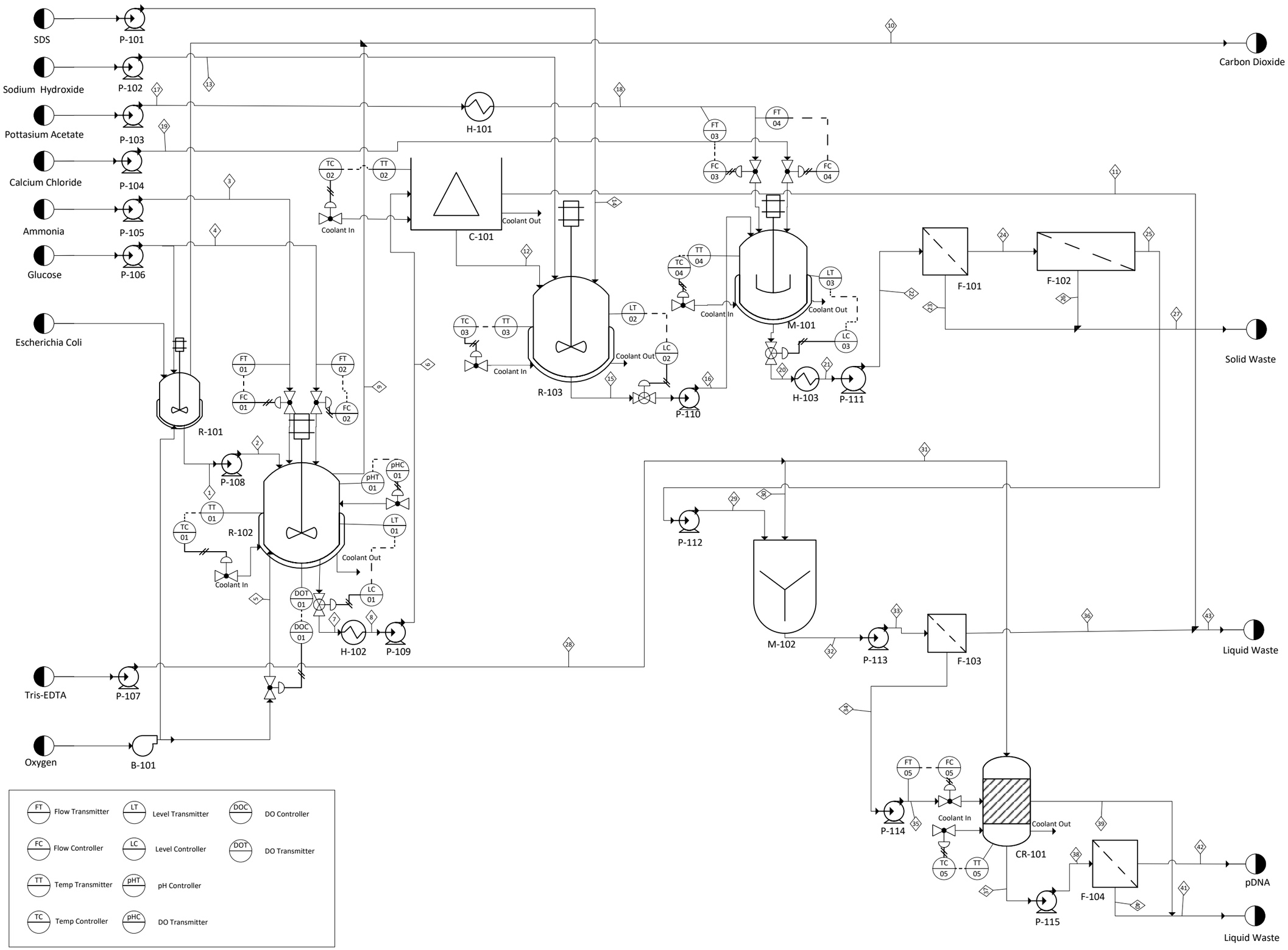
Temperature	To control the temperature of chromatography	Temperature of the chromatography at 30°C	Inlet flowrate of cooling water	When the temperature is higher than 30°C, increase the valve opening to let cooling water in. When the temperature is lower than the setpoint, decrease the valve opening to increase the temperature.	Globe valve

Source: Different Types of Valves used in Piping, 2015

9.4 OVERALL PIPING AND INSTRUMENTATION DIAGRAM

Piping and instrumentation diagram will be shown in the following page.

P-101 Pump 1	P-105 Pump 5	R-101 Reactor 1	P-109 Pump 9	P-110 Pump 10	H-103 STHE 3	F-102 Filter 2	P-115 Pump 15
P-102 Pump 2	P-106 Pump 6	P-108 Pump 8	C-101 Centrifuge 1	P-112 Pump 12	P-111 Pump 11	F-103 Filter 3	F-104 Filter 4
P-103 Pump 3	P-107 Pump 7	R-102 Reactor 2	H-101 STHE 1	M-101 Mixer 1	P-113 Pump 13	P-114 Pump 14	
P-104 Pump 4	B-101 Blower1	H-102 STHE 2	R-103 Reactor 3	M-102 Mixer 2	F-101 Filter 1	CR-101 Chromatography	



Universiti Kebangsaan Malaysia
The National University of Malaysia

Title: Piping and Instrumentation Diagram (P&ID) Production of Plasmid DNA From Escherichia coli

Lecturers:

KKKR3583
Prof. Madya Ts. En. Noorhisham Tan Kofli
Ts. Dr. Ahmad Razi Othman

KKKR3733
Prof. Madya Ts. En. Noorhisham Tan Kofli
Ts. Dr. Darman Nordin
Prof. Madya Ir. Dr. Hassimi Abu Hassan
Prof. Madya Ts. Dr. Rosiah Rohani

KKKR3723
Prof. Madya Ir. Dr. Shuhaida Harun
Prof. Madya Ir. Dr. Teow Yeit Haan

KKKR3713
Prof. Madya Dr. Norliza Abd. Rahman
Dr. Jarinah Mohd Ali

Group: KK1

Group Members:

1. Muhammad Arif Danial Bin Hassan (A173470)

2. Azrul Zulhilmi Bin Ahmad Rosli (A173752)

3. Nuqman Haziq Bin Mohd Ayub Khan (A174188)

4. Ahmad Nawwar Irsyad Bin Moham (A174340)

Input
Output Stream number

Date : 07th July 2022

CONCLUSION

In conclusion, the production of Plasmid DNA from the cultivation of *Escherichia coli* (*e. coli* K12). *E. coli* is a gram-negative bacterium and plasmid DNA is an intermediate chemical to be used in the production of antibiotic and others. By 2027, the market expected to growth to 2344.29 million by 2027, registering CAGR of 23.38% during 2022-2027. Plant capacity in this project is 8.94 kg/hr with 2.5% from the deficient.

In this project, the waste product are solid waste which contains gDNA, RNA and others, liquid waste which contains Tris-EDTA buffer, SDS, and others, and carbon dioxide. In this production, glucose was used as carbon source, and ammonia as nitrogen source. Mass balance for unit operations such as bioreactor, mixer, chromatography, microfiltration, ultrafiltration, and filter are calculated, and the overall mass are balance. The value of entalphy obtained from the energy balance in bioreactor is 048737.49 kJ/hr, hence is an exothermic reaction.

For biochemistry part, the enzyme used is galactose permease (galP) with commission number EC 2.7.1.204. Galactose permease will be used to create the new strain to make the production of acetate low. Furthermore, the main fermentor and CSTR are the pressure vessels designed under respective internal and external pressure according to ASME Code procedure. Then, at utility part, heat exchanger be designed is E-101 and E-102. For separation part, centrifuge is selected as the unit operation. 97.77 % recovery of solid shows that the high efficiency of the centrifuge separation process of solid from mixture. In dynamic and process control, the control configuration (Main fermenter, R-102, Centrifuge, C-101, CSTR, R-103 and Chromatography, CR-101) has been specified before the designing a process control system.

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APPENDIX A
DYNAMIC AND PROCESS CONTROL

Degree of Freedom Analysis

Assumption made for the dynamic model are listed below:

1. Mass in = Mass out

2. Surrounding temperature can be neglected, T*ref* = 0.

3. Specific heat capacity of the mixture is constant, C*p*

4. Density of the mixture is constant.

5. No additional kinetic energy and potential energy, K = P = 0.

6. Heat losses are negligible.

7. The density and heat capacity of the liquid are assumed to be constant

Control Configuration on Main Fermenter (R-102)

The dynamic mathematical modelling for the main fermenter will be derived based on thefollowing assumption:

1. The main fermenter operates at unsteady state.

2. The volume of fermenter and the liquid density are constant.

3. Heat effect is negligible.

4. The feed stream is sterile and thus contains no cells.

5. The cells are growing exponentially.

6. The rate of growth of cell, r_g is given by

$$r_g = \mu X$$

$$\mu = \mu_{max} \frac{S}{K_S + S}$$

7. Rate of product formation per unit volume, $r_P = Y_{P/X} r_g$

Thus,

Equation for Main Fermenter (R-102)

i) Mass Balance

Mass In – Mass Out + Mass Generated = Mass Accumulation

$$(F_2 + F_3 + F_4 + F_5) - F_6 - F_7 = \rho \frac{\delta v}{\delta t}$$

ii) Heat Balance

$$\frac{\delta T}{\delta t} = \frac{F_i}{V}(T_0 - T) - (r_{Av})Hr \pm \frac{Q}{\rho C_P V}$$

$$\rho C_P V \frac{\delta T}{\delta t} = \rho C_P Fi(T_0 - T) - (r_{Av})Hr \pm Q$$

$\rho C_P V \frac{\delta T}{\delta t} = \rho C_P(F_2 + F_3 + F_4 + F_5)(T_0 - T) - (r_{Av})Hr \pm Q$ where,

$$Q = UAC(T_{win} - T_{wout})$$

iii) Height

$$A \frac{\delta h}{\delta t} = \frac{1}{\rho}(Fin - Fout)$$

$$A \frac{\delta h}{\delta t} = \frac{1}{\rho}(F_2 + F_3 + F_4 + F_5) - (F_6 + F_7)$$

Number of variables: F2, F3, F4, F5, F6, F7, T2, T3, T4, T5, T6, T7, V; h, 13

Number of equations: 3

Disturbance: 4 (Flow, Temperature, Pressure)

Degree of freedom: 13 – 3 – 4 = 6

Controller needed: 6

Therefore, 2 flow controls, 1 temperature control, 1 pH control, 1 DO control and 1 level control are required. Flow controller is needed because *E. Coli* needs enough glucose and ammonia at optimum level. At higher temperature, biomass released will

be denatured due to higher temperature. Optimum temperature is needed for biomass. Thus, a temperature controller is required.

Equation of Centrifuge (C-101)

i) Mass balance

$(F_9 - F_{11} - F_{12}) = \rho \frac{\delta v}{\delta t}$, $where\ at\ steady\ state\ \frac{\delta v}{\delta t} = 0$

ii) Efficiency

$Efficiency = 0.90 \times F_i$

Number of variables: F9, F11, F12, V; 4

Number of equations: 2

Disturbance: 1 (Temperature)

Degree of freedom: 4– 2 – 1 = 1

Controller needed: 1

Therefore, 1 temperature control is required.

Equation of CSTR (R-103)

Assumption,

1. Operates at steady state.

2. No accumulation of product.

i) Mass Balance

Mass In = Mass Out

$(F_{12} + F_{13} + F_{14}) - F_{15} = \rho \frac{\delta v}{\delta t}$, $where\ at\ steady\ state\ \frac{\delta v}{\delta t} = 0$

ii) Height

$$A\frac{\delta h}{\delta t} = \frac{1}{\rho}(Fin\ -\ Fout\ )$$

$$A\frac{\delta h}{\delta t} = \frac{1}{\rho}(F_{12}\ +\ F_{13}\ +\ F_{14}\ )\ -\ F_{15}$$

Number of variables: F12, F13, F14, F15, V, h: 6

Number of equations: 2

Disturbance: 2 (Level, Temperature)

Degree of freedom: 6– 2 – 2 = 2

Controller needed: 2

Therefore, 1 temperature control and 1 level control are required.

Equation of Chromatography (CR-101)

i) Mass Balance

Mass In = Mass Out

$$(F_{31} + F_{35}) - F_{37} - F_{39} =\ \rho\frac{\delta v}{\delta t}, where\ at\ steady\ state\ \frac{\delta v}{\delta t} =\ 0$$

ii) Height

$$A\frac{\delta h}{\delta t} = \frac{1}{\rho}(Fin\ -\ Fout\ )$$

$$A\frac{\delta h}{\delta t} = \frac{1}{\rho}((F_{31}\ +\ F_{35})\ -\ F_{37} - F_{39})$$

Number of variables: F31, F35, F37, F39, V, h; 6

Number of equations: 2

Disturbance: 2 (Level, Temperature)

Degree of freedom: 6 – 3 – 2 = 2

Controller needed: 2

Therefore, 1 temperature control, and 1 level control are required.

SAFETY DATA SHEET

Oxygen

Airgas
an Air Liquide company

Section 1. Identification

GHS product identifier : Oxygen

Chemical name : oxygen

Other means of identification : Molecular oxygen; Oxygen molecule; Pure oxygen; O2; UN 1072; Dioxygen; Oxygen USP, Aviator's Breathing Oxygen (ABO)

Product type : Gas.

Product use : Synthetic/Analytical chemistry.

Synonym : Molecular oxygen; Oxygen molecule; Pure oxygen; O2; UN 1072; Dioxygen; Oxygen USP, Aviator's Breathing Oxygen (ABO)

SDS # : 001043

Supplier's details : Airgas USA, LLC and its affiliates
259 North Radnor-Chester Road
Suite 100
Radnor, PA 19087-5283
1-610-687-5253

24-hour telephone : 1-866-734-3438

Section 2. Hazards identification

OSHA/HCS status : This material is considered hazardous by the OSHA Hazard Communication Standard (29 CFR 1910.1200).

Classification of the substance or mixture : OXIDIZING GASES - Category 1
GASES UNDER PRESSURE - Compressed gas

GHS label elements

Hazard pictograms :

Signal word : Danger

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

Precautionary statements

General : Read and follow all Safety Data Sheets (SDS'S) before use. Read label before use. Keep out of reach of children. If medical advice is needed, have product container or label at hand. Close valve after each use and when empty. Use equipment rated for cylinder pressure. Do not open valve until connected to equipment prepared for use. Use a back flow preventative device in the piping. Use only equipment of compatible materials of construction. Open valve slowly. Use only with equipment cleaned for Oxygen service.

Prevention : Keep away from clothing and other combustible materials. Keep reduction valves, valves and fittings free from oil and grease.

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

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

Disposal : Not applicable.

Hazards not otherwise classified : None known.

Section 3. Composition/information on ingredients

Substance/mixture : Substance

Chemical name : oxygen

Other means of identification : Molecular oxygen; Oxygen molecule; Pure oxygen; O2; UN 1072; Dioxygen; Oxygen USP, Aviator's Breathing Oxygen (ABO)

Product code : 001043

CAS number/other identifiers

CAS number : 7782-44-7

Ingredient name	%	CAS number
oxygen	100	7782-44-7

Any concentration shown as a range is to protect confidentiality or is due to batch variation.

There are no additional ingredients present which, within the current knowledge of the supplier and in the concentrations applicable, are classified as hazardous to health or the environment and hence require reporting in this section.

Occupational exposure limits, if available, are listed in Section 8.

Section 4. First aid measures

Description of necessary first aid measures

Eye contact : Immediately flush eyes with plenty of water, occasionally lifting the upper and lower eyelids. Check for and remove any contact lenses. Continue to rinse for at least 10 minutes. Get medical attention.

Inhalation : Remove victim to fresh air and keep at rest in a position comfortable for breathing. If not breathing, if breathing is irregular or if respiratory arrest occurs, provide artificial respiration or oxygen by trained personnel. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation. Get medical attention if adverse health effects persist or are severe. If unconscious, place in recovery position and get medical attention immediately. Maintain an open airway. Loosen tight clothing such as a collar, tie, belt or waistband.

Skin contact : Flush contaminated skin with plenty of water. Remove contaminated clothing and shoes. Get medical attention if symptoms occur. Wash clothing before reuse. Clean shoes thoroughly before reuse.

Ingestion : As this product is a gas, refer to the inhalation section.

Most important symptoms/effects, acute and delayed

Potential acute health effects

Eye contact : Contact with rapidly expanding gas may cause burns or frostbite.

Inhalation : No known significant effects or critical hazards.

Skin contact : Contact with rapidly expanding gas may cause burns or frostbite.

Frostbite : Try to warm up the frozen tissues and seek medical attention.

Ingestion : As this product is a gas, refer to the inhalation section.

Over-exposure signs/symptoms

Eye contact : No specific data.

Inhalation : No specific data.

Skin contact : No specific data.

Ingestion : No specific data.

Indication of immediate medical attention and special treatment needed, if necessary

Notes to physician : Treat symptomatically. Contact poison treatment specialist immediately if large quantities have been ingested or inhaled.

Specific treatments : No specific treatment.

Section 4. First aid measures

Protection of first-aiders : No action shall be taken involving any personal risk or without suitable training. It may be dangerous to the person providing aid to give mouth-to-mouth resuscitation.

See toxicological information (Section 11)

Section 5. Fire-fighting measures

Extinguishing media

Suitable extinguishing media : Use an extinguishing agent suitable for the surrounding fire.

Unsuitable extinguishing media : None known.

Specific hazards arising from the chemical : Contains gas under pressure. Oxidizing material. This material increases the risk of fire and may aid combustion. Contact with combustible material may cause fire. In a fire or if heated, a pressure increase will occur and the container may burst or explode.

Hazardous thermal decomposition products : No specific data.

Special protective actions for fire-fighters : Promptly isolate the scene by removing all persons from the vicinity of the incident if there is a fire. No action shall be taken involving any personal risk or without suitable training. Contact supplier immediately for specialist advice. Move containers from fire area if this can be done without risk. Use water spray to keep fire-exposed containers cool. If involved in fire, shut off flow immediately if it can be done without risk.

Special protective equipment for fire-fighters : Fire-fighters should wear appropriate protective equipment and self-contained breathing apparatus (SCBA) with a full face-piece operated in positive pressure mode.

Section 6. Accidental release measures

Personal precautions, protective equipment and emergency procedures

For non-emergency personnel : No action shall be taken involving any personal risk or without suitable training. Evacuate surrounding areas. Keep unnecessary and unprotected personnel from entering. Shut off all ignition sources. No flares, smoking or flames in hazard area. Avoid breathing gas. Provide adequate ventilation. Wear appropriate respirator when ventilation is inadequate. Put on appropriate personal protective equipment.

For emergency responders : If specialized clothing is required to deal with the spillage, take note of any information in Section 8 on suitable and unsuitable materials. See also the information in "For non-emergency personnel".

Environmental precautions : Ensure emergency procedures to deal with accidental gas releases are in place to avoid contamination of the environment. Inform the relevant authorities if the product has caused environmental pollution (sewers, waterways, soil or air).

Methods and materials for containment and cleaning up

Small spill : Immediately contact emergency personnel. Stop leak if without risk. Use spark-proof tools and explosion-proof equipment.

Large spill : Immediately contact emergency personnel. Stop leak if without risk. Use spark-proof tools and explosion-proof equipment. Note: see Section 1 for emergency contact information and Section 13 for waste disposal.

Section 7. Handling and storage

Precautions for safe handling

Section 7. Handling and storage

Protective measures : Put on appropriate personal protective equipment (see Section 8). Contains gas under pressure. Avoid breathing gas. Do not puncture or incinerate container. Use equipment rated for cylinder pressure. Close valve after each use and when empty. Protect cylinders from physical damage; do not drag, roll, slide, or drop. Use a suitable hand truck for cylinder movement.

Avoid contact with eyes, skin and clothing. Empty containers retain product residue and can be hazardous. Keep away from clothing, incompatible materials and combustible materials. Keep reduction valves free from grease and oil.

Advice on general occupational hygiene : Eating, drinking and smoking should be prohibited in areas where this material is handled, stored and processed. Workers should wash hands and face before eating, drinking and smoking. Remove contaminated clothing and protective equipment before entering eating areas. See also Section 8 for additional information on hygiene measures.

Conditions for safe storage, including any incompatibilities : Store in accordance with local regulations. Store in a segregated and approved area. Store away from direct sunlight in a dry, cool and well-ventilated area, away from incompatible materials (see Section 10). Cylinders should be stored upright, with valve protection cap in place, and firmly secured to prevent falling or being knocked over. Cylinder temperatures should not exceed 52 °C (125 °F). Separate from reducing agents and combustible materials. Store away from grease and oil. Keep container tightly closed and sealed until ready for use. See Section 10 for incompatible materials before handling or use.

Section 8. Exposure controls/personal protection

Control parameters

Occupational exposure limits

Ingredient name	Exposure limits
oxygen	None.

Appropriate engineering controls : Good general ventilation should be sufficient to control worker exposure to airborne contaminants.

Environmental exposure controls : Emissions from ventilation or work process equipment should be checked to ensure they comply with the requirements of environmental protection legislation. In some cases, fume scrubbers, filters or engineering modifications to the process equipment will be necessary to reduce emissions to acceptable levels.

Individual protection measures

Hygiene measures : Wash hands, forearms and face thoroughly after handling chemical products, before eating, smoking and using the lavatory and at the end of the working period. Appropriate techniques should be used to remove potentially contaminated clothing. Wash contaminated clothing before reusing. Ensure that eyewash stations and safety showers are close to the workstation location.

Eye/face protection : Safety eyewear complying with an approved standard should be used when a risk assessment indicates this is necessary to avoid exposure to liquid splashes, mists, gases or dusts. If contact is possible, the following protection should be worn, unless the assessment indicates a higher degree of protection: safety glasses with side-shields.

Skin protection

Hand protection : Chemical-resistant, impervious gloves complying with an approved standard should be worn at all times when handling chemical products if a risk assessment indicates this is necessary. Considering the parameters specified by the glove manufacturer, check during use that the gloves are still retaining their protective properties. It should be noted that the time to breakthrough for any glove material may be different for different glove manufacturers. In the case of mixtures, consisting of several substances, the protection time of the gloves cannot be accurately estimated.

Section 8. Exposure controls/personal protection

Body protection : Personal protective equipment for the body should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Other skin protection : Appropriate footwear and any additional skin protection measures should be selected based on the task being performed and the risks involved and should be approved by a specialist before handling this product.

Respiratory protection : Based on the hazard and potential for exposure, select a respirator that meets the appropriate standard or certification. Respirators must be used according to a respiratory protection program to ensure proper fitting, training, and other important aspects of use. Respirator selection must be based on known or anticipated exposure levels, the hazards of the product and the safe working limits of the selected respirator.

Section 9. Physical and chemical properties

Appearance

Physical state : Gas. [Compressed gas.]

Color : Colorless. Blue.

Odor : Odorless.

Odor threshold : Not available.

pH : Not available.

Melting point : -218.4°C (-361.1°F)

Boiling point : -183°C (-297.4°F)

Critical temperature : -118.15°C (-180.7°F)

Flash point : [Product does not sustain combustion.]

Evaporation rate : Not available.

Flammability (solid, gas) : Extremely flammable in the presence of the following materials or conditions: reducing materials, combustible materials and organic materials.

Lower and upper explosive (flammable) limits : Not available.

Vapor pressure : Not available.

Vapor density : 1.1 (Air = 1)

Specific Volume (ft ^{3}/lb) : 12.0482

Gas Density (lb/ft ^{3}) : 0.083

Relative density : Not applicable.

Solubility : Not available.

Solubility in water : Not available.

Partition coefficient: n-octanol/water : 0.65

Auto-ignition temperature : Not available.

Decomposition temperature : Not available.

Viscosity : Not applicable.

Flow time (ISO 2431) : Not available.

Molecular weight : 32 g/mole

Section 10. Stability and reactivity

Reactivity : No specific test data related to reactivity available for this product or its ingredients.

Chemical stability : The product is stable.

Possibility of hazardous reactions : Hazardous reactions or instability may occur under certain conditions of storage or use.
Conditions may include the following:
contact with combustible materials
Reactions may include the following:
risk of causing fire

Section 10. Stability and reactivity

Conditions to avoid : No specific data.

Incompatible materials : Highly reactive or incompatible with the following materials:
combustible materials
reducing materials
grease
oil

Hazardous decomposition products : Under normal conditions of storage and use, hazardous decomposition products should not be produced.

Hazardous polymerization : Under normal conditions of storage and use, hazardous polymerization will not occur.

Section 11. Toxicological information

Information on toxicological effects

Acute toxicity
Not available.

Irritation/Corrosion
Not available.

Sensitization
Not available.

Mutagenicity
Not available.

Carcinogenicity
Not available.

Reproductive toxicity
Not available.

Teratogenicity
Not available.

Specific target organ toxicity (single exposure)
Not available.

Specific target organ toxicity (repeated exposure)
Not available.

Aspiration hazard
Not available.

Information on the likely routes of exposure : Not available.

Potential acute health effects

Eye contact : Contact with rapidly expanding gas may cause burns or frostbite.

Inhalation : No known significant effects or critical hazards.

Skin contact : Contact with rapidly expanding gas may cause burns or frostbite.

Ingestion : As this product is a gas, refer to the inhalation section.

Symptoms related to the physical, chemical and toxicological characteristics

Section 11. Toxicological information

Eye contact : No specific data.

Inhalation : No specific data.

Skin contact : No specific data.

Ingestion : No specific data.

Delayed and immediate effects and also chronic effects from short and long term exposure

Short term exposure

Potential immediate effects : Not available.

Potential delayed effects : Not available.

Long term exposure

Potential immediate effects : Not available.

Potential delayed effects : Not available.

Potential chronic health effects

Not available.

General : No known significant effects or critical hazards.

Carcinogenicity : No known significant effects or critical hazards.

Mutagenicity : No known significant effects or critical hazards.

Teratogenicity : No known significant effects or critical hazards.

Developmental effects : No known significant effects or critical hazards.

Fertility effects : No known significant effects or critical hazards.

Numerical measures of toxicity

Acute toxicity estimates

Not available.

Section 12. Ecological information

Toxicity

Not available.

Persistence and degradability

Not available.

Bioaccumulative potential

Product/ingredient name	$LogP_{ow}$	BCF	Potential
oxygen	0.65	-	low

Mobility in soil

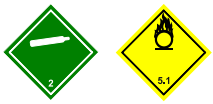
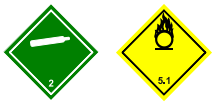
Soil/water partition coefficient (K_{OC}) : Not available.

Other adverse effects : No known significant effects or critical hazards.

Section 13. Disposal considerations

Disposal methods : The generation of waste should be avoided or minimized wherever possible. Disposal of this product, solutions and any by-products should at all times comply with the requirements of environmental protection and waste disposal legislation and any regional local authority requirements. Dispose of surplus and non-recyclable products via a licensed waste disposal contractor. Waste should not be disposed of untreated to the sewer unless fully compliant with the requirements of all authorities with jurisdiction. Empty Airgas-owned pressure vessels should be returned to Airgas. Waste packaging should be recycled. Incineration or landfill should only be considered when recycling is not feasible. This material and its container must be disposed of in a safe way. Empty containers or liners may retain some product residues. Do not puncture or incinerate container.

Section 14. Transport information

	DOT	TDG	Mexico	IMDG	IATA
UN number	UN1072	UN1072	UN1072	UN1072	UN1072
UN proper shipping name	OXYGEN, COMPRESSED	OXYGEN, COMPRESSED	OXYGEN, COMPRESSED	OXYGEN, COMPRESSED	OXYGEN, COMPRESSED
Transport hazard class(es)	2.2 (5.1) 	2.2 	2.2 (5.1) 	2.2 (5.1) 	2.2 (5.1) 
Packing group	-	-	-	-	-
Environmental hazards	No.	No.	No.	No.	No.

"Refer to CFR 49 (or authority having jurisdiction) to determine the information required for shipment of the product."

Additional information

DOT Classification : **Limited quantity** Yes.
Quantity limitation Passenger aircraft/rail: 75 kg. Cargo aircraft: 150 kg.
Special provisions A52

TDG Classification : Product classified as per the following sections of the Transportation of Dangerous Goods Regulations: 2.13-2.17 (Class 2), 2.23-2.25 (Class 5).
Explosive Limit and Limited Quantity Index 0.125
ERAP Index 3000
Passenger Carrying Vessel Index 50
Passenger Carrying Road or Rail Index 75
Special provisions 42

IATA : **Quantity limitation** Passenger and Cargo Aircraft: 75 kg. Cargo Aircraft Only: 150 kg.

Special precautions for user : **Transport within user's premises:** always transport in closed containers that are upright and secure. Ensure that persons transporting the product know what to do in the event of an accident or spillage.

Transport in bulk according to IMO instruments : Not available.

Section 15. Regulatory information

U.S. Federal regulations : **TSCA 8(a) CDR Exempt/Partial exemption**: This material is listed or exempted.

Clean Air Act Section 112 (b) Hazardous Air Pollutants (HAPs) : Not listed

Clean Air Act Section 602 Class I Substances : Not listed

Clean Air Act Section 602 Class II Substances : Not listed

DEA List I Chemicals (Precursor Chemicals) : Not listed

DEA List II Chemicals (Essential Chemicals) : Not listed

SARA 302/304

Composition/information on ingredients

No products were found.

SARA 304 RQ : Not applicable.

SARA 311/312

Classification : Refer to Section 2: Hazards Identification of this SDS for classification of substance.

State regulations

Massachusetts : This material is listed.

New York : This material is not listed.

New Jersey : This material is listed.

Pennsylvania : This material is listed.

California Prop. 65

This product does not require a Safe Harbor warning under California Prop. 65.

International regulations

Chemical Weapon Convention List Schedules I, II & III Chemicals

Not listed.

Montreal Protocol

Not listed.

Stockholm Convention on Persistent Organic Pollutants

Not listed.

Rotterdam Convention on Prior Informed Consent (PIC)

Not listed.

UNECE Aarhus Protocol on POPs and Heavy Metals

Not listed.

Inventory list

Australia : This material is listed or exempted.

Canada : This material is listed or exempted.

China : This material is listed or exempted.

Europe : This material is listed or exempted.

Japan : **Japan inventory (ENCS)**: Not determined.
Japan inventory (ISHL): Not determined.

New Zealand : This material is listed or exempted.

Philippines : This material is listed or exempted.

Section 15. Regulatory information

Republic of Korea : This material is listed or exempted.

Taiwan : This material is listed or exempted.

Thailand : Not determined.

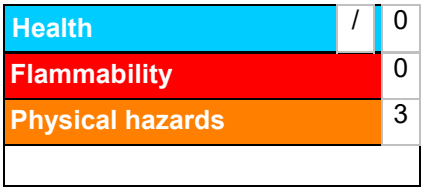
Turkey : Not determined.

United States : This material is active or exempted.

Viet Nam : This material is listed or exempted.

Section 16. Other information

Hazardous Material Information System (U.S.A.)



Caution: HMIS® ratings are based on a 0-4 rating scale, with 0 representing minimal hazards or risks, and 4 representing significant hazards or risks. Although HMIS® ratings and the associated label are not required on SDSs or products leaving a facility under 29 CFR 1910.1200, the preparer may choose to provide them. HMIS® ratings are to be used with a fully implemented HMIS® program. HMIS® is a registered trademark and service mark of the American Coatings Association, Inc.

The customer is responsible for determining the PPE code for this material. For more information on HMIS® Personal Protective Equipment (PPE) codes, consult the HMIS® Implementation Manual.

National Fire Protection Association (U.S.A.)

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Copyright ©2001, National Fire Protection Association, Quincy, MA 02269. This warning system is intended to be interpreted and applied only by properly trained individuals to identify fire, health and reactivity hazards of chemicals. The user is referred to certain limited number of chemicals with recommended classifications in NFPA 49 and NFPA 325, which would be used as a guideline only. Whether the chemicals are classified by NFPA or not, anyone using the 704 systems to classify chemicals does so at their own risk.

Procedure used to derive the classification

Classification	Justification
OXIDIZING GASES - Category 1 GASES UNDER PRESSURE - Compressed gas	Expert judgment According to package

History

Date of printing : 9/22/2020

Date of issue/Date of revision : 9/22/2020

Date of previous issue : 2/3/2018

Version : 1

Key to abbreviations : ATE = Acute Toxicity Estimate
BCF = Bioconcentration Factor
GHS = Globally Harmonized System of Classification and Labelling of Chemicals
IATA = International Air Transport Association
IBC = Intermediate Bulk Container
IMDG = International Maritime Dangerous Goods
LogPow = logarithm of the octanol/water partition coefficient

Section 16. Other information

MARPOL = International Convention for the Prevention of Pollution From Ships, 1973 as modified by the Protocol of 1978. ("Marpol" = marine pollution)
UN = United Nations

References : Not available.

Notice to reader

To the best of our knowledge, the information contained herein is accurate. However, neither the above-named supplier, nor any of its subsidiaries, assumes any liability whatsoever for the accuracy or completeness of the information contained herein.
Final determination of suitability of any material is the sole responsibility of the user. All materials may present unknown hazards and should be used with caution. Although certain hazards are described herein, we cannot guarantee that these are the only hazards that exist.

Ammonia

SECTION 1 : Identification of the substance/mixture and of the supplier

Product name : **Ammonia**

Manufacturer/Supplier Trade name:

Manufacturer/Supplier Article number: **S25164**

Recommended uses of the product and uses restrictions on use:

Manufacturer Details:

AquaPhoenix Scientific
9 Barnhart Drive, Hanover, PA 17331

Supplier Details:

Fisher Science Education
15 Jet View Drive, Rochester, NY 14624

Emergency telephone number:

Fisher Science Education Emergency Telephone No.: 800-535-5053

SECTION 2 : Hazards identification

Classification of the substance or mixture:

Corrosive
Skin corrosion, category 1B

Environmentally Damaging
Acute hazards to the aquatic environment, category 1

Irritant
Specific target organ toxicity following single exposure, category 3

STOT SE 3
AcAq Tox 1
Skin Corr. 1B

Signal word :Danger

Hazard statements:
Causes severe skin burns and eye damage
May cause respiratory irritation
Very toxic to aquatic life
Precautionary statements:
If medical advice is needed, have product container or label at hand
Keep out of reach of children
Read label before use
Do not breathe dust/fume/gas/mist/vapours/spray
Avoid release to the environment
Wear protective gloves/protective clothing/eye protection/face protection
Use personal protective equipment as required

Do not eat, drink or smoke when using this product
Wash skin thoroughly after handling
IF INHALED: Remove victim to fresh air and keep at rest in a position comfortable for breathing
IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses if present and easy to do. Continue rinsing
Immediately call a POISON CENTER or doctor/physician
IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower
IF SWALLOWED: Rinse mouth. Do NOT induce vomiting
Collect spillage
Specific treatment (see supplemental first aid instructions on this label)
Wash contaminated clothing before reuse
Store locked up
Store in a dry place
Store in a well ventilated place. Keep container tightly closed
Dispose of contents/container to ...

Combustible Dust Hazard: :
May form combustible dust concentrations in air (during processing).

Other Non-GHS Classification:

WHMIS

E 

NFPA/HMIS

NFPA SCALE (0-4)

HMIS RATINGS (0-4)

SECTION 3 : Composition/information on ingredients

Ingredients:		
CAS 1336-21-6	Ammonium Hydroxide, ACS	12.32 %
CAS 7732-18-5	Deionized Water	87 %
		Percentages are by weight

SECTION 4 : First aid measures

Description of first aid measures

After inhalation: Move exposed individual to fresh air. Loosen clothing as necessary and position individual in

a comfortable position.Seek medical advice if discomfort or irritation persists.If breathing difficult, give oxygen.

After skin contact: Wash affected area with soap and water. Rinse/flush exposed skin gently using water for 15-20 minutes. Seek medical advice if discomfort or irritation persists.

After eye contact: Protect unexposed eye. Rinse/flush exposed eye(s) gently using water for 15-20 minutes. Remove contact lens(es) if able to do so during rinsing. Seek medical attention if irritation persists or if concerned.

After swallowing: Rinse mouth thoroughly. Do not induce vomiting. Have exposed individual drink sips of water. Seek medical attention if irritation, discomfort or vomiting persists.

Most important symptoms and effects, both acute and delayed:

Irritation, Nausea,Headache, Shortness of breath.;

Indication of any immediate medical attention and special treatment needed:

If seeking medical attention, provide SDS document to physician.

SECTION 5 : Firefighting measures

Extinguishing media

Suitable extinguishing agents: If in laboratory setting, follow laboratory fire suppression procedures. Use appropriate fire suppression agents for adjacent combustible materials or sources of ignition

For safety reasons unsuitable extinguishing agents:

Special hazards arising from the substance or mixture:

Combustion products may include carbon oxides or other toxic vapors.Thermal decomposition can lead to release of irritating gases and vapors.Avoid generating dust; fine dust dispersed in air in sufficient concentrations, and in the presence of an ignition source is a potential dust explosion hazard.

Advice for firefighters:

Protective equipment: Use NIOSH-approved respiratory protection/breathing apparatus.

Additional information (precautions): Move product containers away from fire or keep cool with water spray as a protective measure, where feasible.Use spark-proof tools and explosion-proof equipment.

SECTION 6 : Accidental release measures

Personal precautions, protective equipment and emergency procedures:

Wear protective equipment. Transfer to a disposal or recovery container.Use spark-proof tools and explosion-proof equipment.Use respiratory protective device against the effects of fumes/dust/aerosol. Keep unprotected persons away. Ensure adequate ventilation.Keep away from ignition sources. Protect from heat.Stop the spill, if possible. Contain spilled material by diking or using inert absorbent.

Environmental precautions:

Prevent from reaching drains, sewer or waterway. Collect contaminated soil for characterization per Section 13

Methods and material for containment and cleaning up:

If in a laboratory setting, follow Chemical Hygiene Plan procedures.Place into properly labeled containers for recovery or disposal. If necessary, use trained response staff/contractor.Dust deposits should not be allowed to accumulate on surfaces, as these may form an explosive mixture if they are released into the atmosphere in sufficient concentration. Avoid dispersal of dust in the air (i.e., clearing dust surfaces with compressed air). Collect solids in powder form using vacuum with (HEPA filter)

Reference to other sections:

SECTION 7 : Handling and storage

Precautions for safe handling:

Wash hands after handling. Follow good hygiene procedures when handling chemical materials. Do not eat, drink, smoke, or use personal products when handling chemical substances. If in a laboratory setting, follow Chemical Hygiene Plan.Use only in well ventilated areas.Avoid contact with eyes, skin, and clothing.

Conditions for safe storage, including any incompatibilities:

Provide ventilation for containers. Avoid storage near extreme heat, ignition sources or open flame. Store away from foodstuffs. Store away from oxidizing agents.Store in cool, dry conditions in well sealed containers. Store with like hazards

SECTION 8 : Exposure controls/personal protection



Control Parameters:
1336-21-6, Ammonium Hydroxide, ACGIH TLV: 17 mg/m3
1336-21-6 , Ammonium Hydroxide , OSHA PEL: 35 mg/m3
1336-21-6, Ammonium Hydroxide, OSHA TWA 25 ppm (18 mg/m3) ST 35 ppm (27 mg/m3)
1336-21-6, Ammonium Hydroxide, ACGIH TWA 25 ppm (18 mg/m3) ST 35 ppm (27 mg/m3)

Appropriate Engineering controls:
Emergency eye wash fountains and safety showers should be available in the immediate vicinity of use/handling.Provide exhaust ventilation or other engineering controls to keep the airborne concentrations of vapor or dusts (total/respirable) below the applicable workplace exposure limits (Occupational Exposure Limits-OELs) indicated above.Use under a fume hood. It is recommended that all dust control equipment such as local exhaust ventilation and material transport systems involved in handling of this product contain explosion relief vents or an explosion suppression system or an oxygen deficient environment.Ensure that dust-handling systems (such as exhaust ducts, dust collectors, vessels, and processing equipment) are designed in a manner to prevent the escape of dust into the work area (i.e., there is no leakage from the equipment).

Respiratory protection:
Use suitable respiratory protective device when high concentrations are present. Use suitable respiratory protective device when aerosol or mist is formed. For spills, respiratory protection may be advisable.

Protection of skin:
The glove material has to be impermeable and resistant to the product/ the substance/ the preparation being used/handled.Selection of the glove material on consideration of the penetration times, rates of diffusion and the degradation.

Eye protection:
Safety glasses with side shields or goggles.

General hygienic measures:
The usual precautionary measures are to be adhered to when handling chemicals. Keep away from food, beverages and feed sources. Immediately remove all soiled and contaminated clothing. Wash hands before breaks and at the end of work. Do not inhale gases/fumes/dust/mist/vapor/aerosols. Avoid contact with the eyes and skin.

SECTION 9 : Physical and chemical properties

Appearance (physical state,color):	Clear, colorless liquid.	**Explosion limit lower**: **Explosion limit upper**:	Not Determined Not Determined

Ammonia

Odor:	Ammonia-like	**Vapor pressure**:	115 at 20 C
Odor threshold:	Not Determined	**Vapor density**:	3.38
pH-value:	9	**Relative density**:	0.9
Melting/Freezing point:	- 72 C	**Solubilities**:	Infinite solubility in water.
Boiling point/Boiling range:	36 C	**Partition coefficient (n-octanol/water)**:	Not Determined
Flash point (closed cup):	Not Determined	**Auto/Self-ignition temperature**:	Not Determined
Evaporation rate:	Not Determined	**Decomposition temperature**:	Not Determined
Flammability (solid,gaseous):	Not Determined	**Viscosity**:	a. Kinematic:Not Determined b. Dynamic: Not Determined
Density: 0.9 g/cm3 at 20 °C			

SECTION 10 : Stability and reactivity

Reactivity:
Chemical stability: No decomposition if used and stored according to specifications.
Possible hazardous reactions:
Conditions to avoid: Store away from oxidizing agents, strong acids or bases.
Incompatible materials: Strong oxidizers, acids, gold, mercury, halogens, silv er, calcium hypochlorite bleaches.
Hazardous decomposition products: Ammonia and nitrogen oxides.

SECTION 11 : Toxicological information

Acute Toxicity:		
Oral:	LD50: 350 mg/kg (rat)	Ammonium Hydroxide (1336-21-6)
Chronic Toxicity: No additional information.		
Corrosion Irritation: No additional information.		
Sensitization:		No additional information.
Single Target Organ (STOT):		No additional information.
Numerical Measures:		No additional information.
Carcinogenicity:		No additional information.
Mutagenicity:		No additional information.
Reproductive Toxicity:		No additional information.

SECTION 12 : Ecological information

Ecotoxicity

Fish (acute 1336-21-6): 96 Hr LC50 Pimephales promelas: 8.2 mg/L

Crustacea (acute 1336-21-6): 48 Hr EC50 water flea: 0.66 mg/L; 48 Hr EC50 Daphnia pulex: 0.66 mg/L

Persistence and degradability: Readily degradable in the environment.
Bioaccumulative potential:
Mobility in soil:
Other adverse effects:

SECTION 13 : Disposal considerations

Waste disposal recommendations:

Product/containers must not be disposed together with household garbage. Do not allow product to reach sewage system or open water.It is the responsibility of the waste generator to properly characterize all waste materials according to applicable regulatory entities (US 40CFR262.11). Consult federal state/ provincial and local regulations regarding the proper disposal of waste material that may incorporate some amount of this product.

SECTION 14 : Transport information

UN-Number

2672

UN proper shipping name

Ammonia Solution

Transport hazard class(es)

Class:
8 Corrosive substances

Packing group:III
Environmental hazard:
Transport in bulk:
Special precautions for user:

SECTION 15 : Regulatory information

United States (USA)

SARA Section 311/312 (Specific toxic chemical listings):

Acute, Chronic

SARA Section 313 (Specific toxic chemical listings):

1336-21-6 Ammonium Hydroxide

RCRA (hazardous waste code):

None of the ingredients is listed

TSCA (Toxic Substances Control Act):

All ingredients are listed.

CERCLA (Comprehensive Environmental Response, Compensation, and Liability Act):

1336-21-6 Ammonium Hydroxide, ACS 1000

Proposition 65 (California):

Chemicals known to cause cancer:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for females:

None of the ingredients is listed

Chemicals known to cause reproductive toxicity for males:

None of the ingredients is listed

Chemicals known to cause developmental toxicity:

None of the ingredients is listed

Canada

Canadian Domestic Substances List (DSL):

All ingredients are listed.

Canadian NPRI Ingredient Disclosure list (limit 0.1%):

None of the ingredients is listed

Canadian NPRI Ingredient Disclosure list (limit 1%):

1336-21-6 Ammonium hydroxide

SECTION 16 : Other information

This product has been classified in accordance with hazard criteria of the Controlled Products Regulations and the SDS contains all the information required by the Controlled Products Regulations.Note:. The responsibility to provide a safe workplace remains with the user.The user should consider the health hazards and safety information contained herein as a guide and should take those precautions required in an individual operation to instruct employees and develop work practice procedures for a safe work environment.The information contained herein is, to the best of our knowledge and belief, accurate.However, since the conditions of handling and use are beyond our control, we make no guarantee of results, and assume no liability for damages incurred by the use of this material.It is the responsibility of the user to comply with all applicable laws and regulations applicable to this material.

GHS Full Text Phrases:

Abbreviations and acronyms:

IMDG: International Maritime Code for Dangerous Goods
PNEC: Predicted No-Effect Concentration (REACH)
CFR: Code of Federal Regulations (USA)
SARA: Superfund Amendments and Reauthorization Act (USA)
RCRA: Resource Conservation and Recovery Act (USA)
TSCA: Toxic Substances Control Act (USA)
NPRI: National Pollutant Release Inventory (Canada)
DOT: US Department of Transportation
IATA: International Air Transport Association
GHS: Globally Harmonized System of Classification and Labelling of Chemicals
ACGIH: American Conference of Governmental Industrial Hygienists
CAS: Chemical Abstracts Service (division of the American Chemical Society)
NFPA: National Fire Protection Association (USA)
HMIS: Hazardous Materials Identification System (USA)
WHMIS: Workplace Hazardous Materials Information System (Canada)
DNEL: Derived No-Effect Level (REACH)

Effective date : 12.31.2014
Last updated : 03.19.2015

Safety data sheet

according to Regulation (EC) No. 1907/2006 (REACH)

Calcium chloride ≥94 %, dehydrated

article number: **A119**
Version: **3.2 en**
Replaces version of: 22.05.2020
Version: (3)

date of compilation: 23.05.2016
Revision: 23.03.2021

SECTION 1: Identification of the substance/mixture and of the company/ undertaking

1.1 Product identifier

Identification of the substance	**Calcium chloride** ≥94 %, dehydrated
Article number	A119
Registration number (REACH)	01-2119494219-28-xxxx
Index number in CLP Annex VI	017-013-00-2
EC number	233-140-8
CAS number	10043-52-4

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

Relevant identified uses:	Laboratory chemical Laboratory and analytical use
Uses advised against:	Do not use for products which come into contact with foodstuffs. Do not use for private purposes (household).

1.3 Details of the supplier of the safety data sheet

Carl Roth GmbH + Co KG
Schoemperlenstr. 3-5
D-76185 Karlsruhe
Germany

Telephone:+49 (0) 721 - 56 06 0
Telefax: +49 (0) 721 - 56 06 149
e-mail: sicherheit@carlroth.de
Website: www.carlroth.de

Competent person responsible for the safety data sheet:	:Department Health, Safety and Environment
e-mail (competent person):	**sicherheit@carlroth.de**

1.4 Emergency telephone number

SECTION 2: Hazards identification

2.1 Classification of the substance or mixture

Classification according to Regulation (EC) No 1272/2008 (CLP)

Section	Hazard class	Cat-egory	Hazard class and category	Hazard statement
3.3	Serious eye damage/eye irritation	2	Eye Irrit. 2	H319

For full text of abbreviations: see SECTION 16

2.2 Label elements

Labelling according to Regulation (EC) No 1272/2008 (CLP)

Signal word **Warning**

Pictograms

GHS07

Hazard statements

H319 Causes serious eye irritation

Precautionary statements

Precautionary statements - prevention

P280 Wear protective gloves/eye protection

Precautionary statements - response

P305+P351+P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing

P337+P313 If eye irritation persists: Get medical advice/attention

Labelling of packages where the contents do not exceed 125 ml

Signal word: **Warning**

Symbol(s)

2.3 Other hazards

Results of PBT and vPvB assessment

According to the results of its assessment, this substance is not a PBT or a vPvB.

SECTION 3: Composition/information on ingredients

3.1 Substances

Name of substance	Calcium chloride
Molecular formula	$CaCl_2$
Molar mass	111 $^{g}/_{mol}$
REACH Reg. No	01-2119494219-28-xxxx
CAS No	10043-52-4
EC No	233-140-8
Index No	017-013-00-2

SECTION 4: First aid measures

4.1 Description of first aid measures

General notes

Take off contaminated clothing.

Following inhalation

Provide fresh air. In all cases of doubt, or when symptoms persist, seek medical advice.

Following skin contact

Rinse skin with water/shower. In all cases of doubt, or when symptoms persist, seek medical advice.

Following eye contact

Irrigate copiously with clean, fresh water for at least 10 minutes, holding the eyelids apart. In case of eye irritation consult an ophthalmologist.

Following ingestion

Rinse mouth. Call a doctor if you feel unwell.

4.2 Most important symptoms and effects, both acute and delayed

Irritation

4.3 Indication of any immediate medical attention and special treatment needed

none

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media

co-ordinate firefighting measures to the fire surroundings
water, foam, alcohol resistant foam, dry extinguishing powder, ABC-powder

Unsuitable extinguishing media

water jet

5.2 Special hazards arising from the substance or mixture

Non-combustible.

Hazardous combustion products

In case of fire may be liberated: Hydrogen chloride (HCl)

5.3 Advice for firefighters

In case of fire and/or explosion do not breathe fumes. Fight fire with normal precautions from a reasonable distance. Wear self-contained breathing apparatus.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures

For non-emergency personnel

Avoid contact with skin, eyes and clothes. Do not breathe dust.

6.2 Environmental precautions

Keep away from drains, surface and ground water.

6.3 Methods and material for containment and cleaning up

Advice on how to contain a spill

Covering of drains. Take up mechanically.

Advice on how to clean up a spill

Take up mechanically. Control of dust.

Other information relating to spills and releases

Place in appropriate containers for disposal.

6.4 Reference to other sections

Hazardous combustion products: see section 5. Personal protective equipment: see section 8. Incompatible materials: see section 10. Disposal considerations: see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Avoid dust formation.

Measures to prevent fire as well as aerosol and dust generation

Removal of dust deposits.

Advice on general occupational hygiene

Wash hands before breaks and after work. Keep away from food, drink and animal feedingstuffs.

7.2 Conditions for safe storage, including any incompatibilities

Store in a dry place. Keep container tightly closed. Hygroscopic solid.

Incompatible substances or mixtures

Observe hints for combined storage.

Consideration of other advice

Ventilation requirements

Use local and general ventilation.

Specific designs for storage rooms or vessels

Recommended storage temperature: 15 – 25 °C

7.3 Specific end use(s)

No information available.

SECTION 8: Exposure controls/personal protection

8.1 Control parameters

National limit values

Occupational exposure limit values (Workplace Exposure Limits)

Data are not available.

Human health values

Relevant DNELs and other threshold levels

Endpoint	Threshold level	Protection goal, route of exposure	Used in	Exposure time
DNEL	5 mg/m³	human, inhalatory	worker (industry)	chronic - local effects
DNEL	10 mg/m³	human, inhalatory	worker (industry)	acute - local effects

8.2 Exposure controls

Individual protection measures (personal protective equipment)

Eye/face protection

Use safety goggle with side protection.

Skin protection

• hand protection

Wear suitable gloves. Chemical protection gloves are suitable, which are tested according to EN 374. For special purposes, it is recommended to check the resistance to chemicals of the protective gloves mentioned above together with the supplier of these gloves. The times are approximate values from measurements at 22 ° C and permanent contact. Increased temperatures due to heated substances, body heat etc. and a reduction of the effective layer thickness by stretching can lead to a considerable reduction of the breakthrough time. If in doubt, contact manufacturer. At an approx. 1.5 times larger / smaller layer thickness, the respective breakthrough time is doubled / halved. The data apply only to the pure substance. When transferred to substance mixtures, they may only be considered as a guide.

• type of material

NBR (Nitrile rubber)

• material thickness

>0,11 mm

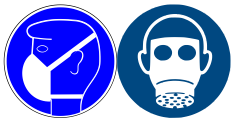
• breakthrough times of the glove material

>480 minutes (permeation: level 6)

• other protection measures

Take recovery periods for skin regeneration. Preventive skin protection (barrier creams/ointments) is recommended.

Respiratory protection



Respiratory protection necessary at: Dust formation. Particulate filter device (EN 143). P1 (filters at least 80 % of airborne particles, colour code: White).

Environmental exposure controls

Keep away from drains, surface and ground water.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

Physical state	solid
Form	acc. to product description
Colour	white
Odour	odourless
Melting point/freezing point	775 °C at 1.013 hPa (ECHA)
Boiling point or initial boiling point and boiling range	1.935 °C at 1.013 hPa (ECHA)
Flammability	non-combustible
Lower and upper explosion limit	not determined
Flash point	not applicable
Auto-ignition temperature	not determined
Decomposition temperature	not relevant
pH (value)	8 – 10 (in aqueous solution: 100 $^{g}/_{l}$, 20 °C)
Kinematic viscosity	not relevant
Solubility(ies)	
Water solubility	745 $^{g}/_{l}$ at 20 °C (ECHA)
Partition coefficient	
Partition coefficient n-octanol/water (log value):	not relevant (inorganic)
Vapour pressure	not determined
Density	2,15 $^{g}/_{cm^3}$ at 25 °C
Particle characteristics	No data available.

Other safety parameters

Oxidising properties	none

9.2 Other information

Information with regard to physical hazard classes:	hazard classes acc. to GHS (physical hazards): not relevant
Other safety characteristics:	There is no additional information.

SECTION 10: Stability and reactivity

10.1 Reactivity

This material is not reactive under normal ambient conditions.

10.2 Chemical stability

Hygroscopic solid.

10.3 Possibility of hazardous reactions

Reacts with water, releasing excess pressure or heat,
Violent reaction with: Strong acid,
Dangerous/dangerous reactions with: Zinc => Hydrogen

10.4 Conditions to avoid

Protect from moisture.

10.5 Incompatible materials

There is no additional information.

10.6 Hazardous decomposition products

Hazardous combustion products: see section 5.

SECTION 11: Toxicological information

11.1 Information on hazard classes as defined in Regulation (EC) No 1272/2008

Classification according to GHS (1272/2008/EC, CLP)

Acute toxicity

Shall not be classified as acutely toxic.

Acute toxicity

Exposure route	Endpoint	Value	Species	Method	Source
oral	LD50	2.120 $^{mg}/_{kg}$	rat		ECHA
dermal	LD50	>5.000 $^{mg}/_{kg}$	rabbit		ECHA

Skin corrosion/irritation

Shall not be classified as corrosive/irritant to skin.

Serious eye damage/eye irritation

Causes serious eye irritation.

Respiratory or skin sensitisation

Shall not be classified as a respiratory or skin sensitiser.

Germ cell mutagenicity

Shall not be classified as germ cell mutagenic.

Carcinogenicity

Shall not be classified as carcinogenic.

Reproductive toxicity

Shall not be classified as a reproductive toxicant.

Specific target organ toxicity - single exposure

Shall not be classified as a specific target organ toxicant (single exposure).

Specific target organ toxicity - repeated exposure

Shall not be classified as a specific target organ toxicant (repeated exposure).

Aspiration hazard

Shall not be classified as presenting an aspiration hazard.

Symptoms related to the physical, chemical and toxicological characteristics

• If swallowed

Data are not available.

• If in eyes

Causes serious eye irritation

• If inhaled

Data are not available.

• If on skin

Frequently or prolonged contact with skin may cause dermal irritation

• Other information

none

11.2 Endocrine disrupting properties

Not listed.

11.3 Information on other hazards

There is no additional information.

SECTION 12: Ecological information

12.1 Toxicity

Shall not be classified as hazardous to the aquatic environment.

Aquatic toxicity (acute)			
Endpoint	**Value**	**Species**	**Exposure time**
LC50	4.630 $^{mg}/_{l}$	fish	96 h
ErC50	>4.000 $^{mg}/_{l}$	algae	72 h

Aquatic toxicity (chronic)			
Endpoint	**Value**	**Species**	**Exposure time**
EC50	610 $^{mg}/_{l}$	aquatic invertebrates	21 d

Biodegradation

The methods for determining the biological degradability are not applicable to inorganic substances.

12.2 Process of degradability

Data are not available.

12.3 Bioaccumulative potential

Data are not available.

12.4 Mobility in soil

Data are not available.

12.5 Results of PBT and vPvB assessment

Data are not available.

12.6 Endocrine disrupting properties

Not listed.

12.7 Other adverse effects

Data are not available.

SECTION 13: Disposal considerations

13.1 Waste treatment methods

This material and its container must be disposed of as hazardous waste. Dispose of contents/container in accordance with local/regional/national/international regulations.

Sewage disposal-relevant information

Do not empty into drains.

13.2 Relevant provisions relating to waste

The allocation of waste identity numbers/waste descriptions must be carried out according to the EEC, specific to the industry and process. Waste catalogue ordinance (Germany).

13.3 Remarks

Waste shall be separated into the categories that can be handled separately by the local or national waste management facilities. Please consider the relevant national or regional provisions.

SECTION 14: Transport information

14.1	**UN number or ID number**	not subject to transport regulations
14.2	**UN proper shipping name**	not assigned
14.3	**Transport hazard class(es)**	none
14.4	**Packing group**	not assigned
14.5	**Environmental hazards**	non-environmentally hazardous acc. to the dangerous goods regulations

14.6 Special precautions for user

There is no additional information.

14.7 Maritime transport in bulk according to IMO instruments

The cargo is not intended to be carried in bulk.

Information for each of the UN Model Regulations

Transport of dangerous goods by road, rail and inland waterway (ADR/RID/ADN) - Additional information

not assigned

International Maritime Dangerous Goods Code (IMDG) - Additional information

Not subject to IMDG.

International Civil Aviation Organization (ICAO-IATA/DGR) - Additional information

Not subject to ICAO-IATA.

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

Relevant provisions of the European Union (EU)

Restrictions according to REACH, Annex XVII

not listed

List of substances subject to authorisation (REACH, Annex XIV)/SVHC - candidate list

Not listed.

Seveso Directive

2012/18/EU (Seveso III)			
No	**Dangerous substance/hazard categories**	**Qualifying quantity (tonnes) for the application of lower and upper-tier requirements**	**Notes**
	not assigned		

Deco-Paint Directive (2004/42/EC)

VOC content	0 % 0 $^{g}/_{l}$

Directive on industrial emissions (VOCs, 2010/75/EU)

VOC content	0 %
VOC content	0 g/l

Directive 2011/65/EU on the restriction of the use of certain hazardous substances in electrical and electronic equipment (RoHS) - Annex II

not listed

Regulation 166/2006/EC concerning the establishment of a European Pollutant Release and Transfer Register (PRTR)

not listed

Water Framework Directive (WFD)

List of pollutants (WFD)

Name of substance	Name acc. to inventory	CAS No	Listed in	Remarks
Calcium chloride	Metals and their compounds		A)	

Legend
A) Indicative list of the main pollutants

Regulation 98/2013/EU on the marketing and use of explosives precursors

not listed

Regulation 111/2005/EC laying down rules for the monitoring of trade between the Community and third countries in drug precursors

not listed

Regulation 1005/2009/EC on substances that deplete the ozone layer (ODS)

not listed

Regulation 649/2012/EU concerning the export and import of hazardous chemicals (PIC)

not listed

National inventories

Country	Inventory	Status
AU	AICS	substance is listed
CA	DSL	substance is listed
CN	IECSC	substance is listed
EU	ECSI	substance is listed
EU	REACH Reg.	substance is listed
JP	CSCL-ENCS	substance is listed
KR	KECI	substance is listed
MX	INSQ	substance is listed
NZ	NZIoC	substance is listed
PH	PICCS	substance is listed
TR	CICR	substance is listed
TW	TCSI	substance is listed

Country	Inventory	Status
US	TSCA	substance is listed

Legend

AICS	Australian Inventory of Chemical Substances
CICR	Chemical Inventory and Control Regulation
CSCL-ENCS	List of Existing and New Chemical Substances (CSCL-ENCS)
DSL	Domestic Substances List (DSL)
ECSI	EC Substance Inventory (EINECS, ELINCS, NLP)
IECSC	Inventory of Existing Chemical Substances Produced or Imported in China
INSQ	National Inventory of Chemical Substances
KECI	Korea Existing Chemicals Inventory
NZIoC	New Zealand Inventory of Chemicals
PICCS	Philippine Inventory of Chemicals and Chemical Substances (PICCS)
REACH Reg.	REACH registered substances
TCSI	Taiwan Chemical Substance Inventory
TSCA	Toxic Substance Control Act

15.2 Chemical Safety Assessment

No Chemical Safety Assessment has been carried out for this substance.

SECTION 16: Other information

Indication of changes (revised safety data sheet)

Alignment to regulation: Regulation (EC) No. 1907/2006 (REACH), amended by 2020/878/EU

Restructuring: section 9, section 14

Abbreviations and acronyms

Abbr.	Descriptions of used abbreviations
ADN	Accord européen relatif au transport international des marchandises dangereuses par voies de navigation intérieures (European Agreement concerning the International Carriage of Dangerous Goods by Inland Waterways)
ADR	Accord européen relatif au transport international des marchandises dangereuses par route (European Agreement concerning the International Carriage of Dangerous Goods by Road)
CAS	Chemical Abstracts Service (service that maintains the most comprehensive list of chemical substances)
CLP	Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures
DGR	Dangerous Goods Regulations (see IATA/DGR)
DNEL	Derived No-Effect Level
EC50	Effective Concentration 50 %. The EC50 corresponds to the concentration of a tested substance causing 50 % changes in response (e.g. on growth) during a specified time interval
EC No	The EC Inventory (EINECS, ELINCS and the NLP-list) is the source for the seven-digit EC number, an identifier of substances commercially available within the EU (European Union)
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of Notified Chemical Substances
ErC50	≡ EC50: in this method, that concentration of test substance which results in a 50 % reduction in either growth (EbC50) or growth rate (ErC50) relative to the control
GHS	"Globally Harmonized System of Classification and Labelling of Chemicals" developed by the United Nations
IATA	International Air Transport Association
IATA/DGR	Dangerous Goods Regulations (DGR) for the air transport (IATA)
ICAO	International Civil Aviation Organization

Abbr.	Descriptions of used abbreviations
IMDG	International Maritime Dangerous Goods Code
index No	The Index number is the identification code given to the substance in Part 3 of Annex VI to Regulation (EC) No 1272/2008
LC50	Lethal Concentration 50%: the LC50 corresponds to the concentration of a tested substance causing 50 % lethality during a specified time interval
LD50	Lethal Dose 50 %: the LD50 corresponds to the dose of a tested substance causing 50 % lethality during a specified time interval
NLP	No-Longer Polymer
PBT	Persistent, Bioaccumulative and Toxic
REACH	Registration, Evaluation, Authorisation and Restriction of Chemicals
RID	Règlement concernant le transport International ferroviaire des marchandises Dangereuses (Regulations concerning the International carriage of Dangerous goods by Rail)
SVHC	Substance of Very High Concern
VOC	Volatile Organic Compounds
vPvB	Very Persistent and very Bioaccumulative

Key literature references and sources for data

Regulation (EC) No 1272/2008 on classification, labelling and packaging of substances and mixtures. Regulation (EC) No. 1907/2006 (REACH), amended by 2020/878/EU.

Transport of dangerous goods by road, rail and inland waterway (ADR/RID/ADN). International Maritime Dangerous Goods Code (IMDG). Dangerous Goods Regulations (DGR) for the air transport (IATA).

List of relevant phrases (code and full text as stated in chapter 2 and 3)

Code	Text
H319	Causes serious eye irritation.

Disclaimer

This information is based upon the present state of our knowledge. This SDS has been compiled and is solely intended for this product.

SAFETY DATA SHEET

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

Product Identifier

Material Name: Escherichia Coli Bacterial Extract

Trade Name: Escherichia Coli Bacterial Extract
Synonyms: USDA veterinary biologic product code 264E.01
Chemical Family: Mixture

Relevant Identified Uses of the Substance or Mixture and Uses Advised Against

Intended Use: Veterinary Vaccine

Details of the Supplier of the Safety Data Sheet

Zoetis Inc.
100 Campus Drive, P.O. Box 651
Florham Park, New Jersey 07932 (USA)
Rocky Mountain Poison and Drug Center Phone: 1-866-531-8896
Product Support/Technical Services Phone: 1-800-366-5288

Zoetis Belgium S.A.
Mercuriusstraat 20
1930 Zaventem
Belgium

Emergency telephone number:
CHEMTREC (24 hours): 1-800-424-9300
Contact E-Mail: VMIPSrecords@zoetis.com

Emergency telephone number:
International CHEMTREC (24 hours): +1-703-527-3887

2. HAZARDS IDENTIFICATION

Appearance: Liquid solution

Classification of the Substance or Mixture

GHS - Classification Not classified as hazardous

EU Classification:

EU Indication of danger: Not classified

Label Elements

Signal Word: Not Classified
Hazard Statements: Non-hazardous in accordance with international standards for workplace safety.

Other Hazards

Short Term: May cause eye and skin irritation . May cause allergic skin reaction . In the event of accidental injection, an allergic reaction may occur. Signs and symptoms might include skin rash, itching, redness or swelling. Respiratory reactions may be characterized by rhinitis, sneezing, scratchy throat, oral mucosal edema, laryngeal mucosal edema, coughing, shortness of breath, wheezing, and chest pain. Asthma like reactions occur with acute exposures in sensitized patients. If an allergic reaction occurs, the worker should be removed to the nearest emergency room and the appropriate therapy instituted.

Australian Hazard Classification (NOHSC): Non-Hazardous Substance. Non-Dangerous Goods.

Note: This document has been prepared in accordance with standards for workplace safety, which require the inclusion of all known hazards of the product or its ingredients regardless of the potential risk. The precautionary statements and warnings included may not apply in all cases. Your needs may vary depending upon the potential for exposure in your workplace.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Hazardous

Ingredient	CAS Number	EU EINECS/ELINCS List	EU Classification	GHS Classification	%
Formaldehyde	50-00-0	200-001-8	T; R23/24/25 C; R34 Carc.Cat.3; R40 R43	Acute Tox. 3 (H301) Skin Corr. 1B (H314) Skin Sens. 1 (H317) Carc. 1A (H350) Acute Tox. 3 (H331)	<0.1

Ingredient	CAS Number	EU EINECS/ELINCS List	EU Classification	GHS Classification	%
Escherichia coli	NOT ASSIGNED	Not Listed	Not Listed	Not Listed	*
Surfactant	NOT APPLICABLE	Not Listed	Not Listed	Not Listed	*
Emulsigen	Proprietary	Not Listed	Not Listed	Not Listed	25

Additional Information: * Proprietary
Ingredient(s) indicated as hazardous have been assessed under standards for workplace safety. In accordance with 29 CFR 1910.1200, the exact percentage composition of this mixture has been withheld as a trade secret.

For the full text of the R phrases and CLP/GHS abbreviations mentioned in this Section, see Section 16

4. FIRST AID MEASURES

Description of First Aid Measures

Eye Contact: Immediately flush eyes with water for at least 15 minutes. If irritation occurs or persists, get medical attention.

Skin Contact: Wash skin with soap and water. If irritation occurs or persists, get medical attention.

Ingestion: Get medical attention. Do not induce vomiting unless directed by medical personnel. Never give anything by mouth to an unconscious person.

Inhalation: Remove to fresh air. If not breathing, give artificial respiration. Get medical attention immediately.

Most Important Symptoms and Effects, Both Acute and Delayed

Symptoms and Effects of Exposure: For information on potential signs and symptoms of exposure, See Section 2 - Hazards Identification and/or Section 11 - Toxicological Information.

Medical Conditions Aggravated by Exposure: None known

Indication of the Immediate Medical Attention and Special Treatment Needed

Notes to Physician: None

5. FIRE-FIGHTING MEASURES

Extinguishing Media: Extinguish fires with CO2, extinguishing powder, foam, or water.

Special Hazards Arising from the Substance or Mixture

Hazardous Combustion Products: Formation of toxic gases is possible during heating or fire.

Fire / Explosion Hazards: Fine particles (such as dust and mists) may fuel fires/explosions.

Advice for Fire-Fighters

During all fire fighting activities, wear appropriate protective equipment, including self-contained breathing apparatus.

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions, Protective Equipment and Emergency Procedures

Personnel involved in clean-up should wear appropriate personal protective equipment (see Section 8). Minimize exposure.

Environmental Precautions

Place waste in an appropriately labeled, sealed container for disposal. Care should be taken to avoid environmental release.

Methods and Material for Containment and Cleaning Up

Measures for Cleaning / Collecting: Contain the source of spill if it is safe to do so. Collect spill with absorbent material. Clean spill area thoroughly.

Additional Consideration for Large Spills: Non-essential personnel should be evacuated from affected area. Report emergency situations immediately. Clean up operations should only be undertaken by trained personnel.

7. HANDLING AND STORAGE

Precautions for Safe Handling

When handling, use proper personal protective equipment as specified in Section 8. Use with adequate ventilation. Avoid contact with eyes, skin and clothing. Avoid breathing vapor or mist. Avoid accidental injection. Wash thoroughly after handling. Releases to the environment should be avoided.

Conditions for Safe Storage, Including any Incompatibilities

Storage Conditions: Store under refrigeration in closed container.
Storage Temperature: 2-7°C
Incompatible Materials: This material can be denatured or inactivated by a variety of organic solvents, salts or heavy metals.
Specific end use(s): No data available

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Control Parameters

Refer to available public information for specific member state Occupational Exposure Limits.

Formaldehyde

ACGIH Ceiling Threshold Limit:	0.3 ppm
ACGIH - Sensitizer Designation	Sensitizer

8. EXPOSURE CONTROLS / PERSONAL PROTECTION

Australia STEL	2 ppm 2.5 mg/m^3
Australia TWA	1 ppm 1.2 mg/m^3
Austria OEL - MAKs	0.5 ppm 0.6 mg/m^3
Bulgaria OEL - TWA	1.0 mg/m^3
Czech Republic OEL - TWA	0.5 mg/m^3
Estonia OEL - TWA	0.5 ppm 0.6 mg/m^3
Finland OEL - TWA	0.3 ppm 0.37 mg/m^3
France OEL - TWA	0.5 ppm
Germany (DFG) - MAK	0.3 ppm 0.37 mg/m^3 no irritation should occur during mixed exposure
Greece OEL - TWA	2 ppm 2.5 mg/m^3
Hungary OEL - TWA	0.6 mg/m^3
Ireland OEL - TWAs	2 ppm 2.5 mg/m^3
Japan - OELs - Ceilings	0.2 ppm 0.24 mg/m^3
Latvia OEL - TWA	0.5 mg/m^3
Lithuania OEL - TWA	0.5 ppm 0.6 mg/m^3
Netherlands OEL - TWA	0.15 mg/m^3
Vietnam OEL - TWAs	0.5 mg/m^3
OSHA - Final PELS - TWAs:	0.75 ppm
OSHA - Specifically Regulated Chemicals	2 ppm 0.5 ppm 0.75 ppm
Poland OEL - TWA	0.5 mg/m^3
Romania OEL - TWA	1 ppm 1.20 mg/m^3
Slovakia OEL - TWA	0.3 ppm 0.37 mg/m^3
Slovenia OEL - TWA	0.5 ppm 0.62 mg/m^3
Sweden OEL - TWAs	0.3 ppm 0.37 mg/m^3
Switzerland OEL -TWAs	0.3 ppm 0.37 mg/m^3

Exposure Controls

Engineering Controls:	Engineering controls should be used as the primary means to control exposures.
Personal Protective Equipment:	Refer to applicable national standards and regulations in the selection and use of personal protective equipment (PPE).
Hands:	Wear impervious gloves if skin contact is possible.
Eyes:	Safety glasses or goggles
Skin:	Use protective clothing (uniforms, lab coats, disposable coveralls, etc.) in both production and laboratory areas.
Respiratory protection:	If the applicable Occupational Exposure Limit (OEL) is exceeded, wear an appropriate respirator with a protection factor sufficient to control exposures to below the OEL.

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State:	Liquid solution	**Color:**	No data available.
Odor:	No data available.	**Odor Threshold:**	No data available.
Molecular Formula:	Mixture	**Molecular Weight:**	Mixture

Solvent Solubility: No data available
Water Solubility: No data available
Solubility: Soluble: Water (based on components)
pH: 7.0 +/- 1.5
Melting/Freezing Point (°C): No data available
Boiling Point (°C): >100
Partition Coefficient: (Method, pH, Endpoint, Value)
No data available
Decomposition Temperature (°C): No data available.

Evaporation Rate (Gram/s): No data available
Vapor Pressure (kPa): Expected to be negligible
Vapor Density (g/ml): No data available
Relative Density: No data available
Specific Gravity: 1.0 +/-0.2
Viscosity: No data available

Flammablity:
- **Autoignition Temperature (Solid) (°C):** No data available
- **Flammability (Solids):** No data available
- **Flash Point (Liquid) (°C):** Non-flammable
- **Upper Explosive Limits (Liquid) (% by Vol.):** No data available
- **Lower Explosive Limits (Liquid) (% by Vol.):** No data available

Polymerization: Will not occur

10. STABILITY AND REACTIVITY

Reactivity: No data available
Chemical Stability: Stable
Possibility of Hazardous Reactions
- **Oxidizing Properties:** No data available
- **Conditions to Avoid:** Store at 2-7°C. Prolonged exposure to higher temperatures may adversely affect potency. Do not freeze.
- **Incompatible Materials:** This material can be denatured or inactivated by a variety of organic solvents, salts or heavy metals.
- **Hazardous Decomposition Products:** None expected under normal conditions.

11. TOXICOLOGICAL INFORMATION

Information on Toxicological Effects
General Information: Toxicological properties of the formulation have not been investigated. The information in this section describes the potential hazards of the individual ingredients and the formulation. The antigens included in this product are non-infectious. All have been prepared from killed or inactivated preparations of microorganisms. Routes of exposure: eye contact , skin contact

11. TOXICOLOGICAL INFORMATION

Acute Toxicity: (Species, Route, End Point, Dose)

Formaldehyde
Rat Oral LD50 800 mg/kg

Irritation / Sensitization: (Study Type, Species, Severity)

Formaldehyde
Eye Irritation Rabbit Severe
Skin Irritation Rabbit Moderate Severe
Skin Sensitization Positive

Repeated Dose Toxicity: (Duration, Species, Route, Dose, End Point, Target Organ)

Formaldehyde
90 Day(s) Dog Inhalation Not Specified Lungs
90 Day(s) Rat Inhalation Not Specified Lungs
90 Day(s) Monkey Inhalation Not Specified Lungs
90 Day(s) Rat Inhalation 15 ppm LOAEL Respiratory system

Reproduction & Developmental Toxicity: (Study Type, Species, Route, Dose, End Point, Effect(s))

Formaldehyde
Embryo / Fetal Development Mouse Oral 185 mg/kg/day Not teratogenic, Maternal toxicity
Embryo / Fetal Development Rat Inhalation 40 ppm Not Teratogenic, Maternal Toxicity

Genetic Toxicity: (Study Type, Cell Type/Organism, Result)

Formaldehyde
In Vitro Bacterial Mutagenicity (Ames) Bacteria Positive
In Vitro Chromosome Aberration Rodent Positive
In Vitro Sister Chromatid Exchange Rodent Positive
In Vivo Chromosome Aberration Not specified Positive

Carcinogenicity: (Duration, Species, Route, Dose, End Point, Effect(s))

Formaldehyde
2 Year(s) Rat Inhalation 6 ppm LOAEL Tumors
2 Year(s) Mouse Inhalation 15 ppm LOAEL Tumors

Carcinogen Status: None of the components present in this material at concentrations equal to or greater than 0.1% are listed by IARC, NTP, OSHA, or ACGIH as a carcinogen.

Formaldehyde

IARC:	Group 1 (Carcinogenic to Humans)
NTP:	Known Human Carcinogen
OSHA:	Listed

12. ECOLOGICAL INFORMATION

Environmental Overview: Environmental properties of the formulation have not been investigated. Releases to the environment should be avoided.

Toxicity: No data available

Persistence and Degradability: No data available

Bio-accumulative Potential: No data available

Mobility in Soil: No data available

13. DISPOSAL CONSIDERATIONS

Waste Treatment Methods: Dispose of waste in accordance with all applicable laws and regulations. Member State specific and Community specific provisions must be considered. Considering the relevant known environmental and human health hazards of the material, review and implement appropriate technical and procedural waste water and waste disposal measures to prevent occupational exposure and environmental release. It is recommended that waste minimization be practiced. The best available technology should be utilized to prevent environmental releases. This may include destructive techniques for waste and wastewater.

Formaldehyde
RCRA - U Series Wastes Listed

14. TRANSPORT INFORMATION

The following refers to all modes of transportation unless specified below.

Not regulated for transport under USDOT, EUADR, IATA, or IMDG regulations.

U.S. DOT Reportable Quantity (RQ), 49 CFR 172.101 Appendix A:

Formaldehyde
CERCLA/SARA Hazardous Substances and their Reportable Quantities: 100 lb / 45.4 kg

15. REGULATORY INFORMATION

Safety, Health and Environmental Regulations/Legislation Specific for the Substance or Mixture

Canada - WHMIS: Classifications

15. REGULATORY INFORMATION

WHMIS hazard class:
Non-controlled
This product has been classified in accordance with the hazard criteria of the CPR and the SDS contains all of the information required by the CPR.

Escherichia coli

CERCLA/SARA 313 Emission reporting	Not Listed
California Proposition 65	Not Listed
EU EINECS/ELINCS List	Not Listed

Formaldehyde

CERCLA/SARA 313 Emission reporting	0.1 %
CERCLA/SARA Hazardous Substances and their Reportable Quantities:	100 lb 45.4 kg
CERCLA/SARA - Section 302 Extremely Hazardous TPQs	500 lb
CERCLA/SARA - Section 302 Extremely Hazardous Substances EPCRA RQs	100 lb
California Proposition 65	carcinogen initial date 1/1/88 gas
OSHA - Specifically Regulated Chemicals	2 ppm 0.5 ppm 0.75 ppm
Inventory - United States TSCA - Sect. 8(b)	Present
Australia (AICS):	Present
Standard for the Uniform Scheduling for Drugs and Poisons:	Schedule 2 Schedule 6
EU EINECS/ELINCS List	200-001-8

Surfactant

CERCLA/SARA 313 Emission reporting	Not Listed
California Proposition 65	Not Listed
EU EINECS/ELINCS List	Not Listed

Emulsigen

CERCLA/SARA 313 Emission reporting	Not Listed
California Proposition 65	Not Listed
EU EINECS/ELINCS List	Not Listed

16. OTHER INFORMATION

Text of R phrases and GHS Classification abbreviations mentioned in Section 3

Acute toxicity, oral-Cat.3; H301 - Toxic if swallowed
Acute toxicity, inhalation-Cat.3; H331 - Toxic if inhaled
Skin corrosion/irritation-Cat.1B; H314 - Causes severe skin burns and eye damage
Sensitization, skin-Cat.1; H317 - May cause an allergic skin reaction
Carcinogenicity-Cat.1A; H350 - May cause cancer

T - Toxic
C - Corrosive
Carcinogenic: Category 3

R34 - Causes burns.
R40 - Limited evidence of a carcinogenic effect
R43 - May cause sensitization by skin contact.
R23/24/25 - Toxic by inhalation, in contact with skin and if swallowed.

Data Sources:	The data contained in this SDS may have been gathered from confidential internal sources, raw material suppliers, or from the published literature.
Reasons for Revision:	Updated Section 1 - Identification of the Substance/Preparation and the Company/Undertaking. Updated Section 2 - Hazard Identification. Updated Section 11 - Toxicology Information.
Prepared by:	Toxicology and Hazard Communication Zoetis Global Risk Management

Zoetis Inc. believes that the information contained in this Safety Data Sheet is accurate, and while it is provided in good faith, it is without warranty of any kind, expressed or implied. If data for a hazard are not included in this document there is no known information at this time.

End of Safety Data Sheet

SAFETY DATA SHEET

Creation Date 06-Nov-2009 **Revision Date** 28-Dec-2021 **Revision Number** 4

1. Identification

Product Name **D(+)-Glucose monohydrate**

Cat No. : **AC450740000; AC450740010; AC450740050; AC450740250**

CAS No 14431-43-7
Synonyms D-Glucose monohydrate (Crystalline Powder/USP/EP/BP)

Recommended Use Laboratory chemicals.
Uses advised against Food, drug, pesticide or biocidal product use.

Details of the supplier of the safety data sheet

Company

Fisher Scientific Company
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Acros Organics
One Reagent Lane
Fair Lawn, NJ 07410

Emergency Telephone Number For information **US** call: 001-800-ACROS-01 / **Europe** call: +32 14 57 52 11
Emergency Number **US:**001-201-796-7100 / **Europe:** +32 14 57 52 99
CHEMTREC Tel. No.**US:**001-800-424-9300 / **Europe:**001-703-527-3887

2. Hazard(s) identification

Classification

Classification under 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

This chemical is not considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Label Elements

None required

Hazards not otherwise classified (HNOC)

None identified

3. Composition/Information on Ingredients

Component	CAS No	Weight %
Dextrose monohydrate	14431-43-7	100
Glucose	50-99-7	-

4. First-aid measures

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Remove to fresh air. Get medical attention immediately if symptoms occur. If not breathing, give artificial respiration.

Ingestion Do NOT induce vomiting. Get medical attention.

Most important symptoms and effects No information available.

Notes to Physician Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media Water spray, carbon dioxide (CO2), dry chemical, alcohol-resistant foam.

Unsuitable Extinguishing Media No information available

Flash Point Not applicable
Method - No information available

Autoignition Temperature No information available
Explosion Limits
Upper No data available
Lower No data available
Sensitivity to Mechanical Impact No information available
Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical
None known.

Hazardous Combustion Products
Carbon monoxide (CO). Carbon dioxide (CO_2).
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.

NFPA

Health	Flammability	Instability	Physical hazards
1	1	0	N/A

6. Accidental release measures

Personal Precautions Use personal protective equipment as required. Ensure adequate ventilation.
Environmental Precautions Should not be released into the environment.

Methods for Containment and Clean Up Sweep up and shovel into suitable containers for disposal. Avoid dust formation.

7. Handling and storage

Handling Wear personal protective equipment/face protection. Ensure adequate ventilation. Avoid dust formation. Do not breathe dust. Avoid contact with skin, eyes or clothing.

Storage. Keep containers tightly closed in a dry, cool and well-ventilated place. Incompatible Materials. Strong oxidizing agents.

8. Exposure controls / personal protection

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

Engineering Measures None under normal use conditions.

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection No protective equipment is needed under normal use conditions.

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

9. Physical and chemical properties

Physical State	Solid
Appearance	White
Odor	Odorless
Odor Threshold	No information available
pH	5.9 (0.5M)
Melting Point/Range	No data available
Boiling Point/Range	No information available
Flash Point	Not applicable
Evaporation Rate	Not applicable
Flammability (solid,gas)	No information available
Flammability or explosive limits	
Upper	No data available
Lower	No data available
Vapor Pressure	negligible
Vapor Density	Not applicable
Specific Gravity	1.54 (H2O=1)
Solubility	Soluble in water
Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	No information available
Decomposition Temperature	No information available
Viscosity	Not applicable
Molecular Formula	C6H12O6.H2O
Molecular Weight	198.18

10. Stability and reactivity

Reactive Hazard None known, based on information available

Stability Stable under normal conditions.

Conditions to Avoid Avoid dust formation. Incompatible products. Excess heat.

Incompatible Materials Strong oxidizing agents

Hazardous Decomposition Products Carbon monoxide (CO), Carbon dioxide (CO_2)

Hazardous Polymerization Hazardous polymerization does not occur.

Hazardous Reactions None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information No acute toxicity information is available for this product
Oral LD50 Based on ATE data, the classification criteria are not met. ATE > 2000 mg/kg.
Dermal LD50 Based on ATE data, the classification criteria are not met. ATE > 2000 mg/kg.
Mist LC50 Based on ATE data, the classification criteria are not met. ATE > 5 mg/l.

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Glucose	25.8 g/kg (Rat)	Not listed	Not listed

Toxicologically Synergistic Products No information available

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

Irritation No information available

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS No	IARC	NTP	ACGIH	OSHA	Mexico
Dextrose monohydrate	14431-43-7	Not listed	Not listed	Not listed	Not listed	Not listed
Glucose	50-99-7	Not listed	Not listed	Not listed	Not listed	Not listed

Mutagenic Effects Mutagenic effects have occurred in experimental animals.

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

STOT - single exposure None known
STOT - repeated exposure None known

Aspiration hazard No information available

Symptoms / effects,both acute and delayed No information available

Endocrine Disruptor Information No information available

Other Adverse Effects Tumorigenic effects have been reported in experimental animals.

12. Ecological information

Ecotoxicity
Do not empty into drains.

Persistence and Degradability Soluble in water Persistence is unlikely based on information available.

Bioaccumulation/ Accumulation No information available.

Mobility Will likely be mobile in the environment due to its water solubility.

13. Disposal considerations

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. Transport information

DOT Not regulated
TDG Not regulated
IATA Not regulated
IMDG/IMO Not regulated

15. Regulatory information

United States of America Inventory

Component	CAS No	TSCA	TSCA Inventory notification - Active-Inactive	TSCA - EPA Regulatory Flags
Dextrose monohydrate	14431-43-7	-	-	-
Glucose	50-99-7	X	ACTIVE	-

Legend:
TSCA US EPA (TSCA) - Toxic Substances Control Act, (40 CFR Part 710)
X - Listed
'-' - Not Listed

TSCA 12(b) - Notices of Export Not applicable

International Inventories
Canada (DSL/NDSL), Europe (EINECS/ELINCS/NLP), Philippines (PICCS), Japan (ENCS), Japan (ISHL), Australia (AICS), China (IECSC), Korea (KECL).

Component	CAS No	DSL	NDSL	EINECS	PICCS	ENCS	ISHL	AICS	IECSC	KECL
Dextrose monohydrate	14431-43-7	-	-	-	X	-		X	X	-
Glucose	50-99-7	X	-	200-075-1	X	X	X	X	X	KE-17727

KECL - NIER number or KE number (http://ncis.nier.go.kr/en/main.do)

U.S. Federal Regulations

SARA 313 Not applicable

SARA 311/312 Hazard Categories See section 2 for more information

CWA (Clean Water Act) Not applicable

Clean Air Act Not applicable

OSHA - Occupational Safety and Health Administration Not applicable

CERCLA Not applicable

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

U.S. State Right-to-Know Regulations Not applicable

U.S. Department of Transportation
Reportable Quantity (RQ): N
DOT Marine Pollutant N
DOT Severe Marine Pollutant N

U.S. Department of Homeland Security This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

Authorisation/Restrictions according to EU REACH

Safety, health and environmental regulations/legislation specific for the substance or mixture

Component	CAS No	OECD HPV	Persistent Organic Pollutant	Ozone Depletion Potential	Restriction of Hazardous Substances (RoHS)
Dextrose monohydrate	14431-43-7	Listed	Not applicable	Not applicable	Not applicable
Glucose	50-99-7	Listed	Not applicable	Not applicable	Not applicable

Component	CAS No	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Major Accident Notification	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Safety Report Requirements	Rotterdam Convention (PIC)	Basel Convention (Hazardous Waste)
Dextrose monohydrate	14431-43-7	Not applicable	Not applicable	Not applicable	Not applicable
Glucose	50-99-7	Not applicable	Not applicable	Not applicable	Not applicable

16. Other information

Prepared By
Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Creation Date 06-Nov-2009
Revision Date 28-Dec-2021
Print Date 28-Dec-2021
Revision Summary This document has been updated to comply with the US OSHA HazCom 2012 Standard replacing the current legislation under 29 CFR 1910.1200 to align with the Globally Harmonized System of Classification and Labeling of Chemicals (GHS).

Disclaimer
The information provided in this Safety Data Sheet 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 guidance for safe handling, use, processing, storage, transportation, disposal and release and is not to be considered 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 materials or in any process, unless specified in the text

End of SDS

Safety Data Sheet

Creation Date 21-Mar-2014

1. PRODUCT AND COMPANY IDENTIFICATION

Product Name **Plasmid DNA**

Cat No. **Plasmid DNA**

Synonyms No information available.

Recommended Use For research use only

Company
Addgene Inc
75 Sidney Street, Suite 550A
Cambridge, MA 02139
Tel: 617-225-9000
Email: help@addgene.org

Emergency Telephone Number
Chemtrec US: (800) 424-9300
Chemtrec EU: (202) 483-7616

2. HAZARDS IDENTIFICATION

CAUTION!

Emergency Overview

Low hazard for usual industrial or commercial handling. Handle in accordance with good industrial hygiene and safety practice.

Appearance Colorless **Physical State** Liquid **odor** No information available

Target Organs None known.

Potential Health Effects

Acute Effects
Principle Routes of Exposure

Eyes	May cause slight irritation.
Skin	Non-irritating during normal use.
Inhalation	Low hazard for usual industrial or commercial handling.
Ingestion	Low hazard for usual industrial or commercial handling.

Chronic Effects No information available.

See Section 11 for additional Toxicological information.

Aggravated Medical Conditions No information available.

3. COMPOSITION/INFORMATION ON INGREDIENTS

Haz/Non-haz

Component	CAS-No	Weight %
Deoxyribonucleic acids	9007-49-2	100

4. FIRST AID MEASURES

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Obtain medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Move to fresh air. If breathing is difficult, give oxygen. Get medical attention immediately if symptoms occur.

Ingestion Do not induce vomiting. Obtain medical attention.

Notes to Physician Treat symptomatically.

5. FIRE-FIGHTING MEASURES

Flash Point Not applicable
Method No information available.

Autoignition Temperature No information available.

Explosion Limits
Upper No data available
Lower No data available

Suitable Extinguishing Media Substance is nonflammable; use agent most appropriate to extinguish surrounding fire..

Unsuitable Extinguishing Media No information available.

Hazardous Combustion Products No information available.

Sensitivity to mechanical impact No information available.
Sensitivity to static discharge No information available.

Specific Hazards Arising from the Chemical
Thermal decomposition can lead to release of irritating gases and vapors.

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.

NFPA **Health** 0 **Flammability** 0 **Instability** 0 **Physical hazards** N/A

6. ACCIDENTAL RELEASE MEASURES

Personal Precautions Ensure adequate ventilation. Use personal protective equipment. Avoid contact with skin, eyes and clothing.

Environmental Precautions Should not be released into the environment.

Methods for Containment and Clean Up Soak up with inert absorbent material. Keep in suitable, closed containers for disposal..

7. HANDLING AND STORAGE

Handling Ensure adequate ventilation. Wear personal protective equipment. Avoid contact with skin, eyes and clothing.

Storage Long term. Keep tightly closed in a dry and cool place. Keep at 4°C.

8. EXPOSURE CONTROLS/PERSONAL PROTECTION

Engineering Measures Ensure adequate ventilation, especially in confined areas. Ensure that eyewash stations and safety showers are close to the workstation location.

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

NIOSH IDLH: *Immediately Dangerous to Life or Health*

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure

Respiratory Protection Follow the OSHA respirator regulations found in 29 CFR 1910.134 or European Standard EN 149. Use a NIOSH/MSHA or European Standard EN 149 approved respirator if exposure limits are exceeded or if irritation or other symptoms are experienced

9. PHYSICAL AND CHEMICAL PROPERTIES

Physical State	Liquid
Appearance	Colorless
odor	No information available
Odor Threshold	No information available.
pH	Not applicable
Vapor Pressure	No information available.
Vapor Density	No information available.
Viscosity	No information available.
Boiling Point/Range	Not applicable
Melting Point/Range	No information available.
Decomposition temperature	No information available.
Flash Point	Not applicable
Evaporation Rate	No information available.
Specific Gravity	No information available.
Solubility	No information available.
log Pow	No data available

10. STABILITY AND REACTIVITY

Stability Stable under normal conditions.

Conditions to Avoid Incompatible products. Excess heat. Exposure to air or moisture over prolonged periods.

Incompatible Materials Alcohols

Hazardous Decomposition Products	None known
Hazardous Polymerization	Hazardous polymerization does not occur.
Hazardous Reactions .	None under normal processing..

11. TOXICOLOGICAL INFORMATION

Acute Toxicity

Product Information	No acute toxicity information is available for this product

Component Information

Irritation	No information available.
Toxicologically Synergistic Products	No information available.

Chronic Toxicity

Carcinogenicity	There are no known carcinogenic chemicals in this product

Sensitization	No information available.
Mutagenic Effects	No information available.
Reproductive Effects	No information available.
Developmental Effects	No information available.
Teratogenicity	No information available.
Other Adverse Effects	The toxicological properties have not been fully investigated.
Endocrine Disruptor Information	No information available

12. ECOLOGICAL INFORMATION

Ecotoxicity

Do not empty into drains.

Persistence and Degradability	No information available
Bioaccumulation/ Accumulation	No information available
Mobility	No information available

13. DISPOSAL CONSIDERATIONS

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification

14. TRANSPORT INFORMATION

DOT Not regulated

TDG Not regulated

IATA Not regulated

IMDG/IMO Not regulated

15. REGULATORY INFORMATION

International Inventories

Component	TSCA	DSL	NDSL	EINECS	ELINCS	NLP	PICCS	ENCS	AICS	CHINA	KECL
Deoxyribonucleic acids	-	X	-	-	-		-	-	X	X	-

Legend:
X - Listed
E - Indicates a substance that is the subject of a Section 5(e) Consent order under TSCA.
F - Indicates a substance that is the subject of a Section 5(f) Rule under TSCA.
N - Indicates a polymeric substance containing no free-radical initiator in its inventory name but is considered to cover the designated polymer made with any free-radical initiator regardless of the amount used.
P - Indicates a commenced PMN substance
R - Indicates a substance that is the subject of a Section 6 risk management rule under TSCA.
S - Indicates a substance that is identified in a proposed or final Significant New Use Rule
T - Indicates a substance that is the subject of a Section 4 test rule under TSCA.
XU - Indicates a substance exempt from reporting under the Inventory Update Rule, i.e. Partial Updating of the TSCA Inventory Data Base Production and Site Reports (40 CFR 710(B).
Y1 - Indicates an exempt polymer that has a number-average molecular weight of 1,000 or greater.
Y2 - Indicates an exempt polymer that is a polyester and is made only from reactants included in a specified list of low concern reactants that comprises one of the eligibility criteria for the exemption rule.

U.S. Federal Regulations

TSCA 12(b) Not applicable

SARA 313
Not applicable

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

Clean Water Act
Not applicable

Clean Air Act
Not applicable

OSHA
Not applicable

CERCLA
Not Applicable

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

State Right-to-Know
Not applicable

U.S. Department of Transportation

Reportable Quantity (RQ):	N
DOT Marine Pollutant	N
DOT Severe Marine Pollutant	N

U.S. Department of Homeland Security
This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade No information available

Canada

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.

WHMIS Hazard Class
Non-controlled

16. OTHER INFORMATION

Prepared By

Addgene Inc
Tel: 617-225-9000
Email: help@addgene.org

Creation Date 21-Mar-2014

Disclaimer

The information provided on this Safety Data Sheet 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

Creation Date 18-Jun-2009 **Revision Date** 24-Dec-2021 **Revision Number** 6

1. Identification

Product Name **Potassium acetate**

Cat No. : **BP364-500; P171-500; P171I-3; P178-3; P181-12; P181-212**

CAS No 127-08-2
Synonyms Acetic acid, potassium salt (Crystalline/Powder/USP/EP/BP/Certified ACS)

Recommended Use Laboratory chemicals.
Uses advised against Food, drug, pesticide or biocidal product use.

Details of the supplier of the safety data sheet

Company
Fisher Scientific Company
One Reagent Lane
Fair Lawn, NJ 07410
Tel: (201) 796-7100

Emergency Telephone Number CHEMTREC®, Inside the USA: 800-424-9300
CHEMTREC®, Outside the USA: 001-703-527-3887

2. Hazard(s) identification

Classification
This chemical is considered hazardous by the 2012 OSHA Hazard Communication Standard (29 CFR 1910.1200)

Combustible dust	Yes

Label Elements

Signal Word
Warning

Hazard Statements
May form combustible dust concentrations in air

Precautionary Statements
Storage

Store in a well-ventilated place. Keep container tightly closed

Hazards not otherwise classified (HNOC)

None identified

3. Composition/Information on Ingredients

Component	CAS No	Weight %
Potassium acetate	127-08-2	>95

4. First-aid measures

Eye Contact Rinse immediately with plenty of water, also under the eyelids, for at least 15 minutes. Get medical attention.

Skin Contact Wash off immediately with plenty of water for at least 15 minutes. Get medical attention immediately if symptoms occur.

Inhalation Remove to fresh air. If breathing is difficult, give oxygen. Get medical attention immediately if symptoms occur.

Ingestion Do NOT induce vomiting. Get medical attention.

Most important symptoms and effects No information available.

Notes to Physician Treat symptomatically

5. Fire-fighting measures

Suitable Extinguishing Media Water spray, carbon dioxide (CO2), dry chemical, alcohol-resistant foam.

Unsuitable Extinguishing Media No information available

Flash Point Not applicable
Method -

Autoignition Temperature No information available

Explosion Limits
Upper No data available
Lower No data available
Sensitivity to Mechanical Impact No information available
Sensitivity to Static Discharge No information available

Specific Hazards Arising from the Chemical

Dust can form an explosive mixture with air. Keep product and empty container away from heat and sources of ignition.

Hazardous Combustion Products

Carbon monoxide (CO). Carbon dioxide (CO_2). Potassium oxides.

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.

NFPA

Health	Flammability	Instability	Physical hazards
1	1	1	N/A

6. Accidental release measures

Personal Precautions Ensure adequate ventilation. Use personal protective equipment as required. Avoid dust formation.

Environmental Precautions Should not be released into the environment. See Section 12 for additional Ecological Information.

Methods for Containment and Clean Up Sweep up and shovel into suitable containers for disposal. Avoid dust formation.

7. Handling and storage

Handling Wear personal protective equipment/face protection. Ensure adequate ventilation. Avoid contact with skin, eyes or clothing. Avoid ingestion and inhalation. Avoid dust formation. Protect from moisture.

Storage. Keep containers tightly closed in a dry, cool and well-ventilated place. Incompatible Materials. Strong oxidizing agents.

8. Exposure controls / personal protection

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

Engineering Measures None under normal use conditions.

Personal Protective Equipment

Eye/face Protection Wear appropriate protective eyeglasses or chemical safety goggles as described by OSHA's eye and face protection regulations in 29 CFR 1910.133 or European Standard EN166.

Skin and body protection Wear appropriate protective gloves and clothing to prevent skin exposure.

Respiratory Protection No protective equipment is needed under normal use conditions.

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

9. Physical and chemical properties

Physical State	Solid
Appearance	White
Odor	Odorless
Odor Threshold	No information available
pH	7.0-8.0 1% aq. sol
Melting Point/Range	292 °C / 557.6 °F
Boiling Point/Range	392 °C
Flash Point	Not applicable
Method -	
Evaporation Rate	Not applicable
Flammability (solid,gas)	No information available
Flammability or explosive limits	
Upper	No data available
Lower	No data available
Vapor Pressure	No information available
Vapor Density	Not applicable
Specific Gravity	No information available
Bulk Density	~1.8 g/cm3
Solubility	Soluble in water

Partition coefficient; n-octanol/water	No data available
Autoignition Temperature	No information available
Decomposition Temperature	No information available
Viscosity	Not applicable
Molecular Formula	C2 H3 K O2
Molecular Weight	98.14

10. Stability and reactivity

Reactive Hazard None known, based on information available

Stability Hygroscopic. Absorbs moisture from air and becomes liquid.

Conditions to Avoid Incompatible products. Excess heat. Avoid dust formation. Exposure to moist air or water.

Incompatible Materials Strong oxidizing agents

Hazardous Decomposition Products Carbon monoxide (CO), Carbon dioxide (CO_2), Potassium oxides

Hazardous Polymerization Hazardous polymerization does not occur.

Hazardous Reactions None under normal processing.

11. Toxicological information

Acute Toxicity

Product Information

Component Information

Component	LD50 Oral	LD50 Dermal	LC50 Inhalation
Potassium acetate	LD50 = 3250 mg/kg (Rat)	LD50 > 20000 mg/kg (Rabbit)	Not listed

Toxicologically Synergistic Products No information available

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

Irritation No information available

Sensitization No information available

Carcinogenicity The table below indicates whether each agency has listed any ingredient as a carcinogen.

Component	CAS No	IARC	NTP	ACGIH	OSHA	Mexico
Potassium acetate	127-08-2	Not listed	Not listed	Not listed	Not listed	Not listed

Mutagenic Effects No information available

Reproductive Effects No information available.

Developmental Effects No information available.

Teratogenicity No information available.

STOT - single exposure None known

STOT - repeated exposure None known

Aspiration hazard No information available

Symptoms / effects,both acute and delayed No information available

Endocrine Disruptor Information No information available

Other Adverse Effects See actual entry in RTECS for complete information. The toxicological properties have not been fully investigated.

12. Ecological information

Ecotoxicity

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Component	Freshwater Algae	Freshwater Fish	Microtox	Water Flea
Potassium acetate	Not listed	LC50: = 6800 mg/L, 96h semi-static (Oncorhynchus mykiss)	Not listed	Not listed

Persistence and Degradability Soluble in water Persistence is unlikely based on information available.

Bioaccumulation/ Accumulation No information available.

Mobility Will likely be mobile in the environment due to its water solubility.

13. Disposal considerations

Waste Disposal Methods Chemical waste generators must determine whether a discarded chemical is classified as a hazardous waste. Chemical waste generators must also consult local, regional, and national hazardous waste regulations to ensure complete and accurate classification.

14. Transport information

DOT Not regulated
TDG Not regulated
IATA Not regulated
IMDG/IMO Not regulated

15. Regulatory information

United States of America Inventory

Component	CAS No	TSCA	TSCA Inventory notification - Active-Inactive	TSCA - EPA Regulatory Flags
Potassium acetate	127-08-2	X	ACTIVE	-

Legend:
TSCA US EPA (TSCA) - Toxic Substances Control Act, (40 CFR Part 710)
X - Listed
'-' - Not Listed

TSCA 12(b) - Notices of Export Not applicable

International Inventories
Canada (DSL/NDSL), Europe (EINECS/ELINCS/NLP), Philippines (PICCS), Japan (ENCS), Japan (ISHL), Australia (AICS), China (IECSC), Korea (KECL).

Component	CAS No	DSL	NDSL	EINECS	PICCS	ENCS	ISHL	AICS	IECSC	KECL
Potassium acetate	127-08-2	X	-	204-822-2	X	X	X	X	X	KE-29069

KECL - NIER number or KE number (http://ncis.nier.go.kr/en/main.do)

U.S. Federal Regulations

SARA 313 Not applicable

SARA 311/312 Hazard Categories See section 2 for more information

CWA (Clean Water Act) Not applicable

Clean Air Act Not applicable

OSHA - Occupational Safety and Health Administration Not applicable

CERCLA Not applicable

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

U.S. State Right-to-Know Regulations Not applicable

U.S. Department of Transportation
Reportable Quantity (RQ): N
DOT Marine Pollutant N
DOT Severe Marine Pollutant N

U.S. Department of Homeland Security This product does not contain any DHS chemicals.

Other International Regulations

Mexico - Grade Slight risk, Grade 1

Authorisation/Restrictions according to EU REACH

Safety, health and environmental regulations/legislation specific for the substance or mixture

Component	CAS No	OECD HPV	Persistent Organic Pollutant	Ozone Depletion Potential	Restriction of Hazardous Substances (RoHS)
Potassium acetate	127-08-2	Listed	Not applicable	Not applicable	Not applicable

Component	CAS No	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Major Accident Notification	Seveso III Directive (2012/18/EC) - Qualifying Quantities for Safety Report Requirements	Rotterdam Convention (PIC)	Basel Convention (Hazardous Waste)
Potassium acetate	127-08-2	Not applicable	Not applicable	Not applicable	Not applicable

16. Other information

Prepared By Regulatory Affairs
Thermo Fisher Scientific
Email: EMSDS.RA@thermofisher.com

Creation Date 18-Jun-2009
Revision Date 24-Dec-2021
Print Date 24-Dec-2021
Revision Summary SDS sections updated. 2.

Disclaimer
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End of SDS

SAFETY DATA SHEET

Version 7.0
Revision Date 27.12.2021
Print Date 20.06.2022

SECTION 1: Identification of the hazardous chemical and of the supplier

1.1 Product identifiers

Product name : Sodium dodecyl sulfate

Product Number : L4509
Brand : Sigma-Aldrich
CAS-No. : 151-21-3

1.2 Other means of identification

Lauryl sulfatesodium salt
Sodium dodecyl sulphate
Sodium lauryl sulfate
Dodecyl sodium sulfate
Dodecyl sulfatesodium salt
SDS
Sodium dodecyl sulfate

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

Identified uses : For R&D use only. Not for pharmaceutical, household or other uses.

1.4 Details of the supplier of the safety data sheet

Company : SIGMA-ALDRICH (M) SDN BHD
Level 3, Menara Sunway Annexe,
Jalan Lagoon Timur, Bandar Sunway,
46150 PETALING JAYA, SELANGOR
MALAYSIA

Telephone : +60 (603)03-563-53321
Fax : +60 (603)03-563-54116

1.5 Emergency telephone

Emergency Phone # : 1-800-815-308 (CHEMTREC) * + 62 0800 140 1253 (Customer Call Centre)

Section 2: Hazard identification

2.1 GHS Classification

Classification according to CLASS regulations 2013
Flammable solids (Category 2), H228
Acute toxicity, Oral (Category 4), H302
Acute toxicity, Inhalation (Category 4), H332
Skin corrosion/irritation (Category 2), H315
Serious eye damage/eye irritation (Category 1), H318
Specific target organ toxicity - single exposure (Category 3), Respiratory system, H335
Hazardous to the aquatic environment - chronic hazard (Category 3), H412

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

2.2 GHS Label elements, including precautionary statements

Labelling according to CLASS regulations 2013

Pictogram

Signal word Danger

Hazard statement(s)

H228	Flammable solid.
H302 + H332	Harmful if swallowed or if inhaled.
H315	Causes skin irritation.
H318	Causes serious eye damage.
H335	May cause respiratory irritation.
H412	Harmful to aquatic life with long lasting effects.

Precautionary statement(s)

Prevention

P210	Keep away from heat/ sparks/ open flames/ hot surfaces. No smoking.
P261	Avoid breathing dust/ fume/ gas/ mist/ vapors/ spray.
P264	Wash skin thoroughly after handling.
P280	Wear protective gloves/ eye protection/ face protection.

Response

P305 + P351 + P338 + P310	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Immediately call a POISON CENTER or doctor/ physician.
P370 + P378	In case of fire: Use dry sand, dry chemical or alcohol-resistant foam for extinction.

2.3 Other hazards - none

SECTION 3: Composition and information of the ingredients of the hazardous chemical

Substance / Mixture : Substance

3.1 Substances

Synonyms : Lauryl sulfatesodium salt
Sodium dodecyl sulphate
Sodium lauryl sulfate
Dodecyl sodium sulfate
Dodecyl sulfatesodium salt
SDS
Sodium dodecyl sulfate

Formula : C12H25O4S.Na
Molecular weight : 288.38 g/mol
CAS-No. : 151-21-3
EC-No. : 205-788-1

Hazardous ingredients

Component	Classification	Concentration
Sodium dodecyl sulphate		
	Flam. Sol. 2; Acute Tox. 4;	<= 100 %

	2; 1; STOT SE 3; Aquatic Chronic 3; H228, H302, H332, H315, H318, H335, H412 Concentration limits: 10 - < 20 %: Eye Irrit. 2, H319; >= 20 %: Eye Dam. 1, H318;	

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

SECTION 4: First aid measures

4.1 Description of first-aid measures

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

If inhaled
After inhalation: fresh air.

In case of skin contact
In case of skin contact: Take off immediately all contaminated clothing. Rinse skin with water/ shower.

In case of eye contact
After eye contact: rinse out with plenty of water. Immediately call in ophthalmologist. Remove contact lenses.

If swallowed
After swallowing: immediately make victim drink water (two glasses at most). Consult a physician.

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

SECTION 5: Firefighting measures

5.1 Extinguishing media

Suitable extinguishing media
Water Foam Carbon dioxide (CO2) Dry powder

Unsuitable extinguishing media
For this substance/mixture no limitations of extinguishing agents are given.

5.2 Special hazards arising from the substance or mixture
Carbon oxides
Sulfur oxides
Sodium oxides
Carbon oxides
Sulfur oxides
Sodium oxides
Combustible.
Vapors are heavier than air and may spread along floors.
Forms explosive mixtures with air on intense heating.

Development of hazardous combustion gases or vapours possible in the event of fire.

5.3 Advice for firefighters
Stay in danger area only with self-contained breathing apparatus. Prevent skin contact by keeping a safe distance or by wearing suitable protective clothing.

5.4 Further information
Suppress (knock down) gases/vapors/mists with a water spray jet. Prevent fire extinguishing water from contaminating surface water or the ground water system.

SECTION 6: Accidental release measures

6.1 Personal precautions, protective equipment and emergency procedures
Advice for non-emergency personnel: Avoid inhalation of dusts. Avoid substance contact. Ensure adequate ventilation. Keep away from heat and sources of ignition. Evacuate the danger area, observe emergency procedures, consult an expert.
For personal protection see section 8.

6.2 Environmental precautions
Do not let product enter drains. Risk of explosion.

6.3 Methods and materials for containment and cleaning up
Cover drains. Collect, bind, and pump off spills. Observe possible material restrictions (see sections 7 and 10). Take up dry. Dispose of properly. Clean up affected area. Avoid generation of dusts.

6.4 Reference to other sections
For disposal see section 13.

SECTION 7: Handling and storage

7.1 Precautions for safe handling

Advice on safe handling
Work under hood. Do not inhale substance/mixture.

Advice on protection against fire and explosion
Keep away from open flames, hot surfaces and sources of ignition.Take precautionary measures against static discharge.

Hygiene measures
Immediately change contaminated clothing. Apply preventive skin protection. Wash hands and face after working with substance.
For precautions see section 2.2.

7.2 Conditions for safe storage, including any incompatibilities

Storage conditions
Tightly closed. Keep away from heat and sources of ignition.

hygroscopic

Storage class
Storage class (TRGS 510): 4.1B: Flammable solid hazardous materials

7.3 Specific end use(s)
Apart from the uses mentioned in section 1.2 no other specific uses are stipulated

SECTION 8: Exposure controls and personal protection

8.1 Control parameters

Ingredients with workplace control parameters
Contains no substances with occupational exposure limit values.

8.2 Exposure controls

Appropriate engineering controls
Immediately change contaminated clothing. Apply preventive skin protection. Wash hands and face after working with substance.

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). Tightly fitting safety goggles

Skin protection
This recommendation applies only to the product stated in the safety data sheet, supplied by us and for the designated use. When dissolving in or mixing with other substances and under conditions deviating from those stated in EN374 please contact the supplier of CE-approved gloves (e.g. KCL GmbH, D-36124 Eichenzell, Internet: www.kcl.de).

Full contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:KCL 741 Dermatril® L

Splash contact
Material: Nitrile rubber
Minimum layer thickness: 0.11 mm
Break through time: 480 min
Material tested:KCL 741 Dermatril® L

Body Protection
Flame retardant antistatic protective clothing.

Respiratory protection
required when dusts are generated.
Our recommendations on filtering respiratory protection are based on the following standards: DIN EN 143, DIN 14387 and other accompanying standards relating to the used respiratory protection system.

Control of environmental exposure
Do not let product enter drains. Risk of explosion.

SECTION 9: Physical and chemical properties

9.1 Information on basic physical and chemical properties

a)	Appearance	Form: Rods Color: white
b)	Odor	odorless

c)	Odor Threshold	No data available
d)	pH	9.1 at 10 g/l
e)	Melting point/freezing point	Melting point/range: 204 - 207 °C - lit.
f)	Initial boiling point and boiling range	No data available
g)	Flash point	170 °C
h)	Evaporation rate	No data available
i)	Flammability (solid, gas)	The substance or mixture is a flammable solid with the category 2.
j)	Upper/lower flammability or explosive limits	No data available
k)	Vapor pressure	0.002 hPa at 20 °C
l)	Vapor density	No data available
m)	Density	0.370 g/cm3
	Relative density	No data available
n)	Water solubility	130 g/l at 20 °C - OECD Test Guideline 105
o)	Partition coefficient: n-octanol/water	log Pow: 0.83 at 22 °C
p)	Autoignition temperature	310.5 °C
q)	Decomposition temperature	No data available
r)	Viscosity	Viscosity, kinematic: No data available Viscosity, dynamic: No data available
s)	Explosive properties	No data available
t)	Oxidizing properties	none

9.2 Other safety information

Surface tension	25.2 mN/m at 23 °C
Dissociation constant	1.31 at 20 °C

SECTION 10: Stability and reactivity

10.1 Reactivity

Forms explosive mixtures with air on intense heating.
A range from approx. 15 Kelvin below the flash point is to be rated as critical.
The following applies in general to flammable organic substances and mixtures: in correspondingly fine distribution, when whirled up a dust explosion potential may generally be assumed.

10.2 Chemical stability

The product is chemically stable under standard ambient conditions (room temperature) .

10.3 Possibility of hazardous reactions
No data available

10.4 Conditions to avoid
Strong heating.

10.5 Incompatible materials
Strong oxidizing agents

10.6 Hazardous decomposition products
In the event of fire: see section 5

SECTION 11: Toxicological information

11.1 Information on toxicological effects

Acute toxicity
LD50 Oral - Rat - female - 977 mg/kg
(OECD Test Guideline 401)
Symptoms: Irritations of mucous membranes in the mouth, pharynx, oesophagus and gastrointestinal tract.
Acute toxicity estimate Inhalation - 4 h - 1.51 mg/l - dust/mist

(Expert judgment)
LD50 Dermal - Rabbit - > 2,000 mg/kg
(OECD Test Guideline 404)

Skin corrosion/irritation
Skin - Rabbit
Result: Irritations
(OECD Test Guideline 404)

Serious eye damage/eye irritation
Eyes - Rabbit
Result: Irreversible effects on the eye
(OECD Test Guideline 405)

Respiratory or skin sensitization
Maximization Test
Result: negative
Remarks: (IUCLID)

Germ cell mutagenicity
Test Type: Ames test
Test system: Salmonella typhimurium
Metabolic activation: with and without metabolic activation
Method: OECD Test Guideline 471
Result: negative
Test Type: Mutagenicity (mammal cell test):
Test system: Mouse lymphoma test
Method: OECD Test Guideline 476
Result: negative

Carcinogenicity
No data available

Reproductive toxicity
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

11.2 Additional Information

RTECS: WT1050000
sneezing, The sodium salt of dodecyl sulfate has been reported to cause pulmonary sensitization resulting in hyperactive airway dysfunction and pulmonary allergy accompanied by fatigue, malaise, and aching. Significant symptoms of exposure can persist for more than two years and can be activated by a variety of nonspecific environmental stimuli such as automobile exhaust, perfumes, and passive smoking.
To the best of our knowledge, the chemical, physical, and toxicological properties have not been thoroughly investigated.

SECTION 12: Ecological information

12.1 Toxicity

Toxicity to fish	flow-through test LC50 - Pimephales promelas (fathead minnow) - 29 mg/l - 96 h (OECD Test Guideline 203)
Toxicity to daphnia and other aquatic invertebrates	flow-through test EC50 - Daphnia dubia (Water flea) - 5.55 mg/l - 48 h Remarks: (ECHA)
	flow-through test NOEC - Ceriodaphnia dubia (water flea) - 0.88 mg/l - 7 d (US-EPA)
Toxicity to algae	ErC50 - Desmodesmus subspicatus (green algae) - > 120 mg/l - 72 h (DIN 38412)
Toxicity to bacteria	EC50 - activated sludge - 130 mg/l - 3 h (OECD Test Guideline 209)

12.2 Persistence and degradability

Biodegradability	aerobic - Exposure time 28 d Result: 95 % - Readily biodegradable. (OECD Test Guideline 301B)

12.3 Bioaccumulative potential

Bioaccumulation	Cyprinus carpio (Carp) - 72 h (Sodium dodecyl sulphate)
	Bioconcentration factor (BCF): 3.9 - 5.3

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 Endocrine disrupting properties
No data available

12.7 Other adverse effects
No data available

SECTION 13: Disposal information

13.1 Waste treatment methods

Product
Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself. See www.retrologistik.com for processes regarding the return of chemicals and containers, or contact us there if you have further questions. According to Quality Environment Regulation (Scheduled Waste) 2005, waste need to be sent to designated premise for recycle, treatment or disposal. Please contact Kualiti Alam for waste classification and correct disposal method. Waste material must be disposed of in accordance with the national and local regulations. Leave chemicals in original containers. No mixing with other waste. Handle uncleaned containers like the product itself.

SECTION 14: Transportation information

14.1 UN number
ADR/RID: 1325 IMDG: 1325 IATA-DGR: 1325

14.2 UN proper shipping name
ADR/RID: FLAMMABLE SOLID, ORGANIC, N.O.S. (dodecyl sulphate sodium salt)
IMDG: FLAMMABLE SOLID, ORGANIC, N.O.S. (dodecyl sulphate sodium salt)
IATA-DGR: Flammable solid, organic, n.o.s. (dodecyl sulphate sodium salt)

14.3 Transport hazard class(es)
ADR/RID: 4.1 IMDG: 4.1 IATA-DGR: 4.1

14.4 Packaging group
ADR/RID: III IMDG: III IATA-DGR: III

14.5 Environmental hazards
ADR/RID: no IMDG Marine pollutant: no IATA-DGR: no

14.6 Special precautions for user
None

14.7 Incompatible materials
Strong oxidizing agents

Other regulations
Hazchem Code : 1Z

SECTION 15: Regulatory information

15.1 Safety, health and environmental regulations/legislation specific for the substance or mixture

Notification status
DSL: All components of this product are on the Canadian DSL

ENCS:	On the inventory, or in compliance with the inventory
ISHL:	On the inventory, or in compliance with the inventory
KECI:	On the inventory, or in compliance with the inventory
NZIoC:	On the inventory, or in compliance with the inventory
PICCS:	On the inventory, or in compliance with the inventory

SECTION 16: Other information

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

H228	Flammable solid.
H302	Harmful if swallowed.
H315	Causes skin irritation.
H318	Causes serious eye damage.
H319	Causes serious eye irritation.
H332	Harmful if inhaled.
H335	May cause respiratory irritation.
H412	Harmful to aquatic life with long lasting effects.

Relevant changes since previous version
8. Exposure controls/personal protection

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.

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Sodium Hydroxide

Safety Data Sheet

according to Federal Register / Vol. 77, No. 58 / Monday, March 26, 2012 / Rules and Regulations

Date of issue: 07/06/1998 Revision date: 02/21/2018 Supersedes: 10/14/2013 Version: 1.1

SECTION 1: Identification

1.1. Identification

Product form	:	Substance
Substance name	:	Sodium Hydroxide
CAS-No.	:	1310-73-2
Product code	:	LC23900
Formula	:	NaOH
Synonyms	:	anhydrous caustic soda / caustic alkali / caustic flake / caustic soda, solid / caustic white / caustic, flaked / hydrate of soda / hydroxide of soda / LEWIS red devil lye / soda lye / sodium hydrate / sodium hydroxide, pellets

1.2. Recommended use and restrictions on use

Use of the substance/mixture	:	Industrial use
Recommended use	:	Laboratory chemicals
Restrictions on use	:	Not for food, drug or household use

1.3. Supplier

LabChem Inc
Jackson's Pointe Commerce Park Building 1000, 1010 Jackson's Pointe Court
Zelienople, PA 16063 - USA
T 412-826-5230 - F 724-473-0647
info@labchem.com - www.labchem.com

1.4. Emergency telephone number

Emergency number : CHEMTREC: 1-800-424-9300 or 011-703-527-3887

SECTION 2: Hazard(s) identification

2.1. Classification of the substance or mixture

GHS-US classification

Skin corrosion/irritation, Category 1A	H314	Causes severe skin burns and eye damage.
Serious eye damage/eye irritation, Category 1	H318	Causes serious eye damage.
Hazardous to the aquatic environment — Acute Hazard, Category 3	H402	Harmful to aquatic life

Full text of H statements : see section 16

2.2. GHS Label elements, including precautionary statements

GHS-US labelling

Hazard pictograms (GHS-US) :

GHS05

Signal word (GHS-US) : Danger

Hazard statements (GHS-US) :
H314 - Causes severe skin burns and eye damage.
H402 - Harmful to aquatic life

Precautionary statements (GHS-US) :
P260 - Do not breathe dust, vapours.
P264 - Wash exposed skin thoroughly after handling.
P273 - Avoid release to the environment.
P280 - Wear eye protection, face protection, protective clothing, protective gloves.
P301+P330+P331 - IF SWALLOWED: rinse mouth. Do NOT induce vomiting.
P303+P361+P353 - IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water/shower.
P304+P340 - IF INHALED: Remove person to fresh air and keep comfortable for breathing.
P305+P351+P338 - IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.
P310 - Immediately call a POISON CENTER/doctor

P363 - Wash contaminated clothing before reuse.
P405 - Store locked up.
P501 - Dispose of contents/container to Comply with applicable regulations

2.3. Other hazards which do not result in classification

Other hazards not contributing to the classification : None under normal conditions.

2.4. Unknown acute toxicity (GHS US)

Not applicable

SECTION 3: Composition/information on ingredients

3.1. Substances

Substance type : Mono-constituent

Name	Product identifier	%	GHS-US classification
Sodium Hydroxide (Main constituent)	(CAS-No.) 1310-73-2	100	Skin Corr. 1A, H314 Eye Dam. 1, H318 Aquatic Acute 3, H402

Full text of hazard classes and H-statements : see section 16

3.2. Mixtures

Not applicable

SECTION 4: First-aid measures

4.1. Description of first aid measures

First-aid measures general : Check the vital functions. Unconscious: maintain adequate airway and respiration. Respiratory arrest: artificial respiration or oxygen. Cardiac arrest: perform resuscitation. Victim conscious with laboured breathing: half-seated. Victim in shock: on his back with legs slightly raised. Vomiting: prevent asphyxia/aspiration pneumonia. Prevent cooling by covering the victim (no warming up). Keep watching the victim. Give psychological aid. Keep the victim calm, avoid physical strain. Depending on the victim's condition: doctor/hospital.

First-aid measures after inhalation : Remove the victim into fresh air. Respiratory problems: consult a doctor/medical service.

First-aid measures after skin contact : Wipe off dry product from skin. Remove clothing before washing. Wash immediately with lots of water (15 minutes)/shower. Do not apply (chemical) neutralizing agents. Do not remove clothing if it sticks to the skin. Cover wounds with sterile bandage. Consult a doctor/medical service. If burned surface > 10%: take victim to hospital.

First-aid measures after eye contact : Rinse immediately with plenty of water for 15 minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Do not apply neutralizing agents. Take victim to an ophthalmologist.

First-aid measures after ingestion : Rinse mouth with water. Immediately after ingestion: give lots of water to drink. Do not induce vomiting. Do not give activated charcoal. Do not give chemical antidote. Immediately consult a doctor/medical service. Call Poison Information Centre (www.big.be/antigif.htm). Ingestion of large quantities: immediately to hospital. Take the container/vomit to the doctor/hospital.

4.2. Most important symptoms and effects (acute and delayed)

Symptoms/effects after inhalation : WHEN PROCESSED: Dry/sore throat. Coughing. Irritation of the respiratory tract. Irritation of the nasal mucous membranes. ON CONTINUOUS EXPOSURE/CONTACT: Respiratory difficulties. FOLLOWING SYMPTOMS MAY APPEAR LATER: Possible oedema of the upper respiratory tract. Possible laryngeal spasm/oedema. Risk of lung oedema.

Symptoms/effects after skin contact : Blisters. Caustic burns/corrosion of the skin. Slow-healing wounds.

Symptoms/effects after eye contact : Corrosion of the eye tissue. Permanent eye damage.

Symptoms/effects after ingestion : Dry/sore throat. Nausea. Abdominal pain. Blood in vomit. Difficulty in swallowing. Possible esophageal perforation. Burns to the gastric/intestinal mucosa. Bleeding of the gastrointestinal tract. Shock.

Chronic symptoms : ON CONTINUOUS/REPEATED EXPOSURE/CONTACT: Dry skin. Skin rash/inflammation. Possible inflammation of the respiratory tract. Gastrointestinal complaints.

4.3. Immediate medical attention and special treatment, if necessary

Obtain medical assistance.

SECTION 5: Fire-fighting measures

5.1. Suitable (and unsuitable) extinguishing media

Suitable extinguishing media : Adapt extinguishing media to the environment for surrounding fires.

5.2. Specific hazards arising from the chemical

Fire hazard	:	DIRECT FIRE HAZARD: Non combustible. INDIRECT FIRE HAZARD: Reactions involving a fire hazard: see "Reactivity Hazard".
Explosion hazard	:	INDIRECT EXPLOSION HAZARD: Reactions with explosion hazards: see "Reactivity Hazard".
Reactivity	:	May be corrosive to metals. Absorbs the atmospheric CO2. Violent to explosive reaction with (some) acids. Reacts violently with many compounds: heat release resulting in increased fire or explosion risk. Violent exothermic reaction with water (moisture): release of corrosive mist. Reacts exothermically on exposure to water (moisture) with combustible materials: risk of spontaneous ignition.

5.3. Special protective equipment and precautions for fire-fighters

Precautionary measures fire	:	Exposure to fire/heat: keep upwind. Exposure to fire/heat: consider evacuation. Exposure to fire/heat: have neighbourhood close doors and windows.
Firefighting instructions	:	Cool tanks/drums with water spray/remove them into safety. When cooling/extinguishing: no water in the substance. Take account of toxic fire-fighting water. Use water moderately and if possible collect or contain it.
Protection during firefighting	:	Heat/fire exposure: compressed air/oxygen apparatus.

SECTION 6: Accidental release measures

6.1. Personal precautions, protective equipment and emergency procedures

General measures : Absorb spillage to prevent material damage. Dike and contain spill.

6.1.1. For non-emergency personnel

Protective equipment	:	Gloves. Face-shield. Corrosion-proof suit. Dust cloud production: compressed air/oxygen apparatus. Contact with moisture/water: compressed air/oxygen apparatus. Contact with moisture/water: gas-tight suit.
Emergency procedures	:	Mark the danger area. Prevent dust cloud formation. Corrosion-proof appliances. Keep containers closed. Avoid ingress of water in the containers. Wash contaminated clothes. On contact with moisture/water: keep upwind. On contact with moisture/water: consider evacuation. In case of hazardous reactions: keep upwind. In case of reactivity hazard: consider evacuation.
Measures in case of dust release	:	In case of dust production: keep upwind. Dust production: have neighbourhood close doors and windows.

6.1.2. For emergency responders

Protective equipment	:	Equip cleanup crew with proper protection. Do not breathe dust.
Emergency procedures	:	Stop release.

6.2. Environmental precautions

Prevent soil and water pollution. Prevent spreading in sewers.

6.3. Methods and material for containment and cleaning up

For containment	:	Contain released product, pump into suitable containers. Plug the leak, cut off the supply. Dam up the solid spill. Hazardous reaction: measure explosive gas-air mixture. Reaction: dilute combustible gas/vapour with water curtain.
Methods for cleaning up	:	Collect the spill only if it is in a dry state. Wetted substance: cover with powdered limestone or dry sand, earth, vermiculite. Scoop solid spill into closing containers. Under controlled conditions: neutralize leftovers with dilute acid solution. Possible violent reaction if you neutralize. Carefully collect the spill/leftovers. Clean contaminated surfaces with an excess of water. Take collected spill to manufacturer/competent authority. Wash clothing and equipment after handling.

6.4. Reference to other sections

No additional information available

SECTION 7: Handling and storage

7.1. Precautions for safe handling

Precautions for safe handling	:	Avoid raising dust. Avoid contact of substance with water. Measure the concentration in the air regularly. Carry operations in the open/under local exhaust/ventilation or with respiratory protection. Comply with the legal requirements. Remove contaminated clothing immediately. Clean contaminated clothing. Keep the substance free from contamination. Use corrosionproof equipment. Thoroughly clean/dry the installation before use. Do not discharge the waste into the drain.

Hygiene measures	:	Wash hands and other exposed areas with mild soap and water before eating, drinking or smoking and when leaving work. Wash contaminated clothing before reuse. Separate working clothes from town clothes. Launder separately.

7.2. Conditions for safe storage, including any incompatibilities

Incompatible products	:	combustible materials. metals. Strong acids. Strong oxidizers. Protect from moisture.
Incompatible materials	:	incompatible materials. Moisture. Heat sources.
Storage temperature	:	20 °C
Heat and ignition sources	:	KEEP SUBSTANCE AWAY FROM: heat sources.
Prohibitions on mixed storage	:	KEEP SUBSTANCE AWAY FROM: combustible materials. oxidizing agents. (strong) acids. metals. organic materials. water/moisture.
Storage area	:	Store in a dry area. Keep container in a well-ventilated place. Keep locked up. Unauthorized persons are not admitted. Store at ambient temperature. Keep only in the original container. Meet the legal requirements.
Special rules on packaging	:	SPECIAL REQUIREMENTS: hermetical. watertight. corrosion-proof. dry. clean. correctly labelled. meet the legal requirements. Secure fragile packagings in solid containers.
Packaging materials	:	SUITABLE MATERIAL: stainless steel. nickel. polyethylene. paper. MATERIAL TO AVOID: lead. aluminium. copper. tin. zinc. bronze. textile.

SECTION 8: Exposure controls/personal protection

8.1. Control parameters

Sodium Hydroxide (1310-73-2)		
ACGIH	ACGIH Ceiling (mg/m³)	2 mg/m³
OSHA	OSHA PEL (TWA) (mg/m³)	2 mg/m³
IDLH	US IDLH (mg/m³)	10 mg/m³
NIOSH	NIOSH REL (ceiling) (mg/m³)	2 mg/m³

8.2. Appropriate engineering controls

Appropriate engineering controls	:	Emergency eye wash fountains and safety showers should be available in the immediate vicinity of any potential exposure. Provide adequate general and local exhaust ventilation.

8.3. Individual protection measures/Personal protective equipment

Personal protective equipment:

Safety glasses. Protective clothing. Gloves. Dust/aerosol mask with filter type P3.

Materials for protective clothing:

GIVE GOOD RESISTANCE: natural rubber. neoprene. nitrile rubber. GIVE LESS RESISTANCE: butyl rubber. polyethylene. PVA. GIVE POOR RESISTANCE: natural fibres

Hand protection:

Gloves

Eye protection:

Face shield. In case of dust production: protective goggles

Skin and body protection:

Corrosion-proof clothing. In case of dust production: head/neck protection

Respiratory protection:

Dust production: dust mask with filter type P3.
High dust production: self-contained breathing apparatus

SECTION 9: Physical and chemical properties

9.1. Information on basic physical and chemical properties

Physical state	:	Solid
Appearance	:	Crystalline solid. Crystalline powder. Little spheres. Lumps. Needles. Scales. Flakes.
Colour	:	White
Odour	:	Odourless
Odour threshold	:	No data available
pH	:	14 (5 %)
Melting point	:	323 °C
Freezing point	:	No data available
Boiling point	:	1388 °C (1013.25 hPa)
Flash point	:	Not applicable
Relative evaporation rate (butylacetate=1)	:	No data available
Flammability (solid, gas)	:	No data available
Vapour pressure	:	< 0.1 hPa (20 °C)
Relative vapour density at 20 °C	:	No data available
Relative density	:	2.13 (20 °C)
Density	:	2130 kg/m³
Molecular mass	:	40 g/mol
Solubility	:	Exothermically soluble in water. Soluble in ethanol. Soluble in methanol. Soluble in glycerol. Water: 100 g/100ml (25 °C) Ethanol: soluble
Log Pow	:	No data available
Auto-ignition temperature	:	Not applicable
Decomposition temperature	:	No data available
Viscosity, kinematic	:	0.53 mm²/s (25 °C, 1 mol/l)
Viscosity, dynamic	:	0.997 mPa.s (25 °C, Test data)
Explosive limits	:	No data available
Explosive properties	:	Not applicable.
Oxidising properties	:	None.

9.2. Other information

Minimum ignition energy	:	Not applicable
Saturation concentration	:	671 g/m³
VOC content	:	Not applicable (inorganic)
Other properties	:	Translucent. Hygroscopic. Substance has basic reaction.

SECTION 10: Stability and reactivity

10.1. Reactivity

May be corrosive to metals. Absorbs the atmospheric CO2. Violent to explosive reaction with (some) acids. Reacts violently with many compounds: heat release resulting in increased fire or explosion risk. Violent exothermic reaction with water (moisture): release of corrosive mist. Reacts exothermically on exposure to water (moisture) with combustible materials: risk of spontaneous ignition.

10.2. Chemical stability

Hygroscopic. Unstable on exposure to air.

10.3. Possibility of hazardous reactions

Reacts violently with acids. Reacts violently with water.

10.4. Conditions to avoid

Moisture. Incompatible materials.

10.5. Incompatible materials

Water. Strong oxidizers. Strong acids. metals. combustible materials.

10.6. Hazardous decomposition products

Sodium oxide.

SECTION 11: Toxicological information

11.1. Information on toxicological effects

Likely routes of exposure	:	Skin and eyes contact
Acute toxicity	:	Not classified
Skin corrosion/irritation	:	Causes severe skin burns and eye damage. pH: 14 (5 %)
Serious eye damage/irritation	:	Causes serious eye damage. pH: 14 (5 %)
Respiratory or skin sensitisation	:	Not classified
Germ cell mutagenicity	:	Not classified
Carcinogenicity	:	Not classified (Based on available data, the classification criteria are not met)
Reproductive toxicity	:	Not classified
Specific target organ toxicity (single exposure)	:	Not classified
Specific target organ toxicity (repeated exposure)	:	Not classified
Aspiration hazard	:	Not classified
Potential adverse human health effects and symptoms	:	Causes severe skin burns. Causes serious eye damage.
Symptoms/effects after inhalation	:	WHEN PROCESSED: Dry/sore throat. Coughing. Irritation of the respiratory tract. Irritation of the nasal mucous membranes. ON CONTINUOUS EXPOSURE/CONTACT: Respiratory difficulties. FOLLOWING SYMPTOMS MAY APPEAR LATER: Possible oedema of the upper respiratory tract. Possible laryngeal spasm/oedema. Risk of lung oedema.
Symptoms/effects after skin contact	:	Blisters. Caustic burns/corrosion of the skin. Slow-healing wounds.
Symptoms/effects after eye contact	:	Corrosion of the eye tissue. Permanent eye damage.
Symptoms/effects after ingestion	:	Dry/sore throat. Nausea. Abdominal pain. Blood in vomit. Difficulty in swallowing. Possible esophageal perforation. Burns to the gastric/intestinal mucosa. Bleeding of the gastrointestinal tract. Shock.
Chronic symptoms	:	ON CONTINUOUS/REPEATED EXPOSURE/CONTACT: Dry skin. Skin rash/inflammation. Possible inflammation of the respiratory tract. Gastrointestinal complaints.

SECTION 12: Ecological information

12.1. Toxicity

Ecology - general	:	Not classified as dangerous for the environment according to the criteria of Regulation (EC) No 1272/2008.
Ecology - air	:	Not included in the list of fluorinated greenhouse gases (Regulation (EU) No 517/2014). Not classified as dangerous for the ozone layer (Regulation (EC) No 1005/2009).
Ecology - water	:	Harmful to crustacea. Harmful to fishes. Groundwater pollutant. pH shift.

Sodium Hydroxide (1310-73-2)	
LC50 fish 1	45.4 mg/l (Other, 96 h, Salmo gairdneri, Static system, Fresh water, Experimental value)
EC50 Daphnia 1	40.4 mg/l (Other, 48 h, Ceriodaphnia sp., Experimental value)

12.2. Persistence and degradability

Sodium Hydroxide (1310-73-2)	
Persistence and degradability	Biodegradability: not applicable.
Biochemical oxygen demand (BOD)	Not applicable (inorganic)
Chemical oxygen demand (COD)	Not applicable (inorganic)
ThOD	Not applicable (inorganic)

12.3. Bioaccumulative potential

Sodium Hydroxide (1310-73-2)	
Bioaccumulative potential	Not bioaccumulative.

12.4. Mobility in soil

Sodium Hydroxide (1310-73-2)	
Ecology - soil	No (test)data on mobility of the substance available.

12.5. Other adverse effects

No additional information available

SECTION 13: Disposal considerations

13.1. Disposal methods

Waste disposal recommendations : Do not discharge into drains or the environment. Remove waste in accordance with local and/or national regulations. Hazardous waste shall not be mixed together with other waste. Different types of hazardous waste shall not be mixed together if this may entail a risk of pollution or create problems for the further management of the waste. Hazardous waste shall be managed responsibly. All entities that store, transport or handle hazardous waste shall take the necessary measures to prevent risks of pollution or damage to people or animals. Should not be landfilled with household waste. Recycle/reuse. Dilute. Neutralize.

Additional information : Hazardous waste according to Directive 2008/98/EC, as amended by Regulation (EU) No 1357/2014 and Regulation (EU) No 2017/997.

SECTION 14: Transport information

Department of Transportation (DOT)

In accordance with DOT

Transport document description : UN1823 Sodium hydroxide, solid, 8, II

UN-No.(DOT) : UN1823

Proper Shipping Name (DOT) : Sodium hydroxide, solid

Transport hazard class(es) (DOT) : 8 - Class 8 - Corrosive material 49 CFR 173.136

Packing group (DOT) : II - Medium Danger

Hazard labels (DOT) : 8 - Corrosive

DOT Packaging Non Bulk (49 CFR 173.xxx) : 212

DOT Packaging Bulk (49 CFR 173.xxx) : 240

DOT Special Provisions (49 CFR 172.102) : IB8 - Authorized IBCs: Metal (11A, 11B, 11N, 21A, 21B, 21N, 31A, 31B and 31N); Rigid plastics (11H1, 11H2, 21H1, 21H2, 31H1 and 31H2); Composite (11HZ1, 11HZ2, 21HZ1, 21HZ2, 31HZ1 and 31HZ2); Fiberboard (11G); Wooden (11C, 11D and 11F); Flexible (13H1, 13H2, 13H3, 13H4, 13H5, 13L1, 13L2, 13L3, 13L4, 13M1 or 13M2).
IP2 - When IBCs other than metal or rigid plastics IBCs are used, they must be offered for transportation in a closed freight container or a closed transport vehicle.
IP4 - Flexible, fiberboard or wooden IBCs must be sift-proof and water-resistant or be fitted with a sift-proof and water-resistant liner.
T3 - 2.65 178.274(d)(2) Normal..... 178.275(d)(2)
TP33 - The portable tank instruction assigned for this substance applies for granular and powdered solids and for solids which are filled and discharged at temperatures above their melting point which are cooled and transported as a solid mass. Solid substances transported or offered for transport above their melting point are authorized for transportation in portable tanks conforming to the provisions of portable tank instruction T4 for solid substances of packing group III or T7 for solid substances of packing group II, unless a tank with more stringent requirements for minimum shell thickness, maximum allowable working pressure, pressure-relief devices or bottom outlets are assigned in which case the more stringent tank instruction and special provisions shall apply. Filling limits must be in accordance with portable tank special provision TP3. Solids meeting the definition of an elevated temperature material must be transported in accordance with the applicable requirements of this subchapter.

DOT Packaging Exceptions (49 CFR 173.xxx) : 154

DOT Quantity Limitations Passenger aircraft/rail (49 CFR 173.27) : 15 kg

DOT Quantity Limitations Cargo aircraft only (49 CFR 175.75) : 50 kg

DOT Vessel Stowage Location : A - The material may be stowed ‘‘on deck’’ or ‘‘under deck’’ on a cargo vessel and on a passenger vessel.

DOT Vessel Stowage Other : 52 - Stow ‘‘separated from’’ acids

Other information : No supplementary information available.

SECTION 15: Regulatory information

15.1. US Federal regulations

Sodium Hydroxide (1310-73-2)	
Listed on the United States TSCA (Toxic Substances Control Act) inventory Not subject to reporting requirements of the United States SARA Section 313	
RQ (Reportable quantity, section 304 of EPA's List of Lists)	1000 lb
SARA Section 311/312 Hazard Classes	Immediate (acute) health hazard

All components of this product are listed, or excluded from listing, on the United States Environmental Protection Agency Toxic Substances Control Act (TSCA) inventory

15.2. International regulations

CANADA

Sodium Hydroxide (1310-73-2)
Listed on the Canadian DSL (Domestic Substances List)

EU-Regulations
No additional information available

National regulations
No additional information available

15.3. US State regulations

California Proposition 65 - This product does not contain any substances known to the state of California to cause cancer, developmental and/or reproductive harm

SECTION 16: Other information

Revision date : 02/21/2018

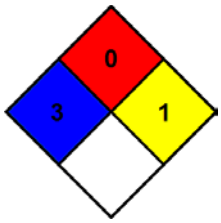
Full text of H-statements: see section 16:

H314	Causes severe skin burns and eye damage.
H318	Causes serious eye damage.
H402	Harmful to aquatic life

NFPA health hazard : 3 - Materials that, under emergency conditions, can cause serious or permanent injury.

NFPA fire hazard : 0 - Materials that will not burn under typical dire conditions, including intrinsically noncombustible materials such as concrete, stone, and sand.

NFPA reactivity : 1 - Materials that in themselves are normally stable but can become unstable at elevated temperatures and pressures.



Hazard Rating

Health : 3 Serious Hazard - Major injury likely unless prompt action is taken and medical treatment is given

Flammability : 0 Minimal Hazard - Materials that will not burn

Physical : 1 Slight Hazard - Materials that are normally stable but can become unstable (self-react) at high temperatures and pressures. Materials may react non-violently with water or undergo hazardous polymerization in the absence of inhibitors.

Personal protection : F

F - Safety glasses, Gloves, Synthetic apron, Dust respirator

SDS US LabChem

Information in this SDS is from available published sources and is believed to be accurate. No warranty, express or implied, is made and LabChem Inc assumes no liability resulting from the use of this SDS. The user must determine suitability of this information for his application.

SAFETY DATA SHEET
Tris-EDTA Stock Solution (10X, pH 7.4)

Revision: 12/24/2016
Supersedes Revision: 06/24/2013

according to Regulation (EC) No. 1907/2006 as amended by (EC) No. 1272/2008

Section 1. Identification of the Substance/Mixture and of the Company/Undertaking

1.1 Product Code: 600223

Product Name: Tris-EDTA Stock Solution (10X, pH 7.4)

Synonyms: TE Buffer;

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

Relevant identified uses: For research use only, not for human or veterinary use.

1.3 Details of the Supplier of the Safety Data Sheet:

Company Name: Cayman Chemical Company
1180 E. Ellsworth Rd.
Ann Arbor, MI 48108

Web site address: www.caymanchem.com

Information: Cayman Chemical Company +1 (734)971-3335

1.4 Emergency telephone number:

Emergency Contact: CHEMTREC Within USA and Canada: +1 (800)424-9300
CHEMTREC Outside USA and Canada: +1 (703)527-3887

Section 2. Hazards Identification

2.1 Classification of the Substance or Mixture:

Skin Corrosion/Irritation, Category 3

2.2 Label Elements:

GHS Signal Word: **Warning**

GHS Hazard Phrases:

H316: Causes mild skin irritation.

GHS Precaution Phrases:

No phrases apply.

GHS Response Phrases:

P332+313: If skin irritation occurs, get medical advice/attention.

GHS Storage and Disposal Phrases:

Please refer to Section 7 for Storage and Section 13 for Disposal information.

2.3 Adverse Human Health Effects and Symptoms: Causes mild skin irritation.
Material may be irritating to the mucous membranes and upper respiratory tract.
May be harmful by inhalation, ingestion, or skin absorption.
May cause eye and respiratory system irritation.
To the best of our knowledge, the toxicological properties have not been thoroughly investigated.

SAFETY DATA SHEET
Tris-EDTA Stock Solution (10X, pH 7.4)

Revision: 12/24/2016
Supersedes Revision: 06/24/2013

Section 3. Composition/Information on Ingredients

CAS # / RTECS #	Hazardous Components (Chemical Name)/ REACH Registration No.	Concentration	EC No./ EC Index No.	GHS Classification
77-86-1 TY2900000	Trizma base	1.211 %	201-064-4 NA	Skin Corr. 2: H315 Eye Damage 2: H319 STOT (SE) 3: H335
60-00-4 AH4025000	Ethylenediamine Tetraacetic Acid	0.292 %	200-449-4 607-429-00-8	Eye Damage 2A: H319
7732-18-5 ZC0110000	Water	98.497 %	231-791-2 NA	No data available.

Section 4. First Aid Measures

4.1 Description of First Aid Measures:

In Case of Inhalation: Remove to fresh air. If not breathing, give artificial respiration or give oxygen by trained personnel. Get immediate medical attention.

In Case of Skin Contact: Immediately wash skin with soap and plenty of water for at least 20 minutes. Remove contaminated clothing. Get medical attention if symptoms occur. Wash clothing before reuse.

In Case of Eye Contact: Hold eyelids apart and flush eyes with plenty of water for at least 20 minutes. Have eyes examined and tested by medical personnel.

In Case of Ingestion: Wash out mouth with water provided person is conscious. Never give anything by mouth to an unconscious person. Get medical attention. Do NOT induce vomiting unless directed to do so by medical personnel.

Section 5. Fire Fighting Measures

5.1 Suitable Extinguishing Media: Use alcohol-resistant foam, carbon dioxide, water, or dry chemical spray.
Use water spray to cool fire-exposed containers.

Unsuitable Extinguishing Media: A solid water stream may be inefficient.

5.2 Flammable Properties and Hazards: No data available.

No data available.

Flash Pt: No data.

Explosive Limits: LEL: No data. UEL: No data.

Autoignition Pt: No data.

5.3 Fire Fighting Instructions: As in any fire, wear self-contained breathing apparatus pressure-demand (NIOSH approved or equivalent), and full protective gear to prevent contact with skin and eyes.

Section 6. Accidental Release Measures

6.1 Protective Precautions, Protective Equipment and Emergency Procedures: Avoid breathing vapors and provide adequate ventilation.
As conditions warrant, wear a NIOSH approved self-contained breathing apparatus, or respirator, and appropriate personal protection (rubber boots, safety goggles, and heavy rubber gloves).

6.2 Environmental Precautions: Take steps to avoid release into the environment, if safe to do so.

6.3 Methods and Material For Containment and Cleaning Up: Contain spill and collect, as appropriate.
Transfer to a chemical waste container for disposal in accordance with local regulations.

Section 7. Handling and Storage

7.1 Precautions To Be Taken in Handling: Avoid breathing dust/fume/gas/mist/vapours/spray.
Avoid prolonged or repeated exposure.

7.2 Precautions To Be Taken in Storing: Keep container tightly closed.
Store in accordance with information listed on the product insert.

Section 8. Exposure Controls/Personal Protection

8.1 Exposure Parameters:

8.2 Exposure Controls:

8.2.1 Engineering Controls (Ventilation etc.): Use process enclosures, local exhaust ventilation, or other engineering controls to control airborne levels below recommended exposure limits.

8.2.2 Personal protection equipment:

Eye Protection: Safety glasses

Protective Gloves: Compatible chemical-resistant gloves

Other Protective Clothing: Lab coat

Respiratory Equipment (Specify Type): NIOSH approved respirator, as conditions warrant.

Work/Hygienic/Maintenance Practices: Do not take internally.
Facilities storing or utilizing this material should be equipped with an eyewash and a safety shower.
Wash thoroughly after handling.

No data available.

Section 9. Physical and Chemical Properties

9.1 Information on Basic Physical and Chemical Properties

Physical States:	[ ] Gas [X] Liquid [ ] Solid
Appearance and Odor:	100 mM Tris-Cl and 10 mM EDTA (pH 7.4)
pH:	7.4
Melting Point:	No data.
Boiling Point:	No data.
Flash Pt:	No data.
Evaporation Rate:	No data.
Flammability (solid, gas):	No data available.
Explosive Limits:	LEL: No data. UEL: No data.
Vapor Pressure (vs. Air or mm Hg):	No data.
Vapor Density (vs. Air = 1):	No data.
Specific Gravity (Water = 1):	No data.
Solubility in Water:	No data.
Octanol/Water Partition Coefficient:	No data.

Autoignition Pt: No data.

Decomposition Temperature: No data.

Viscosity: No data.

9.2 Other Information

Percent Volatile: No data.

Section 10. Stability and Reactivity

10.1 Reactivity: No data available.

10.2 Stability: Unstable [ ] Stable [X]

10.3 Stability Note(s): Stable if stored in accordance with information listed on the product insert.

Polymerization: Will occur [ ] Will not occur [X]

10.4 Conditions To Avoid: No data available.

10.5 Incompatibility - Materials To Avoid: strong oxidizing agents

10.6 Hazardous Decomposition or Byproducts: carbon dioxide, carbon monoxide, nitrogen oxides

Section 11. Toxicological Information

11.1 Information on Toxicological Effects: The toxicological effects of this product have not been thoroughly studied.
Trizma base - Toxicity Data: Oral LDLO (rabbit): 1,000 mg/kg; Oral TDLO (mouse): 3,000 mg/kg; Oral LD50 (rat) >3,000 mg/kg; Oral LD50 (mouse): 5,500 mg/kg; Oral TDLO (rat): 3,000 mg/kg;

Chronic Toxicological Effects: Trizma base - Investigated as a reproductive effector.
Only select Registry of Toxic Effects of Chemical Substances (RTECS) data is presented here. See actual entry in RTECS for complete information.
Trizma base RTECS Number: TY2900000

Carcinogenicity: NTP? No IARC Monographs? No OSHA Regulated? No

CAS #	Hazardous Components (Chemical Name)	NTP	IARC	ACGIH	OSHA
77-86-1	Trizma base	n.a.	n.a.	n.a.	n.a.
60-00-4	Ethylenediamine Tetraacetic Acid	n.a.	n.a.	n.a.	n.a.
7732-18-5	Water	n.a.	n.a.	n.a.	n.a.

Section 12. Ecological Information

12.1 Toxicity: Avoid release into the environment.
Runoff from fire control or dilution water may cause pollution.

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: No data available.

12.6 Other adverse effects: No data available.

SAFETY DATA SHEET
Tris-EDTA Stock Solution (10X, pH 7.4)

Revision: 12/24/2016
Supersedes Revision: 06/24/2013

Section 13. Disposal Considerations

13.1 Waste Disposal Method: Dispose in accordance with local, state, and federal regulations.

Section 14. Transport Information

14.1 LAND TRANSPORT (US DOT):

DOT Proper Shipping Name: Not dangerous goods.

DOT Hazard Class:

UN/NA Number:

14.1 LAND TRANSPORT (European ADR/RID):

ADR/RID Shipping Name: Not dangerous goods.

UN Number:

Hazard Class:

14.3 AIR TRANSPORT (ICAO/IATA):

ICAO/IATA Shipping Name: Not dangerous goods.

Additional Transport Information: Transport in accordance with local, state, and federal regulations.

Section 15. Regulatory Information

EPA SARA (Superfund Amendments and Reauthorization Act of 1986) Lists

CAS #	Hazardous Components (Chemical Name)	S. 302 (EHS)	S. 304 RQ	S. 313 (TRI)
77-86-1	Trizma base	No	No	Yes-Cat. N106
60-00-4	Ethylenediamine Tetraacetic Acid	No	Yes 5000 LB	No
7732-18-5	Water	No	No	No

CAS #	Hazardous Components (Chemical Name)	Other US EPA or State Lists
77-86-1	Trizma base	CAA HAP,ODC: HAP; CWA NPDES: No; TSCA: Yes - Inventory; CA PROP.65: No
60-00-4	Ethylenediamine Tetraacetic Acid	CAA HAP,ODC: No; CWA NPDES: No; TSCA: Yes - Inventory; CA PROP.65: No
7732-18-5	Water	CAA HAP,ODC: No; CWA NPDES: No; TSCA: Yes - Inventory; CA PROP.65: No

Regulatory Information Statement: This SDS was prepared in accordance with 29 CFR 1910.1200 and Regulation (EC) No.1272/2008.

Section 16. Other Information

Revision Date: 12/24/2016

Additional Information About This Product: No data available.

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