An investigation into process limitations in membrane bioreactor (MBR) systems used for lactic acid production

by

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Declaration

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Abstract

Lactic acid is conventionally produced through batch fermentation which suffers a major challenge of low lactic acid productivity due to end-product inhibition and low cell density in the fermenter. To overcome this challenge, several investigations have been focused on continuous lactic acid fermentation using membrane bioreactor (MBR) systems which have proved to be very promising in improving lactic acid productivity by alleviating the end-product inhibitory effects and increasing cell density in the fermenter. However, none of these MBR systems have been upscaled for industrial applications probably due to some process limitations associated with them.

Therefore, the present study postulated and investigated some of these possible process limitations such as membrane fouling limitations, lactate inhibition to bacterial cells once the membrane has fouled limitations, nutrient limitations, and mass transfer limitations. A laboratory scale MBR system that consisted of an ultrafiltration (UF) membrane externally connected to a fermenter was used for continuous lactic acid fermentations at glucose concentrations of 60 g/L, 90 g/L and 120 g/L. The MBR system was operated at sub-critical flux conditions.

Significant membrane fouling indicated by the permeate flux decline throughout the fermentation period was realized at all the glucose concentrations investigated, and it was most severe at glucose concentration of 120 g/L due to high biomass concentrations that blocked the membrane pores. Furthermore, the total volume of permeate at the end of the continuous fermentation runs at each glucose concentration investigated decreased in the order of 60 g/L > 90 g/L > 120 g/L, which confirmed the severe membrane fouling at higher glucose concentrations.

For the investigation of lactate inhibition to bacterial cells once the membrane has fouled limitations, it was observed that lactate productivity decreased at all the glucose concentrations investigated. Similarly, there was noticeable decrease in biomass concentrations at glucose concentration of 120 g/L compared to 90 g/L and 60 g/L. Hence, this process limitation was found to have a significant impact on these MBR systems. The investigation on nutrient limitations was inconclusive since all the glucose concentrations investigated turned out to be above the threshold substrate concentration. On the other hand, mass transfer limitations were not found in these MBR systems.

Since there was significant membrane fouling contrary to minimal fouling that was expected when the MBR system was operated at sub-critical flux conditions, further set of critical flux experiments using lactate fermentation broths were conducted whereby it was found out that the significant membrane fouling was probably due to the low cross-flow velocity that was used to avoid the possibility of bacterial cell damage. These experiments also established that operating below the critical flux conditions can significantly lower membrane fouling in MBR systems used for lactic acid fermentation, but this is only possible at high cross-flow velocities.

The present study, therefore, identified membrane fouling and lactate inhibition to bacterial cells once the membrane has fouled as the main process limitations that have a significant impact on the MBR systems used for lactic acid fermentation. To improve these systems for industrial upscaling, further studies on suitable methods to minimize membrane fouling are necessary.

Opsomming

Melksuur word konvensioneel geproduseer deur lotfermentasie wat die groot uitdaging duld van lae melksuurproduktiwiteit as gevolg van eindprodukinhibisie en lae seldigtheid in die fermenteerder. Om hierdie uitdaging te oorkom, het verskeie ondersoeke op kontinue melksuurfermentasie gefokus deur die gebruik van membraan bioreaktor (MBR) sisteme wat bewys is om ’n belowende verbetering van melksuurproduktiwiteit te hê deur die eindproduk se inhiberende effekte te verlig en seldigtheid in die fermenteerder te verhoog. Geen van hierdie MBR-sisteme is egter opgeskaal vir industriële toepassing nie, tien teen een as gevolg van sommige prosesbeperkinge geassosieer daarmee.

Daarom het die huidige studie sommige van hierdie moontlike prosesbeperkinge soos membraanaanpakkingbeperkinge, laktaatinhibisie op bakteriële selle nadat die membraan aangepak het, nutriëntbeperkinge, en massa-oordragbeperkinge, gepostuleer en ondersoek. ’n Laboratoriumskaal MBR-sisteem wat bestaan uit ’n ultrafiltrasie (UF) membraan wat ekstern aan ’n fermenteerder gekonnekteer is, is gebruik vir kontinue melksuurfermentasies by glukosekonsentrasies van 60 g/L, 90 g/L en 120 g/L. Die MBR-sisteem is bedryf by subkritiese fluks kondisies.

Beduidende membraanaanpakking aangedui deur die deurlaat fluks afname deur die fermentasieperiode is gerealiseer by al die glukosekonsentrasies ondersoek, en dit was ergste by glukosekonsentrasie van 120 g/L as gevolg van hoë biomassakonsentrasies wat die membraanporieë geblok het. Verder, die totale volume deurlaat aan die einde van die kontinue fermentasielopies by elke glukosekonsentrasie ondersoek, het afgeneem in die orde van 60 g/L > 90 g/L >120 g/L, wat die ergste membraanaanpakking by hoër glukosekonsentrasies bevestig.

Vir die ondersoek van laktaatinhibisie op bakteriële selle nadat die membraan aangepak het, is dit waargeneem dat laktaatproduktiwiteit afgeneem het by al die glukosekonsentrasies ondersoek. Soortgelyk was daar opmerklike afname in biomassakonsentrasies by glukosekonsentrasie van 120 g/L in vergelyking met 90 g/L en 60 g/L. Daarom is hierdie prosesbeperking gevind om ’n beduidende impak op hierdie MBR-sisteme te hê. Die ondersoek op nutriëntbeperkinge was onbeslis aangesien al die glukosekonsentrasies ondersoek bo die drempel substraatkonsentrasie was. Aan die anderkant was massa-oordragbeperkinge nie gevind in hierdie MBR-sisteme nie.

Aangesien daar beduidende membraanaanpakking was, anders as wat verwag is toe die MBR-sisteem bedryf is by subkritiese fluks kondisies, is ’n verdere stel kritiese fluks eksperimente uitgevoer deur laktaatfermentasiesop te gebruik waar dit bevind is dat die beduidende membraanaanpakking moontlik as gevolg van die lae kruisvloeisnelheid is wat gebruik word om die moontlikheid van bakteriële selbeskadiging te vermy. Hierdie eksperimente het ook vasgestel dat bedryf onder die kritiese fluks kondisies membraanaanpakking in MBR-sisteme wat gebruik word vir melksuurfermentasie, beduidend kan verlaag, maar dis slegs moontlik by hoë kruisvloeisnelhede.

Die huidige studie, daarom, het membraanaanpakking en laktaatinhibisie op bakteriële selle nadat die membraan aangepak is, geïdentifiseer as die hoof prosesbeperkinge wat ’n beduidende impak het op die MBR-sisteme wat gebruik word vir melksuurfermentasie. Om hierdie sisteme te verbeter vir opskaal vir die industrie, is verdere studies op gepaste metodes om die membraanaanpakking te minimeer, noodsaaklik.

Dedication

I dedicate this thesis to my parents: Peter Obondo and Rose Obondo.

Your prayers and encouragement have always made me believe that nothing is impossible with determination and focus.

You have always motivated me to close my eyes to the negativities surrounding me and see the positive side even in adverse situations. This helped me to overcome the challenges that I faced during my studies.

I love you so much. May God bless you with long life to enjoy the fruits of my hard work.

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Nomenclature

Symbols

A	membrane surface area	(m^2)
J	permeate flux	($L/m^2.h$ / LMH)
Q_p	permeate volumetric flowrate	(L/h)

Acronyms

ADP	Adenosine diphosphate
ATCC	American Type Culture Collection
ATP	Adenosine triphosphate
CDW	Cell dry weight
CEB	Chemical enhanced backwash
CIP	Clean in place
COP	Clean -out- of- place
CT	Capillary tube
CW	Chemical wash
Da	Dalton
DNA	Deoxyribonucleic acid
DOTM	Direct observation through the membrane
EMP	Embden – Meyerof – Parnas

EPS	Extracellular polymeric substance
FT	Flat sheet
HCN	Hydrogen cyanide
HF	Hollow fibre
HPLC	High performance liquid chromatography
kDa	Kilodalton
kPa	Kilopascal
LA	Lactic acid
LAB	Lactic acid bacteria
LMH	Litres per m^2 per hour [$L/(m^2. h)$]
MBR	Membrane bioreactor
MF	Microfiltration
MRS	De Man, Rogosa and Sharpe
MWCO	Molecular weight cut off
NAD^+	Nicotinamide adenine dinucleotide
NADH	Nicotinamide adenine dinucleotide (reduced form)
NF	Nanofiltration
NOM	Natural organic matter
$OD_{620\ nm}$	Optical density at 620 nm
PE	Polyethylene

PES	Polyethersulfone
PK	Phosphoketolase
PLA	Polylactic acid
PS	Polysulfone
PTFE	Polytetrafluoroethylene
PVDF	Polyvinylidene fluoride
PVP	Polyvinyl pyridine
PWF	Pure water flux
RO	Reverse osmosis
rpm	revolutions per minute
TMP	Transmembrane pressure
UF	Ultrafiltration
UPS	Uninterruptible power supply

Chapter 1 Introduction

1.1 General background and problem statement

Lactic acid [2-hydroxypropanoic acid, $CH_3CH(OH)COOH$] is a valuable chemical that has various applications in the food, cosmetic, textile, chemical and pharmaceutical industries (Cui et al., 2011; Tian et al., 2016). The global annual demand for lactic acid was estimated to be 1220.0 kilotonnes in 2016 (Singhvi et al., 2018). Nevertheless, this demand is expected to increase to 1960.1 kilotonnes by 2025 with an estimated annual increase of 16.2 % in the global demand for lactic acid (Alves de Oliveira et al., 2018). This significant increase in the demand for lactic acid is mainly attributed to its application in manufacturing biocompatible and biodegradable polylactic acid (PLA) polymers (Abdel-Rahman et al., 2013; John et al., 2007). These polymers are suitable for producing environmentally friendly biodegradable plastics instead of the currently used synthetic plastics derived from petroleum resources (Garde et al., 2002; Wang et al., 2015).

Lactic acid can be produced commercially by either chemical synthesis or microbial fermentation (Guo et al., 2010; Wee et al., 2006). Chemical synthesis involves a series of steps. First, lactonitrile is produced by adding hydrogen cyanide to acetaldehyde in the presence of a base catalyst under high pressure. This crude lactonitrile is then purified by distillation and subsequently hydrolyzed to lactic acid by either hydrochloric acid or sulphuric acid (John et al., 2009; Pal et al., 2009). On the other hand, microbial fermentation is characterized by the biological degradation of a substrate (e.g., glucose) by a population of microorganisms (biomass) into lactic acid (Komesu et al., 2017).

Chemical synthesis has not been widely used because it produces a racemic mixture of DL- lactic acid which yields a less crystalline PLA with a lower melting point (John et al., 2009; Sikder et al., 2012). Furthermore, the chemicals used in chemical synthesis as raw materials are expensive thus making the method not economically viable (Pal et al., 2009). Therefore, approximately 90 percent of lactic acid is produced by microbial fermentation globally (Boontawan et al., 2011; Wang et al., 2015). Microbial fermentation is preferred because it can produce either optically pure L (+) or D (–)-lactic acid based on the selected lactic acid-producing microorganism (Kadam et al., 2006; Moon et al.,

2012). In addition, inexpensive substrates such as lignocellulosic biomass and starchy materials can be used for microbial fermentation (Abdel-Rahman et al., 2013; Huang et al., 2005).

Conventionally, lactic acid is produced through a batch fermentation mode (Ding et al., 2006; Ghaffar et al., 2014; Xu et al., 2006). In a batch fermentation mode, lactic acid-producing microorganisms (e.g., bacteria) are inoculated to a given fermentation medium volume in a fermenter. As the microorganisms grow, they gradually consume the nutrients and subsequently lead to the accumulation of lactic acid (Ghaffar et al., 2014; Hofvendahl et al., 2000). This lactic acid produced (i.e., end-product) has inhibitory effects on the growth of bacteria (Okano et al., 2010; Wee et al., 2006). The undissociated lactic acid can pass through the bacterial membrane and lead to increased intracellular accumulation of lactic acid, which decreases the intracellular pH and disrupts the cell membrane (Abdel-Rahman et al., 2016; Aljundi et al., 2005). Consequently, much energy is used by the cells to maintain the internal pH constant, leading to inhibition of the bacterial cell growth resulting in low lactic acid productivity (Jantasee et al., 2017; Xu et al., 2006).

To alleviate this inhibitory effect, lactic acid produced is precipitated using neutralizing agents such as calcium hydroxide or calcium carbonate and subsequently recovered in the downstream processing where the fermentation broth is treated with strong sulphuric acid that liberates lactic acid and simultaneously generates calcium sulphate (gypsum), a solid waste (Krzyzaniak et al., 2013; Singhvi et al., 2018). However, this precipitation method is faced with some drawbacks. The huge quantities of calcium sulphate (gypsum) wastes pose disposal challenges (Alves de Oliveira et al., 2018; Milcent et al., 2001). Also, the optical purity of lactic acid can suffer during the purification process because isomerization of lactic acid may occur, which affects the purity requirements for food and pharmaceutical industries (Jantasee et al., 2017). Furthermore, the precipitation method is not suitable from an economic perspective since the high cost of chemicals and many steps involved in purifying lactic acid from the fermentation broth account for up to 50 % of the total lactic acid production costs (Dey et al., 2012b; Lee et al., 2017).

Owing to the abovementioned drawbacks of the conventional precipitation method, other methods such as reactive extraction and adsorption have been investigated to circumvent this inhibitory effect (Abdel-Rahman et al., 2016; Hǒvá et al., 2004). Reactive extraction involves the use of amine extractant which recovers lactic acid from the fermentation broth by reacting with it to form an acid-

amine complex that is solubilized into the extractant phase. Thereafter, regeneration through back extraction recovers the acid into a product (lactic acid) phase and acid-free extractant that can be recycled (Joglekar et al., 2006; Wasewar, 2005). On the other hand, adsorption involves the sorption of lactic acid on solid adsorbents or ion exchange resins for *in situ* removal of lactic acid from the fermentation broth (Aljundi et al., 2005; Boonmee et al., 2016).

Nevertheless, these methods have some significant drawbacks that limit their industrial applications. For instance, *in situ* removal of lactic acid during fermentation using reactive extraction is limited by the toxicity of most extractants to microorganisms that hinder the completion of the fermentation process (Boontawan et al., 2011; Wasewar, 2005). Similarly, the adsorption method requires regeneration of an ion exchange resin and adjustment of the feed pH to increase the sorption efficiency; hence, large amounts of chemicals are required (Boontawan et al., 2011; Joglekar et al., 2006).

Recently, several investigations have focused on continuous lactic acid fermentation using membrane bioreactor (MBR) systems (Fan et al., 2017a; Giorno et al., 2002; Lu et al., 2012; Xu et al., 2006). A membrane bioreactor (MBR) system refers to a system whereby a membrane is submerged in a fermenter or externally connected to a fermenter to achieve either *in situ* lactic acid recovery or continuous removal of lactic acid produced thus alleviating lactic acid inhibitory effects to the bacterial cell growth (Fan et al., 2017a; Tejayadi et al., 1995). Since the membranes are used *in situ* or externally connected to the fermenter, these MBR systems can also retain or recycle bacterial cells back to the fermenter and subsequently improve lactic acid productivity (Choudhury et al., 2006; Lu et al., 2012). From these investigations, both on laboratory and pilot scales, the performance of the MBR systems have proved to be very promising in alleviating the inhibitory effects and improving lactic acid productivity. However, none of these systems has been upscaled for industrial applications. Seemingly, there are some process limitations associated with them that need to be identified and solved.

Therefore, the present study postulated some possible process limitations associated with the membrane bioreactor (MBR) systems used for lactic acid fermentation. These include:

(i) membrane fouling that results due to improper membrane operating conditions

Although membrane fouling cannot be avoided in membrane bioreactor (MBR) systems, its severity can be minimized through proper membrane operating conditions (Mohammad et al., 2012; Zhang et al., 2015). Membrane fouling has been reported in MBR systems used for lactic acid fermentation (Giorno et al., 2002; Zhang et al., 1994), however, it was postulated that this had been the case probably due to improper membrane operating conditions applied in running such MBR systems.

(ii) possibility of lactate inhibition for bacterial cells once the membrane has fouled

During the fermentation process, the lactic acid produced is neutralized with bases such as sodium hydroxide to the less inhibitory lactate form (Abdel-Rahman et al., 2013). However, high concentrations of the accumulated lactate in the fermenter inhibit bacterial cell growth (Hetényi et al., 2011; Rault et al., 2009). Therefore, one main reason for integrating the membrane to the fermenter is to continuously remove the lactate from the fermenter to avoid such inhibitory effects (Tejayadi et al., 1995). Nonetheless, it was postulated that once the membrane has fouled and the removal of lactate has reduced, the accumulated lactate can inhibit bacterial cell growth and lower lactic acid productivity.

(iii) nutrient limitations

In lactic acid fermentation, nutrients are provided in the form of carbon sources, nitrogen sources and mineral salts (Castillo Martinez et al., 2013; Oh et al., 2003). As the fermentation progresses, the nutrients provided are consumed by bacteria to enhance their growth and produce lactic acid (Hofvendahl et al., 2000; Monteagudo et al., 1997). However, when one or more nutrients become limiting, there will be a sharp drop in the specific growth rate of the bacteria (Leroy et al., 2001). Therefore, it was postulated that due to the increase in cell density in the fermenter as a result of cell recycling in membrane bioreactor systems, there would be rapid consumption of the available nutrients and probably result in nutrient limitations.

(iv) mass transfer limitations

Cell recycling in membrane bioreactor systems results in high cell density in the fermenter (Lu et al., 2012). Therefore, it was postulated that the increased cell concentrations in the fermenter could

result in mass transfer limitations and subsequently affect substrate utilization leading to an unutilized substrate in the fermenter.

Hence, the present study focused on identifying some of these postulated process limitations with the intention of providing possible solutions from an engineering perspective.

1.2 Objectives of the present study

The main aim of the present study was to improve the performance of membrane bioreactor (MBR) systems used for lactic acid fermentation by identifying their process limitations. To address this aim, the following five specific objectives were formulated:

(i) To investigate whether membrane fouling limitations dominate the MBR systems used for lactic acid fermentation.

(ii) To investigate whether lactate inhibition for bacterial cells once the membrane has fouled limits the MBR systems used for lactic acid fermentation.

(iii) To investigate whether nutrient limitations dominate the MBR systems used for lactic acid fermentation.

(iv) To investigate whether mass transfer limitations dominate the MBR systems used for lactic acid fermentation.

(v) To establish whether operating MBR systems, used for lactic acid fermentation, below the critical flux conditions can significantly lower membrane fouling in those systems.

1.3 Approach

The approach adopted in the present study involved conducting investigations that served as preliminary experiments. Afterwards, the main investigations that addressed the objectives of the present study were conducted.

First, batch fermentation runs were conducted to understand glucose consumption trends and bacterial cell growth trends during lactic acid fermentation. These investigations of batch fermentation served as preliminary experiments before starting the continuous fermentation runs.

After that, a laboratory-scale membrane bioreactor (MBR) system was built. The MBR system consisted of an ultrafiltration (UF) membrane externally connected to a fermenter.

To determine the operating conditions for the MBR system, critical flux experiments using bacterial cells were conducted. This provided the transmembrane pressure and the cross-flow velocity used in running the MBR system. Continuous fermentation runs using the MBR system were then carried out to address the objectives of the present study.

After the continuous fermentation runs, it turned out that an additional set of critical flux experiments was necessary. These experiments were to establish whether operating MBR systems used for lactic acid fermentation below the critical flux conditions can significantly lower membrane fouling in such systems. Therefore, these critical flux experiments were carried out using lactate fermentation broths of different biomass concentrations.

1.4 Thesis outline

This thesis consists of five chapters. Chapter 1 presents the general background and problem statement of the present study, the objectives that this study aimed to address and the approach that was taken to address these objectives.

Chapter 2 provides a broad literature review on lactic acid and its production methods, basic concepts of the membrane separation technology and insights into membrane bioreactor systems used for lactic acid fermentation.

Chapter 3 outlines the experimental apparatus and protocols used to obtain the data to address the objectives of the present study. The analytical methods and calculations adopted during the present study are also outlined in this chapter.

In Chapter 4, the focus is on discussing the results obtained in the present study. The results obtained from the batch fermentation runs, continuous fermentation runs using the membrane bioreactor system, and the critical flux experiments are discussed.

Chapter 5 summarizes the conclusions drawn from the investigations carried out in line with meeting the objectives of the present study. It also provides some recommendations for future studies.

Chapter 2 Literature review

2.1 Introduction

This chapter starts by discussing lactic acid and its production methods. After that, the focus shifts to membrane separation technology where the basic concepts of membrane separation processes and membrane bioreactors are discussed. Finally, the application of membrane bioreactor systems for lactic acid fermentation and the research gaps in the literature that the present study aimed to investigate are discussed before concluding the whole chapter.

2.2 Lactic acid

2.2.1 Historical background

Lactic acid (LA) was first discovered in sour milk by Swedish Chemist Carl Wilhelm Scheele in 1780. In 1789, Antoine Lavoisier gave this milk component the name ‘acide lactique’, which possibly generated the current name ‘lactic acid’ (Wee et al., 2006). Later in 1857, Louis Pasteur discovered that lactic acid was not a component of milk but a metabolite that some microorganisms produced by fermentation. In 1881, a French scientist Edmond Frémy used fermentation to produce lactic acid resulting in its industrial production (Ghaffar et al., 2014).

2.2.2 Physical and chemical properties

Lactic acid [2-hydroxypropanoic acid, $CH_3CH(OH)COOH$] is considered the simplest hydroxycarboxylic acid with an asymmetric carbon atom. It exists in two optical isomers i.e., L (+) and D (–)-lactic acids as shown in Figure 2.1 (Castillo Martinez et al., 2013).

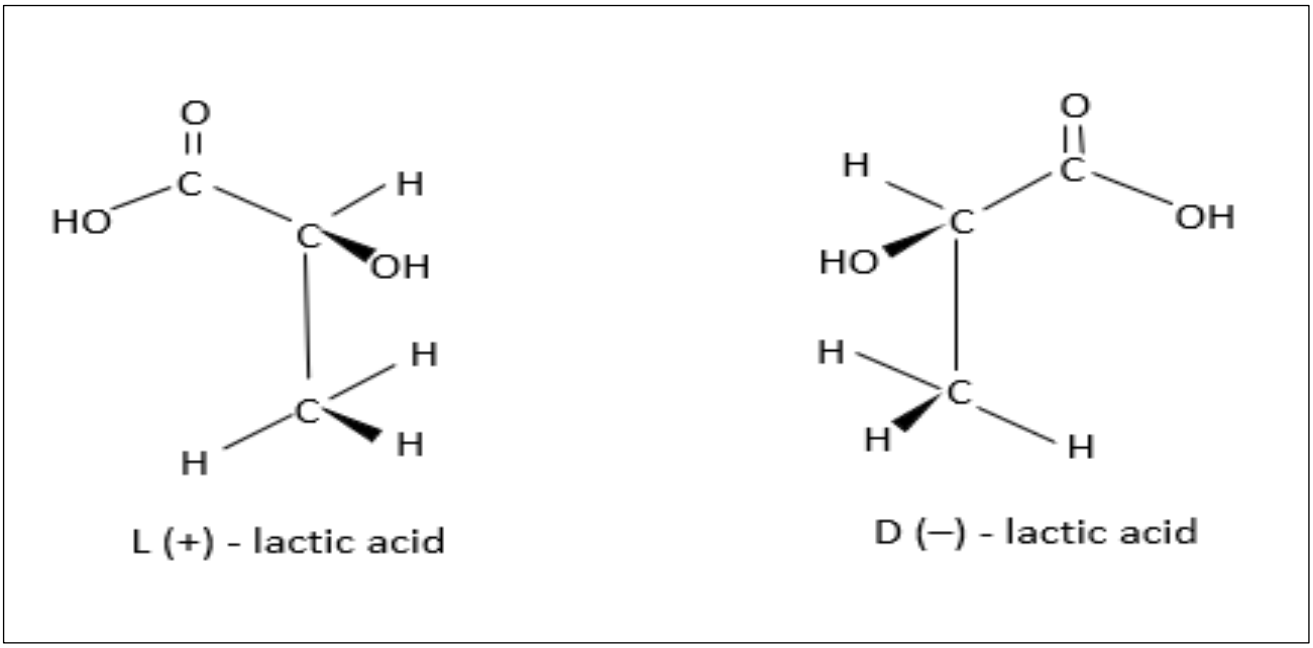


Figure 2.1: Structure of L (+) and D (–) - lactic acid isomers, redrawn from Castillo Martinez et al., 2013.

Lactic acid has carboxyl and hydroxyl reactive functional groups hence can participate in various chemical reactions (Castillo Martinez et al., 2013). It can readily form a linear dimer lactoyl lactate and higher linear polymers in aqueous solutions in which the hydroxyl group of one molecule is esterified with the carboxyl group of another molecule (Litchfield, 1996). The asymmetric carbon that confers optical activity in lactic acid also determines its chemical behaviour, especially in reactions where other compounds also have optical activity (Komesu et al., 2017). The physical properties of lactic acid are shown in Table 2.1.

Table 2.1: Physical properties of lactic acid (Narayanan et al., 2004)

Property	Value
Molecular weight	90.08 g/mol
Melting point	16.8 °C
Boiling point	82°C at 0.5 mmHg 122°C at 14 mmHg
Dissociation constant K_a, at 25°C	1.37×10^{-4}
Heat of combustion, ΔH_c	1361 kJ/mol
Specific heat, C_p at 20°C	190 J/mol/°C

2.2.3 Market demand and applications

The worldwide annual demand for lactic acid was estimated to be 1220.0 kilotonnes in 2016 (Singhvi et al., 2018). However, this demand is expected to increase to 1960.1 kilotonnes by 2025 with an estimated annual increase of 16.2 % in the global demand for lactic acid (Alves de Oliveira et al., 2018). The significant increase in the demand for lactic acid is majorly due to its application in the manufacture of polylactic acid (PLA) polymer, a biodegradable and biocompatible polymer with various applications (Abdel-Rahman et al., 2013). Furthermore, lactic acid has many uses in the food, pharmaceutical, cosmetic, textile and chemical industries, as discussed in this section. The food and pharmaceutical industries only use L (+)-lactic acid isomer because it is the one that the human body can metabolize (Gezae Daful et al., 2017). On the other hand, one of the optically pure isomers of lactic acid or a racemic mixture can be used in other industries depending on the nature of the application (Castillo Martinez et al., 2013; Vijayakumar et al., 2008).

In the food industries, lactic acid is used as an acidulant in pickled vegetables, baked foods, salads and beverages due to its mild acidic taste (Wee et al., 2006). Moreover, it is used as a pH regulator, flavouring agent and inhibitor of residual bacteria in food processing for soft drinks, beer, dairy products, bread, jams and jellies among others (Datta et al., 2006). In the pharmaceutical industry, lactic acid is used as an electrolyte in various parenteral/intravenous solutions intended to replenish bodily fluids. They include Lactated Ringer's solutions and dialysis solutions for conventional artificial kidney machines (Vijayakumar et al., 2008). In addition, lactic acid is used in mineral preparations such as prostheses, tablets, controlled drug delivery systems and surgical sutures (Wee et al., 2006).

Due to its rejuvenating, antimicrobial and moisturizing effects on the skin, lactic acid is used in the cosmetic industry to manufacture hygiene and aesthetic products (Castillo Martinez et al., 2013). Lactic acid is used as a fixative for dyeing in the textile industry. It also results in lower cost and higher efficiency when used instead of ethylene glycol in antifreeze (Komesu et al., 2017).

The two reactive functional groups; a carboxylic group and a hydroxyl group in lactic acid, can allow it to undergo several chemical conversions into useful products (Fan et al., 2009). Therefore, lactic acid is a raw material for the production of acrylic acid through dehydration; 2,3-pentanedione through condensation; propanoic acid through reduction; acetaldehyde through decarboxylation,

and propylene oxide through hydrogenation in the chemical industries (Fan et al., 2009; Gao et al., 2011). Moreover, polylactic acid (PLA) polymer is manufactured from a lactic acid monomer. Due to the biocompatibility and biodegradability of PLA polymers, they are used to manufacture food packagings, rigid containers, extruded films for wrappings, trash bags, protective clothing, and thermo-formed plastics like eating utensils (Vaidya et al., 2005; Vijayakumar et al., 2008). The major lactic acid applications are summarized in Table 2.2.

Table 2.2: Summary of the major lactic acid applications (Wee et al., 2006)

Industry	Lactic acid applications
Food	Acidulants Preservatives Flavours pH regulators
Pharmaceutical	Parenteral/intravenous solution Dialysis solution Tabletting Prostheses Surgical sutures Controlled drug delivery systems
Cosmetic	Moisturizers Skin-lightening agents Skin-rejuvenating agents pH regulators Anti-acne agents Humectants Anti-tartar agents
Chemical	Production of propylene oxide Production of acetaldehyde Production of acrylic acid Production of propanoic acid Production of 2,3-pentanedione Production of ethyl lactate Production of polylactic acid (PLA)

2.2.4 Commercial manufacturers

The major commercial manufacturers of lactic acid include NatureWorks LLC - Cargill (USA), Corbion-Purac (Netherlands), Archer Daniels Midland (ADM) Company (USA), Galactic (Belgium) and other companies such as Teijin, Cellulac, Uhde Inventa-Fischer, Musashino Chemical, Jiuding Biological Engineering, Henan Jindan Lactic Acid Technology, Danimer Scientific, Direvo Industrial Biotechnology, Futerro, Tongjieliang, Hisun Biomaterials, Pyramid Bioplastics, Piaoan, Dow, Myriant, Shenzhen BrightChina Industrial, ThyssenKrupp Industrial Solutions, Synbra Technology, Yangtzelabre, and Zhejiang Hisun Chemical among others (Alves de Oliveira et al., 2018; Castillo Martinez et al., 2013; John et al., 2009). NatureWorks LLC is also the leading producer of polylactic acid (PLA) polymers globally (Abdel-Rahman et al., 2013).

2.3 Lactic acid production methods

Lactic acid can be produced commercially by either chemical synthesis or microbial fermentation (Guo et al., 2010). However, the microbial fermentation process is preferred hence being used to produce approximately 90 percent of lactic acid worldwide (Joglekar et al., 2006; Wang et al., 2015). This section explains these two processes in detail, giving insights into their advantages and disadvantages.

2.3.1 Chemical synthesis

This lactic acid production process is based on the hydrolysis of lactonitrile, a derivative of petrochemicals, by strong acids leading to the formation of a racemic mixture DL-lactic acid (John et al., 2009). The lactonitrile is produced when hydrogen cyanide (HCN) is added to liquid acetaldehyde in the presence of a base catalyst under high atmospheric pressures. Lactonitrile is then recovered, purified by distillation, and hydrolyzed using strong sulphuric acid or hydrochloric acid to obtain lactic acid and ammonium salt. Lactic acid produced is esterified with methanol forming methyl lactate which is recovered, purified by distillation, and hydrolyzed with acidified water to produce methanol and lactic acid. The distillation method is then used to separate methanol which is recycled back into the process (Ghaffar et al., 2014; Komesu et al., 2017; Pal et al., 2009). The reactions involved in the chemical synthesis process can be illustrated by the equations 2.1 – 2.4 (Ghaffar et al., 2014):

a. Addition of hydrogen cyanide (HCN) to acetaldehyde

$$CH_3CHO + HCN \rightarrow CH_3CHOHCN \quad (2.1)$$

Acetaldehyde Hydrogen cyanide Lactonitrile

b. Hydrolysis by sulphuric acid

$$CH_3CHOHCN + H_2O + \frac{1}{2}H_2SO_4 \rightarrow CH_3CHOHCOOH + \frac{1}{2}(NH_4)2SO_4 \quad (2.2)$$

Lactonitrile Sulphuric acid Lactic acid Ammonium sulphate

c. Esterification

$$CH_3CHOHCOOH + CH_3OH \rightarrow CH_3CHOHCOOCH_3 + H_2O \quad (2.3)$$

Lactic acid Methanol Methyl lactate

d. Hydrolysis by water

$$CH_3CHOHCOOCH_3 + H_2O \rightarrow CH_3CHOHCOOH + CH_3OH \quad (2.4)$$

Methyl lactate Lactic acid Methanol

There are other possible routes that can be used to produce lactic acid by chemical synthesis such as oxidation of propylene glycol, nitric acid oxidation of propylene, hydrolysis of chloropropionic acid, and base-catalyzed degradation of sugars. Nevertheless, none of these chemical process routes is technically and economically feasible due to high costs, energy, and material (Gao et al., 2011; Pal et al., 2013). The chemical synthesis process has some drawbacks such as producing a racemic mixture DL- lactic acid even though most industrial applications only need pure isomers of lactic acid (Pal et al., 2009). The dependence on raw materials from other industries also makes the process unreliable. Moreover, the chemicals used as raw materials are expensive thus making the process not economically viable (Komesu et al., 2017; Pal et al., 2009).

In 1963, Monsanto in the United States of America (USA) was the first company to use chemical synthesis to produce 4,500 tonnes of lactic acid, which was 40 % of the total lactic acid consumed in the USA at that time (Komesu et al., 2017). Other companies that used chemical synthesis include

Sterling Chemicals and Musashino Chemicals. However, these companies have changed their production processes to microbial fermentation (Komesu et al., 2017).

2.3.2 Microbial fermentation

Microbial fermentation has gained much attention over chemical synthesis because it can produce an optically pure isomer of either L (+) or D (–)-lactic acid when an appropriate microorganism is selected (Hofvendahl et al., 2000; Kadam et al., 2006). It is worth mentioning that the optical purity of lactic acid directly contributes to the physical properties of polylactic acid (PLA) polymer because an optically pure L (+) or D (–)-lactic acid produces high crystalline PLA suitable for commercial applications (Dumbrepatil et al., 2008; Ghaffar et al., 2014). Some other advantages of microbial fermentation include the possibility of using renewable and inexpensive fermentation substrates such as lignocellulosic biomass and some agro-industrial byproducts which makes the process cost-effective and eco-friendly (Huang et al., 2005). Moreover, microbial fermentation requires low production temperatures and low energy consumption (Abdel-Rahman et al., 2011; Okano et al., 2010). The present study was based on lactic acid production through microbial fermentation.

2.4 The lactic acid fermentation process

2.4.1 Efficiency of the lactic acid fermentation process

The lactic acid fermentation process is characterized by the biological degradation of a substrate (e.g., glucose) by a population of microorganisms (biomass) into lactic acid (Komesu et al., 2017). The efficiency of the lactic acid fermentation process can be measured as the concentration of lactic acid produced, as the lactic acid yield based on the substrate consumed and as the lactic acid productivity (i.e., lactic acid production rate) (Hofvendahl et al., 2000; Mussatto et al., 2007).

For the lactic acid fermentation process to be considered efficient and economical, the following goals should be met (Tejayadi et al., 1995):

(i) maximum lactic acid yield should be achieved by minimizing by-product formation and subsequently reducing the separation costs in the downstream processing

(ii) the substrate should be completely utilized to minimize feedstock costs

(iii) the lactic acid concentration should be high to minimize concentration costs during downstream processing

(iv) maximum lactic acid productivity should be achieved to minimize capital and operating costs during the production processes.

The subsequent sections will deal with the factors that may influence the efficiency of the lactic acid fermentation process such as lactic acid-producing microorganisms, nutritional requirements of the microorganisms, fermentation conditions and fermentation modes.

2.4.2 Lactic acid - producing microorganisms

Microorganisms that can be used in lactic acid fermentation include bacteria, fungi, algae, cyanobacteria and yeast. The bacterial and fungal fermentations are commonly adopted (Abdel-Rahman et al., 2013; Guo et al., 2010). The choice of a microorganism to use majorly depends on the substrate to be fermented because a microorganism's metabolism differs with different carbon sources (Komesu et al., 2017). However, the chosen microorganism should be able to ferment cheap raw materials, produce large amounts of lactic acid, have low nitrogen requirements and produce the least amount of by-products (Alves de Oliveira et al., 2018).

Different types of bacteria such as lactic acid bacteria (LAB), *Escherichia coli*, *Bacillus* strains and *Corynebacterium glutamicum* have been used to produce lactic acid. However, lactic acid bacteria (LAB) are the most preferred among them because they can produce lactic acid with high yield and high productivity (Abdel-Rahman et al., 2013; Wang et al., 2015). Lactic acid bacteria are Gram-positive, non-motile, non-spore-forming rods and cocci. They do not use oxygen in their energy production hence grow under anaerobic conditions but they are also capable of growing in the presence of oxygen (Vijayakumar et al., 2008). Most LAB species belong to genera *Lactobacillus*, *Lactococcus, Carnobacterium, Streptococcus, Enterococcus, Vagococcus, Tetragenococcus, Pediococcus* and *Aerococcus* (Reddy et al., 2008). Among them, the genus *Lactobacillus* are commonly used to produce lactic acid because they do have a high tolerance to acid environments. Moreover, they can be easily genetically modified to produce a specific lactic acid isomer (De Oliveira et al., 2016).

Despite bacterial fermentation using lactic acid bacteria (LAB) being common, fungal fermentation has also been used to produce lactic acid (Tay et al., 2002). Filamentous fungus like *Rhizopus oryzae* can utilize glucose aerobically to produce L (+)-lactic acid (Guo et al., 2010; Wee et al., 2006). Nevertheless, *Rhizopus oryzae* requires vigorous aeration because it is an obligate aerobe. Furthermore, the mycelial growth of the *Rhizopus* species causes increased broth viscosity, leading to mass transfer limitations and low lactic acid productivity (Liu et al., 2008; Tay et al.,, 2002). Other by-products such as fumaric acid and ethanol are also formed during fungal fermentation which lower the lactic acid yield (Wee et al., 2006). Due to these drawbacks of fungal fermentation, their applications for industrial lactic acid production have been limited as compared to bacterial fermentation (Wee et al., 2006). The present study used *Lactobacillus casei* as the lactic acid-producing microorganism.

2.4.3 Nutritional requirements of lactic acid bacteria (LAB) for lactic acid fermentation

Lactic acid bacteria have complex nutritional requirements due to their inability to synthesize their own growth factors (Narayanan et al., 2004). Therefore, to achieve efficient lactic acid fermentation, various nutrients which include the carbon sources, nitrogen sources and mineral salts are provided to support their cell growth and viability (Castillo Martinez et al., 2013; Oh et al., 2003).

2.4.3.1 Carbon sources

Carbon sources refer to the different substrates used in lactic acid fermentation. Substrate consumption is essential for all cells to maintain life in the absence of growth (Gerson et al., 1988; Monteagudo et al., 1997). The maintenance energy derived from these substrates provides the cell with resources for various physiological and biochemical functions such as maintaining intracellular ionic concentrations and DNA repair (Gerson et al., 1988; Vijayakumar et al., 2008).

The substrates are categorized into refined sugars, starchy materials, and lignocellulosic biomass (Hofvendahl et al., 2000; John et al., 2007). The refined sugars such as glucose and sucrose ease the production processes because they do not require the pretreatment step, but the high costs of these sugars render them unfavorable for the commercial production of lactic acid (Hofvendahl et al.,

2000). Therefore, cheap raw materials like lignocellulosic biomass and starchy materials are of keen interest. Starchy materials including wheat, barley, rye, corn, potato, cassava, sweet sorghum, carrot and rice have been used for lactic acid production (John et al., 2007; Komesu et al., 2017). Considering that most of these starchy materials are food crops, they do get competition from human consumption (Alves de Oliveira et al., 2018). Hence, to avoid what seems to hinder food availability, lignocellulosic biomass has been regarded as the promising alternative raw material for lactic acid fermentation. They include cassava bagasse, sugarcane bagasse, corncob, wastepaper and agricultural residues such as wheat bran, corn stover, wheat straw, alfalfa fibre among others (Abdel-Rahman et al., 2011; Hu et al., 2016).

Lignocellulosic biomass contains a persistent polymer (cellulose) which requires physico-chemical pretreatments and several enzymatic reactions for degradation purposes (Gao et al., 2011; Okano et al., 2010). Nonetheless, these pretreatment steps produce inhibitory compounds like furfural, 5 – hydroxymethyl furfural, inorganic ions, bi-alcohols, acetic acid and formic acid that inhibits the fermentation process by affecting the growth of microorganisms, enzyme activities and lactic acid production (Abdel-Rahman et al., 2016). Moreover, the complete hydrolysates derived from lignocellulosic biomass usually contain different ratios of glucose and xylose which limit the effective utilization of lignocellulosic biomass because most lactic acid bacteria (LAB) cannot utilize xylose (Abdel-Rahman et al., 2015). Sugar-containing industrial waste products such as molasses and cheese whey which contain sucrose and lactose respectively, are other promising substrates for lactic acid production (Dumbrepatil et al., 2008; Sun et al., 2019).

Lactic acid fermentation can be economically feasible if cheap substrates are used. Furthermore, the substrates should also possess some of the following characteristics: (i) should have the ability to be fermented with little or no pretreatment, (ii) should ensure high yield of lactic acid, (iii) should ensure rapid production rate, (iv) should ensure little or no by-product formation and (v) should be available all year-round to sustain lactic acid production (Oh et al., 2005; Wee et al., 2006). Therefore, choosing a specific substrate to be used for lactic acid fermentation primarily depends on its availability, its cost and the desired purity of lactic acid (John et al., 2007).

Among the refined sugars, the optimal lactic acid production was obtained from glucose, fructose, lactose, and galactose (Vijayakumar et al., 2008). In the present study, glucose was used as the carbon

source. Glucose is regarded as one of the simplest carbon sources used in the fermentation industry to manufacture various industrial chemicals (Gerson et al., 1988). Furthermore, from the previous studies using *Lactobacillus casei*, the bacteria preferred glucose to other refined sugars such as lactose and sucrose for their growth and lactic acid production (Alonso et al., 2010).

2.4.3.2 Nitrogen sources

For efficient lactic acid fermentation, the substrate should be supplemented with a source of metabolizable nitrogen and vitamins (Willem Schepers et al., 2002). The nitrogen sources used in lactic acid fermentation include peptone, tryptone, soybean meal, cotton seed extract and yeast extract (Castillo Martinez et al., 2013; Gerson et al., 1988). However, among these nitrogen sources, yeast extract has been proven to be the most effective nitrogen source because it ensures high cell growth and high lactic acid productivity (Oh et al., 2003; Olmos-Dichara et al., 1997). Yeast extract provides nitrogen for the formation of cell bodies thus enhancing the growth of various *Lactobacilli* such as *Lactobacillus casei* used in the present study (Yoo et al., 1997). Additionally, yeast extract can be used as a source of vitamin B in the fermentation media for the growth and maintenance of lactic acid bacteria (Vijayakumar et al., 2008). In the present study both yeast extract and peptone were used as nitrogen sources.

2.4.3.3 Mineral salts

Lactic acid bacteria require varying amounts of phosphorous, sulphur, potassium, magnesium, sodium, calcium, iron, copper, manganese, molybdenum, cobalt, zinc, and chloride (Castillo Martinez et al., 2013; Gerson et al., 1988). These mineral elements are provided in the fermentation medium in the form of mineral salts such as magnesium sulphate, manganese (II) sulphate, iron (II) sulphate, di-potassium hydrogen phosphate, sodium acetate and Tween-80 (Polysorbate 80) among others (Castillo Martinez et al., 2013; Vijayakumar et al., 2008). They enhance cell growth, and thus influence lactic acid production. For instance, Tween-80 is significant for the production of enzymes; zinc is necessary for the function of alcohol dehydrogenase; iron is associated with the cytochromes; magnesium activates enzymes such as hexokinase and regulates the degree of association of ribosomes (Gerson et al., 1988; Vijayakumar et al., 2008).

2.4.4 Metabolic pathways of lactic acid bacteria (LAB)

Lactic acid bacteria (LAB) can be classified as homofermentative or heterofermentative depending on the fermentation end-products formed through different metabolic pathways (Abdel-Rahman et al., 2013; Reddy et al., 2008). The homofermentative LAB metabolize glucose through the Embden-Meyerof-Parnas (EMP) pathway to produce more than 85 % lactic acid from glucose. They ferment one mole of glucose to two moles of lactic acid, generating a net yield of two moles of ATP per molecule of glucose metabolized. Lactic acid is, therefore, the primary end product of the EMP pathway (Reddy et al., 2008; Wee et al., 2006). On the other hand, heterofermentative LAB use the phosphoketolase (PK) pathway to produce only 50 % lactic acid from glucose. They ferment one mole of glucose to one mole of lactic acid, one mole of ethanol and one mole of carbondioxide. Since only one mole of ATP is generated per mole of glucose, there is less growth per mole of glucose metabolized (Alves de Oliveira et al., 2018; Reddy et al., 2008). Therefore, to maximize the yield of lactic acid, homofermentative LAB are commonly used for commercial lactic acid production (Abdel-Rahman et al., 2013). Table 2.3 shows some homofermentative and heterofermentative LAB together with the corresponding lactic acid isomers they produce.

Table 2.3: Examples of homofermentative and heterofermentative LAB (Litchfield, 1996; Vijayakumar et al., 2008)

Category	Examples	Lactic acid isomer produced
Homofermentative LAB	*Lactobacillus delbrueckii*	D (–)
	Lactobacillus lactis	D (–)
	Lactobacillus bulgaricus	D (–)
	Lactobacillus casei	L (+)
	Lactobacillus plantarum	DL [i.e., mixture of L (+) and D (–)]
	Lactobacillus curvatus	DL
	Lactobacillus amylophilus	L (+)
	Lactobacillus amylovorus	DL
Heterofermentative LAB	*Lactobacillus brevis*	DL
	Lactobacillus fermentum	DL
	Lactobacillus rhamnosus	L (+)

The metabolic pathways of homofermentative and heterofermentative LAB are shown in Figure 2.2. Most LAB strains are anaerobic in nature. They utilize pyruvic acid, the end product of the Embden-Meyerof-Parnas (EMP) pathway (Singhvi et al., 2018). L-lactate dehydrogenase or D-lactate dehydrogenase enzyme converts pyruvic acid to lactate, thus producing lactic acid by oxidizing NADH (nicotinamide adenine dinucleotide) generated during glycolysis with pyruvate as the electron acceptor (Singhvi et al., 2018).

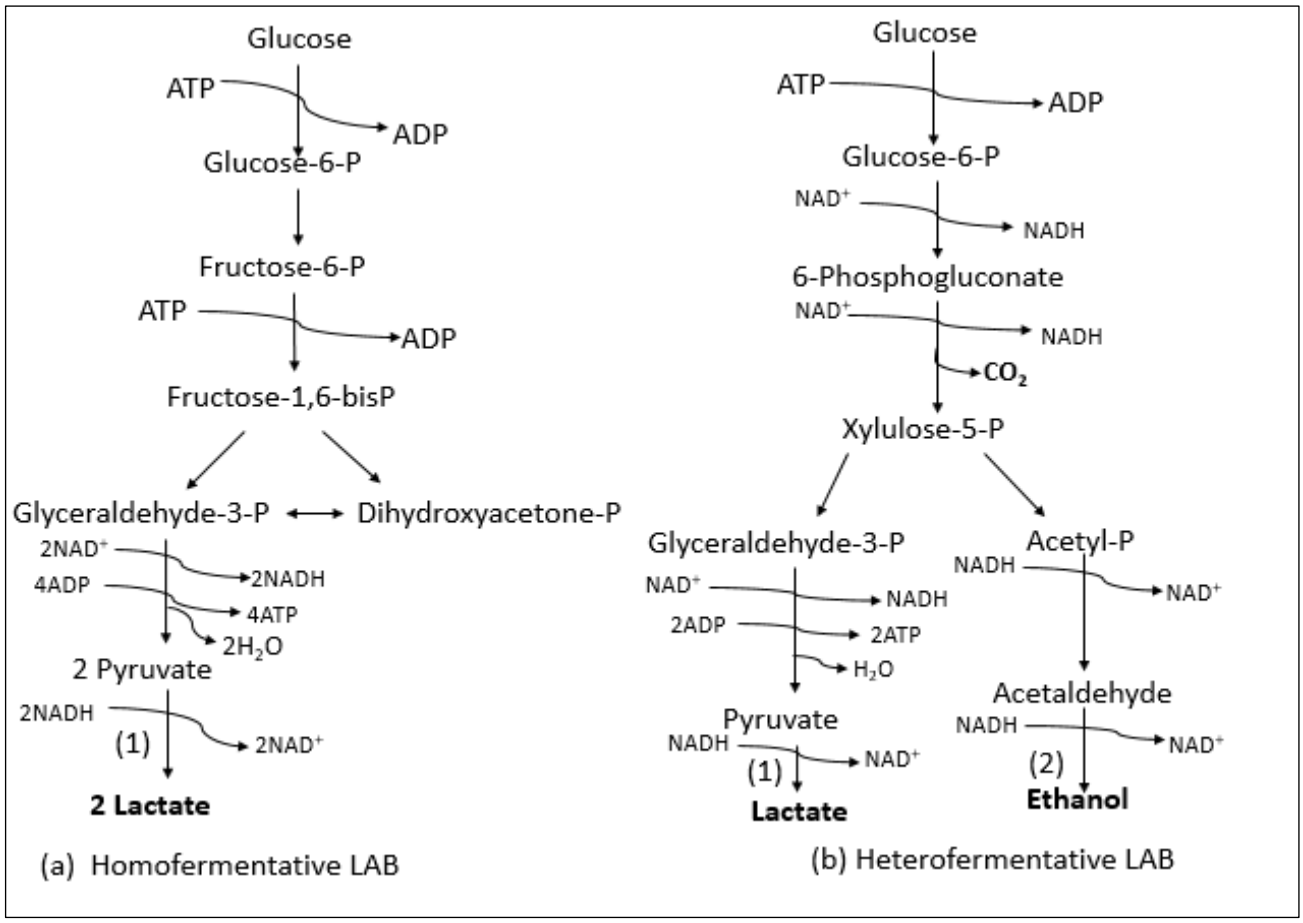


Figure 2.2: Metabolic pathways of (a) homofermentative and (b) heterofermentative LAB, redrawn from Wee et al., 2006.

Note: In Figure 2.2**,** P is phosphate; ADP is adenosine 5'-diphosphate; ATP is adenosine 5'-triphosphate; NAD^+ is nicotinamide adenine dinucleotide; NADH is nicotinamide adenine dinucleotide (reduced form); and (1) represents lactate dehydrogenase enzyme; (2) represents alcohol dehydrogenase enzyme.

2.4.5 Effects of process parameters on lactic acid fermentation

The main process parameters that are important in lactic acid fermentation include pH, temperature, inoculum size and initial sugar concentration (Kaavessina et al., 2017; Wang et al., 2015). As discussed in this section, they influence cell growth, lactic acid productivity, lactic acid yield and lactic acid concentration.

2.4.5.1 pH

The bacterial growth depends on the fermentation pH. Generally, the optimal pH for lactic acid fermentation varies between pH 5 and pH 7 depending on the microorganism used (Hetényi et al., 2011; Hofvendahl et al., 2000). Nevertheless, the optimal pH for the growth of most lactic acid bacteria (LAB) was found to be pH 6.5. Hence, most lactic acid fermentations by LAB are carried out at pH 6.5 (Komesu et al., 2017; Panesar et al., 2010).

During the lactic acid fermentation process, the increase in lactic acid concentrations lowers the pH of the fermentation broth thus causing inhibitory effects to the metabolism of bacteria (Okano et al., 2010). Moreover, the undissociated lactic acid can pass through the bacterial membrane and lead to increased intracellular accumulation of lactic acid, which decreases the intracellular pH and disrupts the cell membrane (Abdel-Rahman et al., 2016; Aljundi et al., 2005). Consequently, much energy is used by the cells to maintain the internal pH constant leading to cell growth rate inhibition which reduces the rate of lactic acid production (Jantasee et al., 2017).

To alleviate these inhibitory effects, a neutralizing agent (a base) is usually added to control the pH during fermentation (Krzyzaniak et al., 2013). The added base converts the undissociated lactic acid to its dissociated lactate form which is less inhibitory to bacteria (Abdel-Rahman et al., 2016). Traditionally, calcium hydroxide and calcium carbonate have been used as neutralizing agents due their affordability. However, other neutralizing agents such as sodium hydroxide and ammonium hydroxide can also be used (Nakano et al., 2012; Singhvi et al., 2018).

Most *Lactobacillus* species cannot grow and produce lactic acid below pH 4 (i.e. below the pKa of lactic acid, which is 3.86) (Okano et al., 2010). Therefore, in cases where the fermentation pH was not controlled in the previous studies, low yield, low productivity and low lactic acid concentrations

were reported due to the aforementioned inhibitory effects (Hofvendahl et al., 2000). In the present study, a pH of 6.5, optimal pH for *Lactobacillus casei,* was used for lactic acid fermentation, and sodium hydroxide was used as the neutralizing agent.

2.4.5.2 Temperature

Temperature is an essential factor in lactic acid fermentation because it influences the microbial activity, substrate consumption rate and lactic acid production rate (Panesar et al., 2010; Tang et al., 2016). Lactic acid bacteria can be categorized as either mesophilic or thermophilic bacteria depending on their optimal growth temperature ranges (Idris et al., 2006). The optimal growth of mesophilic lactic acid bacteria occurs within a temperature range of 28 ℃ to 45 ℃ while that of thermophilic lactic acid bacteria occurs within a temperature range of 45 ℃ to 62 ℃ (Vaidya et al., 2005).

Usually, if bacteria are grown below or above the optimal growth temperature range, the growth occurs slowly resulting in a decreased rate of cellular production and product formation (Panesar et al., 2010; Wang et al., 2014a). However, the industrially applicable lactic acid bacteria (LAB) are mesophilic with an optimal growth temperature of 37 ℃, hence this temperature has been adopted for lactic acid fermentation by LAB (Abdel-Rahman et al., 2016). Since this temperature is below 45 ℃, the fermenter and fermentation media must be sterilized to prevent the growth of extraneous microorganisms that can cause contamination risks and decrease the fermentation efficiency (Litchfield, 1996). The present study used mesophilic *Lactobacillus casei* with an optimal growth temperature of 37 ℃, hence, this temperature was adopted for lactic acid fermentation.

2.4.5.3 Inoculum size

The inoculum size has a significant effect on the lag phase of the bacterial growth. Basically, the duration of a lag phase decreases with an increase in the inoculum size (Taleghani et al., 2016). Consequently, the reduced lag phase period results in a decreased total fermentation time thus improving the lactic acid productivity (Wang et al., 2014). On the other hand, a long lag phase period is not required because it is time consuming and the fermentation medium is used to maintain a viable culture before bacteria enter the exponential growth phase (Taleghani et al., 2016).

The industrial lactic acid fermentation uses inoculum size range between 3 to 10 % (v/v) of the fermentation broth volume (Taleghani et al., 2016). It has been reported that higher inoculum sizes can cause drastic decrease of nutrients necessary for the cell growth while lower inoculum sizes lead to insufficient biomass for lactic acid production (Panesar et al., 2010; Wang et al., 2015). In an investigation by Juan Wang et al., 2014 to determine the optimal inoculum size of *Lactobacillus casei* during lactic acid production, they used 5, 10, 15, and 20 % (v/v) inoculum sizes. From their investigation, the inoculum size of 10 % (v/v) was determined as the optimal inoculum size for *Lactobacillus casei*. They also found out that a low inoculum size was not beneficial for the accumulation of the target product. On the other hand, minimal increase in lactic acid production was obtained at inoculum sizes higher than 10 % (v/v) (Wang et al., 2014a). In the present study, 10 % (v/v) inoculum size which is optimal for *Lactobacillus casei* was used in batch fermentation experiments.

2.4.5.4 Initial sugar concentration

The initial sugar concentration has effects on the cell growth, substrate utilization and lactic acid production (Taleghani et al., 2016). High initial sugar concentration in a batch process restricts the diffusion of sugar and nutrition into the cell thus inhibiting cell growth. Additionally, the total lactic acid productivity is reduced due to the substrate inhibition that occurs at high initial sugar concentration (Kaavessina et al., 2017; Rojas-Garbanzo et al., 2012; Taleghani et al., 2016). On the other hand, low initial sugar concentration leads to the formation of by-products such as ethanol, acetic acid and carbon dioxide alongside the lactic acid which reduces the product yield (Åkerberg et al., 1998). The formation of these by-products is attributed to the shift from homofermentation to mixed-acid fermentation by lactic acid bacteria under substrate-limited conditions (Åkerberg et al., 1998).

In a study to determine the effect of initial sugar concentration on lactic acid fermentation using soybean straw enzymatic hydrolyzate and *Lactobacillus casei*, Juan Wang et al., 2014 concluded that low initial sugar concentration resulted in low lactic acid production. Also, an initial sugar concentration of up to 35 g/L did not restrict the metabolism of the bacteria (Wang et al., 2014a). Therefore, in the present study, an initial glucose concentration of 30 g/L was used to avoid restricting the metabolism of *Lactobacillus casei* during lactic acid fermentation.

2.4.6 Fermentation modes

Lactic acid fermentation can be carried out in different modes including batch, fed-batch, repeated batch, and continuous modes (Hofvendahl et al., 2000; Jantasee et al., 2017). These fermentation modes influence lactic acid concentrations, yields and productivities obtained (Litchfield, 1996). Batch fermentation mode is the most commonly used approach in industrial lactic acid production (Ding et al., 2006; Ghaffar et al., 2014; Xu et al., 2006).

2.4.6.1 Batch fermentation

In a batch fermentation mode, lactic acid-producing microorganisms are inoculated to a given fermentation medium volume in a fermenter. As the microorganisms grow, they gradually consume the nutrients and subsequently lead to the accumulation of the lactic acid (Ghaffar et al., 2014). The fermentation process is left to proceed for the desired duration without adding any nutrients except for the neutralizing agents that control the pH of the fermentation medium (Hofvendahl et al., 2000; Jantasee et al., 2017).

The bacterial cell growth in a batch fermenter is divided into lag, exponential, stationary and death phases (Yates et al., 2007). During the lag phase, cells undergo intracellular changes in an effort to adjust to a new environment, hence, little or no cell reproduction occurs (Yates et al., 2007). During the exponential phase, cells reproduce at a rate proportional to the number of cells leading to an exponential increase in the number of cells (Yates et al., 2007). During the stationary phase, the bacteria stop replicating due to the unfavorable conditions for growth (Rolfe et al., 2012; Zwietering et al., 1990). It has been explained that at the stationary phase either the nutrients are limited or the end-product inhibits the bacterial cell growth and thus restrict the number of cells that can be supported (Yates et al., 2007). Finally, in the death phase, the cells lose their viability and a population decline is realized. At this phase the surroundings cannot maintain the population, hence, the population declines (Rolfe et al., 2012; Yates et al., 2007). An illustration of a bacterial cell growth curve in a batch fermentation mode is shown in Figure 2.3.

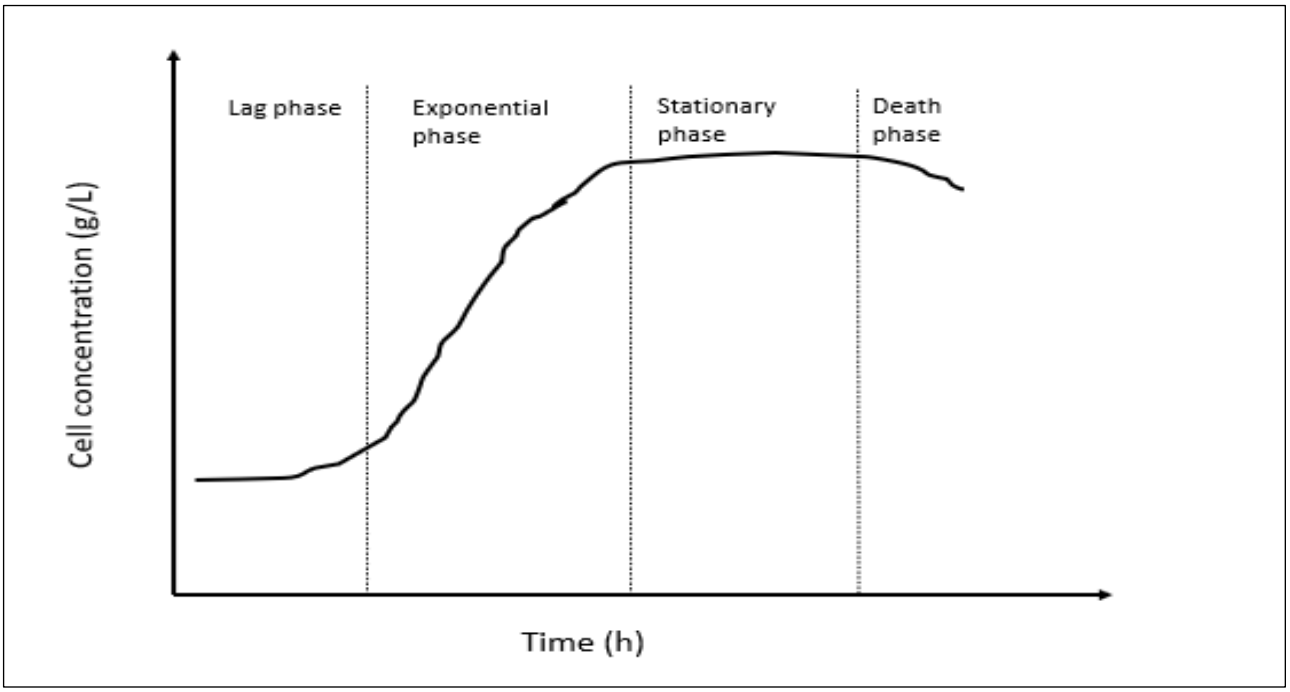


Figure 2.3: Illustration of a bacterial cell growth curve in a batch fermentation mode modified after Yates et al., 2007.

The batch fermentation mode has been hailed for its advantages such as ease of operation, reduced risk of contaminations and high lactic acid concentrations (Hofvendahl et al., 2000; Jantasee et al., 2017). However, low lactic acid productivity due to end-product inhibition, which affects bacterial cell growth and metabolism, remains a major challenge in the batch fermentation mode (Senthuran et al., 1997; Xu et al., 2006). The low lactic acid productivity in batch fermentation has also been associated with low cell density in the fermenter (John et al., 2007; Zhang et al., 1994).

2.4.6.2 Continuous fermentation

In a continuous fermentation mode, a fresh medium is continuously added to the fermenter while simultaneously removing the products formed from the fermenter. The fermenter volume usually stays the same when the addition of the fresh medium and removal of the products is done at the same rate (Abdel-Rahman et al., 2013; Jantasee et al., 2017).

The continuous fermentation mode overcomes some of the challenges associated with the batch fermentation mode such as the end-product inhibition by removing the lactic acid produced from the fermentation medium. However, its major limitation is the washout of cells from the fermenter as

the products are being removed (John et al., 2007; Tejayadi et al., 1995). Furthermore, unutilized carbon sources can also flow from the fermenter as the products are being withdrawn thus reducing the fermentation efficiency (Hofvendahl et al., 2000; Tejayadi et al., 1995).

2.5 Challenges that hinder efficient and economical lactic acid fermentation

Although different challenges associated with the lactic acid fermentation have been directly or indirectly captured in some of the sections above, this section summarizes the major challenges that hinder the possibility of achieving efficient and economical lactic acid fermentation.

2.5.1 High cost of raw materials

The main raw materials used in lactic acid fermentation are the carbon and nitrogen sources whose costs pose a challenge to efficient and economical lactic acid production. Generally, it is estimated that the cost of raw materials in lactic acid fermentation can account for around 40 % to 70 % of the total production costs (López-Gómez et al., 2019). Glucose and starchy materials are the commonly used carbon sources for commercial lactic acid production (Abdel-Rahman et al., 2013). However, the high costs of glucose render large scale production of lactic acid less economically feasible (Hofvendahl et al., 2000). On the other hand, most starchy materials are food crops, and thus face competition from human consumption. Therefore, various studies have focused on the exploitation of lignocellulosic biomass and agro-industrial wastes as alternative carbon sources (Hu et al., 2016). Similarly, the commonly used nitrogen sources such as yeast extract and peptone are costly (Jantasee et al., 2017). Hence, various studies have focused on alternative cheap nitrogen sources such as silkworm larvae, corn steep liquor, yeast autolyzate, and white rice bran hydrolysate among others. Nevertheless, the lactic acid yield and productivity from these cheap nitrogen sources are very low hence they have not been widely adopted for lactic acid fermentation (Alves de Oliveira et al., 2018; John et al., 2007).

2.5.2 Carbon catabolite repression

This is a phenomenon in which glucose limits the ability to effectively utilize other sugars for lactic acid production during co-fermentation by mixed sugars as the carbon source (Wang et al., 2015). Usually, most lactic acid-producing microorganisms prefer glucose as the carbon source. Therefore, carbon catabolite repression becomes a major challenge when using lignocellulosic biomass hydrolysates that contains mixed sugars (Singhvi et al., 2018). This non-simultaneous utilization of sugars causes some of them to remain unused hence decreases lactic acid yield and productivity (Abdel-Rahman & Sonomoto, 2016). In order to address this challenge, various studies have aimed at developing metabolically engineered bacterial strains that can effectively utilize all the sugars (Okano et al., 2010). Other studies have also proposed the use of mixed cultures, where each bacterial strain can be able to convert a specific sugar to lactic acid (Eş et al., 2018).

2.5.3 End-product inhibition

Lactic acid (i.e., the end-product) in the fermentation broth inhibits bacterial cell growth as described in section 2.4.5.1. Conventionally, neutralizing agents such as calcium carbonate and sodium hydroxide are added to convert the lactic acid produced into a less inhibitory lactate form (Abdel-Rahman et al., 2013). The need to address this major challenge in lactic acid fermentation provided a strong motivation for the present study.

2.6 Methods available to relieve the end-product inhibition during lactic acid fermentation

The conventional method to relieve the end-product inhibition is the precipitation method (Lee et al., 2017). Nevertheless, there are other methods to remove lactic acid from the fermentation broth through reactive extraction, adsorption, and the use of membrane bioreactor systems in order to reduce the inhibitory effects (Abdel-Rahman & Sonomoto, 2016; Wee et al., 2006). This section focuses briefly on these methods. However, in the subsequent sections, membrane bioreactor systems will be dealt with in detail since this was the main focus of the present study.

2.6.1 Precipitation

During the lactic acid fermentation process, calcium carbonate or calcium hydroxide is usually added as a neutralizing agent to precipitate the produced lactic acid as described in section 2.4.5.1. Therefore, the resultant broth contains calcium lactate among other components (Jantasee et al., 2017). To recover lactic acid from this calcium lactate containing broth, centrifugation or filtration is used to separate the microbial cells in the initial step. The biomass free broth is then treated with strong sulphuric acid (H_2SO_4) that liberates lactic acid and simultaneously generates calcium sulphate (gypsum), a solid waste. The broth is then filtered to separate the gypsum waste and crude lactic acid solution (Boonmee et al., 2016; Singhvi et al., 2018). The crude lactic acid is further subjected to other numerous purification steps such as activated carbon treatment for the removal of colorants impurities followed by a combination of several methods which include extraction with an appropriate solvent, ion exchange, adsorption , reactive distillation or electrodialysis in order to improve on the purity of the desired lactic acid (Boonmee et al., 2016).

The precipitation method has major drawbacks such as consumption of high amounts of sulphuric acid and production of large quantities of solid waste (gypsum) leading to high costs of chemicals and waste disposal challenges (Milcent et al., 2001; Wasewar, 2005). The optical purity of lactic acid can also suffer during the purification process because isomerization of lactic acid may occur which in turn affects the purity requirements for food industries, pharmaceutical industries or polylactic acid (PLA) production (Jantasee et al., 2017). Moreover, the precipitation method is not suitable from an economic perspective since the many steps involved account for up to 50 % of the total lactic acid production costs (Dey et al., 2012b; Lee et al., 2017).

For every ton of lactic acid recovered through the precipitation method, one ton of gypsum solid waste is also generated (Gezae Daful et al., 2017). Hence, different attempts to have gypsum-free lactic acid fermentation using other neutralizing agents such as magnesium hydroxide, sodium hydroxide and ammonium hydroxide have been suggested (Gezae Daful et al., 2017; Nakano et al., 2012). However, these neutralizing agents have some negative impacts either on the microbial growth or total lactic acid production costs. When ammonium hydroxide is used, the ammonium lactate formed results in osmotic stress on the microbial cells, which reduces lactic acid production rates (Singhvi et al., 2018). Furthermore, ammonium lactate is difficult to thermally split to get the

desired lactic acid (Gezae Daful et al., 2017). On the other hand, using sodium hydroxide as a neutralizing agent for large scale lactic acid production is not suitable from an economic standpoint due to its high costs compared to calcium hydroxide or calcium carbonate (Nakano et al., 2012).

2.6.2 Reactive extraction

This method involves lactic acid being extracted from the fermentation broth by the extractant and then recovered from the solvent by back extraction into another solvent (Joglekar et al., 2006; Wasewar, 2005). Therefore, high molecular mass aliphatic amines (e.g. dodecyl amine) and phosphorous-bonded oxygen-bearing solvents (e.g. tributyl phosphate) exhibiting good selectivity for lactic acid can be used to extract lactic acid from the fermentation broth (Vaidya et al., 2005). Nonetheless, high molecular mass aliphatic amines are regarded as the most effective extractants. This is due to the high basicity of amines that results in reactive extraction of lactic acid with increased extraction efficiency (Vaidya et al., 2005).

During reactive extraction, the amine extractant recovers lactic acid from the fermentation broth by reacting with it to form an acid-amine complex solubilized into the extractant phase. After that, regeneration through back extraction recovers the acid into a product phase and acid-free extractant that can be recycled (Joglekar et al., 2006; Wasewar, 2005). The back extraction of lactic acid from the loaded organic phase processes includes the use of hydrochloric acid, sodium hydroxide, trimethylamine, diluent swing regeneration, temperature swing regeneration and gas antisolvent induced regeneration (Joglekar et al., 2006; Wasewar, 2005). Among them, the gas antisolvent induced regeneration method is preferred because it does not require any toxic material and it has no distillation step compared to other processes thus low energy requirement (Wasewar, 2005). The primary criteria used for solvent selection include solvents with high distribution coefficients for lactic acid, solvents that allow easy regeneration and solvents with a low tendency to an emulsion. However, other factors such as non-toxicity of the solvents to the microorganisms used in the fermentation process, high capacity of the solvents, affordable prices of the solvents and high selectivity for lactic acid also play a significant role in solvent selection (Vaidya et al., 2005; Wasewar, 2005).

Reactive extraction has been investigated for the *in situ* removal of lactic acid from the fermentation broth simultaneously as it is formed in an attempt to alleviate end-product inhibition to the microorganisms (Harington et al., 2008). Solvents such as water-insoluble amines (e.g., tri-n-octylamine), ketone (e.g., methyl isobutyl ketone), ester (e.g., tri-n-butyl phosphate) or quaternary ammonium salts (e.g., Tri(C_8C_{10})methylammonium chloride, [Aliquat 336]) are commonly used (Jantasee et al., 2017). Tertiary amines with long hydrocarbon chains have been found to be suitable extractants due to their high efficiency and selectivity as well as poor solubility in the aqueous phase (Jantasee et al., 2017; Tik et al., 2001).

Although reactive extraction has proven to be a suitable method to alleviate end-product inhibition, it faces some challenges that limit its adoption for the industrial lactic acid fermentation. These include the unfavorable distribution coefficients of solvents and high toxicity of extractants to the lactic acid-producing microorganisms (Boontawan et al., 2011; Gao et al., 2009).

2.6.3 Adsorption

Several researchers have investigated the adsorption of lactic acid on solid adsorbents or ion exchange resins for the *in situ* removal of lactic acid from the fermentation broth to minimize end-product inhibition to the microorganisms (Boonmee et al., 2016; Wasewar, 2005). Several adsorbents and ion exchange resins, including weak base polymer adsorbents MWA-1, Amberlite IRA-35, Amberlite IRA-92 resin, polyvinyl pyridine (PVP) resin, Amberlite IRA-400 resin among others have been used for lactic acid removal (Joglekar et al., 2006). The choice of any of these adsorbents or ion exchange resins is influenced by the high adsorption capacity for the acid, high selectivity of the acid as opposed to water and substrate, biocompatibility with microorganisms and regenerability (Joglekar et al., 2006; Wasewar, 2005). Amberlite IRA-400 resin has been reported to be the suitable adsorbent for the lactic acid recovery from fermentation broth because it has a proper pore size and high adsorption capacity to adsorb lactic acid within a wide pH range (Joglekar et al., 2006).

The adsorption method has not been successful for industrial applications due to some of its major challenges. This method requires regeneration of an ion exchange resin and adjustment of the feed pH to increase the sorption efficiency thus requiring large amounts of chemicals (Boontawan et al., 2011). In addition, ion exchange resins remove essential anions (e.g., sulphate ions) other than

lactate from the fermentation broth. This does not only lower the purity of lactic acid recovered but also interferes with the available nutrients necessary for the growth of the microorganisms during the fermentation process (Aljundi et al., 2005; Wasewar, 2005).

2.6.4 Membrane bioreactor systems

A membrane bioreactor (MBR) system refers to a system whereby a membrane is submerged in a fermenter or externally connected to a fermenter to achieve either *in situ* lactic acid recovery or continuous removal of the lactic acid produced thus alleviating its inhibitory effects on the bacterial cell growth (Fan et al., 2017a; Tejayadi et al., 1995). In MBR systems, the bacterial cells are either retained in the fermenter or recycled back to the fermenter by the membrane while lactic acid is continuously removed (Zhang et al., 1994). Since the membrane bioreactor systems do not only alleviate the end-product inhibition but also ensure high cell density in the fermenter, they are regarded as very promising method in improving lactic acid productivity (Fan et al., 2017a; Giorno et al., 2002; Xu et al., 2006). The present study focused on the membrane bioreactor systems used for lactic acid fermentation, hence, the subsequent sections will deal with it in detail.

2.7 Membrane separation technology

2.7.1 Overview

A membrane is defined as an interphase between two adjacent phases acting as a selective barrier, regulating the transport of substances between the two compartments (Ulbricht, 2006). Since a membrane acts as a selective barrier, it is mainly used for separation and purification (Zhang et al., 2015). On the other hand, membrane separation technology is defined as a separation technique using a film which is specially manufactured, and with selective transmission for separation, purification and concentration of the mixture driven by an external force (Gao, 2016).

Membrane separation technology has gained interests in several industrial sectors due to its promising features such as (Chevereau et al., 2010; Gao, 2016; Zhang et al., 2015):

i. low energy consumption. Membrane separation processes do not have a phase change hence low energy consumption

ii. high separation efficiency

iii. easy control and operation. The separation devices used in membrane separation technology are very simple, easy to operate and easy to be controlled

iv. ease in scale-up and integration with other separation processes.

In a typical membrane separation process, the feed stream to a membrane module is divided into the retentate and permeate streams (Cui et al., 2010). The retentate is the stream that has been retained by the membrane, containing the material that the membrane has rejected, whereas permeate is the stream that has passed through the membrane, containing particles/molecules less in size than the membrane pores (Cui et al., 2010). The schematic diagram of a typical membrane separation process is shown in Figure 2.4.

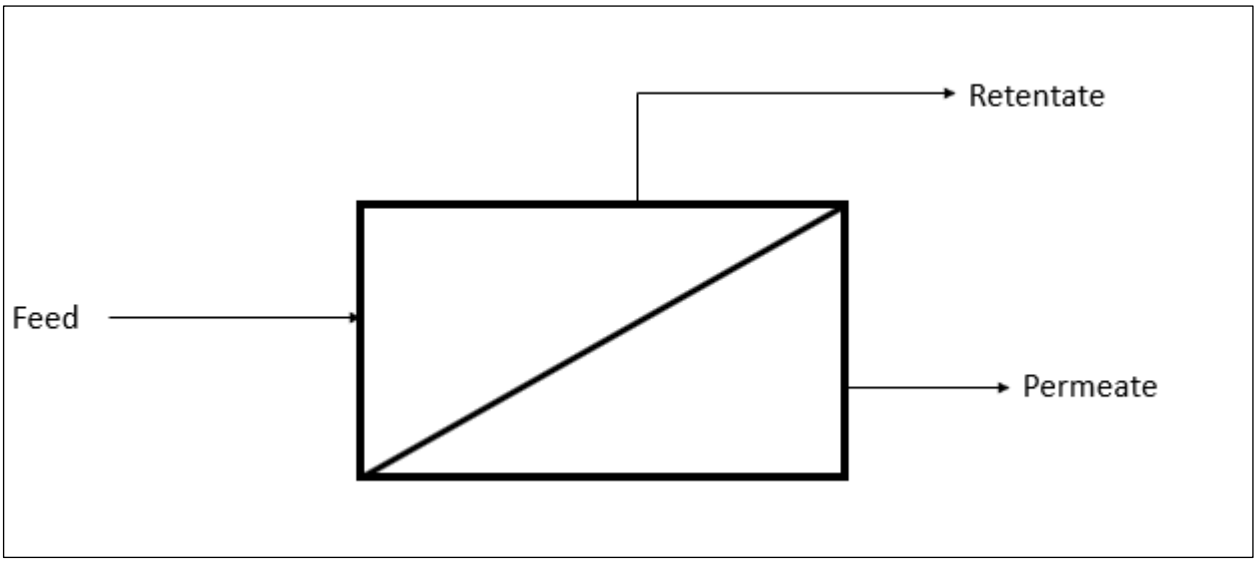


Figure 2.4: Schematic diagram of a typical membrane separation process redrawn from Judd, 2011.

The pressure-driven membrane processes such as microfiltration (MF), ultrafiltration (UF), nanofiltration (NF) and reverse osmosis (RO) utilize pressure difference as the driving force for the separation purposes (Oonkhanond et al., 2017; Zhang et al., 2015). Among these, MF and UF are commonly used in biotechnology for the removal of microorganisms (Milcent et al., 2001). Their separation mechanism is mainly by size exclusion which depends on their pore sizes or molecular weight cut off (MWCO), which is defined as the smallest molecular weight of species of which the

membrane has more than 90 % rejection (Cui et al., 2010). The typical pore sizes and MWCO of the four major pressure-driven membrane processes are: MF (pore size of 0.1 – 5 µm), UF (MWCO of 500 – 100,000 Da and pore size of 1 – 100 nm), NF (MWCO of 100 – 500 Da and pore size of 0.5 – 10 nm), and RO (pore size less than 0.5 nm) (Cui et al., 2010).

2.7.2 Membrane materials

In terms of materials, membranes can be categorized into ceramic and polymeric membranes. The ceramic membranes are usually manufactured from inorganic materials such as silica (SiO_2), zirconia (ZrO_2), alumina (Al_2O_3) and titania (TiO_2) (Fujioka et al., 2014; Mancinelli et al., 2015), whereas the polymeric membranes are made of organic materials such as cellulose, polyethersulfone (PES), polyvinylidene fluoride (PVDF), polysulfone (PS), polytetrafluoroethylene (PTFE) or polyethylene (PE) (Li et al., 2008).

Owing to the advantages of ceramic membranes compared to the polymeric membranes, ceramic membranes have been used in many industries for the purposes of membrane separation (Murić et al., 2014). These advantages include (Chevereau et al., 2010; Hofs et al., 2011; Murić et al., 2014):

(i) higher mechanical and thermal stability. The higher mechanical stability of ceramic membranes is evident when high backwash pressures are applied without their breakage Also, due to the higher mechanical and thermal stability, ceramic membranes can be operated at higher flow rates

(ii) a relatively narrow pore size distribution and high porosity which results in better separation characteristics and a higher flux

(iii) higher chemical stability resulting in longer membrane lifetimes. Also, due to the higher chemical stability, the effects of changes in pH are less significant with ceramic membranes

(iv) higher hydrophilicity resulting in high fluxes at low pressures.

In spite of the aforementioned advantages of ceramic membranes, their production costs are still higher than those of polymeric membranes thus resulting in higher investment costs. However, it has been reported that the investment costs can be balanced by the longer membrane lifespan (Mancinelli et al., 2015). Ceramic membranes are widely used in many industries such as chemical

manufacturing, food processing, beverage production and biotechnology which require specialized applications under harsh environments like high temperatures, aggressive organic chemicals, and extreme pH (Fujioka et al., 2014; Qi et al., 2013). The present study used a ceramic membrane owing to its advantages stated above.

2.7.3 Membrane modules

A membrane module is defined as the way a membrane is arranged into devices and hardware to separate the feed stream into permeate and retentate streams (Cui et al., 2010). Different configurations of membrane modules such as flat sheet, spiral wound, capillary tube/tubular, and hollow fiber modules strongly influence the flux behaviour in the membrane processes (Judd, 2011; Zhang et al., 2015). Therefore, to increase the mass transfer, reduce concentration polarization and ensure better flux performance, a proper membrane module is necessary (Zhang et al., 2015).

The capillary tube operates with the flow passing from the inside-to-outside the tube while in the hollow fibre module, the flow pass from outside-to-inside the tube (Judd, 2011). On the other hand, the flat sheet module consists of a selective flat sheet membrane on the top and a flat plate at the bottom with a net-like material placed in between them to provide space for the permeate removal (Cui et al., 2010). Flat sheet, hollow fibre and capillary tube/tubular modules are suitable for the membrane bioreactor systems since they permit turbulence promotion and regular effective cleaning (Judd, 2011). Nevertheless, tubular membrane modules are preferred due to their good ability to minimize fouling even for a feed with high solid content and high concentrations because of their open channel design (Xavier et al., 1995; Zhang et al., 2015). The present study adopted a tubular membrane module**.** The schematic diagrams of these modules are given in Figure 2.5.

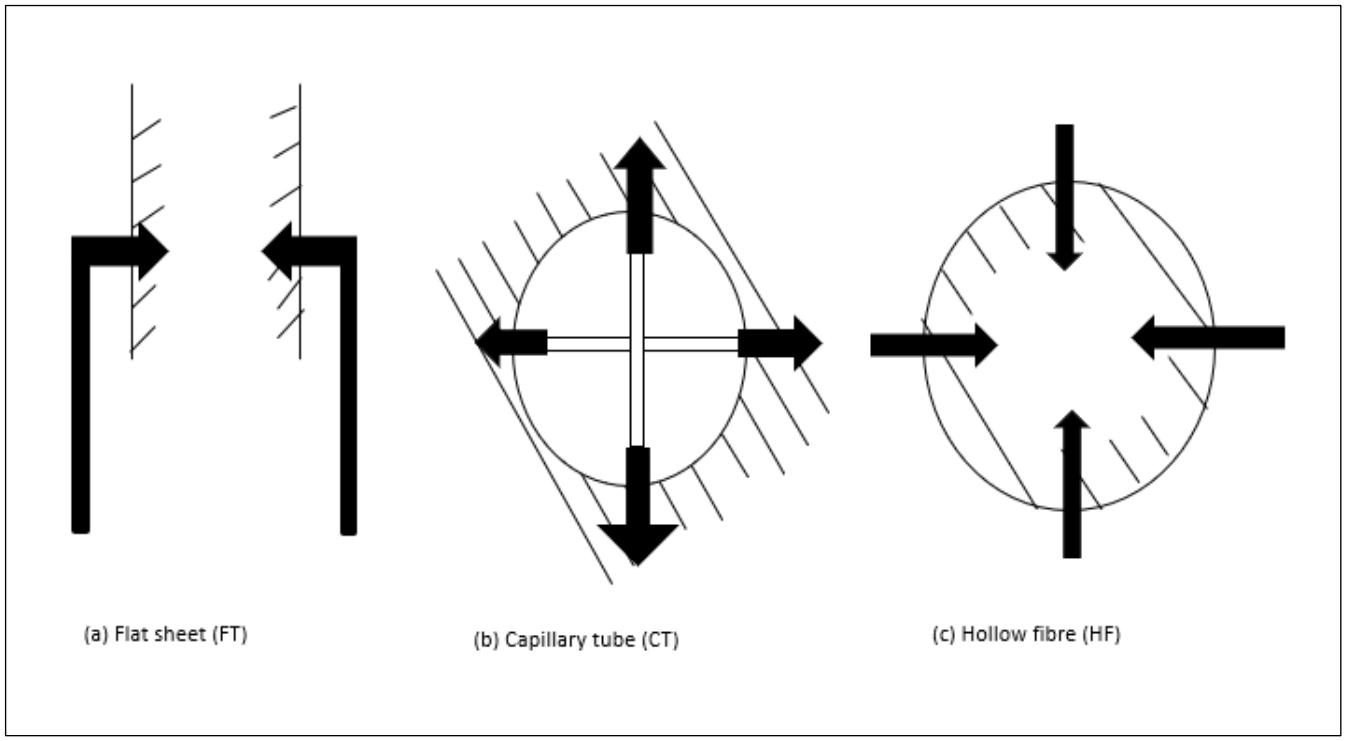


Figure 2.5: Schematic diagrams of (a) flat sheet (b) capillary tube and (c) hollow fibre modules, redrawn from Judd , 2011.

2.7.4 Membrane operating modes

There are two standard operating modes for membranes: dead-end and cross-flow (Charcosset, 2006). In dead-end operating mode, the entire fluid to be filtered is forced through the membrane using an applied perpendicular pressure. This leads to increased accumulation of particles on the membrane surface, hence, higher pressures are needed to maintain constant flow (Carstensen et al., 2012).

For cross-flow mode, the fluid to be filtered flows parallel to the membrane surface and permeates through the membrane due to the pressure difference. The cross-flow mode reduces cake layer formation by keeping it at low levels (Charcosset, 2006). Therefore, most membrane bioreactors for product recovery processes use the cross-flow mode (Carstensen et al., 2012). The present study employed the cross-flow mode of operation due to its advantage of minimizing cake layer formation. The illustration of the two membrane operating modes is shown in Figure 2.6.

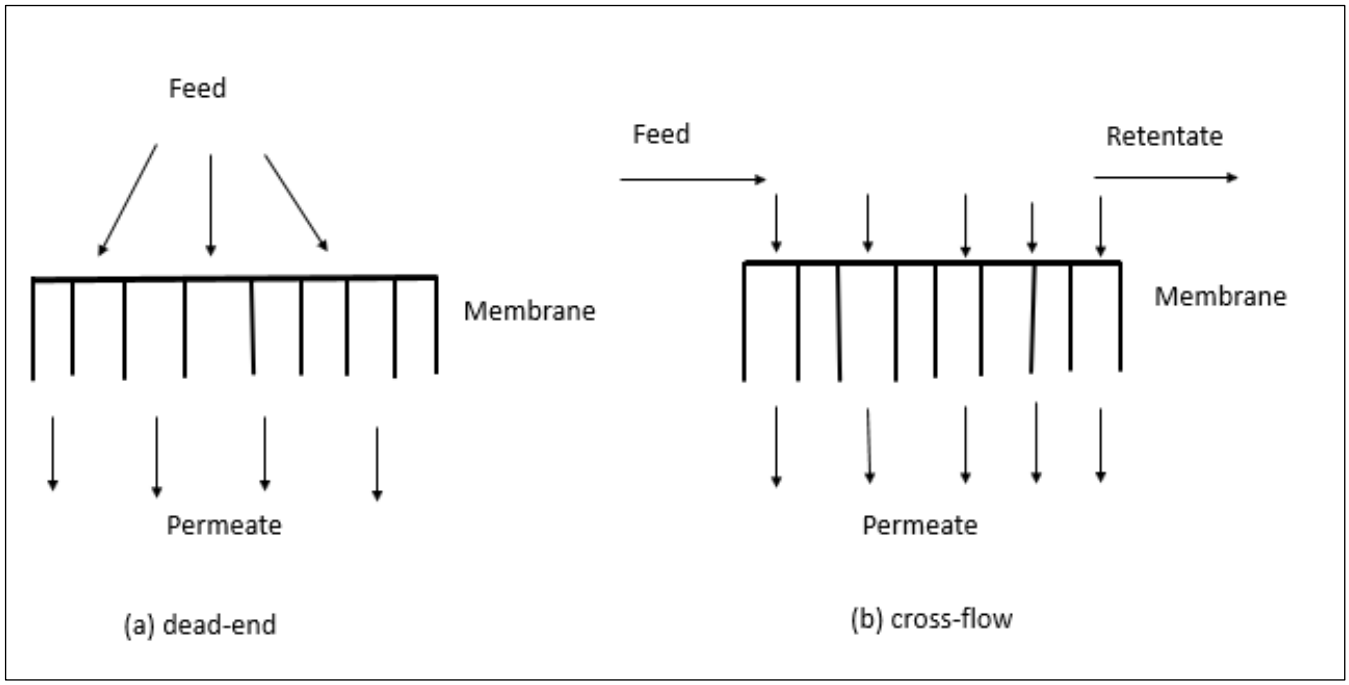


Figure 2.6: Illustration of the membrane operating modes (a) dead-end mode and (b) cross-flow mode redrawn from Charcosset, 2006.

2.7.5 Membrane operational parameters

This section covers the membrane operational parameters: flux, transmembrane pressure (TMP) and cross-flow velocity.

(i) Flux (J) is defined as the quantity of material passing through a unit area of membrane per unit time. It is usually referred to as permeate flux described by equation 2.5 (Judd, 2011; Mancinelli et al., 2015):

$$J = \frac{Q_p}{A}, \qquad (2.5)$$

where Q_p is the permeate flow rate (in litres per hour), and A is the membrane surface area (m^2). Therefore, J is expressed as litres per m^2 per hour ($L/(m^2h)$ denoted as LMH (Judd, 2011). Although the SI units of J should be $m^3/(m^2.s)$ (Judd, 2011), LMH has been commonly adopted in membrane technology.

In simpler terms, permeate flux just refers to the volume of liquid permeating per m^2 of membrane surface area in an hour time period (Mancinelli et al., 2015).

The membrane performance is usually measured in terms of the permeate flux (Cui et al., 2010).

(ii) Transmembrane pressure (TMP) is the pressure difference between the feed and the permeate. It is the driving force in pressure-driven membrane separation process (Cui et al., 2010).

(iii) Cross-flow velocity has been considered to influence the permeate flux. Higher fluid velocities increase the mass transfer coefficient and thus the permeate flux (Wojtyniak et al., 2015).

2.8 Membrane fouling

2.8.1 Overview

In pressure-driven membrane separation processes, permeate flux decline over time results due to concentration polarization and membrane fouling (Wojtyniak et al., 2015; Zhang et al., 2015). Concentration polarization is a phenomenon in which the solutes or particles rejected by the membrane accumulate on the boundary layer adjacent to the membrane surface thus reducing the permeate flux, increasing the risk of membrane fouling and deteriorating the permeate quality (Giorno et al., 2002; Zhang et al., 2015). On the other hand, membrane fouling is defined as the blockage of membrane pores during filtration caused by the combination of sieving and adsorption of particulates and compounds onto the membrane surface or within the membrane pores (Li et al., 2008). This blockage of pores causes decline of permeate flux over time when all other operating parameters such as temperature, flow rate, pressure and feed concentration are kept constant (Li et al., 2008). The fouling rate is influenced by different parameters such as pore size distribution, nature and concentration of solutes and solvents, membrane surface characteristics and membrane types among others (Cui et al., 2010).

2.8.2 Types of fouling

The different types of membrane fouling in pressure-driven membrane separation processes can be classified according to the solutes or particles causing the fouling and according to the degree of fouling (Zhang et al., 2015).

According to the solutes or particles causing fouling, the following types of membrane fouling exist:

(i) **Biofouling**: This occurs due to the deposition, accumulation, growth, and metabolism of microorganisms on a membrane surface. When a membrane adsorbs microorganisms, a biofilm grows on the membrane surface and hence provides suitable living conditions for the microorganisms and subsequently leads to irreversible fouling (Zhang et al., 2015).

(ii) **Organic fouling**: This is generally caused by natural organic matter (NOM), proteins, and polysaccharides which adsorb on the membrane surface (Judd, 2008; Zhang et al., 2015).

(iii) **Inorganic fouling**: This is caused by inorganic materials that deposit on the membrane surface. Calcium sulphate and calcium carbonate are regarded as the main components of inorganic fouling (Zhang et al., 2015).

According to the degree of fouling, the following types of membrane fouling can be distinguished:

(i) **Reversible fouling**: This occurs when the foulants loosely attach to the membrane surface, and thus they can be easily removed by physical cleaning methods. This type of fouling is also known as removable or temporary fouling (Wang et al., 2014b).

(ii) **Irreversible fouling**: This results when a strong matrix of fouling layer with solutes is formed during continuous filtration. Usually, physical cleaning methods cannot easily remove the irreversible foulants, hence, chemical cleaning methods are used (Judd, 2011; Wang et al., 2014b).

(iii) **Irrecoverable fouling**: This is also known as permanent fouling, and it determines the life span of a given membrane. Usually for this type of fouling, the original membrane permeability cannot be fully recovered even through chemical cleaning methods (Judd, 2011).

2.8.3 Membrane fouling mechanisms

In order to control the permeate flux decline over time in pressure-driven membrane separation processes, it is necessary to understand the membrane fouling mechanisms (Zhang et al., 2015). According to Cui et al. (2010) and Zhang et al. (2015), the different fouling mechanisms for porous membranes can be summarized as:

(i) complete pore blocking which occurs when the membrane pores are fully blocked by the particles

(ii) internal pore-blocking which occurs when particles with smaller sizes than the membrane pore size enter the pores and deposit on the internal pore surfaces hence narrowing the pore sizes

(iii) partial pore-blocking which occurs when the pores are not fully blocked by the particles but narrow their sizes

(iv) cake filtration which occurs when particles with larger diameter than the membrane pore size form a uniform cake layer on the entire membrane surface.

The membrane fouling mechanisms are illustrated in Figure 2.7.

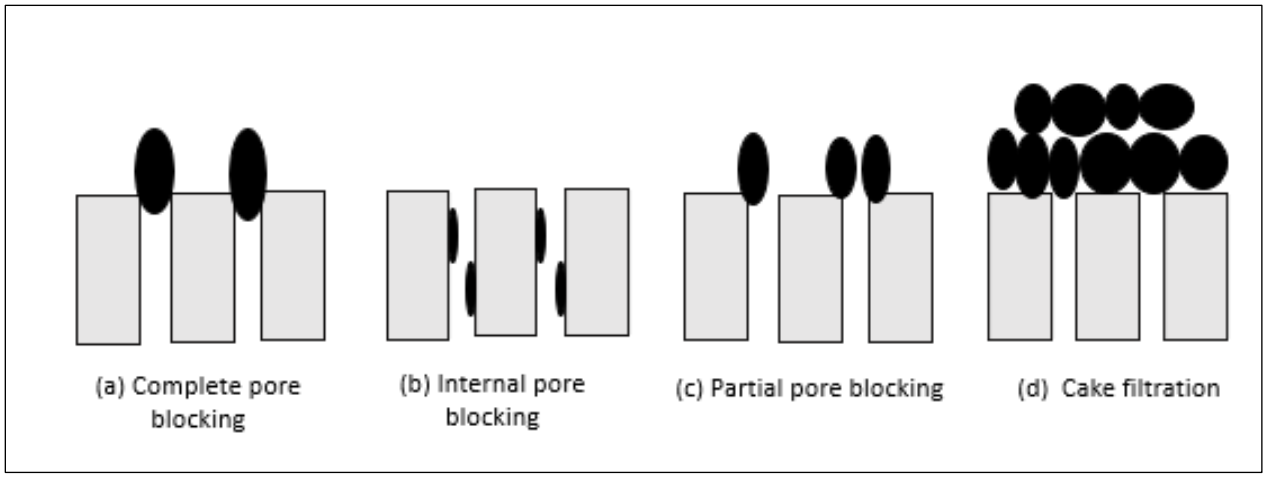


Figure 2.7: Illustration of membrane fouling mechanisms of porous membranes: (a) complete pore blocking, (b) internal pore blocking, (c) partial pore blocking and (d) cake filtration, redrawn from Cui et al., 2010.

2.8.4 Membrane fouling control measures

2.8.4.1 Overview

Membrane fouling is a major problem in pressure-driven membrane processes because it results in deterioration of the membranes as well as decreased membrane performance in terms of the permeate flux decline over time (Lin et al., 2010). Although membrane fouling cannot be avoided in membrane processes, its severe effects can be minimized through proper control measures put in place (Mohammad et al., 2012). A number of membrane control measures exist, however, the choice of a given control measure to use mainly depends on the mechanisms and factors contributing to the specific type of fouling (Field et al., 1995; Mancinelli et al., 2015).

Some of the membrane fouling control measures include the pretreatment of feed, optimization of operating parameters, modification of membrane surface, and regular membrane cleaning as described in Table 2.4 (Field et al., 1995; Mohammad et al., 2012; Zhang et al., 2015). The present study employed the route of minimizing membrane fouling through proper membrane operating conditions i.e., it considered operations below the critical flux. The subsequent sections will focus on the critical flux concept and membrane cleaning.

Table 2.4: Summary of the membrane fouling control measures

Fouling control measure	Description	References
Pretreatment of feed	• Involves removing of the potential foulants in the feed passing through the membrane module. • Methods such as coagulation, adsorption and filtration among others can be used to remove the foulants and improve the filterability. • The pH and concentration of the feed can also be adjusted to change the charge effect.	Cui et al., 2010; Zhang et al., 2015
Membrane surface modification	• Involves adding hydrophilic groups into polymeric structure to achieve hydrophilicity and thus minimal organic fouling. • It can be through methods such as surface coating, surface grafting or surface blending.	Mohammad et al., 2012; Zhang et al., 2015
Optimization of operating parameters	• Entails flow manipulation by controlling the hydrodynamics such as transmembrane pressure and permeate flux to minimize fouling. • Understanding the critical flux concept for minimal fouling. • Increasing cross-flow velocity to enhance shear stress and thus low fouling.	Cui et al., 2010; Meng et al., 2009; Mohammad et al., 2012; Zhang et al., 2015
Membrane cleaning	• Physical cleaning may include hydraulic cleaning methods such as back flushing and air scouring. • Air scouring is used in submerged membrane bioreactors where aeration provides oxygen to the microbes and maintains the solids in suspension and hence lowering the fouling. • Back flushing is where the transmembrane pressure is inverted and part of the permeate flows backwards into the cross-flow channel to remove the foulants. • Chemical cleaning can be carried out *in situ* or *ex situ*. *In situ* chemical cleaning includes cleaning in place (CIP) and chemically enhanced backflush (CEB). *Ex situ* cleaning is majorly performed when the membranes are severely fouled.	Meng et al., 2009, 2017; Mohammad et al., 2012; Wang et al., 2014b

2.8.4.2 The critical flux concept

The concept of critical flux was first introduced by Field et al.,1995 in an attempt to minimize membrane fouling in pressure-driven membrane processes (Miller et al., 2014). Critical flux hypothesis states that: "on the start-up of a given membrane operation (e.g., microfiltration or ultrafiltration), there exists a flux below which a decline of flux with time does not occur (i.e., slight or no fouling is observed) while above it, fouling is observed." This flux is the critical flux (Field et al., 1995).

The exact value of the critical flux depends on membrane properties (e.g., pore size and membrane material), foulant properties (e.g., biomass concentration), and cross-flow velocity (Miller et al., 2014; Wu et al., 1999). In order to minimize the membrane fouling and avoid frequent membrane cleaning, membranes are operated below the critical flux usually referred to as sub-critical flux operations (Pollice et al., 2005; Wu et al., 1999).

Although the concept of critical flux has been accepted as a guide to membrane operations in order to minimize membrane fouling, its practical applications sometimes turn out to be difficult due to the following reasons (Cui et al., 2010):

(i) the critical flux value may be too low to be practically applied

(ii) the critical flux cannot be predicted largely because the feed is often a complex mixture.

2.8.4.3 Methods of determining the critical flux

The critical flux can be determined by either flux or pressure stepping methods. In a flux stepping method (also known as constant flux operation), flux is controlled and the pressure is monitored whereas in a pressure stepping method (also known as constant pressure operation), pressure is controlled and the flux is monitored (Bacchin et al., 2006; Miller et al., 2013).

(i) Constant flux operation

In a constant flux operation, the permeate flux is step-changed while transmembrane pressure (TMP) is monitored. The TMP will follow the step-increase, but at some stage the TMP will start increasing

although the permeate flux remains constant. At this stage, the critical flux has been passed, and the increase in TMP indicates the presence of fouling (Bacchin et al., 2006; Miller et al., 2013). This increase in TMP observed although the permeate flux remains constant is believed to happen in order to compensate the increase in the resistance to permeation. However, below the critical flux there is no presence of this increase in resistance to permeation, hence, the TMP remains constant (Kwon et al., 2000). It is important to note that the chosen initial flux has a great impact on the constant flux operation method. If the initial flux is too high, then irreversible fouling will occur and affect any subsequent measurements. Hence, the chosen initial flux should be sub-critical then increased until the critical flux is obtained (Bacchin et al., 2006; Field et al., 1995). An illustration of the constant flux operation method used in determining the critical flux is shown in Figure 2.8.

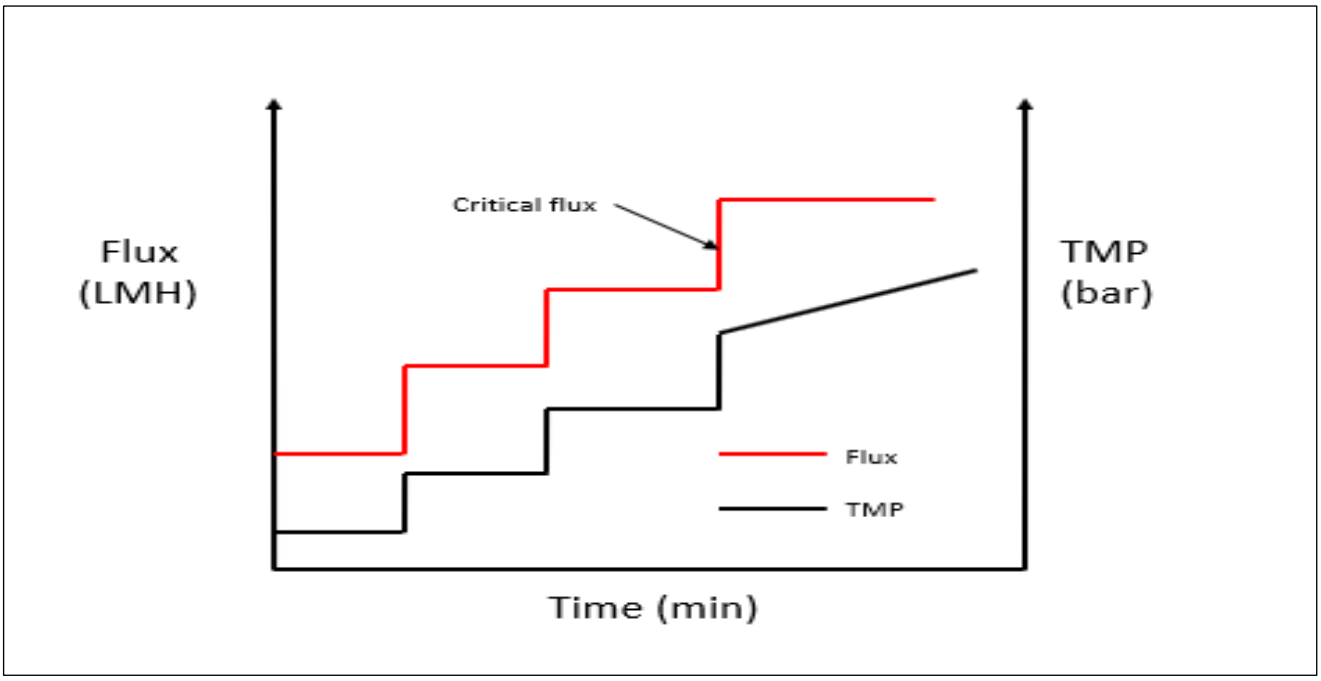


Figure 2.8: Illustration of the constant flux operation method used in determining the critical flux, modified after Bacchin et al., 2006.

Compared to constant pressure operations, the constant flux operations are complex and more challenging for the laboratory scale experiments (Miller et al., 2013). The present study, therefore, employed constant pressure operations.

(ii) Constant pressure operation

In a constant pressure operation, the transmembrane pressure (TMP) is step-changed while the permeate flux is monitored. The permeate flux will follow the step-increase in TMP, but at some stage the permeate flux will start decreasing although the TMP remains constant. Hence, at this stage the critical flux has been passed. The decrease in permeate flux at constant TMP is an indication of fouling that occurs when the critical flux has been passed (Bacchin et al., 2006; Miller et al., 2013). The initial TMP chosen should be below the resultant critical flux. It has been reported that a lower starting TMP with small increments of TMP until the critical flux is obtained, reduces the degree of irreversible fouling (Field et al., 1995). Figure 2.9 shows an illustration of the constant flux operation method used in determining the critical flux.

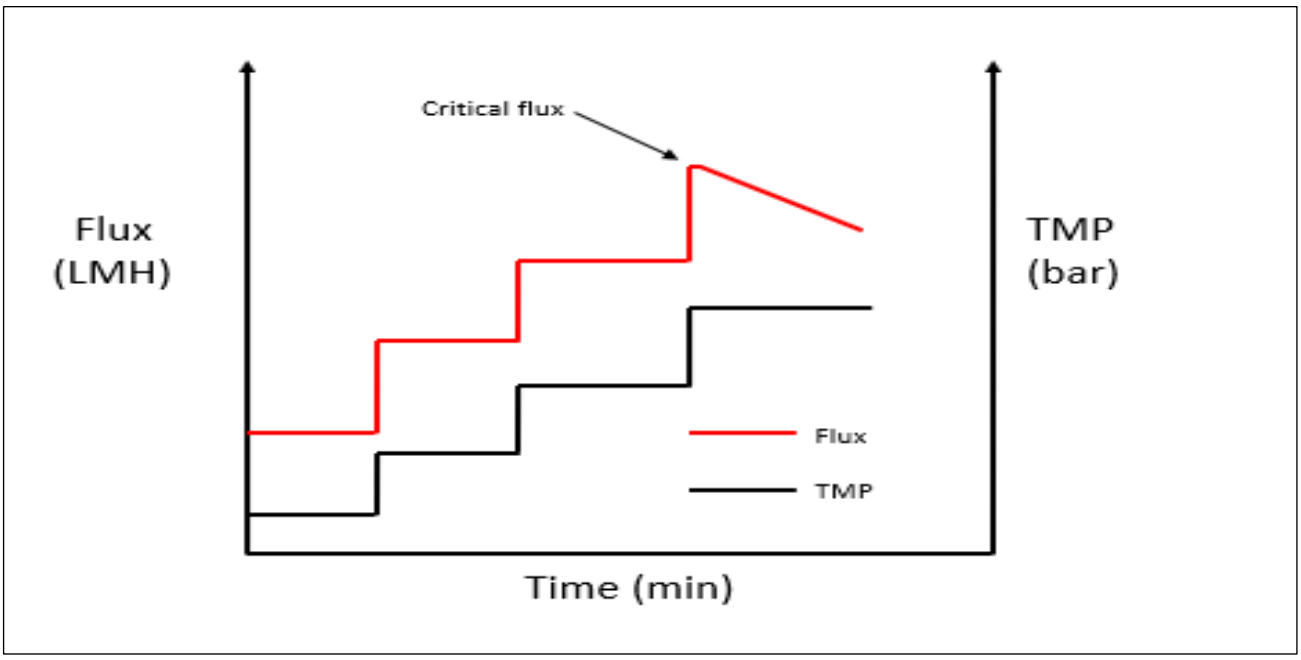


Figure 2.9: Illustration of the constant pressure operation method used in determining the critical flux, modified after Tay et al., 2007.

Two other methods used to determine the critical flux exist, but they are not as common as constant flux operation or constant pressure operation methods. They include:

(i) **Particle mass balance method** which involves monitoring the change of particle concentration in the fluid phase at the outlet stream. The extent and rate of particle deposition at the membrane surface is determined at various flux values, and the highest flux value at which no particle deposition is observed, is taken as the critical flux (Kwon et al., 2000).

(ii) **Direct observation through the membrane (DOTM) method** which involves the use of a microscope to look through transparent membranes to observe either the presence of deposited particles on the membrane surface or their absence. It is believed that there will be no deposited particles at operations below the critical flux. This method is restricted to membranes that are transparent, and the particles that can be observed must have large sizes of approximately 10 µm (in diameter) (Bacchin et al., 2006).

2.9 Membrane cleaning

Membrane cleaning is a method used to restore the permeability of fouled membranes. Therefore, cleaning of fouled membranes can be described as a process whereby the membrane module is relieved of a substance which is not its integral part (Lin et al., 2010). The cleaning processes are classified as physical and chemical cleaning. However, physical cleaning followed by chemical cleaning is widely applied in membrane applications in order to control the extent of membrane fouling and prolong the lifespan of membranes (Lin et al., 2010; Mancinelli et al., 2015).

2.9.1 Physical cleaning

Physical cleaning of porous membranes is categorized as hydraulic, pneumatic and ultrasound cleaning methods. Hydraulic cleaning is the most common and easiest method used to control the membrane fouling. It includes forward flushing and backwashing (Lin et al., 2010; Mancinelli et al., 2015). Forward flushing is usually undertaken during the filtration cycle to remove the particle concentration build-ups on the membrane (Lin et al., 2010). On the other hand, during backwashing (backpulsing) the transmembrane pressure is inverted and part of the permeate flows backward into

the cross-flow channel. This method is suitable for the removal of the surface deposits from the membrane but may be ineffective when the deposits adhere strongly or if the membrane pores were fouled (Mohammad et al., 2012).

2.9.2 Chemical cleaning

The chemical cleaning agents commonly used are categorized as (Lin et al., 2010; Mancinelli et al., 2015; Mohammad et al., 2012):

i. alkalis such as sodium hydroxide used to clean organic and microbial fouled membranes by hydrolysis and solubilization
ii. acids such as sulphuric, nitric, hydrochloric, and phosphoric citric acids used to remove precipitated salts or scalants such as calcium carbonate
iii. surfactants which include surface-active agents like anionic, cationic, non-ionic, and amphoteric electrolytes. They form micelles with fat, oil, and proteins in water and help to clean the membranes fouled by these materials. Also, surfactants can disrupt functions of bacteria cell walls and hence remove biofilms
iv. oxidants that clean membranes by reducing the adhesion of fouling materials to membranes.

The cleaning efficiency depends on the membrane applications, feed characteristics (e.g., pH, temperature, ionic strength, concentration), cleaning time and frequency, and the membrane materials (Mohammad et al., 2012). For instance, temperature is believed to have a significant impact on both the efficiency and rate of membrane cleaning by changing the reaction equilibrium, improving the reaction kinetics, and by increasing the solubility of solutes (Mancinelli et al., 2015; Mohammad et al., 2012)**.** The various ways of chemical cleaning that can be used include (Lin et al., 2010):

i. adding chemicals in the feed stream, i.e., chemical wash (CW)
ii. soaking the fouled membrane in a separate tank with higher concentration cleaning agents i.e., “clean-out-of-place (COP)”
iii. directly immersing the fouled membranes in the chemicals, i.e., “clean-in-place (CIP)”
iv. cleaning in conjunction with the physical cleaning step, i.e., a chemical enhanced backwash (CEB).

During the cleaning process, a cleaning agent can affect fouling materials present on the membrane surface in the following ways: (i) the foulants may be removed, (ii) the morphology of foulants may be changed (e.g., through swelling or compaction), and (iii) the surface chemistry of the deposit may be altered such that the hydrophobicity or charge is modified (Lin et al., 2010).

Besides the economic concerns of the chemical cleaning of membranes, there are other concerns related to environmental challenges as cleaning usually discharges chemical wastes. Hence, there is a need to implement properly designed and optimized cleaning procedures (Mohammad et al., 2012). According to Brepols et al., 2008, a superior cleaning strategy should take into account the right cleaning agents, sequence of cleaning steps and frequencies of applying each method while achieving high cleaning efficiency as well as fulfilling some objectives such as (Brepols et al., 2008):

i. being compatible with the whole treatment process
ii. reducing risks of damaging membranes
iii. minimizing plant downtime during cleaning
iv. lowering labour input
v. reducing chemical cost
vi. avoiding any hazards to plant operators.

Although chemical cleaning can be very effective in the removal of foulants as compared to other methods, it can potentially severely damage the membrane materials and thus reduces membrane lifespan (Mohammad et al., 2012). Hence, determining the cleaning requirements and frequency is vital during membrane cleaning. It has been reported that the cleaning procedures should be applied when the flux decline is higher than 10 % – 30 % (Mancinelli et al., 2015).

2.10 Membrane bioreactors (MBRs)

2.10.1 Overview

A membrane bioreactor (MBR) is a term that refers to the integration of membrane separation with bioreactor systems (e.g. fermenters) to separate microorganisms from the reaction substrates or product (Coutte et al., 2017). Membrane bioreactors have many advantages such as increased

product yield and volumetric productivity due to the possibility of retaining or recycling the microorganisms (Choudhury et al., 2006; Giorno et al., 2002). Moreover, they have low energy requirements and can be easily scaled up (Carstensen et al., 2012). Depending on how the membrane modules are connected to the bioreactors, either immersed or side-stream MBR configurations can be used (Carstensen et al., 2012).

2.10.2 Immersed membrane bioreactors

In an immersed/submerged MBR, the membrane modules are placed directly into the suspension to be filtered and air bubbles are supplied from the bioreactor bottom to control the cake layer formation (Carstensen et al., 2012; Drews et al., 2005). Less energy is needed to operate the immersed MBR compared to the side-stream MBR because the energy required for pumping is eliminated (Coutte et al., 2017). Hence, the immersed MBR has been successfully used in wastewater treatment (Ramchandran et al., 2012). The illustration of an immersed MBR configuration is shown in Figure 2.10.

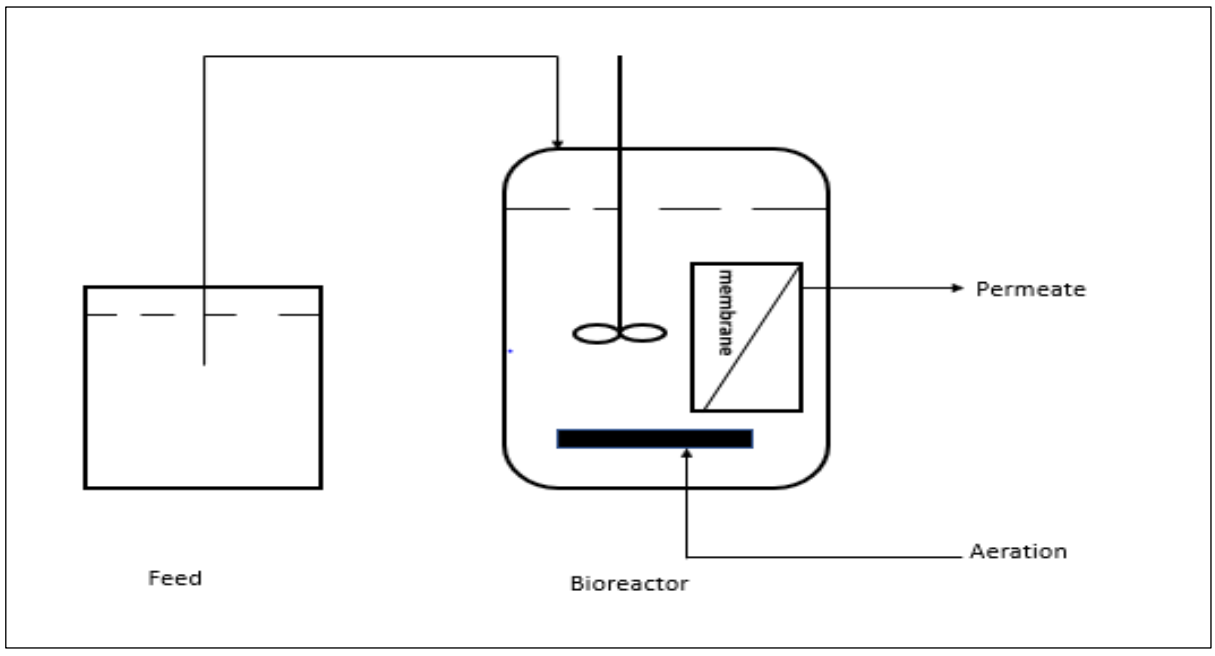


Figure 2.10: Illustration of an immersed MBR configuration, redrawn from Coutte et al., 2017.

2.10.3 Side-stream membrane bioreactors

In a side-stream/external MBR configuration, the membrane module is installed outside the bioreactor. The liquid to be filtered is then pumped through the membrane module. After that, the concentrate is recycled to the bioreactor (Coutte et al., 2017; Drews et al., 2005). The side-stream MBR configurations are preferred in biotechnology for product recovery due to the reduced membrane fouling realized by these configurations (Coutte et al., 2017). The illustration of a side-stream membrane bioreactor is shown in Figure 2.11. The present study employed a side-stream membrane bioreactor for the investigations.

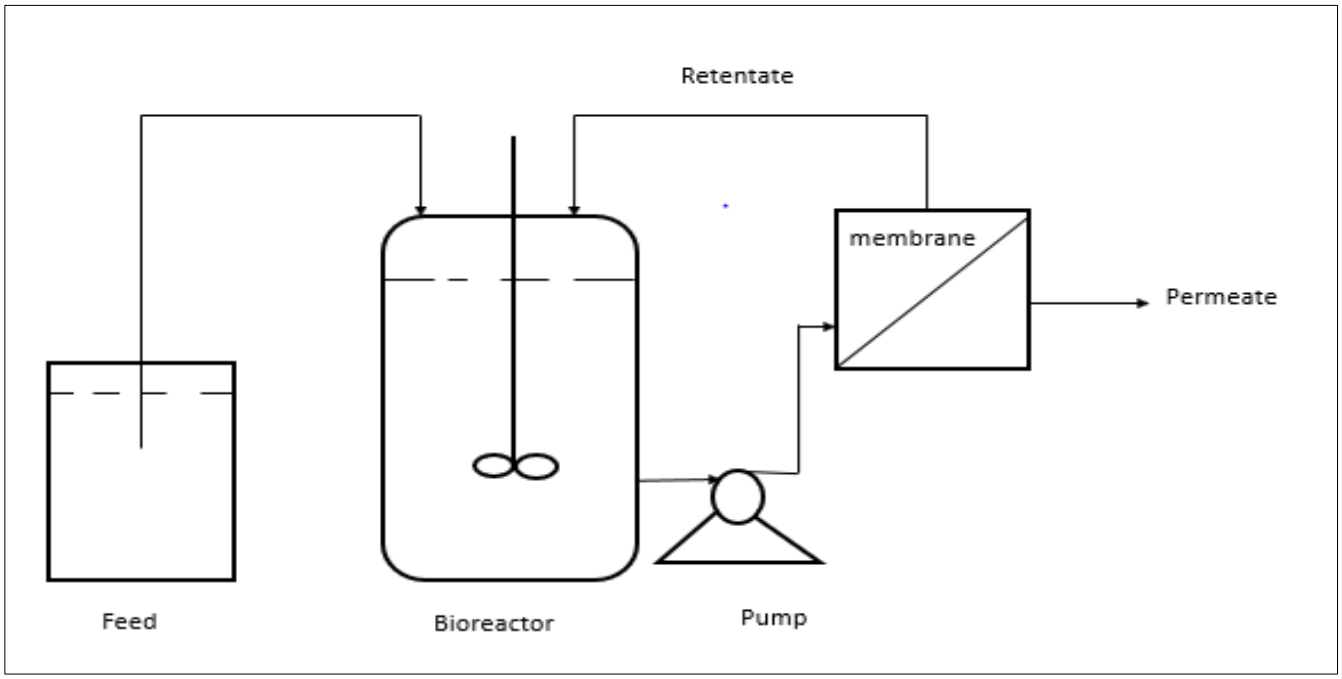


Figure 2.11: Illustration of a side-stream MBR configuration, redrawn from Coutte et al., 2017.

2.10.4 Fouling in membrane bioreactors (MBRs) and its control

Biofouling caused by extracellular polymeric substances (EPS) is the most common type of fouling in MBRs. When the microorganisms used in MBRs die, they result in EPS which are major foulants (Meng et al., 2009). Generally, fouling reduces membrane performance, reduces separation efficiency, shortens membrane lifespan and leads to more membrane cleaning or replacement increasing maintenance and operating costs (Ladewig et al., 2017). Even though biofouling cannot be avoided

entirely in MBRs, it can be controlled to a level that does not severely hinder the MBR operations. Different approaches which are used separately or in combination to control fouling include backflushing and pulsing, hydrodynamic management, membrane surface modification, flux control, feed pretreatment and effective membrane cleaning (Cui et al., 2010).

Flux control as an approach used to control fouling in MBRs involves understanding the concept of critical flux i.e., a level of flux under which fouling is minimal as stated in section 2.8.4.2. Basically, subcritical flux in MBRs can be defined as the flux where the rate of transmembrane pressure (TMP) rises at an allowable rate such that chemical cleaning is not needed (Ladewig et al., 2017).

Cleaning procedures are necessary to restore the permeability of membranes once they have fouled. Physical cleaning, chemical cleaning or a combination of both can be used (Judd, 2008; Ladewig et al., 2017). Backwashing, which is a physical cleaning technique in MBR systems, is where the effluent is delivered back through the membrane into the feed channel, removing the deposited particles on the surface of the membrane (Ladewig et al., 2017).

Chemical cleaning, usually performed at a lower frequency e.g., once per week, is used when backwashing cannot restore the flux. The chemicals to be used primarily depends on the type of foulants and the resistance of membrane to the cleaning agent (Li et al., 2008). The different chemicals that can be used are categorized into alkalis (e.g., sodium hydroxide), oxidants/disinfectants (e.g., sodium hypochlorite, hydrogen peroxide, peroxyacetic acid), acids (e.g., citric acid, nitric acid, hydrochloric acid) and surfactants/detergents. These chemicals are used to aid in hydrolyzing, solubilizing, emulsifying, oxidizing or dispersing the foulants as stated also in section 2.9.2 (Li et al., 2008). Nevertheless, in some MBR systems, chemical cleaning is limited to alkaline hypochlorite (approximately pH 12) followed by citric acid or oxalic acid (approximately pH 3) where either low strength sodium hypochlorite (i.e., 100 – 500 mg/L) is used 2 to 8 times monthly to maintain the membrane permeability, or sometimes high-strength sodium hypochlorite (i.e., 0.3 – 0.5 wt. %) is used 1 to 2 times yearly to recover the membrane permeability (Judd, 2008).

2.11 Application of membrane bioreactors (MBR) systems for lactic acid fermentation

2.11.1 Overview

Since a membrane bioreactor system allows for the integration of both fermentation and separation processes, it can simultaneously (Jeantet et al., 1996; Tejayadi et al., 1995):

(i) maintain high cell density in the fermenter
(ii) recycle the bacterial cells back to the fermenter for further use
(iii) remove the inhibitory product (lactic acid) from the fermenter.

Owing to the abovementioned benefits of membrane bioreactor systems, they have attracted great attention to ensure high lactic acid productivities in continuous lactic acid fermentations (Kwon, 2001; Senthuran et al., 1997). The nominal molecular weight cut-offs (MWCO) are used to characterize the membranes used in membrane bioreactor systems such that all the molecules larger than the MWCO of a particular membrane are retained by the membrane whereas molecules smaller than the MWCO can pass through the membrane (Li et al., 2006). Therefore, the product removal and retention of the microorganisms both depend on the molecular weight cut off (MWCO) of a particular membrane (Marques et al., 2017). Basically, an ideal membrane to be used in a membrane bioreactor system should possess a molecular weight cut-off (MWCO) large enough to allow the free passage of product and yet small enough to retain the microorganisms (Zhang et al., 1994).

In lactic acid fermentation, either microfiltration (MF) or ultrafiltration (UF) membranes with molecular weight cut-off (MWCO) values of at least 100 – 300 kDa can be used to recover bacterial cells from the lactic acid fermentation broth with none of these cells being detected in the permeate (Milcent et al., 2001). However, UF membranes are usually preferred to MF membranes since the internal fouling tendency is more significant in the MF membrane processes than in the UF membrane processes (Lee et al., 2017). Owing to the larger membrane pore sizes in MF membrane than those of the UF membrane (Richard Baker, 2012), the tendency of more particles being

adsorbed into the MF membrane's inner pores increases, and thus causing the significant internal fouling (Lee et al., 2017).

2.11.2 Previous studies on the application of MBR systems for lactic acid fermentation

Membrane bioreactor (MBR) systems have been widely used in aerobic wastewater treatments; hence, they can be considered as a mature technology in that field (Van Hecke et al., 2017). Nonetheless, their full-scale applications for anaerobic treatments such as those applied in the production of organic acids are still limited (Van Hecke et al., 2017). In view of the promising benefits of MBR systems stated in section 2.11.1, several researchers have investigated the possibility of applying these MBR systems for lactic acid fermentation. This section provides some of those previous studies which focused mainly on improving the lactic acid (LA) productivity as shown in Table 2.5.

From Table 2.5, it is clearly that high lactic acid productivity can be achieved using MBR systems compared to batch fermentation. For instance, the maximum lactic acid productivity obtained by Mehaia et al., 1986 when they externally connected an ultrafiltration membrane to a fermenter was ten times higher than that of a batch fermentation (Mehaia et al., 1986). Similarly, Lu et al., 2012 used a microfiltration membrane externally connected to a fermenter and obtained a maximum lactic acid productivity which was 315.64 % higher than that of a batch fermentation (Lu et al., 2012). The high lactic acid productivity obtained in MBR systems has been attributed to the fact that they can alleviate the end-product inhibition that dominate the conventional batch fermentation as well as maintain high cell density in the fermenter (Tejayadi et al., 1995; Xu et al., 2006). These previous studies, therefore, provide strong evidence that MBR systems could greatly contribute to efficient and economical lactic acid fermentation compared to the conventional batch fermentation.

Table 2.5: Previous studies on the application of MBR systems for lactic acid (LA) fermentation

	MBR configuration	Microorganism	Substrate	Maximum LA concentration (g/L)	Maximum LA productivity [g/(L.h)]	Reference
1	Hollow fibre UF [(1)] membrane externally connected to a fermenter	*Lactobacillus bulgaricus*	Lactose in whey permeate	–	85 (*10 times higher than that of batch fermentation*)	Mehaia et al. (1986)
2	MF [(2)] membrane externally connected to a fermenter	*Lactobacillus paracasei*	Glucose	76	31.5 *(10 times greater than that of fed-batch fermentation)*	Xu et al. (2006)
3	UF membrane externally connected to a fermenter	*Lactobacillus* sp. RKY 2	Glucose	69.8	6.7 *(1.6 times higher than that of continuous fermentation without cell recycling)*	Wee et al. (2009)
4	MF membrane externally connected to 3000 litre-fermenter (*pilot-scale study*)	*Lactobacillus rhamnosus*	Yam tuber and persimmon	157	8.77 *(315.64 % higher than those of batch fermentation)*	Lu et al. (2012)
5	Hollow fibre UF membrane externally connected to a fermenter	*Lactobacillus rhamnosus*	Sweet sorghum juice	–	13.77 *(15.47 times higher than that of batch fermentation)*	Yong Wang et al. (2016)
6	Tubular UF membrane externally connected to a fermenter	*Bacillus coagulans* PS5	Glucose	42	8.4 *(5 times higher than that of batch fermentation)*	Fan et al. (2017a)

Note: UF [(1)] = Ultrafiltration , MF [(2)] = Microfiltration and (–) means not provided by the authors.

2.11.3 Research gaps in the literature and what the present study aimed to investigate

For more than three decades, membrane bioreactor (MBR) systems have proved to be efficient in improving lactic acid productivity as shown in Table 2.5. Nevertheless, with these vast improvements in lactic acid productivity by MBR systems, none of them have been upscaled for industrial applications. Seemingly, there are some process limitations that hinder their upscaling, and hence they need to be identified and solved. Therefore, the present study focused on identifying some of these process limitations with the intention of providing possible solutions from an engineering perspective.

Based on the information available in the literature concerning lactic acid fermentation coupled with the knowledge on the operations of a typical MBR system, possible process limitations of the MBR systems used for lactic acid fermentation may be postulated. These include:

(i) Membrane fouling limitations

In membrane separation processes, membrane fouling is inevitable. However, its severity can be minimized through proper membrane operating conditions (Mohammad et al., 2012; Zhang et al., 2015). Although membrane fouling has been reported in the previous studies involving the MBR systems used for lactic acid fermentation (Fan et al., 2017a; Giorno et al., 2002), it was postulated that this had been the case probably as a result of employing improper membrane operating conditions.

(ii) Limitations attributed to lactate inhibition of bacterial cells once the membrane has fouled

During continuous lactic acid fermentation, the pH of the fermentation broth is usually controlled by adding neutralizing agents such as sodium hydroxide as discussed in section 2.4.5.1. This enables the conversion of inhibitory lactic acid to the less inhibitory lactate form. Nevertheless, it has been reported that the accumulation of both undissociated lactic acid and dissociated lactate in the fermenter can lead to inhibition during lactic acid fermentation (Hetényi et al., 2011; Rault et al., 2009). Therefore, one main reason for integrating the membrane to the fermenter during lactic acid

fermentation is to continuously remove the lactate from the fermenter to avoid such inhibitory effects (Tejayadi et al., 1995). Since membrane fouling cannot be avoided in membrane separation processes, it was therefore postulated that once the membrane has fouled and the removal of lactate has reduced, then the bacterial cell growth can be inhibited by the accumulated lactate and subsequently lead to reduced lactic acid production rate.

(iii) Nutrient limitations

Lactic acid bacteria have complex nutritional requirements due to their inability to synthesize their own growth factors (Narayanan et al., 2004). Therefore, during lactic acid fermentation, various nutrients which include the carbon sources, nitrogen sources and mineral salts are provided as discussed in section 2.4.3. As the fermentation progresses, these nutrients are consumed by the bacteria to enhance their growth and produce lactic acid (Hofvendahl et al., 2000; Monteagudo et al., 1997). The bacterial cell growth is expected to increase when there are no nutrient limitations in the fermenter, however, when one or more nutrients become limiting, there will be a sharp drop in the specific growth rate of the bacteria (Leroy et al., 2001). Due to the complexity of nutrient requirements by the bacteria, it remains unclear which nutrients specifically are responsible for the limitations of the bacterial cell growth, but sugars and amino acids have been regarded as the main nutrients that may cause these limitations (Leroy et al., 2001). In the present study, it was postulated that the increase in cell density in the fermenter as a result of cell recycling would probably lead to rapid consumption of the available nutrients and result in nutrient limitations.

(iv) Mass transfer limitations

One major advantage of continuous lactic acid fermentation using MBR systems is the high cell concentrations achieved in the fermenter as a result of cell recycling (Lu et al., 2012; Tejayadi et al., 1995). Therefore, it was postulated that this increased cell concentration in the fermenter could possibly result in mass transfer limitations and subsequently affect the substrate utilization leading to unutilized substrate in the fermenter.

2.12 Conclusions

In recent years, the demand for lactic acid has greatly increased due to its application in the manufacture of biodegradable and biocompatible polylactic acid (PLA) polymers. Nonetheless, efficient and economical lactic acid fermentation still remains a challenge. The conventional batch fermentation is limited by end-product inhibition to the bacterial cells, low cell density in the fermenter, and consequently low lactic acid productivity.

Several investigations have therefore focused on the possibility of avoiding these limitations of the conventional batch fermentation to ensure efficient and economical lactic acid fermentation. These investigations have focused on reactive extraction, adsorption, and membrane bioreactor (MBR) systems specifically with the aim of alleviating end-product inhibition and subsequently improving the lactic acid productivity. From these investigations, MBR systems have proved to be efficient in not only removing the inhibitory product from the fermenter but also maintaining high cell density in the fermenter. Hence, the lactic acid productivity obtained from these MBR systems has improved significantly.

Although the significant improvements in lactic acid productivity by the MBR systems have been evident, none of these systems have been upscaled for industrial applications. The present study postulated that there are some process limitations such as membrane fouling limitations, lactate inhibition to bacterial cells once the membrane has fouled limitations, nutrients limitations, and mass transfer limitations that could possibly hinder the upscaling of these MBR systems for lactic acid fermentation at industrial scale. Hence, the focus of the present study was to identify these postulated process limitations with the intention of providing possible solutions from an engineering perspective.

Chapter 3 Materials and methods

3.1 Membrane bioreactor (MBR) system

The membrane bioreactor (MBR) system used in this study did not exist previously, but it was set up specifically for the purposes of conducting the investigations of the present study on a laboratory scale. Different components such as a fermentation vessel, a suitable membrane, a temperature control system, an agitation system, a pH control system, pressure gauges, diaphragm valves and peristaltic pumps among others were required to set up the MBR system. Hence, this section provides the details of these components and how some of them were built.

3.1.1 Selection of a suitable membrane

Following the explanations given in Chapter 2, that either a microfiltration (MF) or an ultrafiltration (UF) membrane with a molecular weight cut off (MWCO) value of at least 100 – 300 kDa can be used for the bacterial cell recovery from lactic acid fermentation broth, and that UF membranes are superior to MF membranes in terms of the minimal tendency of internal fouling, the present study used a 100 kDa ceramic UF membrane. This choice was further dictated by the fact that lactic acid has a molecular weight of 90.1 g/mol (approximately 0.09 kDa) (Marques et al., 2017) and most lactic acid-producing microorganisms have a size of 1 – 5 µm, which is larger than the UF membrane pore sizes (Lee et al., 2017). Hence, lactic acid can permeate the membrane whereas all the bacterial cells can be recovered from the broth. To confirm this further, the permeate was analyzed for the bacterial cells during the preliminary experiments and it turned out that there were no bacterial cells detected, hence, this confirmed that the 100 kDa ceramic UF membrane was suitable to use for total cell recovery. The characteristics of the UF membrane used in the present study are summarized in Table 3.1.

Table 3.1: Characteristics of the ultrafiltration membrane used in the present study

Membrane characteristic	Specification
Manufacturer	atech innovations Gmbh (Gladbeck, Germany)
Type of membrane module	Mono-channel tubular
MWCO	100 kDa
Membrane material	Ceramic (Al_2O_3, TiO_2)
Pore size	0.05 µm
Membrane dimensions	Internal diameter = 6 mm ; External diameter = 10 mm ; Length = 300 mm
Membrane surface area	0.005655 m^2
pH range	0 – 14
Maximum inlet pressure	10 bar

3.1.2 Fermentation vessel

An autoclavable 1.8-litre glass vessel was used as a fermenter. During the batch fermentation runs, the vessel's lid had ports for the pH probe and neutralizing agent. The lid was then modified during the continuous fermentation runs by adding more ports. In other words, the port for fresh feed into the fermenter, the port for removing the fermentation broth from the fermenter to the membrane module and the port to allow retentate back to the fermenter were added on the lid. Afterward, autoclavable stainless steel pipes were fitted on those ports to allow for pumping of the fermentation broth from the fermenter to the membrane module, feeding of the fresh nutrients into the fermenter and recycling of the cells back into the fermenter (i.e., the retentate side).

3.1.3 Temperature control and agitation systems

To maintain the required temperature during the fermentation runs, the fermenter was placed in a water bath connected to continuous cold water circulating through a heater. The heater was adjusted to the required temperature and sterilized thermometer was used to confirm this temperature inside the fermenter. The water bath was placed on a magnetic stirrer which was used for agitation purposes. The magnetic stirrer allowed for the adjustment of the required agitation speed depending on the demands of the investigation because its agitation speed range was 0 to 1500 rpm. To achieve this agitation, an autoclavable magnetic stirrer rod (approximately 48 mm in length) was placed inside the fermenter.

3.1.4 pH control system

pH control was necessary during the fermentation runs as described in Chapter 2. Therefore, a pH control system was built. First, the components of the pH control system were purchased as follows:

(i) BL 931700 pH controller and pH probes from Hanna Instruments (Cape Town, South Africa)
(ii) pH controller dosing pump from Micro Robotics (Stellenbosch, South Africa)
(iii) plastic enclosures from ElectroMechanica (Pty) Ltd (Cape Town, South Africa).

These components were then assembled in the workshop as per the instruction manual that accompanied each of them. Before any fermentation run was started, the pH controller was calibrated as indicated in the instruction manual and the set point value was adjusted to the required pH. Also, to ensure that the pH control system operated in an automatic mode, the " OFF/Auto/ON" switch was always put at the "Auto" position. The calibration procedure for the pH controller and the solutions used for the calibration, cleaning and storage of the pH probes are summarized in Appendix C.

The neutralizing agent (sodium hydroxide solution) was placed in a 500 mL Schott bottle and connected to the pH controller dosing pump using autoclavable silicon tubing.

3.1.5 Other components of the membrane bioreactor system

The membrane bioreactor system consisted of the ultrafiltration membrane module externally connected to the fermenter. A Masterflex peristaltic pump (model 77800-50) was used to pump the fermentation broth from the fermenter through the membrane module, whereby the cells were recovered and recycled back to the fermenter through the retentate side while the lactate and other components of the broth were collected in the permeate.

In order to determine the pump flow rates at different pump setting positions, and the corresponding cross-flow velocities, the peristaltic pump was calibrated by pumping reverse osmosis water for a given period of time through tubing and recording the volume of the water collected in a measuring cylinder placed at the end of the tubing. The volume of the water collected and the time recorded using the stopwatch were then used to calculate the flow rates. These flow rates were further used

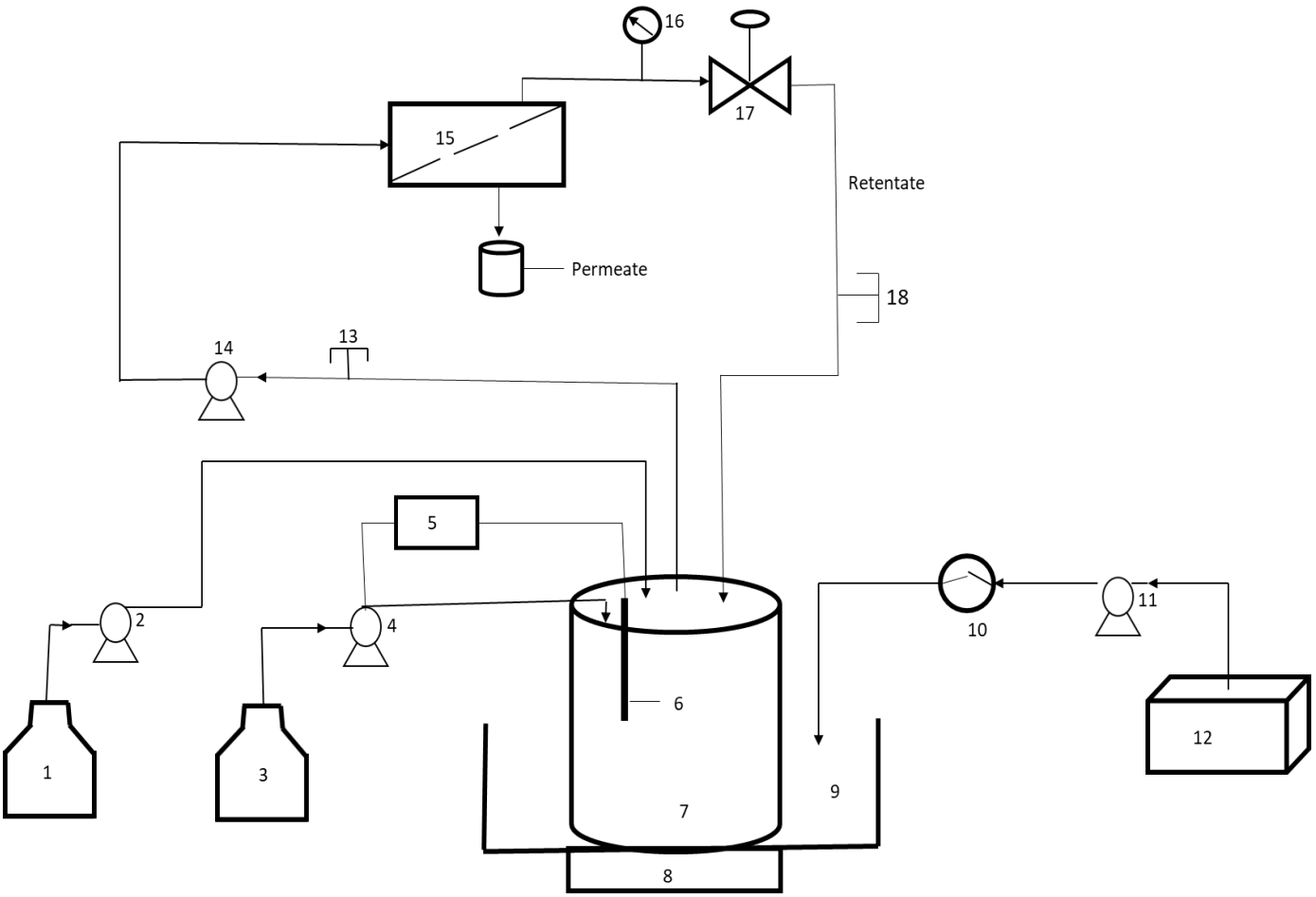
to calculate the cross-flow velocities using the formula indicated in section 3.5. The pump calibration data and the corresponding cross-flow velocities are shown in Appendix A Table A.1.

A pressure gauge was necessary to monitor the transmembrane pressure (TMP) during the operation of the membrane bioreactor system. Therefore, the pressure gauge (0 – 100 kPa) used in the present study was purchased from WIKA instruments (Cape Town, South Africa). Autoclavable stainless steel fittings were then used for the pressure gauge. However, since the pressure gauge itself could not be autoclaved, it was sterilized using 70 % ethanol before any fermentation run could be carried out to prevent any possible contamination of the sterile fermentation broth. To adjust the TMP during the experiments, a manually operated autoclavable diaphragm valve (made of stainless steel) and purchased from GEMU Valves Africa (Pty) Ltd (Johannesburg, South Africa) was used.

The fresh feed into the fermenter during the continuous fermentation runs was pumped using a small peristaltic dosing pump (BT 100 MH pump). This pump was suitable since it allowed the adjustment of the flow rate to as low as 0.05 mL/min (with the maximum flow rate being 19.8 mL/min). This made it possible to adjust the feed flow rate to match the permeate flow rate so as to maintain the constant working volume of the fermenter.

It is important to mention that some of the equipment used in the set-up of the MBR system could be affected by the scheduled or unexpected power loss (either as a result of loadshedding or any other technical difficulties). Therefore, an uninterruptible power supply (UPS) (Capacity: 3000 VA/2700 W) was used to ensure that the fermentations could continue without any interruption. The dosing pump for the fresh feed into the fermenter and the magnetic stirrers were connected to the UPS system because they were not able to restart once the generator started in case of the power loss. All the other equipment could automatically restart once the generator had started.

Figure 3.1 shows the schematic diagram of the membrane bioreactor system used for the continuous fermentation runs. A photo of the membrane bioreactor system is shown in Appendix C.



1. Fresh feed (nutrients)
2. Peristaltic dosing pump
3. Neutralizing agent (6 M NaOH)
4. pH controller dosing pump
5. pH controller
6. pH probe
7. Fermenter
8. Magnetic stirrer
9. Water bath
10. Heater
11. Pump
12. Cold water
13. Sampling point for the feed to the membrane module
14. Masterflex peristaltic pump
15. Ultrafiltration (UF) membrane
16. Pressure gauge
17. Diaphragm valve
18. Sampling point for the retentate

Figure 3.1: Schematic diagram of the membrane bioreactor system used in continuous fermentation runs

3.2 Lactic acid-producing microorganism

3.2.1 Type of the microorganism used

Lactobacillus casei subsp. *casei* ATCC 393 was used throughout the present study. *Lactobacillus casei* is a homofermentative *Lactobacillus* strain that produces mainly the L (+)-lactic acid (Büyükkileci et al., 2004; Hujanen et al., 2001). *Lactobacillus casei* subsp. *casei* ATCC 393 was purchased from American Type Culture Collection (ATCC) (United Kingdom) through the LGC Standards offices (Johannesburg, South Africa) after obtaining a permit for the importation of controlled goods from the Department of Agriculture, Forestry and Fisheries (Republic of South Africa).

Lactobacillus casei subsp. *casei* ATCC 393 is a suitable bacterial strain for lactic acid fermentation using glucose as a substrate as described in Chapter 2. Furthermore, some of the previous studies that used this bacterial strain obtained more than 75 % of lactic acid yield on the substrate consumed (Alonso et al., 2010; Kaavessina et al., 2017).

3.2.2 Storage of the microorganism

Lactobacillus casei subsp. *casei* ATCC 393 was purchased as a freeze-dried culture. To revive the freeze-dried culture, an instructional guide that accompanied the product from the supplier was used. 0.5 mL of sterile De Man, Rogosa and Sharpe (MRS) broth (Merck, Darmstadt, Germany) was used to rehydrate the freeze-dried culture. The rehydrated culture was transferred aseptically into a test tube containing 5 mL of sterile MRS broth and then incubated at 37 ℃ for 48 h under stationary conditions. MRS broth is a medium introduced by De Man, Rogosa and Sharpe for the cultivation of *Lactobacillus* species (Rodrigues et al., 2006), with the following composition (g/L): peptone 10.0; meat extract 5.0; yeast extract 5.0; dextrose 20.0; di-potassium hydrogen phosphate 2.0; Tween – 80: 1.0; tri-ammonium citrate 2.0; magnesium sulphate 0.1; manganese sulphate 0.05 and sodium acetate 5.0. The MRS broth was sterilized by autoclaving at 121 ℃ for 20 min prior to use. After reviving the freeze-dried culture, 2 mL aliquots of the broth culture were maintained in 30 % (v/v) sterile glycerol at – 80℃ for further use.

3.2.3 Inoculum preparation

The stock cultures from the freezer (– 80 ℃) were used throughout the study. To revive the frozen culture, 0.2 mL of the thawed culture was mixed with 5 mL of MRS broth in a test tube and incubated on a rotating wheel at 37 ℃ for 24 h. Using a sterilized inoculation loop, a drop of the test tube culture was then streaked aseptically onto the surface of a MRS agar plate (MRS broth solidified with 1.5 % w/v bacteriological agar after autoclaving). The petri dish was sealed with parafilm and incubated for 2 to 3 days at 37 ℃ until the colonies formed on the surface.

To prepare the fermenter inoculum, a sterile pipette tip was used to transfer 3 to 4 colonies from the MRS agar plate into each 250 mL Erlenmeyer flask containing 150 mL sterile MRS broth. Each flask was capped with a cotton wool plug covered with aluminium foil prior to autoclaving. The flasks were then incubated at 37 ℃ for 24 h under stationary conditions. This 24 h-old culture was then used as the fermenter inoculum to initiate the fermentation process.

The bacterial colonies on the MRS agar plates only remained viable for approximately 2 weeks when stored at room temperature. To maintain viable cells, a sterilized inoculation loop was used to transfer 3 to 4 colonies to a fresh MRS plate every 2 weeks. When the colonies on the MRS agar plate were no longer viable i.e., streaking onto a new plate did not result into visible growth within 2 to 3 days, a new freezer stock culture was revived as described previously. This occurred on average every 6 to 8 weeks.

3.3 Experimental procedures

The experimental procedures for batch fermentation runs, critical flux experiments, pure water flux experiments and continuous fermentation runs employed in the present study are discussed in this section.

3.3.1 Batch fermentation

3.3.1.1 Planned schedule of the experimental runs

The initial plan was to have a single set of batch fermentation runs started at a particular time and stopped after 24 h, whereby samples were to be taken at regular time intervals of 3 h. However, this was not possible due to the challenges mentioned below.

3.3.1.2 Challenges that hindered the implementation of the planned schedule of the experimental runs

The COVID-19 pandemic that led to a national lockdown and a nationwide curfew running from 10 pm to 4 am hindered the implementation of the planned schedule of the experimental runs because there was no access to the laboratory between those times (i.e., 10 pm to 4 am). Therefore, the planned schedule had to be modified to obtain the data for the 24 h batch fermentation runs.

3.3.1.3 Modified schedule of the experimental runs adopted

To obtain the data for the 24 h batch fermentation runs, two sets of batch fermentation experiments were carried out and the results obtained from each set of the experiments were combined to generate the batch fermentation curve.

- The first set of experiments was started at **9 am** and the samples were taken from 12 noon (at the interval of 3 h) after 3, 6 and 9 hours of fermentation.
- The second set of experiments was started at **7 pm** and the samples were taken the following day from 7 am (at the interval of 3 h) after 12, 15, 18, 21 and 24 hours of fermentation.

It is worth mentioning that the same inoculum was used for both sets of experiments started on a particular day (i.e., the one started at 9 am and the other one started at 7 pm used an inoculum incubated at the same time), and each set of the experiments was carried out in triplicate to ensure repeatability. Table 3.2 shows the sampling times for the 1st set and 2nd set of the batch fermentation experiments.

Table 3.2: The sampling times for the 1st set and 2nd set of the batch fermentation experiments

Time (h)	1st set of experiments (sampling times)	2nd set of experiments (sampling times)
0	-	-
3	12:00 PM	
6	3:00 PM	
9	6:00 PM	
12		7:00 AM
15		10:00 AM
18		1:00 PM
21		4:00 PM
24		7:00 PM

3.3.1.4 Batch fermentation runs

Before starting any batch fermentation run, the fermentation medium was prepared. The components of the fermentation medium were based on the description given in Chapter 2 section 2.4.3. However, the typical concentrations of these components of the fermentation medium (as shown in Table 3.3) were modified from those previously suggested by Alonso et al. (2010), Ding et al. (2006) and Pérez et al. (2019) to suit the present study except for the initial glucose concentration which was 30 g/L to avoid restricting the metabolism of the bacterial cells as described in Chapter 2 section 2.4.5.4. All the components of the fermentation medium were purchased from Merck (Darmstadt, Germany) except for the peptone, ammonium citrate tribasic and Tween-80, which were purchased from Sigma Aldrich (Johannesburg, South Africa). These chemicals were of analytical grade with a purity of more than 97 %, hence they were used as purchased. Glucose was used as the substrate throughout the present study.

Table 3.3: Concentrations of the components of the fermentation medium for batch fermentation runs

Component of the fermentation medium	Concentration (g/L of deionized water)
D (+) – glucose	30
Peptone from animal tissue	10
Yeast extract	10
Ammonium citrate tribasic	2
Sodium acetate trihydrate	5
Dipotassium hydrogen phosphate	2
Magnesium sulphate heptahydrate	0.2
Manganese sulphate tetrahydrate	0.05
Tween – 80 (*in liquid form*)	1 mL

The batch fermentation runs were carried out in a 1.8 litre fermenter with a total working volume of 1.5 litres. Once the fermentation medium was prepared, it was autoclaved at 121 ℃ for 20 minutes for sterilization purposes and left to cool before aseptically transferring it to the sterilized fermenter in a laminar flow hood. The fermentation medium in the fermenter was then aseptically inoculated with 10 % (v/v) of a 24 h-old inoculum (in a laminar flow hood) to initiate the fermentation process. This inoculum had a biomass concentration of 4.41 ± 0.18 g/L (determined by measuring optical density of the inoculum at 620 nm and converting it to cell dry weight using a calibration curve as described in section 3.4.2). The laminar flow hood is equipped with ultraviolet (UV) light which helps to reduce the contamination of the bacterial cells by inducing DNA damage to the potential contaminants e.g., viruses, bacteria or any other extra microorganisms that may be introduced in the working area by any means (Bykowski et al., 2008). Hence, the present study ensured strict adherence to aseptic conditions when handling the sterilized fermentation medium, fermenter, and inoculum with the help of a laminar flow hood.

Throughout the fermentation process, the fermentation medium was maintained at a pH of 6.5 by the automatic addition of 6 M sodium hydroxide (NaOH) solution as a neutralizing agent using the pH controller system. A strong and concentrated base (i.e., 6 M NaOH) was used as the neutralizing agent since the fermenter volume was small (i.e., 1.8 litres) compared to the total working volume (i.e., 1.5 litres), and thus only a small volume of the neutralizing agent was feasible during the fermentation process to avoid overflow which was possible if a weak and dilute base had been used. The fermenter was placed on a water bath connected to continuous cold water circulating through a

heater to maintain the temperature at 37 ℃. The temperature and pH used during the batch fermentation runs were the optimal conditions for the growth of *Lactobacillus casei* ATCC 393 as previously described in Chapter 2.

The water bath was placed on a magnetic stirrer, and a magnetic stirrer rod was placed inside the fermenter to aid in agitation for proper mixing of the fermentation medium. The agitation speed was set at 150 rpm (Ding et al., 2006). The batch fermentation runs were terminated once the automatic addition of sodium hydroxide had stopped. In a typical lactic acid fermentation, the automatic termination of significant neutralization indicates the absence of active fermentation (Kuznetsov et al., 2017; Milcent et al., 2001). For the present study, the batch fermentation runs lasted for 24 hours. Throughout the batch fermentation runs, 2 mL samples were taken every 3 hours for the analysis of glucose, lactate, and biomass concentrations. The fermentation runs were carried out in triplicate and the average values of the experimental data were presented with the standard deviation.

3.3.2 Critical flux experiments

As previously mentioned in Chapter 2, the constant pressure operation method was used to determine the critical flux in the present study. Two sets of critical flux experiments were carried out:

(a) critical flux experiments using bacterial cells

In this set of experiments, a 24 h-old bacterial culture was prepared as described in section 3.2.3 and used. The bacterial cells of biomass concentrations of 3.99 ± 0.02 g/L were investigated at three cross-flow velocities which included both the low and high cross-flow velocities i.e., 0.27, 0.50 and 0.90 m/s. The experiments were carried out in duplicate to ensure repeatability.

(b) critical flux experiments using lactate fermentation broths of different biomass concentrations

The lactate fermentation broths used for these experiments were obtained from batch fermentation runs. The experimental procedure for the batch fermentation runs was similar to that described in section 3.3.1.4. All of the batch fermentation runs were carried out on different days to obtain lactate fermentation broths of different biomass concentrations as follows:

(i) started the first batch fermentation run (with inoculum having biomass concentration of 3.42 g/L), stopped it after 6 h and used the broth for critical flux experiments

(ii) started the second batch fermentation run (with inoculum having biomass concentration of 3.48 g/L), stopped it after 15 h and used the broth for critical flux experiments

(iii) started the third batch fermentation run (with inoculum having biomass concentration of 3.45 g/L), stopped it after 22 h and used the broth for critical flux experiments.

The biomass concentrations obtained were 3.82 g/L, 5.37 g/L and 6.03 g/L for the 6 h, 15 h and 22 h lactate fermentation broths respectively. Each lactate fermentation broth was investigated at three cross-flow velocities i.e., 0.27, 0.50 and 0.81 m/s**.** Moreover, to make the experiments comparable, a similar ultrafiltration membrane module was used for all the critical flux experiments.

For each set of the critical flux experiments, the following experimental procedure was adopted:

1. The first step was to select the cross-flow velocity to operate at. The bacterial cells/lactate fermentation broths in the fermenter were then pumped through the membrane module.
2. The measuring cylinder was placed at the outlet of the permeate flow of the membrane.
3. The diaphragm valve was adjusted to attain a pressure of 0.1 bar.
4. The stopwatch was started and the permeate was allowed to flow for 2 minutes.
5. Using the volume of permeate collected in the measuring cylinder and the stopwatch reading, the permeate flux was calculated as shown in section 3.5.
6. Steps 4 and 5 were repeated at 4, 6, 8 and 10 minutes.
7. The diaphragm valve was then adjusted to attain a pressure of 0.2 bar.
8. Steps 4 – 6 were repeated.
9. The pressure was increased in small increments in a similar manner up until the permeate flux started to decrease over time. In the present study, the experimental rig could not withstand a transmembrane pressure of more than 0.5 bar.
10. The critical flux point was identified as the point where the flux decreased for the increasing pressure**.**
11. The membrane used was thoroughly cleaned until the initial pure water flux of the membrane was obtained and then steps 1 – 10 were repeated for the other remaining cross-flow velocities.

Since the concentration in the fermenter needed to remain constant, the permeate was returned back into the fermenter during the critical flux experiments using the lactate fermentation broths. However, for those of bacterial cells, fresh culture was added into the fermenter instead of returning the permeate to avoid any possible contamination.

One major challenge experienced with the critical flux experiments using the lactate fermentation broths was the repeatability of the experiments. As previously mentioned in Chapter 2 section 2.8.4.2; the exact value of the critical flux depends on a specific feed concentration (i.e., biomass concentration), it was not possible to start another batch fermentation for a repeat run, stop it at a particular time and get exactly similar biomass concentration in that broth as it was in the first run. To overcome this challenge, the broths used in the critical flux experiments were therefore chosen to accommodate all the possible biomass concentrations that could result in a batch fermenter. In other words, the broths used were extracted after 6 h, 15 h and 22 h to accommodate all the possible biomass concentrations in a batch fermenter, and each broth was investigated at three cross-flow velocities.

Nevertheless, from the results of the critical flux experiments using bacterial cells that were carried out in duplicate, the results obtained were nearly the same for the two runs since the membrane was thoroughly cleaned between the runs and the biomass concentrations were kept constant for both runs. Hence, it was assumed that the similar trend could possibly apply to the critical flux experiments using the lactate fermentation broths provided the feed concentration remained similar. To confirm this further, the trends in the literature were checked. All the results of the critical flux experiments using the lactate fermentation broths followed the trends in the literature as discussed in the results and discussion chapter.

3.3.3 Pure water flux (PWF) experiments and membrane cleaning

The PWF experiments were carried out to:

(i) determine the initial performance of the ultrafiltration membrane before being used. The PWF curve obtained here served as the basis for the future membrane cleaning processes

(ii) confirm if the membrane cleaning process was effective. After a particular experimental run, the fouled membrane was cleaned and PWF experiments performed to confirm if the membrane was able to regain its initial permeability before starting the subsequent experiments.

The experimental procedure adopted for the pure water flux (PWF) experiments was almost similar to that of critical flux experiments except that for PWF experiments only one cross-flow velocity of 0.38 m/s (i.e., pump setting position 2) was employed. In the present study, the term “pure water” referred to reverse osmosis (RO) water. The experimental procedure for the PWF experiments was as follows:

1. The RO water was pumped through the membrane module.
2. The measuring cylinder was placed at the outlet of the permeate flow of the membrane.
3. The diaphragm valve was adjusted to attain a pressure of 0.1 bar.
4. The stopwatch was started and the permeate was allowed to flow for 2 minutes.
5. Using the volume of permeate collected in the measuring cylinder and the stopwatch reading, the permeate flux was calculated as shown in section 3.5.
6. Steps 4 and 5 were repeated at 4, 6, 8 and 10 minutes.
7. The diaphragm valve was then adjusted to attain a pressure of 0.2 bar.
8. Steps 4 – 6 were repeated.
9. The pressure was increased in small increments in a similar manner up to 0.6 bar.
10. The whole experimental procedure was then repeated for the decreasing pressure i.e., starting from 0.6 bar until 0.1 bar.

A plot of permeate flux and transmembrane pressure (TMP) for the pure water resulted in a linear relationship (as shown in Appendix A Figure A.2) because the permeate flux increased with the increasing TMP.

The membrane cleaning method used in the present study was based on the descriptions of Chapter 2 section 2.9 as well as the recommendations from the supplier of the membrane on the cleaning protocol to adopt. After a particular experimental run, the fouled membrane was cleaned as follows:

(i) reverse osmosis (RO) water was flushed through the membrane for 15 min

(ii) backflushing with RO water was then carried out for 10 min

(iii) the membrane was then soaked overnight in 0.5 M sodium hydroxide (NaOH) solution (pH 12)

(iv) the membrane was then cleaned by circulating 200 ppm sodium hypochlorite (NaOCl) solution for 10 min

(v) finally, the membrane was rinsed with RO water for 15 min to remove any residual NaOCl.

All the membrane cleaning processes were carried out at a cross-flow velocity of 0.50 m/s (i.e., pump setting position 3). Pure water flux (PWF) experiments were then performed on the cleaned membrane to confirm if the cleaning process was effective.

3.3.4 Continuous fermentation using the membrane bioreactor system

3.3.4.1 Planned sampling times and the challenges that hindered it

As mentioned in section 3.3.1.2, the national lockdown and nationwide curfew (due to the COVID-19 pandemic) did not allow for the access to the laboratory at night so that many samples could be taken, hence, the samples were only taken during the day throughout the continuous fermentation runs.

3.3.4.2 Continuous fermentation runs

The continuous fermentation runs using the membrane bioreactor (MBR) system were carried out with a total working volume of 1.5 litres which included both the volume of the fermenter and the volume lost in the connectors, tubings and membrane module. Approximately, between 210 and 220 mL of the working volume was lost as dead volume in the connectors, tubings and membrane module.

The working volume of the fermenter was maintained at a constant value by continuously adding the fresh feed into the fermenter at a rate similar to the permeate flow of the membrane as previously proposed by Xu et al. (2006). Therefore, during the continuous fermentations runs, the permeate flow rate readings were taken at different time intervals (i.e., after every 9 h), and the feed flow rate

adjusted accordingly. All the autoclavable components of the MBR system i.e., connectors, tubing, diaphragm valves, membrane module holder and the fermentation vessel were autoclaved to avoid any contamination risks that could compromise the growth of the bacterial cells.

Depending on the investigation, the glucose concentrations in the fresh feed during the continuous fermentation runs were varied between 60 and 120 g/L. However, the concentrations of other components of the fermentation medium remained similar to those of the batch fermentation runs as shown in Table 3.3. Similarly, other fermentation factors such as autoclaving conditions, pH, temperature, agitation speed and neutralizing agent were similar to those of the batch fermentation runs. The inoculum size for the continuous fermentation runs was 1 % (v/v). This low inoculum size was used to ensure that there was some residual glucose in the fermenter before starting the continuous fermentation.

The choice of 1 % (v/v) inoculum size was dictated by the results of the preliminary experiments of batch fermentation that were carried out using 30 g/L glucose and different inoculum sizes which included: 1 %, 0.5 % and 0.1 % (v/v). From these experiments (see Appendix A Table A.8), after 15 h of batch fermentation, the 1 % (v/v) inoculum size gave a residual glucose concentration of between 7 to 10 g/L whereas both 0.5 % and 0.1 % (v/v) inoculum sizes had more than 16 g/L residual glucose concentrations. The high residual glucose concentrations at both 0.5 % and 0.1 % (v/v) inoculum sizes indicated that there was low substrate utilization at these inoculum sizes. Hence, a 1 % (v/v) inoculum size was chosen for further continuous fermentation runs using the MBR system.

Usually, a batch fermentation mode is required before starting the continuous fermentation runs to ensure that sufficient biomass has accumulated in the fermenter to convert the freshly added nutrients (Fan et al., 2017b; Giorno et al., 2002). The duration of the batch fermentation mode is determined from the cell growth kinetics (Fan et al., 2017b), and hence differs for each microorganism used for a particular investigation. From some of the previous investigations, different durations for the batch fermentation mode before starting the continuous fermentation runs have been reported as between 10 h and 12 h using *Lactobacillus rhamnosus* NRRL B445 (Choudhury et al., 2006), between 12 h and 14 h using *Lactobacillus bulgaricus* (Giorno et al., 2002), and 15 h using *Lactobacillus delbrueckii* NCIM-2025 (Dey et al., 2012a). Based on the batch fermentation runs and other preliminary experiments of continuous fermentation carried out in the present study, sufficient

biomass concentrations of *Lactobacillus casei* ATCC 393 were obtained after 15 h, hence this duration was adopted for the batch fermentation mode before starting the continuous fermentation runs.

The fermenter containing a glucose concentration of 30 g/L and other components of the fermentation medium (with concentrations similar to those of the batch fermentation runs) was aseptically inoculated with 1 % (v/v) of a 24 h inoculum (biomass concentration of 3.42 ± 0.03 g/L), and the fermentation proceeded in a batch fermentation mode for 15 h. Thereafter, the continuous fermentation was started. The lactate fermentation broth was pumped from the fermenter through the ultrafiltration membrane module whereby the bacterial cells were recovered from the broth and recycled back to the fermenter, while the lactate and other components of the broth were collected in the permeate flow. The fresh feed (with glucose concentrations between 60 and 120 g/L depending on the particular investigation) was continuously added to the fermenter at a rate similar to that of the permeate flow.

The MBR system was operated at a constant transmembrane pressure of 0.2 bar and a low cross-flow velocity of 0.27 m/s to avoid bacterial cell damage as well as to ensure no air ingress into the system since lactic acid fermentation is an anaerobic process. From a previous study, it was noted that high cross-flow velocities can damage the bacterial cells and reduce their efficiency as well as lead to aeration which negatively affects the anaerobic lactic acid fermentation (Giorno et al., 2002). The choice of these membrane operating conditions (i.e., a transmembrane pressure of 0.2 bar and a cross-flow velocity of 0.27 m/s) was based on the critical flux experiments carried out using bacterial cells (results shown in Chapter 4).

The continuous fermentation runs using the MBR system lasted for 68 h and they were terminated when there was negligible or no permeate flow i.e., when the membrane pores were fully blocked as a result of membrane fouling**.** 2 mL samples were collected from the feed and retentate for the analysis of glucose, lactate, and biomass concentrations at regular intervals of 3 h (only during the day) up to 68 h. The samples collected from the feed were analyzed for glucose and lactate concentrations whereas the retentate samples were used for the analysis of biomass concentrations. All the continuous fermentation runs were carried out in triplicate and the experimental data (excluding the outliers) were presented with the standard deviation.

3.4 Analytical methods

3.4.1 Determination of glucose and lactate concentrations

The 2 mL samples (in Eppendorf tubes) that were taken during the fermentation process were centrifuged (in a mini centrifuge) at 10,000 rpm for 10 min to separate the cells. The cell free-supernatant was then filtered with a nylon syringe filter of pore size: 0.22 µm and 13 mm diameter (purchased from Lasec, Cape Town, South Africa) for the glucose and lactate concentrations analysis. The centrifuged residue was used for the measurement of optical density.

The glucose and lactate concentrations were analyzed using a high performance liquid chromatography (HPLC) system (Dionex Ultimate 3000 HPLC) equipped with Biorad HPX – 87H column (250 x 7.8 mm with guard cartridge) and refractive index detector (ERC RefractoMax 520). The mobile phase of 0.005 M H_2SO_4 at a flow rate of 0.6 mL /min was used. The temperature of the column was maintained at 65 ℃ while the injection volume of the sample was 20 µL.

All the samples that were taken for the HPLC analysis were diluted with deionized water to ensure that their concentrations did not exceed 10 g/L. However, after getting the HPLC results, they were multiplied by the dilution factor to obtain the actual concentration.

Since the submission of the samples to the analytical laboratory for HPLC analysis could sometimes be delayed due to the booking schedules, the prepared samples were frozen until the submission date. From previous studies, lactic acid fermentation broth can be frozen at – 20 ℃ without any negative effect on the lactic acid or sugars concentrations (Garde et al., 2002; Milcent et al., 2001).

It is worth noting that the present study used the term “lactate concentration” instead of “lactic acid concentration” since the produced lactate (after neutralization with sodium hydroxide) was not subjected to complete downstream processing where it could be converted to lactic acid. Nevertheless, in cases where lactic acid fermentation is carried out without neutralization, the term ‘’lactic acid concentration’’ can be used.

Similarly, the terms “lactate yield” and “lactate productivity” were used in the present study.

3.4.2 Measurement of biomass concentrations

The biomass concentrations were determined by optical density measurements using a UV-Vis spectrophotometer at a wavelength of 620 nm ($OD_{620\ nm}$) (Demirci et al., 1998; Senthuran et al., 1997). The $OD_{620\ nm}$ values were then converted to cell dry weight (CDW) using a calibration curve generated in the present study. The fermentation medium was used as the blank for optical density measurements (Sun et al., 2019).

The calibration curve was generated using dilutions of a bacterial culture whose optical density is known as previously suggested by Rodrigues et al. (2006). A fixed volume of the dilutions (5 mL) was filtered through a 0.45 µm membrane filter (dry cellulose nitrate filter) and left to dry in an oven at 105 ℃ for 24 h. The filter papers were weighed before the filtration and after drying, and the difference in their mass was the cell dry weight. This difference in mass obtained (in grams) for the 5 mL dilutions was converted to grams per litre. Hence, the biomass concentration was routinely obtained as:

$$\mathbf{CDW\ (g/L) = 2.3312 \times OD_{620\ nm} + 0.1944\ (R^2 = 0.9841)} \quad (3.1)$$

The calibration curve for *Lactobacillus casei* ATCC 393 generated in the present study is shown in Appendix A Figure A.1.

3.5 Calculated parameters

As previously mentioned in Chapter 2 section 2.4.1, the efficiency of the lactic acid fermentation process is evaluated by lactate yield per substrate consumed, lactate concentration and lactate productivity. This section describes how some of these parameters were calculated in the present study. Sample calculations for each of these parameters are shown in Appendix B.

(i) Lactate yield (g/g)

Lactate yield per substrate consumed was defined as the ratio of lactate produced to glucose consumed (Hofvendahl et al., 2000; Mussatto et al., 2007; Tashiro et al., 2011).

Lactate yield was calculated as follows:

$$\text{Lactate yield (g/g)} = \frac{\text{Lactate produced (g)}}{\text{Glucose consumed (g)}} \quad (3.2)$$

(ii) Lactate productivity [g/(L.h)]

The calculations of lactate productivity for the batch fermentation runs were different from those of the continuous fermentation runs.

- **Batch fermentation runs**

Lactate productivity of a batch fermentation run was defined as the ratio of the concentration of lactate produced to the indicated fermentation time (Tashiro et al., 2011; Tejayadi et al.,1995). The overall productivity was therefore defined as the ratio of final lactate concentration (obtained at the end of the fermentation process) to the total fermentation time (Fan et al., 2017a).

Lactate productivity was thus calculated as follows:

$$\text{Lactate productivity [g/(L.h)]} = \frac{\text{Lactate concentration (g/L)}}{\text{Fermentation time (h)}} \quad (3.3)$$

- **Continuous fermentation runs**

For a continuous fermentation run, lactate productivity was defined as the product of lactate concentration and dilution rate (Dey et al., 2012a; Tejayadi et al., 1995).

It was calculated as follows:

$$\text{Lactate productivity } [\text{g/(L.h)}] = \text{Lactate concentration (g/L)} \times \text{Dilution rate } (\text{h}^{-1}) \quad (3.4)$$

(iii) Dilution rate (h^{-1})

The dilution rate was defined as the ratio of the feed flow rate to the total working volume of the fermenter (Dey et al., 2012a; Fan et al., 2017a; Tejayadi et al., 1995).

Therefore, it was calculated as follows:

$$\text{Dilution rate } (\text{h}^{-1}) = \frac{\text{Feed flow rate (L/h)}}{\text{Working volume of the fermenter (L)}} \quad (3.5)$$

(iv) Permeate flux, J (LMH)

Based on the definition of permeate flux given in Chapter 2 section 2.7.5, the permeate flux (J) was calculated by the quotient of the volumetric flow rate of the permeate and the membrane surface area (Fan et al., 2017a).

The volumetric flow rate of the permeate was obtained by measuring the volume of permeate collected during a given period of time (Li et al., 2006).

$$\text{Permeate flow rate (L/h)} = \frac{\text{Volume of the permeate collected (L)}}{\text{Time taken to collect the permeate (h)}} \quad (3.6)$$

Hence,

$$\text{Permeate flux, J (LMH)} = \frac{\text{Permeate flow rate (L/h)}}{\text{Membrane surface area } (\text{m}^2)} \quad (3.7)$$

(v) Membrane surface area (m^2)

The membrane surface area used in the calculations of the permeate flux was calculated from the inner diameter of the membrane and its length as follows:

$$\text{Membrane surface area } (m^2) = \pi \times \text{Inner diameter } (m) \times \text{Length } (m) \quad (3.8)$$

(vi) Cross-flow velocity (m/s)

In the present study, the cross-flow velocities were varied by varying the pump flow rates.

The cross-flow velocity was calculated as follows (Dey et al., 2012a; Fan et al., 2017a):

$$\text{Cross-flow velocity } (m/s) = \frac{\text{Volumetric flow rate of fluid through the membrane module } (\mathbf{m^3}/s)}{\text{Inner cross-sectional area of the membrane module } (\mathbf{m^2})} \quad (3.9)$$

Chapter 4 Results and discussion

4.1 Introduction

The results that were obtained in the present study are discussed in this chapter. Section 4.2 discusses the results of the batch fermentation runs, section 4.3 discusses the results of the critical flux experiments using bacterial cells, section 4.4 discusses the results of the different process limitations investigated, and section 4.5 discusses the results obtained from the critical flux experiments using lactate fermentation broths.

4.2 Batch fermentation

The batch fermentation runs were carried out to:

(i) understand the glucose consumption trends in a batch fermentation mode

(ii) determine the possible maximum lactate yield obtained in a batch fermentation mode

(iii) understand biomass concentration trends in a batch fermentation mode

(iv) establish typical lactate productivities obtained in a batch fermentation mode.

Figure 4.1 shows the profiles of lactate, residual glucose, and biomass concentrations of a 24-hour batch fermentation run with 30 g/L glucose as the substrate.

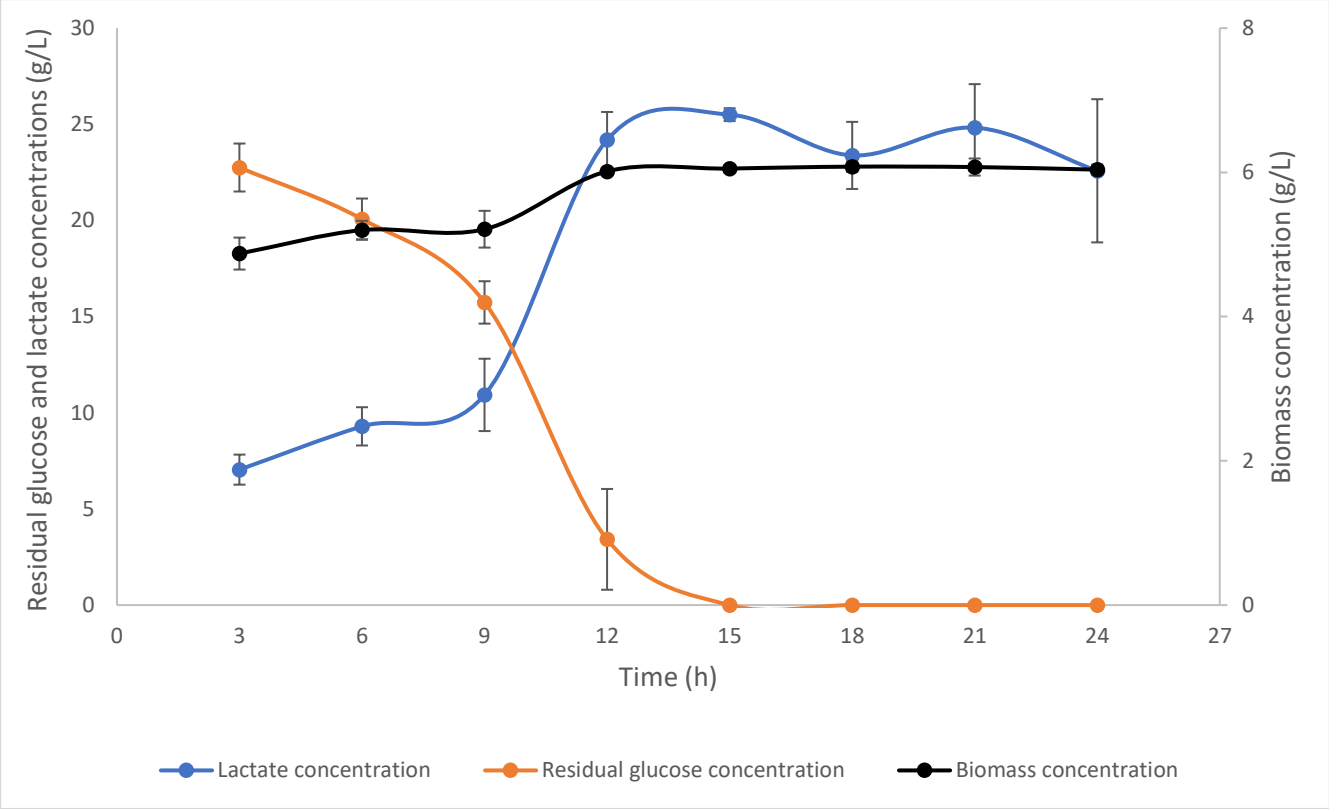


Figure 4.1: Profiles of lactate, residual glucose, and biomass concentrations of a 24 h batch fermentation run with 30 g/L glucose as the substrate

(*Three repeated runs, average presented with error bars*)

From Figure 4.1, it can be seen that glucose was rapidly consumed in the initial stages of the fermentation and after 15 h of the fermentation, all the glucose had been consumed. It can also be seen that lactate production was minimal in the first 9 hours, but rapidly increased thereafter up to 15 h of fermentation before a noticeable decrease was observed. Also, it was observed that the biomass concentration increased up to 15 h of fermentation and afterwards remained nearly constant until the end of the fermentation. These trends are in agreement with those reported by Moon et al. in their study using *Lactobacillus paracasei* subsp. *paracasei* CHB2121 and glucose as the substrate for batch fermentation runs (Moon et al., 2012). Furthermore, the same observations were reported by B. Ali et al., 2004 in an investigation whereby L (+)-lactic acid was produced in a batch fermentation mode using *Lactobacillus casei* NRRL B-441 and whey (containing lactose) as the substrate. They noted that the time needed by the bacteria to completely utilize the substrate

depended mainly on the initial substrate concentration, whereby the time was longer for high initial substrate concentrations than for low initial substrate concentrations (Büyükkileci et al., 2004).

In a typical lactic acid fermentation process, glucose consumption is mainly for biomass formation and its maintenance as well as for lactic acid production (Monteagudo et al., 1997). The bacterial cells consume glucose to obtain energy required for the formation of new bacterial protoplasm, and at the same time they do so as a normal metabolic activity irrespective of growth (Luedeking et al., 2000). Therefore, the rapid consumption of glucose in the first 15 h of fermentation (i.e., from an initial glucose concentration of 30 g/L to 0 g/L) observed in Figure 4.1, can be attributed to the fact that glucose consumption at the beginning of the fermentation was mainly used for cell growth and maintenance purposes, and less of the glucose was used for lactate production (Fu et al., 1999). At this stage when the glucose consumed was mainly used for cell growth and maintenance processes, lactate production was therefore determined by these processes leading to low lactate yield in the initial stages of the fermentation process (Fu et al., 1999; Timmer et al., 1994). However, once adequate biomass had accumulated in the fermenter, lactate production increased because lactic acid fermentation is considered both a growth and non-growth associated process (Fu et al., 1999; Monteagudo et al., 1997; Xu et al., 2006).

Lactic acid fermentation being both a growth and non-growth associated process simply means that it depends on both the cell growth and the final biomass concentrations in the fermentation broth (Fu et al., 1999; Xu et al., 2006). Basically, lactic acid fermentation can be considered different from other types of fermentations such as alcohol fermentation and penicillin fermentation whose kinetics are described as ‘’growth associated’’ and ‘’non-growth associated’’ respectively. In alcohol fermentation, the product formation depends only on the cell growth while in penicillin fermentation, the product formation does not occur until relatively late in batch cultivation, mostly in the stationary phase (Monteagudo et al., 1997).

It can be seen in Figure 4.1 that the biomass concentrations remained nearly constant between 18 h and 24 h (i.e., biomass concentration was 6.08 g/L, 6.07 g/L and 6.04 g/L at 18 h, 21 h and 24 h respectively) whereas lactate production continued. This can be explained by the fact that at this stage although the viability of the cells decreased when the amount of biomass became constant since no new cells were being produced, lactate production was able to continue because some old

cells were still viable, and thus were metabolically active for lactate production (Giorno et al., 2002; Timmer et al., 1994). Similarly, it can be explained that the biomass concentrations remained nearly constant between 18 h and 24 h probably due to the depletion of glucose as well as accumulation of lactate that inhibited the bacterial cell growth (Leroy et al., 2001; Nakano et al., 2012).

Using the formula previously stated in Chapter 3, for the calculation of lactate yield on substrate consumed (sample calculations shown in Appendix B), the maximum lactate yield in the batch fermentation run after 15 h of fermentation was 85 % (i.e., 0.85 g of lactate was produced per g of glucose consumed) whereas the overall lactate yield at the end of fermentation process was 75 % (i.e., 0.75 g of lactate was produced per g of glucose consumed). These values of lactate yield on substrate consumed confirmed those reported by Alonso et al. (2010) and Kaavessina et al. (2017), who obtained between 71 % and 90 % lactate yields on substrate using the same bacterial strain used in the present study i.e., *Lactobacillus casei* ATCC 393.

Table 4.1 shows the lactate productivities obtained during the 24 h batch fermentation run. These lactate productivities were calculated as previously described in Chapter 3, and sample calculations are shown in Appendix B.

Table 4.1: Lactate productivities obtained from a 24 h batch fermentation run using 30 g/L glucose as a substrate

Time (h)	Lactate productivity [g/(L.h)]
0	-
3	2.35 ± 0.26
6	1.55 ± 0.17
9	1.21 ± 0.21
12	2.02 ± 0.12
15	1.70 ± 0.02
18	1.30 ± 0.10
21	1.18 ± 0.11
24	0.94 ± 0.16

(*Three repeated runs, average presented with standard deviation*)

From Table 4.1, it can be seen that all the lactate productivities obtained throughout the 24 h batch fermentation run did not exceed 2.4 g/(L.h), and the overall lactate productivity of 0.94 g/(L.h) was obtained at the end of the fermentation. These results are in agreement with the literature which

reported that the lactate productivities in batch fermentation runs are very low ranging from 1 to 7.5 g/(L.h) (Tejayadi et al., 1995), with most of them rarely higher than 5 g/(L.h) (Fan et al., 2017b).

It is worth noting that lactate productivity is an important factor in a typical lactic acid production process because it is closely related to the economics of the whole process (Moon et al., 2012). Therefore, the low lactate productivities obtained in the batch fermentation runs practically imply that a batch fermentation mode is not suitable for efficient and economical lactic acid production. These low lactate productivities in the batch fermentation runs have been associated with lactate inhibition to the bacterial cell growth (Fan et al., 2017a; Xu et al., 2006). Hence, the use of membrane bioreactor (MBR) systems could potentially improve these lactate productivities by removing the inhibiting lactate from the fermentation broth as well as enabling cell-recycling (Tejayadi et al., 1995). This provided a strong motivation for the present study.

In summary, from the batch fermentation results it was observed that:

- all the glucose had been consumed after 15 h of fermentation when an initial glucose concentration of 30 g/L was used
- the biomass concentration reached its maximum after 15 h of fermentation and remained nearly constant between 18 h to 24 h of fermentation
- the maximum lactate yield on substrate consumed was 85 % after 15 h of fermentation, and the overall lactate yield on substrate consumed was 75 % after 24 h of fermentation
- the overall lactate productivity was only 0.94 g/(L.h) after the 24 h of fermentation.

Implications of the batch fermentation runs

The batch fermentation runs had important implications for the design of the continuous fermentation experiments. As previously stated in Chapter 3, the continuous fermentation runs would be started up in a batch mode to ensure adequate biomass and some residual glucose in the fermenter before switching over to continuous fermentation mode. Figure 4.1 above implies that all the glucose would be consumed overnight, before switching over to the continuous fermentation mode.

Hence, based on these batch fermentation results and other preliminary experiments described in Chapter 3, the following subsequent changes were adopted during the continuous fermentation runs using the membrane bioreactor (MBR) system:

- 30 g/L of glucose and 1 % (v/v) inoculum size were used during the initial batch fermentation period of 15 h. This inoculum size was reduced from the 10 % (v/v) (used in the batch fermentation runs) to 1 % (v/v), to ensure some glucose remained in the fermenter before starting the continuous fermentation runs. Nevertheless, it should be noted that the glucose concentrations in the fresh feed to the fermenter were varied between 60 to 120 g/L depending on the investigation during the continuous fermentation runs.
- The continuous fermentation runs using the MBR system were started after 15 h when adequate biomass had accumulated in the fermenter to consume the added nutrients.

4.3 Critical flux experiments using bacterial cells

The critical flux experiments using bacterial cells were carried out to determine proper membrane operating conditions for the membrane bioreactor (MBR) system used in the present study. It has been previously reported that when MBR systems are used for lactic acid production, the lactic acid-producing microorganisms do not only influence lactate yields and lactate productivity but they also have a great influence on the filtration efficiency of the membranes used in such systems (Fan et al., 2015).

As described earlier in Chapter 3, during these experiments, the transmembrane pressure (TMP) was fixed at a constant value for 10 min during which five permeate flux readings were taken. After the 10 min, transmembrane pressure was increased. This was done until the maximum pressure that the experimental rig was able to withstand.

It is worth mentioning that in these critical flux experiments, the exact values of the critical flux were not obtained because the experimental rig used was not able to withstand a transmembrane pressure (TMP) higher than 0.5 bar, yet at this pressure the critical flux conditions had not been reached.

Nevertheless, a limiting flux was reached in some of the cross-flow velocities investigated. Limiting flux has been defined as the maximum flux beyond which a further increase in TMP does not increase the flux (Bacchin et al., 2006). In some cases, the limiting flux had been reported to be equivalent to the critical flux (Wu et al., 1999). Therefore, based on these explanations, all the trends observed in these critical flux experiments were considered to be in agreement with the literature.

These experiments were carried out using bacterial cells (with biomass concentrations of 3.99 ± 0.02 g/L) at three cross-flow velocities i.e., 0.27 m/s, 0.50 m/s and 0.90 m/s. The data obtained in these experiments are plotted as shown in Figure 4.2 and Figure 4.3.

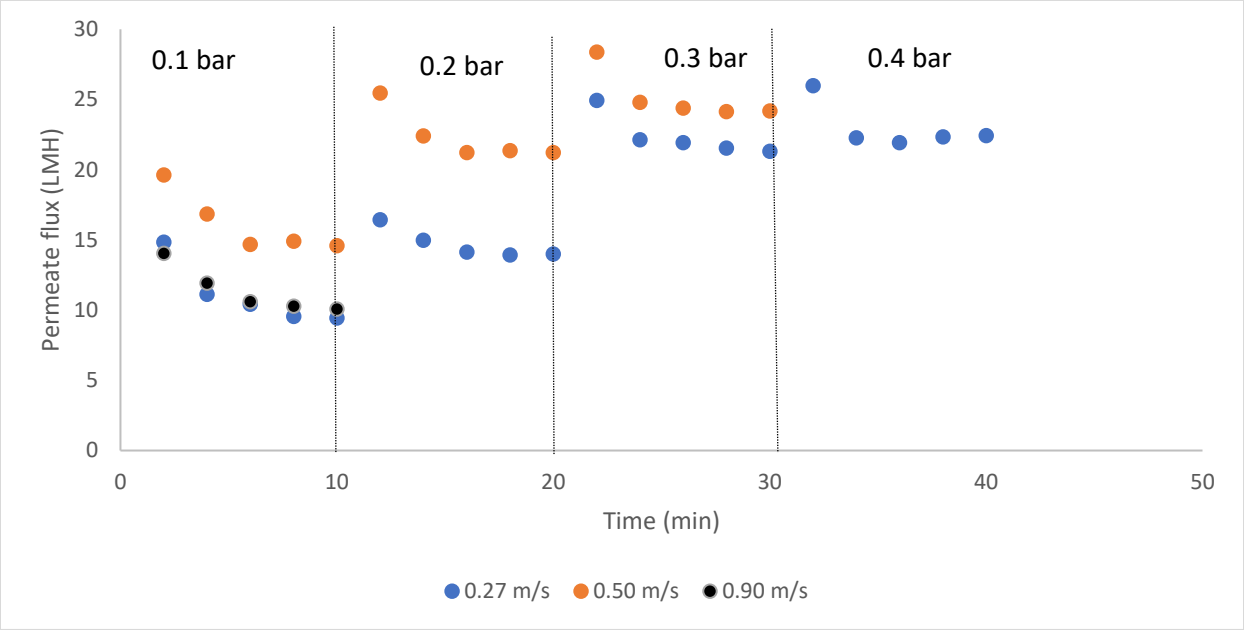


Figure 4.2: Profiles of five permeate flux readings taken during the 10 min of constant TMP at cross flow velocity of 0.27 m/s, 0.50 m/s and 0.90 m/s

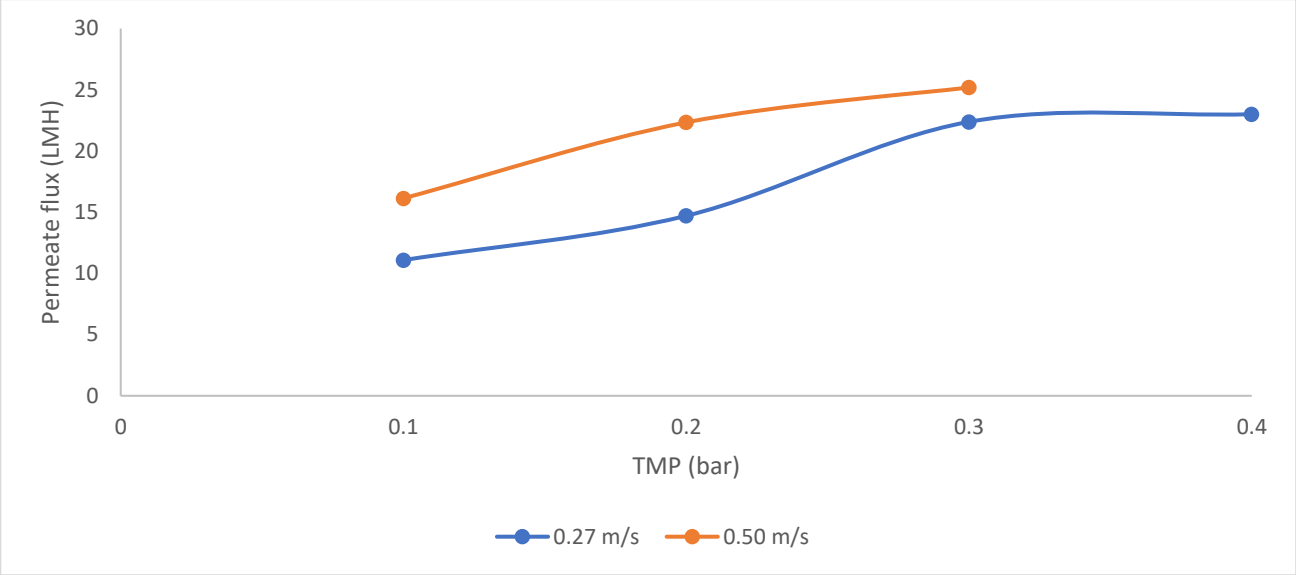


Figure 4.3: Profile of permeate flux – TMP relationships at cross flow velocity of 0.27 m/s and 0.50 m/s

(The permeate flux used in plotting this graph is the arithmetic mean of the 5 permeate flux readings taken during the 10 min of the constant TMP)

Observations made at each cross-flow velocity

- **Cross-flow velocity of 0.27 m/s**

From Figure 4.3, it was observed that the average permeate flux increased from 11.08 LMH at 0.1 bar to approximately 22.38 LMH at 0.3 bar. The permeate flux then became independent of the transmembrane pressure (TMP) after 0.3 bar.

- **Cross-flow velocity of 0.50 m/s**

The maximum TMP that was investigated at this cross-flow velocity was 0.3 bar. From Figure 4.3, it can be seen that the permeate flux continued to increase as the TMP was increased and the limiting flux was not obtained. As was expected the permeate flux at this cross-flow velocity was higher than that at a cross-flow velocity of 0.27 m/s.

- **Cross-flow velocity of 0.90 m/s**

Only a transmembrane pressure of 0.1 bar could be investigated at this high cross-flow velocity of 0.90 m/s. The experimental rig was not able to withstand a pressure higher than 0.1 bar. Beyond this pressure, the piping of the membrane bioreactor system rig could break or disconnect thus causing leakages. From Figure 4.2, it can be seen that the permeate flux suddenly decreased from 14 LMH to 10 LMH. This sudden decrease in the permeate flux can be explained by rapid adsorption of bacterial cells on the membrane surface and blockage of the membrane pores at high cross-flow velocity (Dey et al., 2012a).

Conclusions from the investigation on critical flux experiments using bacterial cells

This investigation was aimed at determining proper membrane operating conditions for the MBR system employed in continuous fermentation runs. From the investigation, it was not possible to use a high cross-flow velocity of 0.90 m/s because the experimental rig was not able to withstand a pressure higher than 0.1 bar at this cross-flow velocity. Although high permeate flux was obtained at a cross-flow velocity of 0.50 m/s, the present study opted for the low cross-flow velocity of 0.27 m/s. This was chosen to avoid bacterial cell damage that occurs at high cross-flow velocities as previously explained in Chapter 3.

Cross-flow velocity and transmembrane pressure are some of the important process parameters that are normally considered to achieve low membrane fouling, high permeate flux, and low operating costs (Cuperus et al., 1993). For the cross-flow velocity of 0.27 m/s, the permeate flux became independent of pressure after 0.3 bar, therefore, to operate below this pressure, a transmembrane pressure of 0.2 bar was chosen for the present study. For all the cross-flow velocities investigated, the critical flux conditions were not reached, hence, the operations at a cross-flow velocity of 0.27 m/s and a transmembrane pressure of 0.2 bar used in the present study were considered to be below the critical flux conditions (i.e., sub-critical flux operations).

4.4 Investigation of the process limitations that dominate the membrane bioreactor (MBR) systems used for lactic acid fermentation

As previously described in Chapter 2, the four process limitations investigated were:

- membrane fouling limitations
- limitations attributed to lactate inhibition of bacterial cells once the membrane has fouled
- nutrient limitations
- mass transfer limitations.

4.4.1 Membrane fouling limitations

Continuous lactic acid fermentation runs were carried out using an ultrafiltration (UF) membrane system (MBR system) at various glucose concentrations (in the feed) of 120 g/L, 90 g/L and 60 g/L in order to investigate whether membrane fouling limitations dominate the MBR systems used for lactic acid fermentation when these systems are operated below the critical flux (i.e., at sub-critical flux operations). The permeate flux decline over time and the total volume of permeate at the end of the continuous fermentation runs were used to describe the occurrence of fouling. The profiles of permeate flux decline over time for the continuous lactic acid fermentation runs at glucose concentrations of 120 g/L, 90 g/L and 60 g/L are shown in Figure 4.4.

From Figure 4.4, it can be seen that the permeate flux declined throughout the fermentation period for all the glucose concentrations investigated. This trend is in agreement with that reported by Giorno et al., 2002 in an investigation using an ultrafiltration cell-recycle membrane fermenter for the production of lactic acid by *Lactobacillus bulgaricus* (Giorno et al., 2002). Furthermore, Yebo Li et al., 2006 observed a similar trend in a study that involved the separation of cells and proteins from a fermented cheese whey broth using an ultrafiltration membrane system and they concluded that the permeate flux decline over time was attributed to membrane fouling (Li et al., 2006).

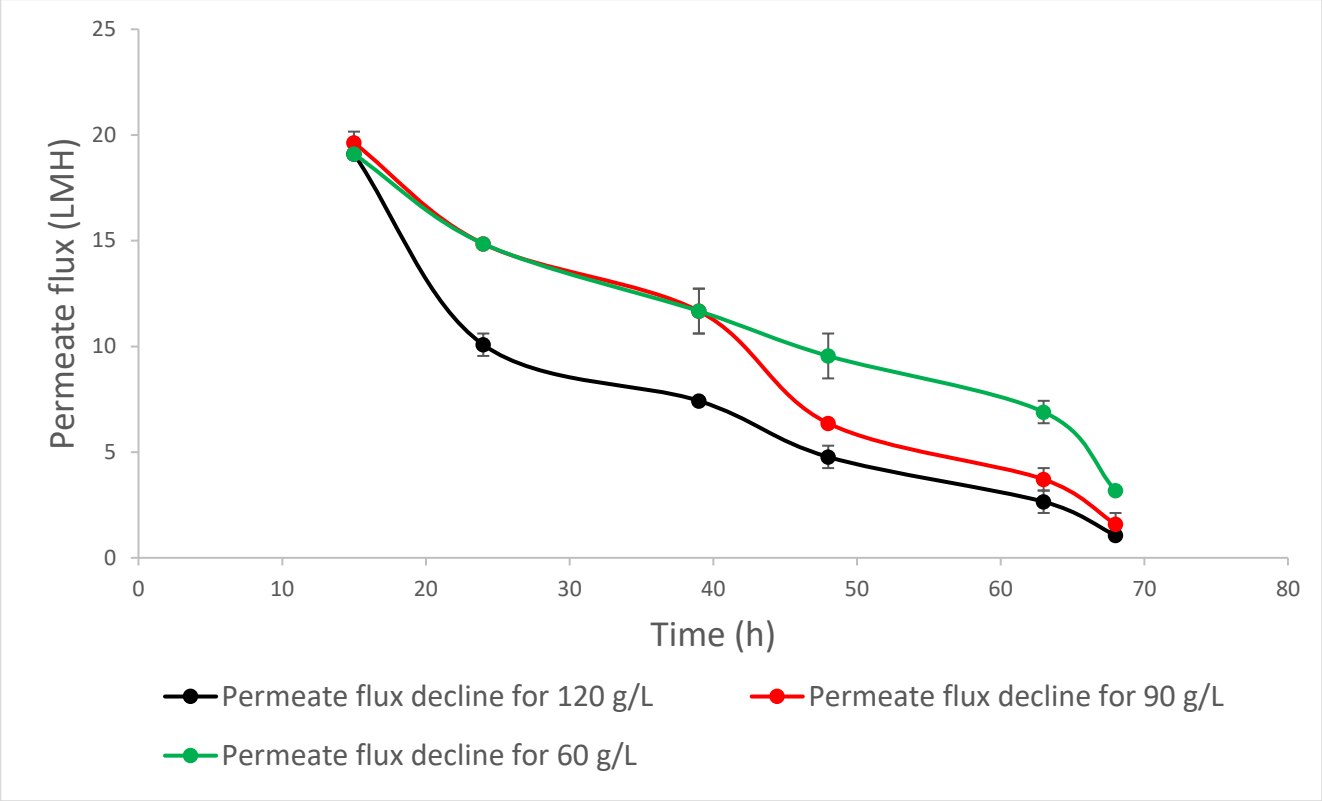


Figure 4.4: Profiles of permeate flux decline over time for the continuous lactic acid fermentations at glucose concentrations (in the feed) of 120 g/L, 90 g/L and 60 g/L

(*Two repeated runs, average presented with error bars*)

Although the continuous fermentations at all the glucose concentrations were carried out at similar membrane operating conditions (i.e., similar transmembrane pressure and cross-flow velocity), the permeate flux decline trends were different as can be seen in Figure 4.4. The permeate flux decline over time was most severe at a glucose concentration of 120 g/L compared to those of 90 g/L and 60 g/L.

Traditionally, membrane fouling phenomena have been described by correlating them to the operating conditions. However, for membrane bioreactor (MBR) systems it is important to consider the effect of bacterial cells because they result in membrane plugging and subsequently lead to a continuous decline in permeate flux over time (Fan et al., 2015; Giorno et al., 2002). Therefore, the differences in permeate flux decline evident in Figure 4.4 were probably as a result of the different bacterial cell concentrations obtained at different glucose concentrations.

It was expected that higher glucose concentrations would lead to higher bacterial growth (Choudhury et al., 2006), hence, at a glucose concentration of 120 g/L there would be more bacterial cells formed which could probably contribute to severe membrane plugging compared to those at 90 g/L and 60 g/L. For instance, after 24 h of fermentation, the biomass concentration at glucose concentration of 120 g/L was 5.79 g/L while that at a glucose concentration of 60 g/L was only 5.42 g/L. The biomass concentrations at each glucose concentration investigated are shown in a tabular form in Appendix A.

From Figure 4.4, the permeate flux at glucose concentration of 90 g/L was almost similar to that at 60 g/L, however after 40 h of fermentation, it drastically decreased and almost became similar to that of 120 g/L. This can be explained by the fact that after 40 h, there were more bacterial cells at a glucose concentration of 90 g/L which seriously plugged the membrane compared to that experienced at 60 g/L. The biomass concentration was used to confirm this further, and it was evident that after 42 h, the biomass concentrations were 5.89 g/L and 5.76 g/L at the glucose concentration of 90 g/L and 60 g/L respectively. Similarly, after 63 h of the fermentation, the biomass concentration at glucose concentration of 90 g/L was 4.6 % higher than that at a glucose concentration of 60 g/L, which translated to further membrane plugging, and a subsequent notable permeate flux decline. Fan et al., 2017 had also reported that the permeate flux declined gradually with increasing biomass concentration during lactic acid fermentation in a membrane bioreactor system (Fan et al., 2017b).

Other fermentation medium components e.g., proteins have also been reported to contribute to membrane fouling (Zhang et al., 1994). Nevertheless, in the present study all the concentrations of other components of the fermentation medium were the same at all the glucose concentrations investigated, hence, the difference in permeate flux decline over time at each glucose concentration can be described in terms of the difference in biomass concentrations.

The total volume of permeate at the end of the continuous fermentation at glucose concentrations (in the feed) of 120 g/L, 90 g/L and 60 g/L are shown in Table 4.2.

Table 4.2: The total volume of permeate at the end of continuous fermentation at 120 g/L, 90 g/L and 60 g/L glucose concentrations (in the feed)

Glucose concentration in the feed	Total volume of the permeate at the end of the continuous fermentation run
120 g/L	925 ± 55 mL
90 g/L	1440 ± 110 mL
60 g/L	1891.5 ± 46.5 mL

(*Two repeated runs, average presented with standard deviation*)

Due to the blocking of the membrane pores by the bacterial cells, it was expected that the permeate flow rate will decrease resulting in a reduced total volume of permeate at the end of the fermentation (Milcent et al., 2001). As can be seen in Table 4.2, the total volume of the permeate decreased with the increasing glucose concentrations confirming the severe membrane fouling at higher glucose concentrations.

In conclusion, this investigation was aimed at identifying whether membrane fouling is a process limitation that dominates the performance of membrane bioreactor (MBR) systems used for lactic acid fermentation when they are operated below the critical flux (i.e., sub-critical flux operations). The permeate flow rate readings taken at regular time intervals of 9 h (during the day) throughout the continuous fermentation as previously described in Chapter 3 were used for the calculation of the permeate flux. In constant transmembrane pressure (TMP) operations, as was the case in this investigation, the permeate flux decline over time in an ultrafiltration membrane system is attributed to membrane fouling (Wojtyniak et al., 2015).

Usually, there would be no or minimal membrane fouling observed when the ultrafiltration membrane system is operated below the critical flux (i.e., sub-critical flux operations) as previously described in Chapter 2. Nevertheless, from the results obtained in this investigation, there was significant membrane fouling. Hence, it was concluded that membrane fouling is a process limitation that dominates membrane bioreactor systems used for lactic acid fermentation.

Membrane fouling necessitates increased energy expenditure or larger membrane area to maintain a reasonable permeate flux, frequent membrane cleaning and frequent membrane replacement (Miller et al., 2014). In the present study, physical cleaning methods alone were not able to remove the foulants, but they were removed by chemical cleaning methods depicting that irreversible fouling had occurred in the ultrafiltration membrane system.

The results obtained in this investigation practically imply that high biomass concentrations realized at high glucose concentrations largely contribute to membrane fouling in membrane bioreactor (MBR) systems used for lactic acid fermentation. Therefore, if some online monitoring and control systems can be connected to the fermenter to monitor the bacterial cell growth (i.e., the increase in biomass concentrations), and subsequently carry out cell bleeding at a suitable rate that would not compromise the lactic acid production process, then MBR systems could have great potential in the future for lactic acid production. Rong Fan et al., 2015 had previously investigated a system with an online monitoring system (optical sensor) for the bacterial cell growth (Fan et al., 2015), however, more focus needs to be put into similar systems so as to minimize membrane fouling observed in the MBR system at sub-critical flux operations.

Summary of the investigation on membrane fouling limitations

- The permeate flux declined throughout the fermentation period at all the glucose concentrations investigated i.e., at 120 g/L, 90 g/L and 60 g/L.
- The trends observed in this investigation were in agreement with the literature.
- The permeate flux decline was more severe at a glucose concentration of 120 g/L than at glucose concentrations of 90 g/L and 60 g/L.
- High biomass concentrations realized at a glucose concentration of 120 g/L were responsible for the membrane plugging, and subsequent severe membrane fouling.
- Chemical cleaning methods were able to remove the foulants at all the glucose concentrations investigated depicting that it was an irreversible fouling.
- Membrane fouling was thus concluded to be a process limitation that dominates the performance of membrane bioreactor systems used for lactic acid fermentation despite employing the sub-critical flux operations.

4.4.2 Limitations attributed to lactate inhibition of bacterial cells once the membrane has fouled

In order to investigate this possible process limitation, continuous fermentations at glucose concentrations (in the feed) of 120 g/L, 90 g/L and 60 g/L were carried out using a membrane bioreactor system (ultrafiltration membrane system), and the corresponding lactate productivity as well as bacterial growth trends were studied after 40 h of fermentation when fouling was significant in the system. The significant fouling, in the present study, was considered after 40 h of fermentation when the permeate flux had decreased to between 40 % – 60 % of the initial permeate flux. The profiles of lactate productivity, biomass concentration and permeate flux decline after 40 h of fermentation at glucose concentrations of 120 g/L, 90 g/L and 60 g/L are shown in Figure 4.5. It is important to note that Figure 4.5b was redrawn from Figure 4.4 to indicate when the membrane had significant fouling (i.e., from 40 h to 68 h of fermentation).

It was observed from Figure 4.5, that the lactate productivity decreased from 45 h until the end of the fermentation process (i.e., 68 h) at all the glucose concentrations investigated. Also, the biomass concentrations nearly remained constant at glucose concentrations of 90 g/L and 60 g/L, except for the glucose concentration of 120 g/L which had a noticeable decrease towards the end of the fermentation. These results are in agreement with those reported in the literature by Wang et al., 1988 who observed similar trends using *Streptococcus cremoris* SBT 1306 (Wang et al., 1988).

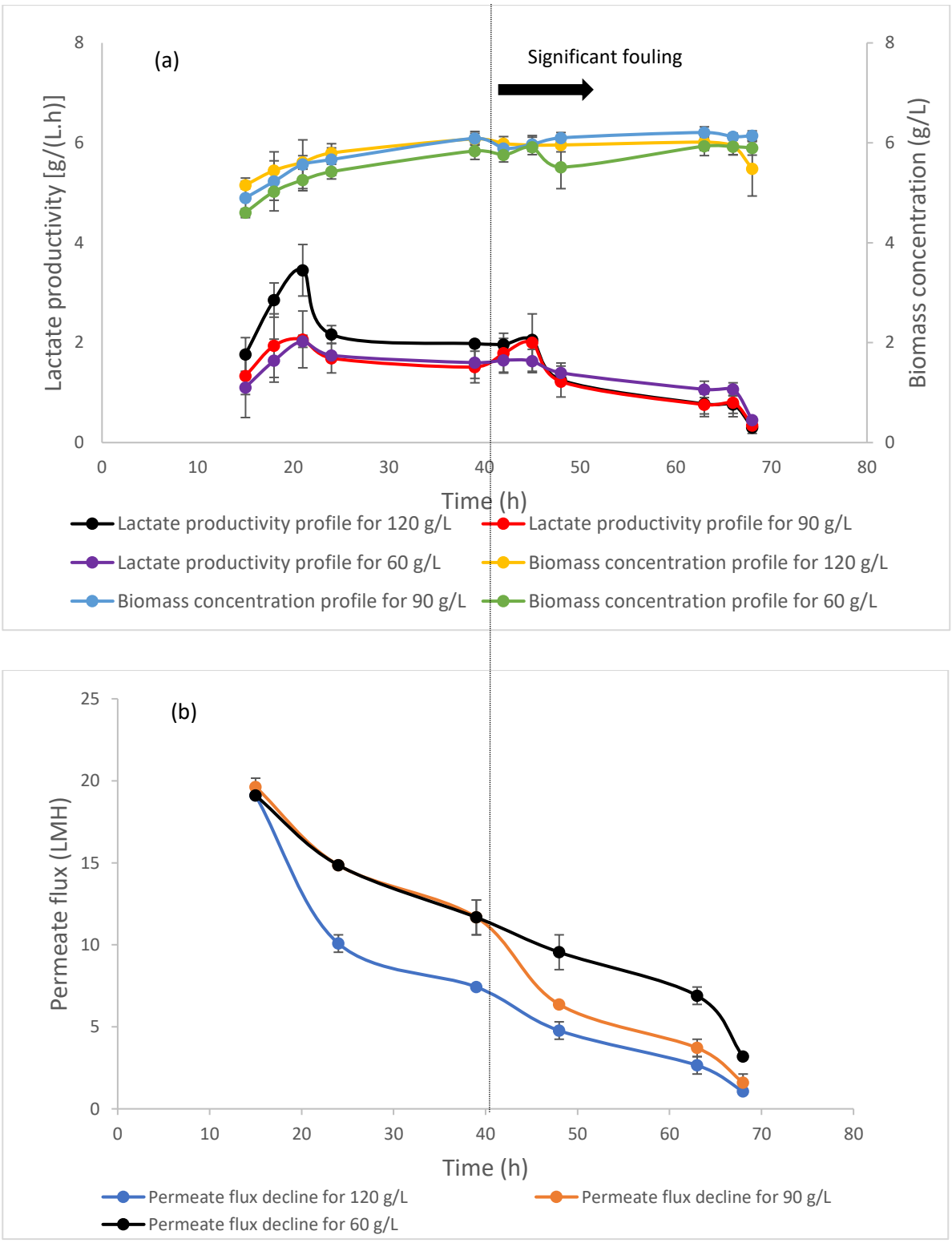


Figure 4.5: Profiles of (a) lactate productivity, biomass concentration and (b) permeate flux decline, after 40 h of fermentation at glucose concentrations of 120 g/L, 90 g/L and 60 g/L

(*Two repeated runs, average presented with error bars*)

From Figure 4.5, the lactate productivity had decreased by 62.5 %, 62.0 % and 34.8 % at glucose concentrations of 120 g/L, 90 g/L and 60 g/L respectively between 45 h and 63 h of fermentation. The decrease in lactate productivity was significantly lower at a glucose concentration of 60 g/L than at glucose concentrations of 120 g/L and 90 g/L. Similarly, the increase in biomass concentrations between this period (45 h and 63 h) was less than 4% at all the glucose concentrations investigated. The biomass concentrations further remained nearly constant at the glucose concentrations of 90 g/L and 60 g/L in the last 5 h of the fermentation. This decrease in lactate productivity and the decreased biomass concentrations at all the glucose concentrations investigated, despite substrate being added to the fermenter, can be attributed to lactate inhibition to the bacterial cells. However, it was so rapid at glucose concentrations of 120 g/L and 90 g/L due to the severe membrane fouling at these concentrations that led to more lactate accumulating in the fermenter.

In Chapter 2, it was mentioned that the bacterial cell growth can be inhibited by the accumulated lactate in the fermenter. To help understand further the results obtained in this investigation, the mechanisms of this inhibition can be described as follows: lactic acid in the fermentation broth dissociates into lactate ion and proton, and the protons are the ones neutralized by the added OH^- (NaOH) whereas the lactate ions continue to increase in the fermentation broth as shown by equations 4.1 and 4.2 (Wang et al., 1995):

$$HL \rightarrow L^- + H^+ \quad (4.1)$$

$$L^- H^+ + OH^- \rightarrow H_2O + L^- \quad (4.2)$$

where; HL is undissociated lactic acid,

L^- is dissociated lactate ion and H^+ is the hydrogen ion (proton)

These lactate ions have an inhibitory effect on the growth of lactic acid bacteria when they accumulate to higher concentrations, thus leading to reduced lactate productivity (Chen et al., 2002; Nakano et al., 2012; Rault et al., 2009).

The mechanism of dissociated lactate inhibition to bacterial cells has been associated with cellular mortality and cell membrane damage. The dissociated lactate induces a high level of negative charges inside and outside the cell, thus leading to damaged or dead cells (Nakano et al., 2012; Ramchandran

et al., 2012; Rault et al., 2009). Owing to the reduced cell activity, the lactate productivity thus decreases as the fermentation continues as observed in this investigation (Wang et al., 1988).

In conclusion, the removal of lactate from the fermentation broth using the ultrafiltration membrane system during the continuous fermentations was necessary to alleviate the inhibitory effects associated with the lactate to ensure proper bacterial cell growth as well as to improve lactate productivity. Nevertheless, once the membrane had fouled, it was not possible to achieve this because the accumulated lactate inhibited the bacterial cell growth and subsequently reduced the lactate production rate. The inhibitory effects were more evident at higher glucose concentrations where there was severe membrane fouling at early stages. From this investigation, it was therefore concluded that lactate inhibition for the bacterial cells once the membrane has fouled is a process limitation that has a significant impact on the membrane bioreactor systems used for lactic acid fermentation.

The results obtained in this investigation imply that the full potential of side-stream membrane bioreactor systems for lactic acid fermentation cannot be achieved once the membrane has fouled. Therefore, much focus should be put on the possible methods that may extract lactic acid from the fermentation broth as soon as it is produced such as adsorption, reactive extraction, and submerged membrane bioreactor systems (i.e., extractive fermentation methods). Similarly, mechanisms to have automatic membrane cleaning methods for the side-stream membrane bioreactor systems probably before severe fouling occurs should be considered. Nevertheless, care must be taken to avoid any contamination of the bacterial cells during the cleaning methods.

Giorno et al., 2002 in their study had tried a method whereby the fouled membrane module was replaced with a new membrane module, and this enabled the fermentation to last for 22.5 hours longer (Giorno et al., 2002). This shows that through proper mechanisms, limitations attributed to lactate inhibition of bacterial cells once the membrane has fouled can be minimized for efficient and economical lactic acid fermentation in membrane bioreactor systems.

Summary of the investigation on limitations attributed to lactate inhibition of bacterial cells once the membrane has fouled

- Lactate productivity decreased from 45 h until the end of the fermentation (i.e., 68 h) at all the glucose concentrations investigated.
- Between 45 h and 68 h, the biomass concentrations nearly remained constant at glucose concentrations of 90 g/L and 60 g/L, but there was a noticeable decrease in biomass concentrations at a glucose concentration of 120 g/L towards the end of fermentation.
- The trends observed in this investigation were in agreement with the literature.
- Decrease in lactate productivity was higher at glucose concentrations of 120 g/L and 90 g/L, than at a glucose concentration of 60 g/L due to the severe membrane fouling at higher glucose concentrations that caused more lactate to accumulate in the fermenter.
- Lactate inhibition for bacterial cells once the membrane has fouled was therefore concluded to be a process limitation that has a significant impact on the membrane bioreactor system used for lactic acid fermentation.

4.4.3 Nutrient limitations

Continuous lactic acid fermentations were carried out using an ultrafiltration (UF) membrane system (membrane bioreactor system) at various glucose concentrations (in the feed) of 120 g/L, 90 g/L and 60 g/L to investigate whether nutrient limitations dominate membrane bioreactor systems used for lactic acid fermentation. The concentrations of other nutrients (i.e., components of the fermentation medium as listed in Table 3.3) remained similar at all the glucose concentrations investigated, therefore only substrate limitations were considered in this investigation. It has been reported that substrate limitations in lactic acid fermentation can only be evident before end-product inhibition starts i.e., in the absence of the inhibition by the accumulated lactate, the assumption is that cessation in bacterial growth is due to deficiency in some of the nutrients required by the bacteria (Amrane et al., 1997; Balannec et al., 2007), hence, possible nutrient limitations were investigated between 15 h and 40 h of fermentation. This was dictated by the explanations given in section 4.4.2 above, that the end-product inhibition was evident after 40 h of fermentation in the present study.

As previously discussed in Chapter 2, when nutrients become limiting during lactic acid fermentation, bacterial cell growth declines and this affects the lactate production rate. Therefore, the profiles of biomass concentrations and lactate productivity for the continuous fermentation between 15 h and 40 h of fermentation are shown in Figure 4.6. It should be noted that Figure 4.6 was redrawn from Figure 4.5a with relevant sections partitioned to show the possible nutrient limitations period.

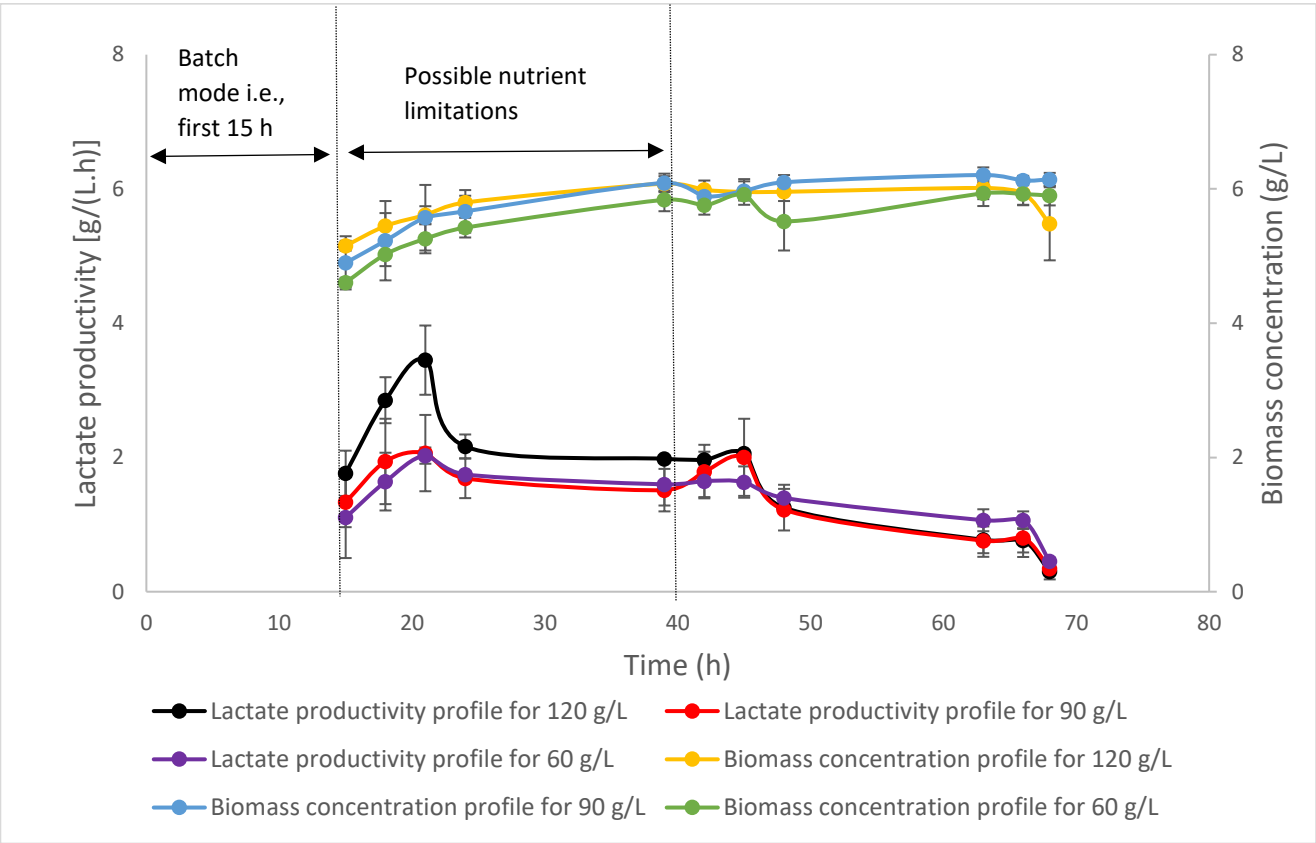


Figure 4.6: Profiles of biomass concentrations and lactate productivity for the continuous fermentation between 15 h and 40 h at glucose concentrations of 120 g/L, 90 g/L and 60 g/L (redrawn from Figure 4.5a but partitioned for this investigation)

(*Two repeated runs, average presented with error bars*)

From Figure 4.6, it can be seen that lactate productivity increased in the first 6 hours (i.e., from 15 h to 21 h of fermentation), and then started to decrease at all the glucose concentrations investigated. On the other hand, biomass concentration increased from 15 h up to 39 h of fermentation before a slight decrease could be observed at all the glucose concentrations investigated. The trends observed

in this investigation are contrary to those reported by Amrane et al. (1997) and Timmer et al. (1994). They reported cessation in the bacterial cell growth during substrate limitations even though lactic acid production continued through maintenance processes.

In this investigation, the biomass concentrations continued to increase from 15 h up to 39 h of fermentation whereas the lactate productivity started to decrease after 21 h up to 39 h of fermentation. Although these findings differ from those reported in the literature, there are two possible reasons that may be given to explain them:

(i) all the glucose concentrations investigated were probably above the threshold substrate concentration. The concept of threshold (minimum) substrate concentration has been described as that substrate concentration necessary to support both the bacterial cell growth and maintenance processes (Konopka, 2000). This simply means that below the threshold substrate concentration, the substrate is not enough to support both the bacterial cell growth and maintenance processes, and could possibly results in reduced biomass concentrations. From these explanations, it can therefore be assumed that the effect of substrate limitations can only be observed below the threshold substrate concentration.

(ii) since the amount of consumed nutrients is related to the amount of biomass synthesized (Leroy et al., 2001), then probably a larger portion of the substrate consumed between 21 h and 39 h of fermentation was mainly used for both bacterial cell growth and maintenance processes rather than lactate synthesis, which then led to increased biomass concentrations (Fu et al., 1999). The maintenance processes are postulated to represent a high fraction of total energy obtained from the substrate (Konopka, 2000). Therefore, it can be reasonably assumed that the lactate production between 21 h to 39 h was only determined by these maintenance processes, and that is why the lactate productivity decreased.

In conclusion, this investigation aimed at identifying whether nutrient limitations dominate membrane bioreactor systems used for lactic acid fermentation. It turned out that nutrient limitations were not realized probably because all the glucose concentrations investigated were above the threshold substrate concentration. Therefore, the investigation on nutrient limitations was regarded as inconclusive.

To achieve optimal bacterial cell growth in lactic acid fermentation using membrane bioreactor (MBR) systems, availability of nutrients as well as lactate removal from the fermenter must be given great consideration since lactate productivity depends on these two factors (Ramchandran et al., 2012). Although the results obtained in this investigation were inconclusive on whether nutrient limitations dominate the MBR systems used for lactic acid fermentation, further studies that involve nitrogen sources e.g., peptone and yeast extracts as well as mineral salts should be carried out to completely understand nutrient limitations in MBR systems.

Timmer et al., 1994 investigated the possibility of nutrient limitations to bacterial cell growth caused by deficiency of yeast extract on a batch fermentation of whey permeate (containing lactose) using *Lactobacillus helveticus* (Timmer et al., 1994). Similarly, Hurok Oh et al., 2003 investigated the effect of omitting yeast extract on a batch fermentation of glucose using *Enterococcus faecalis* RKY1 (Oh et al., 2003). In both investigations, the authors realized significant decrease in lactate productivity in the absence or low yeast extract concentrations. Even though these investigations were carried out in batch fermentation modes, they provide a basis for similar investigations in MBR systems to further understand nutrient limitations and conclude whether it dominates such systems.

Summary of the investigation on nutrient limitations

- Lactate productivity increased in the first 6 hours (i.e., from 15 h to 21 h of fermentation) before it started to decrease at all the glucose concentrations investigated.
- Biomass concentration increased from 15 h up to 39 h of fermentation before a slight decrease was observed at all the glucose concentrations investigated.
- The trends observed in this investigation were contrary to those reported in the literature.
- The glucose concentrations investigated i.e., 120 g/L, 90 g/L and 60 g/L were probably above the threshold substrate concentration (i.e., above the minimum substrate concentration for nutrient limitations to be observed).
- The investigation was therefore inconclusive on whether nutrient limitations dominate the membrane bioreactor systems used for lactic acid fermentation.

4.4.4 Mass transfer limitations

To investigate whether mass transfer limitations dominate membrane bioreactor systems used for lactic acid fermentation, continuous fermentations were conducted using an ultrafiltration (UF) membrane system at glucose concentrations (in the feed) of 120 g/L, 90 g/L and 60 g/L. As previously stated in Chapter 3, the continuous fermentation runs were started up in a batch fermentation mode with an initial glucose concentration of 30 g/L for the first 15 h to ensure that some glucose remained in the fermenter before switching over to the continuous mode. However, lactic acid fermentation being a biological process, was too unpredictable. In some experimental runs the glucose was completely depleted before switching over to the continuous fermentation mode whereas in some cases the glucose remained in the fermenter. To overcome this unpredictability, more runs had to be done and then a decision was made on which runs to use in plotting the graphs. The decision was based on the standard deviations of the residual glucose concentrations obtained from the different runs. The tables of the residual glucose concentrations are in Appendix A.

The profiles of residual glucose concentrations in the fermenter throughout the continuous fermentation period at the glucose concentrations of 120 g/L, 90 g/L and 60 g/L are shown in Figure 4.7. From Figure 4.7, it can be seen that the residual glucose concentrations in the fermenter were as low as 5 g/L, with most of them being zero at all the glucose concentrations investigated. These trends are in agreement with the observations made by Choudhury et al., 2006 who concluded that the increased cell density in the fermenter as a result of cell recycling enhances substrate to product conversion rate (Choudhury et al. 2006). Hence, for this investigation it can be reasonably assumed that the glucose that was fed during the continuous fermentation runs was rapidly consumed by the accumulated biomass in the fermenter leading to low residual glucose concentrations.

In this investigation it was postulated that the increased cell concentrations in the fermenter as a result of cell recycling could possibly affect the consumption of glucose by the bacteria due to mass transfer limitations, and thus significant amount of glucose could remain in the fermenter throughout the fermentation period. Nonetheless, from the results obtained, this was not the case. Therefore, it was concluded that mass transfer limitations do not have a significant impact on membrane bioreactor systems used for lactic acid fermentation since there was no consistent and significant

residual glucose in the fermenter throughout the fermentation period at all the glucose concentrations investigated.

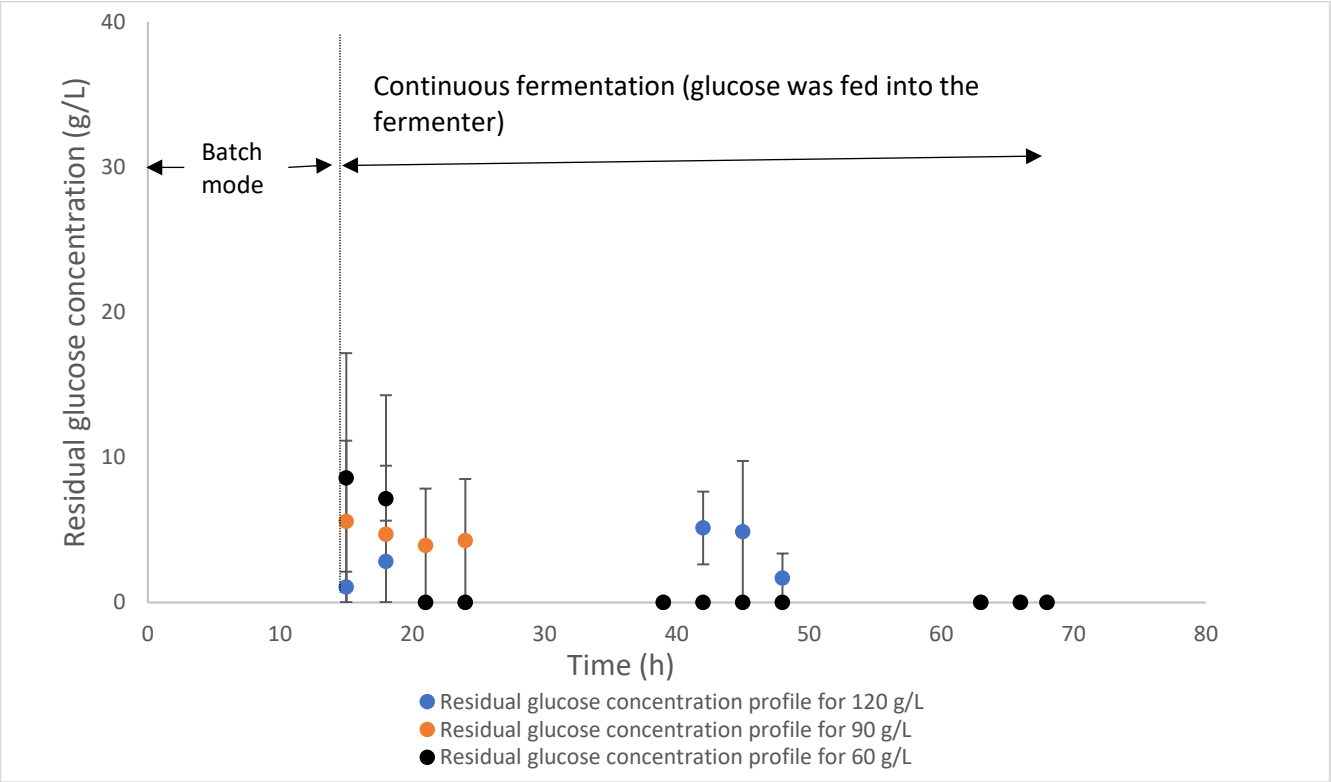


Figure 4.7: Profiles of residual glucose concentration in the fermenter throughout the continuous fermentation period at glucose concentrations of 120 g/L, 90 g/L and 60 g/L

(*Two repeated runs, average presented with error bars*)

The results obtained in this investigation imply that the mass transfer limitations which seem to be a major challenge in immobilized cell systems used for lactic acid fermentation (Wang et al., 1995; Zhang et al., 1994), are not present in the membrane bioreactor systems using free cells in the fermentation broth.

Summary of the investigation on mass transfer limitations

- The residual glucose concentrations in the fermenter were as low as 5 g/L, with most of them being zero throughout the investigation period at all the glucose concentrations investigated.
- The trends observed in this investigation were in agreement with the literature.

- From the results obtained in this investigation, it was concluded that mass transfer limitations do not have a significant impact on the membrane bioreactor systems used for lactic acid fermentation.

4.5 Critical flux experiments using lactate fermentation broths

From the investigations of the different process limitations that dominate membrane bioreactor systems used for lactic acid fermentation conducted in the present study, it turned out that membrane fouling was the major process limitation despite operating the ultrafiltration membrane system below the critical flux conditions (i.e., sub-critical flux operations).

Nonetheless, since the maximum biomass concentrations in the fermenter (i.e., approximately 6.0 g/L) during the continuous fermentation runs using the ultrafiltration membrane system exceeded those of bacterial cells (i.e., 3.99 ± 0.02 g/L) used in determining the membrane operating conditions, the critical flux experiments using bacterial cells alone were not able to establish whether operating below the critical flux conditions could significantly lower the membrane fouling. Therefore, a further set of critical flux experiments was designed using fermentation broths extracted from batch fermentation runs at different fermentation times. The fermentation broths extracted at 6 h, 15 h and 22 h from batch fermentation runs were used for this investigation. The choice of these fermentation times was dictated by the fact that:

- at 6 h, not all the nutrients (that could possibly lead to membrane fouling) had been consumed and the biomass concentrations had not increased significantly.
- at 15 h, most of the nutrients had been consumed and biomass concentrations had increased significantly.
- at 22 h, the fermentation broth possibly contained both the live and dead cells plus other by-products from the bacteria, and the biomass concentrations had increased significantly.

Each lactate fermentation broth was investigated at 3 cross-flow velocities i.e., 0.27, 0.50 and 0.81 m/s. The data obtained from these experiments are plotted in Figure 4.8 and Figure 4.9.

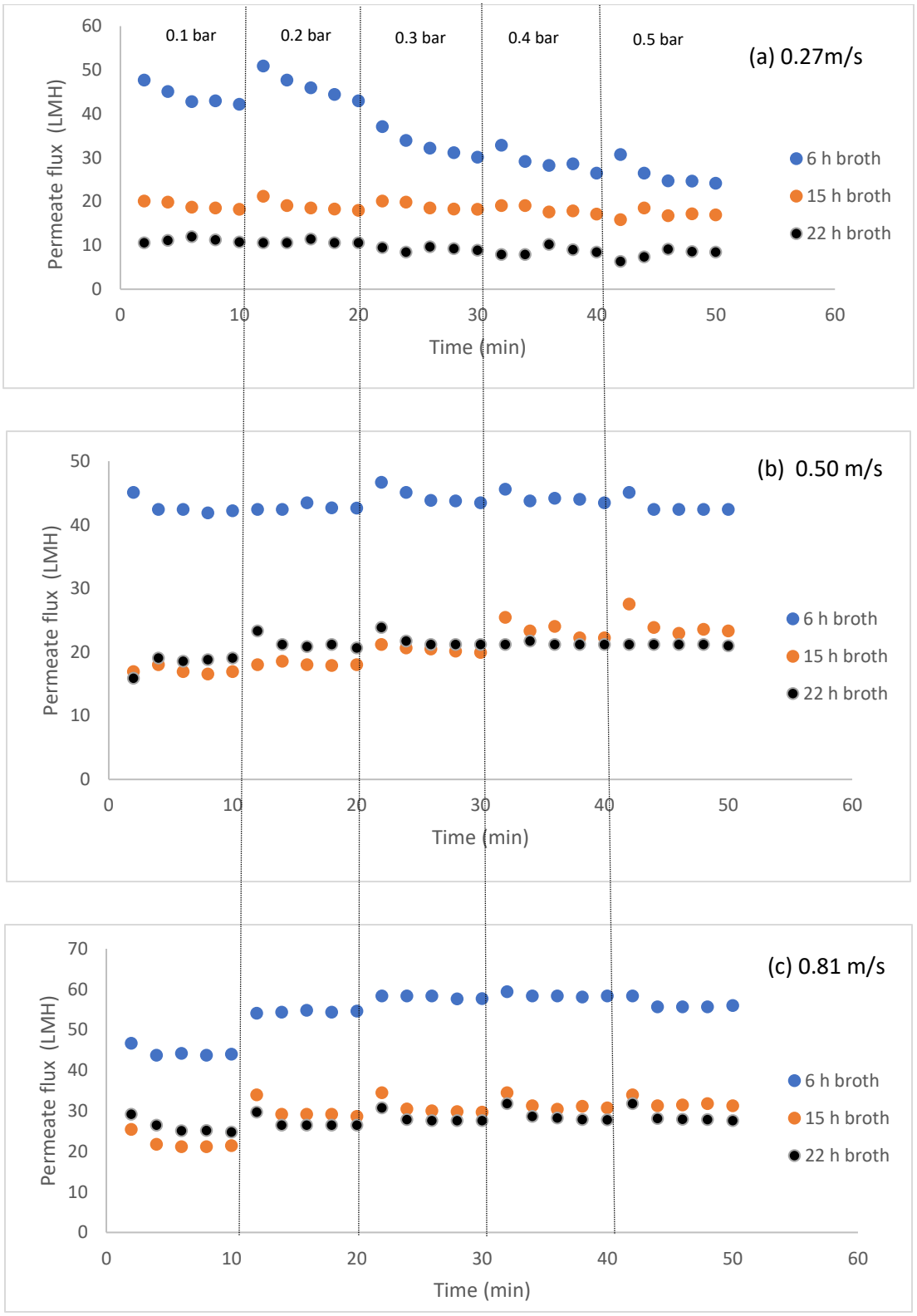


Figure 4.8: Profiles of five permeate flux readings taken during the 10 min of constant TMP at cross-flow velocity of: (a) 0.27 m/s, (b) 0.50 m/s and (c) 0.81 m/s

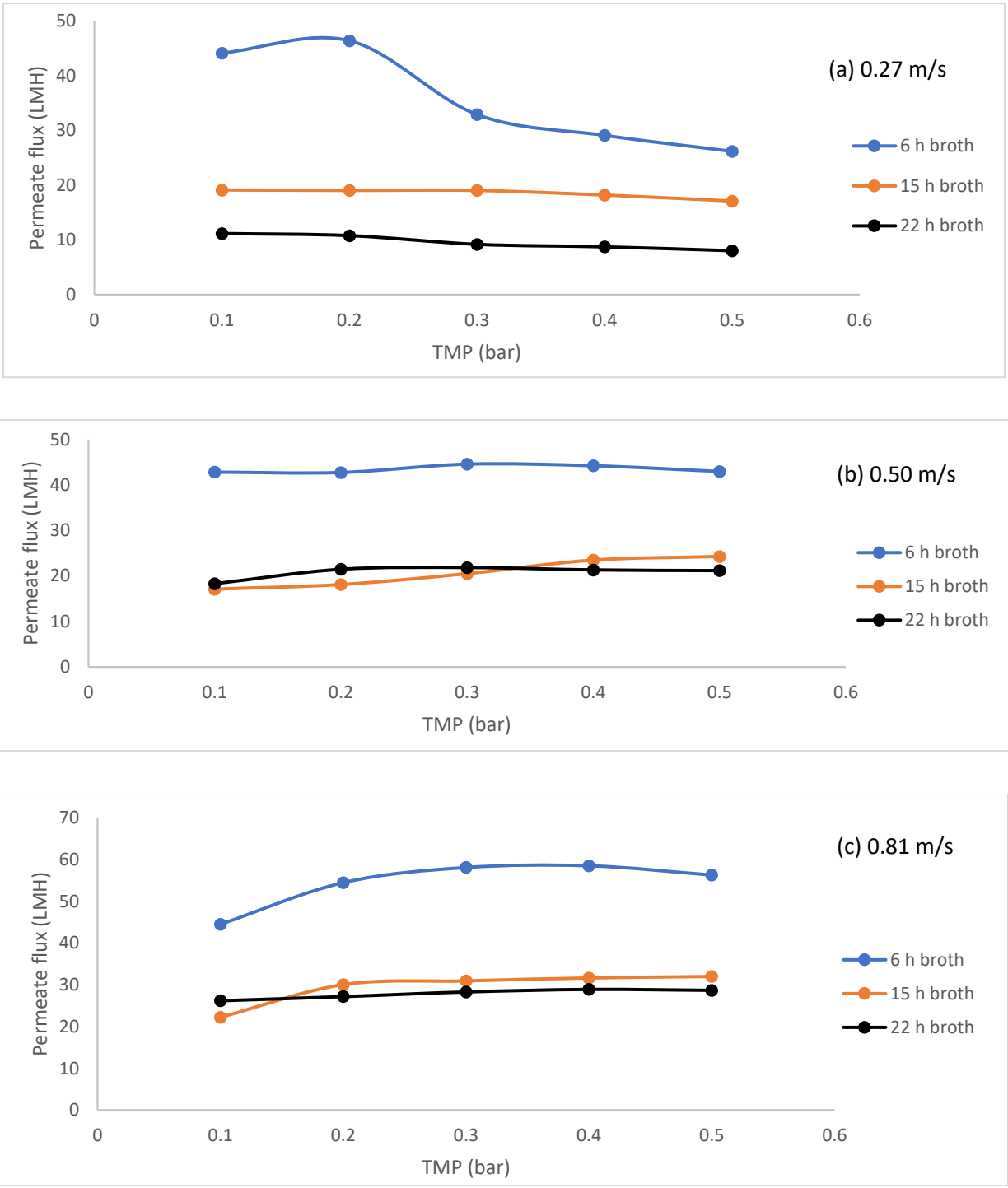


Figure 4.9: Profiles of permeate flux – TMP relationship at cross flow velocity of: (a) 0.27 m/s, (b) 0.50 m/s and (c) 0.81 m/s

(The permeate flux used in plotting these graphs is the arithmetic mean of the 5 permeate flux readings taken during the 10 min of the constant TMP)

From Figure 4.9, it can be seen that the trends of the permeate flux – TMP relationship for 15 h broth and 22 h broth runs were almost similar at high cross-flow velocities (i.e., 0.50 m/s and 0.81 m/s). Similarly, it can be seen that the permeate flux for 6 h broth runs was the highest at all the cross-flow velocities investigated compared to those for the 15 h and 22 h broth runs. Considering the fact that 6 h broth, 15 h broth and 22h broth had biomass concentrations of 3.82 g/L, 5.37 g/L and 6.03 g/L respectively, these trends are in agreement with the literature which had reported that the permeate flux decline increases with the increasing biomass concentrations (Fan et al., 2017b). This has been attributed to the fact that the bacterial cells cause membrane pore blocking, and subsequently lower the membrane permeability (Wang et al., 2013).

For the 6 h broth runs, a noticeable decrease in permeate flux was observed as the TMP was increased at a low cross-flow velocity of 0.27 m/s. However, at high cross-flow velocities of 0.50 m/s and 0.81 m/s, the permeate flux stabilized at some point. The rapid decrease in permeate flux observed at low cross-flow velocity when TMP was increased can be explained that the bacterial cells from the fermentation broth were adsorbed on the membrane surface and led to membrane pore blocking. The bacterial cell cake layer then became more compact at higher pressures resulting in high resistance to the permeate flow (Wang et al., 2013). On the other hand, at high cross-flow velocities, the shear force on the membrane surface was increased and this reduced the accumulation of bacterial cells on the membrane surface hence lowering the membrane fouling (Wang et al., 2013). This can be said in simple terms that at high cross-flow velocities, there was high “sweeping action” (Dey et al., 2012a).

From all the trends observed in Figure 4.9, the exact values of critical flux were not obtained. The 15 h and 22 h broths had high biomass concentrations, and thus had almost identical permeate flux – TMP relationship curves at high cross-flow velocities. Nevertheless, to establish whether operating below the critical flux conditions can significantly lower the membrane fouling in membrane bioreactor systems used for lactic acid fermentation, the critical flux experiments using 22 h broth which had high biomass concentrations similar to those obtained during the continuous fermentation runs were considered.

From Figure 4.9, for the 22 h broth runs, it was observed that:

i. at a cross-flow velocity of 0.27 m/s, the average permeate flux at 0.1 bar was 11.17 LMH, however, it continued to decrease as the TMP was increased to an average permeate flux value of 8.02 LMH at 0.5 bar. This shows that the bacterial cells from the fermentation broth formed a bacterial cell cake layer on the membrane surface and this cake layer became more compact at higher pressures reducing the membrane permeability (Wang et al., 2013).

ii. at a cross-flow velocity of 0.50 m/s, the average permeate flux at 0.1 bar was 18.30 LMH. This flux increased to 21.47 LMH at 0.2 bar, and thereafter remained constant as it became independent of the pressure. As was expected, the increase in cross-flow velocity increased the permeate flux. However, after 0.2 bar the permeate flux became independent of pressure because bacterial cells accumulated on the membrane surface and probably there was pore-blocking causing resistance to the permeate flow (Zhang et al., 1994).

iii. at a cross-flow velocity of 0.81 m/s, the permeate flux remained approximately constant at 28 LMH after the TMP of 0.3 bar.

Drawing conclusions from the above considerations of the 22 h broth runs

The critical flux experiments using lactate fermentation broths were carried out as a result of the significant membrane fouling observed in the continuous fermentation runs using the MBR system despite employing sub-critical flux operations. The initial permeate flux during continuous fermentation runs was 19.10 LMH, however at the end of the fermentation it reduced to below 5 LMH as shown in Figure 4.4. The biomass concentrations during these continuous fermentation runs were 6.0 g/L on average. Since these biomass concentrations were similar to those of the 22 h broth (i.e., 6.03 g/L), conclusions can be drawn from the above considerations of the 22 h broth runs to establish why significant membrane fouling was realized during the continuous fermentation runs using the MBR system. These may include:

(i) the high biomass concentrations in the fermenter were responsible for the significant membrane fouling during the continuous fermentation runs. From the considerations of the

22 h broth runs, membrane fouling (indicated by the permeate flux decline) was realized at all the three cross-flow velocities.

(ii) operations at a low cross-flow velocity of 0.27 m/s to avoid bacterial cell damage during the continuous fermentation runs contributed to significant membrane fouling. Although there was fouling at all the cross-flow velocities investigated for the 22 h broth, it was observed that significant membrane fouling occurred at a low cross-flow velocity of 0.27 m/s compared to that at high cross-flow velocities of 0.50 m/s and 0.81 m/s.

Similarly, it can be concluded from the considerations of the 22 h broth runs that operating below the critical flux conditions can significantly lower membrane fouling in membrane bioreactor systems used for lactic acid fermentation, however, this is only possible at high cross-flow velocities where the permeate flux becomes independent of transmembrane pressure. This simply means that at high cross-flow velocity, a particular transmembrane pressure can be used, and the permeate flux will remain relatively constant for a longer period as opposed to a low cross-flow velocity where the permeate flux does not become independent of pressure. Nevertheless, if the operations below the critical flux conditions are to be considered in MBR systems at high cross-flow velocities, proper measures should be put in place to monitor the bacterial cell damage because high cross-flow velocities can damage the bacterial cells and compromise their performance (Dey et al., 2012a; Giorno et al., 2002).

In another investigation carried out by Milcent et al., 2001 extended filtration times of lactic acid fermentation broths were observed when they operated below the critical flux, and they concluded that operating below the critical flux can extend the MBR operating times (Milcent et al., 2001). Although they used constant flux operations and the present study used constant pressure operations, the results obtained in the present study supported their conclusion that operations below the critical flux can be very promising when applied in MBR systems used for lactic acid fermentation in minimizing membrane fouling.

Summary of the investigation on critical flux experiments using lactate fermentation broths

- The permeate flux – TMP relationship curves for 15 h and 22 h broths runs were almost identical at high cross-flow velocities due to their close biomass concentrations.
- For the 6 h broth runs, there was noticeable decrease in the permeate flux at a low cross-flow velocity of 0.27 m/s due to significant fouling.
- The trends observed in this investigation were in agreement with the literature.
- At some point, the permeate flux became independent of TMP at higher cross-flow velocities (i.e., 0.50 m/s and 0.81 m/s) for the 22 h broth runs. This was not the case at a low cross-flow velocity of 0.27 m/s where the permeate flux continued to decrease as the TMP was increased throughout the filtration period.
- It was therefore concluded that the significant membrane fouling realized during the continuous fermentation runs was probably due to the low cross-flow velocity that was used.
- It was also concluded that operating below the critical flux conditions can significantly lower membrane fouling in membrane bioreactor systems used for lactic acid fermentation. However, this should be done at high cross-flow velocities where the permeate flux becomes independent of TMP, and proper measures to monitor bacterial cell damage should be put in place.

Chapter 5 Conclusion and recommendations

5.1 Conclusion

The overall aim of the present study was to improve the performance of membrane bioreactor (MBR) systems used for lactic acid fermentation by identifying their process limitations. To address this aim, five specific objectives were formulated:

(i) To investigate whether membrane fouling limitations dominate the MBR systems used for lactic acid fermentation.

(ii) To investigate whether lactate inhibition for bacterial cells once the membrane has fouled limits the MBR systems used for lactic acid fermentation.

(iii) To investigate whether nutrient limitations dominate the MBR systems used for lactic acid fermentation.

(iv) To investigate whether mass transfer limitations dominate the MBR systems used for lactic acid fermentation.

(v) To establish whether operating the MBR systems, used for lactic acid fermentation, below the critical flux conditions can significantly lower membrane fouling in those systems.

In line with meeting the aforementioned specific objectives, the conclusions drawn from the investigations that were carried out are summarized in this section.

5.1.1 Investigation of membrane fouling limitations

To investigate membrane fouling limitations, an ultrafiltration (UF) membrane system (MBR system) was operated below the critical flux conditions (i.e., sub-critical flux operations). The membrane operating conditions were determined by conducting critical flux experiments using bacterial cells. Continuous lactic acid fermentation runs were carried out using the UF membrane system (MBR system) at various glucose concentrations of 120 g/L, 90 g/L and 60 g/L. The permeate flux decline over time and the total volume of permeate at the end of the continuous fermentation runs were used to describe the occurrence of fouling.

From this investigation, it turned out that the permeate flux declined throughout the fermentation period at all the glucose concentrations of 120 g/L, 90 g/L and 60 g/L investigated. The permeate flux decline was more severe at a glucose concentration of 120 g/L than at glucose concentrations of 90 g/L and 60 g/L due to the high biomass concentrations realized at a glucose concentration of 120 g/L. The bacterial cells were responsible for the blocking of the membrane pores, and consequently led to severe membrane fouling. The total volume of permeate at the end of the fermentation runs at each glucose concentration investigated decreased in the order of 60 g/L > 90 g/L > 120 g/L, and this confirmed the severe membrane fouling at a glucose concentration of 120 g/L.

It was expected that there would be no or minimal membrane fouling observed when the ultrafiltration membrane system was operated below the critical flux (i.e., sub-critical flux operations). However, from the results obtained in this investigation, the permeate flux decline over time that was evident at all the glucose concentrations of 120 g/L, 90 g/L and 60 g/L indicated that there was significant membrane fouling. Physical cleaning methods alone were not able to remove the foulants but they were removed by chemical cleaning methods at all the glucose concentrations investigated depicting that irreversible fouling had occurred.

From this investigation, it was concluded that membrane fouling is a process limitation that dominates the performance of membrane bioreactor systems used for lactic acid fermentation despite employing the sub-critical flux operations.

5.1.2 Investigation of limitations attributed to lactate inhibition of bacterial cells once the membrane has fouled

Continuous lactic acid fermentation runs were carried out using an ultrafiltration (UF) membrane system (membrane bioreactor system) at glucose concentrations of 120 g/L, 90 g/L and 60 g/L in order to investigate this possible process limitation. Significant fouling, in this investigation, was considered after 40 h of fermentation when the permeate flux had decreased to between 40 % – 60 % of the initial flux. Hence, the corresponding lactate productivity as well as bacterial growth trends were studied after 40 h of fermentation when fouling was significant in the system.

From this investigation, it was observed that the lactate productivity decreased from 45 h until the end of the fermentation (i.e., 68 h) at all the glucose concentrations investigated. The decrease in lactate productivity was higher at glucose concentrations of 120 g/L and 90 g/L, than at a glucose concentration of 60 g/L due to the severe membrane fouling at high glucose concentrations which hindered the removal of the accumulated lactate because the membrane pores were blocked. Also, between 45 h and 68 h of fermentation, the biomass concentrations nearly remained constant at glucose concentrations of 90 g/L and 60 g/L, but there was a noticeable decrease in biomass concentrations at glucose concentration of 120 g/L towards the end of fermentation.

The removal of lactate from the fermentation broth using the ultrafiltration membrane system during continuous fermentations alleviated the inhibitory effects associated with the lactate to ensure proper bacterial cell growth as well as to improve lactate productivity. Nevertheless, once the membrane had fouled, it was not possible to achieve this because the accumulated lactate inhibited the bacterial cell growth and subsequently reduced the lactate production rate. Hence, from the investigation, it was concluded that lactate inhibition for the bacterial cells once the membrane has fouled is a process limitation that has a significant impact on the membrane bioreactor systems used for lactic acid fermentation.

5.1.3 Investigation of nutrient limitations

To investigate nutrient limitations, the present study only considered substrate limitations. Therefore, continuous lactic acid fermentation runs were carried out using an ultrafiltration (UF) membrane system at glucose concentrations of 120 g/L, 90 g/L and 60 g/L whereas the concentrations of other components of the fermentation medium remained the same for all the glucose concentrations investigated. The possible nutrient limitations were investigated between 15 h and 40 h of fermentation before the inhibitory effects of the accumulated lactate (end-product inhibition) were evident.

It was observed from the investigation that the lactate productivity increased in the first 6 hours (i.e., from 15 h to 21 h of fermentation) before it started to decrease at all the glucose concentrations investigated. On the other hand, biomass concentrations increased from 15 h up to 39 h of fermentation before a slight decrease was observed at all the glucose concentrations investigated.

Nevertheless, from the investigation, it turned out that possibly all the glucose concentrations investigated were above the threshold substrate concentration (i.e., above the minimum substrate concentration for nutrient limitations to be observed). Hence, the investigation on nutrient limitations was not able to conclude on whether nutrient limitations have a significant impact on the membrane bioreactor systems used for lactic acid fermentation.

5.1.4 Investigation of mass transfer limitations

To investigate whether mass transfer limitations dominate membrane bioreactor systems used for lactic acid fermentation, continuous fermentations were conducted using an ultrafiltration (UF) membrane system at glucose concentrations of 120 g/L, 90 g/L and 60 g/L. In this investigation, it was postulated that the increased cell concentrations in the fermenter as a result of cell recycling could possibly affect the consumption of glucose by the bacteria due to mass transfer limitations and hence significant amount of glucose could remain in the fermenter throughout the fermentation period.

However, from the investigation, the residual glucose concentrations in the fermenter turned out to be as low as 5 g/L, with most of them being zero at all the glucose concentrations investigated. This was attributed to the rapid consumption of glucose by the increased bacterial cells in the fermenter. It was therefore concluded that mass transfer limitations do not have a significant impact on membrane bioreactor systems used for lactic acid fermentation because there was no consistent and significant residual glucose in the fermenter throughout the fermentation period.

5.1.5 Establishing whether operating membrane bioreactor (MBR) systems, used for lactic acid fermentation, below the critical flux conditions can significantly lower membrane fouling in those MBR systems

This investigation was conducted because there was significant membrane fouling realized during the continuous fermentation runs despite operating the membrane bioreactor systems below the critical flux conditions. Lactate fermentation broths of different biomass concentrations extracted at 6 h, 15 h and 22 h from batch fermentation runs were used for the critical flux experiments, and each

lactate fermentation broth was investigated at three cross-flow velocities which included both the low and high cross-flow velocities i.e., 0.27, 0.50 and 0.81 m/s.

To establish whether operating below the critical flux conditions can significantly lower membrane fouling in membrane bioreactor systems used for lactic acid fermentation, the critical flux experiments of the lactate fermentation broth extracted after 22 h were considered because they had high biomass concentrations similar to those obtained during the continuous fermentation runs. From the results obtained, the permeate flux decreased as the transmembrane pressure (TMP) was increased at low cross-flow velocity of 0.27 m/s whereas at high cross-flow velocities of 0.50 m/s and 0.81 m/s, the average permeate flux became independent of the TMP at pressures higher than 0.2 bar and 0.3 bar respectively.

Following the considerations of the critical flux experiments using the lactate fermentation broth extracted after 22 h, it was concluded that the significant membrane fouling realized during the continuous fermentation runs in the present study was due to the low cross-flow velocity of 0.27 m/s that was used to avoid the bacterial cell damage. Also, it was concluded that operating below the critical flux conditions can significantly lower membrane fouling in membrane bioreactor systems used for lactic acid fermentation, however, this is only possible at high cross-flow velocities. Hence, proper measures should be put in place to monitor bacterial cell damage because high cross-flow velocities can damage the cells and compromise their performance.

5.2 Recommendations

From the investigations of process limitations that dominate membrane bioreactor systems used for lactic acid fermentation, membrane fouling and lactate inhibition for bacterial cells once the fouling has occurred proved to be the main process limitations. In addition, the investigation on nutrient limitations was inconclusive. Therefore, the following investigations are recommended in the future.

i. In the present study, monitoring and control of the biomass concentrations were not possible because there was no access to the laboratory at night, hence, investigations that involve automated monitoring and control systems to monitor the bacterial cell growth in the fermenter and subsequently carry out cell bleeding to regulate the number of cells in the fermenter are recommended. It has been postulated that by regulating the number of bacterial cells in a manner that does not compromise the efficiency of lactic acid fermentation can help in minimizing membrane fouling because an increase in biomass concentrations led to severe membrane fouling in the present study.

ii. Since the present study only focused on substrate limitations, investigations on limitations of other nutrients such as yeast extract and peptone to the bacterial cell growth are recommended for future studies.

iii. The present study did not study bacterial cell damage owing to the fact that running at low cross-flow velocity was not able to result in high shear effects that could damage the cells. However, this led to significant membrane fouling; hence, future studies can try to vary the operating conditions to include high cross-flow velocities and monitor their side effects on bacteria cell growth.

iv. The present study did not put in place measures to have automated physical and chemical cleaning methods as the fermentation progresses. This should be pursued further since it has been postulated that regular backflushing of the membrane can alleviate the long term effects of membrane fouling.

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Appendix A: Experimental raw data and processed results

A.1 Calibration data for Masterflex peristaltic pump

Table A.1: Calibration data for Masterflex peristaltic pump and the corresponding cross-flow velocity

Pump setting position	Flow rate in L/h	Flow rate in m^3/s	Corresponding cross-flow velocity in m/s
0.5	22.20	6.1667E-06	0.22
1	27.60	7.6667E-06	0.27
1.5	34.80	9.6667E-06	0.34
2	39.00	1.0833E-05	0.38
2.5	43.80	1.2167E-05	0.43
3	50.40	1.4000E-05	0.50
4	60.00	1.6667E-05	0.59
5	69.00	1.9167E-05	0.68
6	82.50	2.2917E-05	0.81
7	91.20	2.5333E-05	0.90
Maximum	104.40	2.9000E-05	1.03

A.2 Calibration curve for *Lactobacillus casei* ATCC 393 generated in the present study

Table A.2: Raw data generated for the calibration curve for *Lactobacillus casei* ATCC 393

Dilution	Mass of dry filter paper (g)			Mass of filter paper with dried biomass (g)			Volume of sample filtered (mL)
	Run 1	Run 2	Run 3	Run 1	Run 2	Run 3	
1	0.0768	0.0767	0.0770	0.0810	0.0821	0.0822	5
2	0.0768	0.0770	0.0770	0.0854	0.0823	0.0846	5
3	0.0769	0.0769	0.0767	0.0889	0.0892	0.0879	5
4	0.0769	0.0771	0.0768	0.0923	0.0921	0.0931	5
5	0.0770	0.0770	0.0767	0.0948	0.0952	0.0938	5

Table A.3: Processed data used in generating the calibration curve for *Lactobacillus casei* ATCC 393

Dilution	Optical density at 620 nm				Cell dry weight (CDW) in g/L				
	Run 1	Run 2	Run 3	Average	Run 1	Run 2	Run 3	Average	Standard deviation
1	0.261	0.296	0.284	0.280	0.84	1.08	1.04	0.99	0.10
2	0.592	0.617	0.679	0.629	1.72	1.06	1.52	1.43	0.28
3	0.904	0.885	0.971	0.920	2.40	2.46	2.24	2.37	0.09
4	1.260	1.205	1.232	1.232	3.08	3.00	3.26	3.11	0.11
5	1.387	1.438	1.460	1.428	3.56	3.64	3.42	3.54	0.09

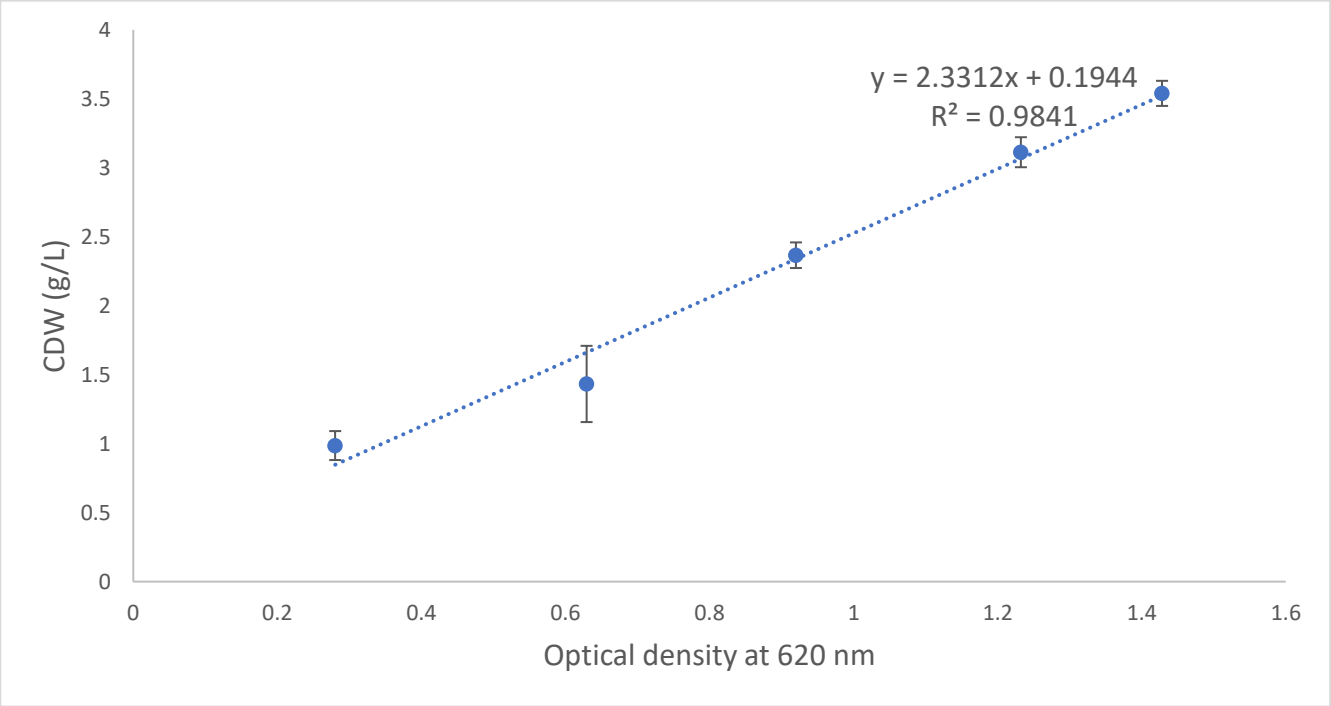


Figure A.1: Calibration curve for *Lactobacillus casei* ATCC 393

(Three repeated runs, average presented with error bars)

A.3 Batch fermentation runs

The batch fermentation experiments were carried out in triplicate. All the 3 runs were used for the average.

Table A.4: Experimental data for lactate concentrations (g/L) obtained from batch fermentation runs

Lactate concentration (g/L)					
Time (h)	Run 1	Run 2	Run 3	Average	Standard deviation
3	6.89	6.17	8.07	7.04	0.78
6	8.52	8.66	10.70	9.29	0.99
9	9.96	9.27	13.56	10.93	1.88
12	22.28	25.80	24.49	24.19	1.45
15	25.36	25.96	25.18	25.50	0.33
18	25.82	21.83	22.50	23.38	1.74
21	27.60	24.83	22.06	24.83	2.26
24	25.38	25.05	17.32	22.58	3.72

Table A.5: Experimental data for biomass concentrations (g/L) obtained from batch fermentation runs

Biomass concentration (g/L)					
Time (h)	Run 1	Run 2	Run 3	Average	Standard deviation
3	4.96	4.57	5.09	4.87	0.22
6	5.08	5.14	5.38	5.20	0.13
9	4.93	5.16	5.55	5.21	0.25
12	6.06	6.01	5.96	6.01	0.04
15	5.98	6.10	6.08	6.05	0.05
18	6.03	6.18	6.02	6.08	0.07
21	6.11	6.19	5.91	6.07	0.12
24	5.95	6.12	6.05	6.04	0.07

Table A.6: Experimental data for residual glucose concentrations (g/L) obtained from batch fermentation runs

Residual glucose concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average**	**Standard deviation**
3	20.99	23.56	23.70	22.75	1.25
6	19.61	21.55	19.04	20.07	1.07
9	16.86	16.12	14.24	15.74	1.10
12	3.89	0.00	6.37	3.42	2.62
15	0.00	0.00	0.00	0.00	0.00
18	0.00	0.00	0.00	0.00	0.00
21	0.00	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00

Table A.7: Calculated data for lactate productivity [g/(L.h)] from batch fermentation runs

Lactate productivity [g/(L.h)]					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average**	**Standard deviation**
3	2.30	2.06	2.69	2.35	0.26
6	1.42	1.44	1.78	1.55	0.17
9	1.11	1.03	1.51	1.21	0.21
12	1.86	2.15	2.04	2.02	0.12
15	1.69	1.73	1.68	1.70	0.02
18	1.43	1.21	1.25	1.30	0.10
21	1.31	1.18	1.05	1.18	0.11
24	1.06	1.04	0.72	0.94	0.16

Table A.8: Results of the 15 h-batch fermentation run with 30 g/L glucose as substrate and different inoculum sizes

	Residual glucose concentration (g/L)			**Biomass concentration (g/L)**		
Inoculum size	Run 1	Run 2	Average	Run 1	Run 2	Average
1 % (v/v)	10.07	7.04	8.55	5.19	5.27	5.23
0.5 % (v/v)	19.48	14.30	16.89	4.35	4.49	4.42
0.1 % (v/v)	24.74	24.80	24.77	4.02	3.92	3.97

A.4 Continuous fermentation runs

Although the continuous fermentation experiments were carried out in triplicate, some runs were outliers based on the standard deviations of glucose, lactate and biomass concentrations obtained, and hence they were not used to determine the average.

(a) Continuous fermentation runs at glucose concentration of 120 g/L

*(Run 1 was an outlier therefore average of Run 2 and Run 3 presented with the standard deviatio*n).

Table A.9: Experimental data for lactate concentrations (g/L) obtained from continuous fermentation runs at glucose concentration of 120 g/L

Lactate concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	18.94	29.18	19.82	24.50	4.68
18	30.27	44.38	34.86	39.62	4.76
21	33.88	55.07	40.74	47.91	7.16
24	31.69	65.06	49.63	57.35	7.71
39	52.07	71.93	69.47	70.70	1.23
42	55.42	74.49	65.97	70.23	4.26
45	55.79	74.55	72.50	73.53	1.02
48	55.93	76.56	64.02	70.29	6.27
63	55.52	80.98	71.73	76.36	4.63
66	59.66	78.87	73.10	75.98	2.89
68	55.01	80.52	70.14	75.33	5.19

Table A.10: Experimental data for biomass concentrations (g/L) obtained from continuous fermentation runs at glucose concentration of 120 g/L

Biomass concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	4.49	5.08	5.23	5.15	0.07
18	4.99	5.26	5.64	5.45	0.19
21	5.09	5.48	5.74	5.61	0.13
24	5.24	5.70	5.90	5.80	0.10
39	5.90	5.97	6.19	6.08	0.11
42	5.93	5.85	6.12	5.99	0.14
45	5.73	5.76	6.15	5.95	0.19
48	5.69	5.82	6.09	5.96	0.14
63	5.72	5.85	6.18	6.01	0.17
66	5.80	5.76	6.12	5.94	0.18
68	5.77	4.94	6.02	5.48	0.54

Table A.11 Experimental data for residual glucose concentrations (g/L) obtained from continuous fermentation runs at glucose concentration of 120 g/L

Residual glucose concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	3.80	0.00	2.12	1.06	1.06
18	17.65	5.64	0.00	2.82	2.82
21	17.59	0.00	0.00	0.00	0.00
24	15.45	0.00	0.00	0.00	0.00
39	2.47	0.00	0.00	0.00	0.00
42	7.91	7.64	2.63	5.13	2.50
45	10.73	9.75	0.00	4.88	4.88
48	16.58	0.00	3.38	1.69	1.69
63	16.29	0.00	0.00	0.00	0.00
66	12.52	0.00	0.00	0.00	0.00
68	10.95	0.00	0.00	0.00	0.00

Table A.12: Calculated data for lactate productivity [g/(L.h)] from continuous fermentation runs at glucose concentration of 120 g/L

Lactate productivity [g/(L.h)]					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	1.36	2.10	1.43	1.76	0.34
18	2.18	3.20	2.51	2.85	0.34
21	2.44	3.97	2.93	3.45	0.52
24	0.89	2.34	1.99	2.16	0.18
39	0.83	2.01	1.95	1.98	0.03
42	0.89	2.09	1.85	1.97	0.12
45	0.89	2.09	2.03	2.06	0.03
48	0.67	1.22	1.28	1.25	0.03
63	0.22	0.97	0.57	0.77	0.20
66	0.24	0.95	0.58	0.77	0.18
68	0.22	0.32	0.28	0.30	0.02

Table A.13: Calculated data for permeate flux (LMH) from continuous fermentation runs at glucose concentration of 120 g/L

Permeate flux (LMH)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	19.10	19.10	19.10	19.10	0.00
24	7.43	9.55	10.61	10.08	0.53
39	4.24	7.43	7.43	7.43	0.00
48	3.18	4.24	5.31	4.77	0.53
63	1.06	3.18	2.12	2.65	0.53
68	1.06	1.06	1.06	1.06	0.00

Table A.14: Total volume of permeate (mL) at the end of continuous fermentation runs at glucose concentration of 120 g/L

Total volume of permeate (mL)				
Run 1	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
584	870	980	925	55

(b) Continuous fermentation runs at glucose concentration of 90 g/L

(Run 2 was an outlier therefore average of Run 1 and Run 3 presented with the standard deviation).

Table A.15: Experimental data for lactate concentrations (g/L) obtained from continuous fermentation runs at glucose concentration of 90 g/L

Lactate concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 1 and Run 3**	**Standard deviation**
15	13.36	14.39	22.45	17.90	4.55
18	18.12	27.47	33.90	26.01	7.89
21	20.79	38.80	34.65	27.72	6.93
24	24.89	45.33	35.45	30.17	5.28
39	29.92	49.99	38.12	34.02	4.10
42	34.77	57.91	45.60	40.18	5.41
45	35.69	54.45	53.67	44.68	8.99
48	38.01	57.66	63.83	50.92	12.91
63	43.30	53.02	62.54	52.92	9.62
66	43.12	48.94	67.85	55.48	12.37
68	45.84	50.90	63.55	54.69	8.86

Table A.16: Experimental data for biomass concentrations (g/L) obtained from continuous fermentation runs at glucose concentration of 90 g/L

Biomass concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 1 and Run 3**	**Standard deviation**
15	5.30	3.81	4.50	4.90	0.40
18	5.82	4.37	4.64	5.23	0.59
21	6.06	4.53	5.08	5.57	0.49
24	5.98	4.59	5.35	5.67	0.32
39	6.23	4.95	5.95	6.09	0.14
42	6.03	4.76	5.75	5.89	0.14
45	6.11	4.92	5.83	5.97	0.14
48	6.21	5.02	5.99	6.10	0.11
63	6.32	5.22	6.09	6.21	0.11
66	6.19	5.16	6.06	6.12	0.07
68	6.24	5.12	6.05	6.14	0.10

Table A.17: Experimental data for residual glucose concentrations (g/L) obtained from continuous fermentation runs at glucose concentration of 90 g/L

Residual glucose concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 1 and Run 3**	**Standard deviation**
15	11.15	2.73	0.00	5.57	5.57
18	9.42	39.39	0.00	4.71	4.71
21	7.85	38.18	0.00	3.92	3.92
24	8.51	21.69	0.00	4.25	4.25
39	0.00	0.00	0.00	0.00	0.00
42	0.00	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00	0.00
48	0.00	0.00	0.00	0.00	0.00
63	0.00	0.00	0.00	0.00	0.00
66	0.00	0.00	0.00	0.00	0.00
68	0.00	0.00	0.00	0.00	0.00

Table A.18: Calculated data for lactate productivity [g/(L.h)] from continuous fermentation runs at glucose concentration of 90 g/L

Lactate productivity [g/(L.h)]					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 1 and Run 3**	**Standard deviation**
15	0.96	1.04	1.71	1.33	0.37
18	1.30	1.98	2.58	1.94	0.64
21	1.50	2.79	2.63	2.06	0.57
24	1.39	2.18	1.99	1.69	0.30
39	1.20	1.60	1.83	1.51	0.32
42	1.39	1.85	2.19	1.79	0.40
45	1.43	1.74	2.58	2.00	0.57
48	0.91	1.38	1.53	1.22	0.31
63	0.52	0.64	1.00	0.76	0.24
66	0.52	0.59	1.09	0.80	0.28
68	0.18	0.20	0.51	0.35	0.16

Table A.19: Calculated data for permeate flux (LMH) from continuous fermentation runs at glucose concentration of 90 g/L

Permeate flux (LMH)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 1 and Run 3**	**Standard deviation**
15	19.10	19.10	20.16	19.63	0.53
24	14.85	12.73	14.85	14.85	0.00
39	10.61	8.49	12.73	11.67	1.06
48	6.37	6.37	6.37	6.37	0.00
63	3.18	3.18	4.24	3.71	0.53
68	1.06	1.06	2.12	1.59	0.53

Table A.20: Total volume of permeate (mL) at the end of continuous fermentation runs at glucose concentration of 90 g/L

Total volume of permeate (mL)				
Run 1	**Run 2**	**Run 3**	**Average of Run 1 and Run 3**	**Standard deviation**
1330	930	1550	1440	110

(c) Continuous fermentation runs at glucose concentration of 60 g/L

(Run 1 was an outlier therefore average of Run 2 and Run 3 presented with the standard deviation).

Table A.21: Experimental data for lactate concentrations (g/L) obtained from continuous fermentation runs at glucose concentration of 60 g/L

Lactate concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	15.52	6.96	23.74	15.35	8.39
18	14.39	16.81	28.76	22.78	5.97
21	15.83	26.48	29.84	28.16	1.68
24	20.77	32.17	30.23	31.20	0.97
39	22.84	39.90	32.10	36.00	3.90
42	23.59	39.28	35.24	37.26	2.02
45	23.37	38.88	35.00	36.94	1.94
48	22.55	39.82	37.51	38.67	1.16
63	25.61	43.86	37.56	40.71	3.15
66	27.99	42.74	38.96	40.85	1.89
68	28.70	39.29	36.04	37.66	1.62

Table A.22: Experimental data for biomass concentrations (g/L) obtained from continuous fermentation runs at glucose concentration of 60 g/L

Biomass concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	4.86	4.53	4.68	4.61	0.07
18	5.12	5.20	4.85	5.03	0.18
21	5.73	5.47	5.04	5.26	0.22
24	5.64	5.57	5.28	5.42	0.15
39	5.95	5.67	6.01	5.84	0.17
42	6.05	5.62	5.90	5.76	0.14
45	6.02	5.85	5.98	5.91	0.07
48	6.00	5.08	5.95	5.52	0.43
63	6.26	5.74	6.12	5.93	0.19
66	6.30	5.76	6.08	5.92	0.16
68	6.34	5.75	6.05	5.90	0.15

Table A.23: Experimental data for residual glucose concentrations (g/L) obtained from continuous fermentation runs at glucose concentration of 60 g/L

Residual glucose concentration (g/L)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	10.30	17.18	0.00	8.59	8.59
18	16.03	14.28	0.00	7.14	7.14
21	3.06	0.00	0.00	0.00	0.00
24	0.00	0.00	0.00	0.00	0.00
39	0.00	0.00	0.00	0.00	0.00
42	0.00	0.00	0.00	0.00	0.00
45	0.00	0.00	0.00	0.00	0.00
48	0.00	0.00	0.00	0.00	0.00
63	0.00	0.00	0.00	0.00	0.00
66	0.00	0.00	0.00	0.00	0.00
68	0.00	0.00	0.00	0.00	0.00

Table A.24: Calculated data for lactate productivity [g/(L.h)] from continuous fermentation runs at glucose concentration of 60 g/L

Lactate productivity [g/(L.h)]					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	1.18	0.50	1.71	1.11	0.60
18	1.09	1.21	2.07	1.64	0.43
21	1.20	1.91	2.15	2.03	0.12
24	1.00	1.80	1.69	1.75	0.05
39	0.91	1.92	1.28	1.60	0.32
42	0.94	1.89	1.41	1.65	0.24
45	0.93	1.87	1.40	1.63	0.23
48	0.63	1.59	1.20	1.40	0.20
63	0.51	1.23	0.90	1.06	0.16
66	0.56	1.20	0.93	1.07	0.13
68	0.34	0.47	0.43	0.45	0.02

Table A.25: Calculated data for permeate flux (LMH) from continuous fermentation runs at glucose concentration of 60 g/L

Permeate flux (LMH)					
Time (h)	**Run 1**	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
15	20.16	19.10	19.10	19.10	0.00
24	12.73	14.85	14.85	14.85	0.00
39	10.61	12.73	10.61	11.67	1.06
48	7.43	10.61	8.49	9.55	1.06
63	5.31	7.43	6.37	6.90	0.53
68	3.18	3.18	3.18	3.18	0.00

Table A.26: Total volume of permeate (mL) at the end of continuous fermentation runs at glucose concentration of 60 g/L

Total volume of permeate (mL)				
Run 1	**Run 2**	**Run 3**	**Average of Run 2 and Run 3**	**Standard deviation**
1560	1938	1845	1891.5	46.5

A.5 Critical flux experiments

(a) Critical flux experiments using bacterial cells

- Cross-flow velocity of 0.27 m/s

Table A.27: Results of critical flux experiments using bacterial cells at cross-flow velocity of 0.27 m/s

TMP (bar)	Time (min)	Volume of permeate (mL) Run 1	Run 2	Average (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	2.8	2.8	2.8	1.400	0.084	14.85	
	4	4.2	4.2	4.2	1.050	0.063	11.14	
0.1	6	6	5.8	5.9	0.983	0.059	10.43	11.08
	8	7.2	7.2	7.2	0.900	0.054	9.55	
	10	8.8	9	8.9	0.890	0.053	9.44	
	2	3.2	3	3.1	1.550	0.093	16.45	
	4	5.8	5.5	5.65	1.413	0.085	14.99	
0.2	6	8	8	8	1.333	0.080	14.15	14.70
	8	10.5	10.5	10.5	1.313	0.079	13.93	
	10	13	13.4	13.2	1.320	0.079	14.01	
	2	4.8	4.6	4.7	2.350	0.141	24.93	
	4	8.5	8.2	8.35	2.088	0.125	22.15	
0.3	6	12.8	12	12.4	2.067	0.124	21.93	22.38
	8	16.5	16	16.25	2.031	0.122	21.55	
	10	20	20.2	20.1	2.010	0.121	21.33	
	2	5	4.8	4.9	2.450	0.147	25.99	
	4	8.8	8	8.4	2.100	0.126	22.28	
0.4	6	12.8	12	12.4	2.067	0.124	21.93	22.99
	8	17.2	16.5	16.85	2.106	0.126	22.35	
	10	21.5	20.8	21.15	2.115	0.127	22.44	

- Cross-flow velocity of 0.50 m/s

Table A.28: Results of critical flux experiments using bacterial cells at cross-flow velocity of 0.50 m/s

TMP (bar)	Time (min)	Volume of permeate (mL) Run 1	Run 2	Average (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	3.8	3.6	3.7	1.850	0.111	19.63	
	4	6.5	6.2	6.35	1.588	0.095	16.84	
0.1	6	8.4	8.2	8.3	1.383	0.083	14.68	16.13
	8	11.5	11	11.25	1.406	0.084	14.92	
	10	14	13.5	13.75	1.375	0.083	14.59	
	2	4.8	4.8	4.8	2.400	0.144	25.46	
	4	8.4	8.5	8.45	2.113	0.127	22.41	
0.2	6	12	12	12	2.000	0.120	21.22	22.33
	8	16.2	16	16.1	2.013	0.121	21.35	
	10	20.2	19.8	20	2.000	0.120	21.22	
	2	5.5	5.2	5.35	2.675	0.161	28.38	
	4	9.5	9.2	9.35	2.338	0.140	24.80	
0.3	6	14	13.6	13.8	2.300	0.138	24.40	25.18
	8	18.4	18	18.2	2.275	0.137	24.14	
	10	23	22.6	22.8	2.280	0.137	24.19	

- Cross-flow velocity of 0.90 m/s

Table A.29: Results of critical flux experiments using bacterial cells at cross-flow velocity of 0.90 m/s

TMP (bar)	Time (min)	Volume of permeate (mL) Run 1	Run 2	Average (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	2.5	2.8	2.65	1.325	0.080	14.06	
	4	4.5	4.5	4.5	1.125	0.068	11.94	
0.1	6	6	6	6	1.000	0.060	10.61	11.39
	8	7.5	8	7.75	0.969	0.058	10.28	
	10	9.2	9.8	9.6	0.950	0.057	10.08	

(b) Critical flux experiments using lactate fermentation broths

i. 6 h broth

- Cross-flow velocity of 0.27 m/s

Table A.30: Results of critical flux experiments using 6 h broth at cross-flow velocity of 0.27 m/s

TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	9	4.500	0.270	47.75	
	4	17	4.250	0.255	45.09	
0.1	6	24.2	4.033	0.242	42.79	44.17
	8	32.4	4.050	0.243	42.97	
	10	39.8	3.980	0.239	42.23	
	2	9.6	4.800	0.288	50.93	
	4	18	4.500	0.270	47.75	
0.2	6	26	4.333	0.260	45.98	46.41
	8	33.5	4.188	0.251	44.43	
	10	40.5	4.050	0.243	42.97	
	2	7	3.500	0.210	37.14	
	4	12.8	3.200	0.192	33.95	
0.3	6	18.2	3.033	0.182	32.18	32.91
	8	23.5	2.938	0.176	31.17	
	10	28.4	2.840	0.170	30.13	
	2	6.2	3.100	0.186	32.89	
	4	11	2.750	0.165	29.18	
0.4	6	16	2.667	0.160	28.29	29.11
	8	21.6	2.700	0.162	28.65	
	10	25	2.500	0.150	26.53	
	2	5.8	2.900	0.174	30.77	
0.5	4	10	2.500	0.150	26.53	26.18
	6	14	2.333	0.140	24.76	
	8	18.6	2.325	0.140	24.67	
	10	22.8	2.280	0.137	24.19	

- Cross-flow velocity of 0.50 m/s

Table A.31: Results of critical flux experiments using 6 h broth at cross-flow velocity of 0.50 m/s

TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	8.5	4.250	0.255	45.09	
	4	16	4.000	0.240	42.44	
0.1	6	24	4.000	0.240	42.44	42.82
	8	31.6	3.950	0.237	41.91	
	10	39.8	3.980	0.239	42.23	
	2	8	4.000	0.240	42.44	
	4	16	4.000	0.240	42.44	
0.2	6	24.6	4.100	0.246	43.50	42.75
	8	32.2	4.025	0.242	42.71	
	10	40.2	4.020	0.241	42.65	
	2	8.8	4.400	0.264	46.68	
	4	17	4.250	0.255	45.09	
0.3	6	24.8	4.133	0.248	43.85	44.58
	8	33	4.125	0.248	43.77	
	10	41	4.100	0.246	43.50	
	2	8.6	4.300	0.258	45.62	
	4	16.5	4.125	0.248	43.77	
0.4	6	25	4.167	0.250	44.21	44.23
	8	33.2	4.150	0.249	44.03	
	10	41	4.100	0.246	43.50	
	2	8.5	4.250	0.255	45.09	
0.5	4	16	4.000	0.240	42.44	42.97
	6	24	4.000	0.240	42.44	
	8	32	4.000	0.240	42.44	
	10	40	4.000	0.240	42.44	

- Cross-flow velocity of 0.81 m/s

Table A.32: Results of critical flux experiments using 6 h broth at cross-flow velocity of 0.81 m/s

TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	8.8	4.400	0.264	46.68	
	4	16.5	4.125	0.248	43.77	
0.1	6	25	4.167	0.250	44.21	44.49
	8	33	4.125	0.248	43.77	
	10	41.5	4.150	0.249	44.03	
	2	10.2	5.100	0.306	54.11	
	4	20.5	5.125	0.308	54.38	
0.2	6	31	5.167	0.310	54.82	54.47
	8	41	5.125	0.308	54.38	
	10	51.5	5.150	0.309	54.64	
	2	11	5.500	0.330	58.36	
	4	22	5.500	0.330	58.36	
0.3	6	33	5.500	0.330	58.36	58.10
	8	43.5	5.438	0.326	57.69	
	10	54.4	5.440	0.326	57.72	
	2	11.2	5.600	0.336	59.42	
	4	22	5.500	0.330	58.36	
0.4	6	33	5.500	0.330	58.36	58.51
	8	43.8	5.475	0.329	58.09	
	10	55	5.500	0.330	58.36	
	2	11	5.500	0.330	58.36	
0.5	4	21	5.250	0.315	55.70	56.30
	6	31.5	5.250	0.315	55.70	
	8	42	5.250	0.315	55.70	
	10	52.8	5.280	0.317	56.02	

ii. 15 h broth

- Cross-flow velocity of 0.27 m/s

Table A.33: Results of critical flux experiments using 15 h broth at cross-flow velocity of 0.27 m/s

TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	3.8	1.900	0.114	20.16	
	4	7.5	1.875	0.113	19.89	
0.1	6	10.6	1.767	0.106	18.74	19.12
	8	14	1.750	0.105	18.57	
	10	17.2	1.720	0.103	18.25	
	2	4	2.000	0.120	21.22	
	4	7.2	1.800	0.108	19.10	
0.2	6	10.5	1.750	0.105	18.57	19.05
	8	13.8	1.725	0.104	18.30	
	10	17	1.700	0.102	18.04	
	2	3.8	1.900	0.114	20.16	
	4	7.5	1.875	0.113	19.89	
0.3	6	10.5	1.750	0.105	18.57	19.03
	8	13.8	1.725	0.104	18.30	
	10	17.2	1.720	0.103	18.25	
	2	3.6	1.800	0.108	19.10	
	4	7.2	1.800	0.108	19.10	
0.4	6	10	1.667	0.100	17.68	18.19
	8	13.5	1.688	0.101	17.90	
	10	16.2	1.620	0.097	17.19	
	2	3	1.500	0.090	15.92	
0.5	4	7	1.750	0.105	18.57	17.10
	6	9.5	1.583	0.095	16.80	
	8	13	1.625	0.098	17.24	
	10	16	1.600	0.096	16.98	

- Cross-flow velocity of 0.50 m/s

Table A.34: Results of critical flux experiments using 15 h broth at cross-flow velocity of 0.50 m/s

TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	3.2	1.600	0.096	16.98	
	4	6.8	1.700	0.102	18.04	
0.1	6	9.6	1.600	0.096	16.98	17.11
	8	12.5	1.563	0.094	16.58	
	10	16	1.600	0.096	16.98	
	2	3.4	1.700	0.102	18.04	
	4	7	1.750	0.105	18.57	
0.2	6	10.2	1.700	0.102	18.04	18.12
	8	13.5	1.688	0.101	17.90	
	10	17	1.700	0.102	18.04	
	2	4	2.000	0.120	21.22	
	4	7.8	1.950	0.117	20.69	
0.3	6	11.6	1.933	0.116	20.51	20.51
	8	15.2	1.900	0.114	20.16	
	10	18.8	1.880	0.113	19.95	
	2	4.8	2.400	0.144	25.46	
	4	8.8	2.200	0.132	23.34	
0.4	6	13.6	2.267	0.136	24.05	23.48
	8	16.8	2.100	0.126	22.28	
	10	21	2.100	0.126	22.28	
	2	5.2	2.600	0.156	27.59	
0.5	4	9	2.250	0.135	23.87	24.28
	6	13	2.167	0.130	22.99	
	8	17.8	2.225	0.134	23.61	
	10	22	2.200	0.132	23.34	

- Cross-flow velocity of 0.81 m/s

Table A.35: Results of critical flux experiments using 15 h broth at cross-flow velocity of 0.81 m/s

TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	4.8	2.400	0.144	25.46	
	4	8.2	2.050	0.123	21.75	
0.1	6	12	2.000	0.120	21.22	22.22
	8	16	2.000	0.120	21.22	
	10	20.2	2.020	0.121	21.43	
	2	6.4	3.200	0.192	33.95	
	4	11	2.750	0.165	29.18	
0.2	6	16.5	2.750	0.165	29.18	30.03
	8	22	2.750	0.165	29.18	
	10	27	2.700	0.162	28.65	
	2	6.5	3.250	0.195	34.48	
	4	11.5	2.875	0.173	30.50	
0.3	6	17	2.833	0.170	30.06	30.92
	8	22.5	2.813	0.169	29.84	
	10	28	2.800	0.168	29.71	
	2	6.5	3.250	0.195	34.48	
	4	11.8	2.950	0.177	31.30	
0.4	6	17.2	2.867	0.172	30.42	31.63
	8	23.5	2.938	0.176	31.17	
	10	29	2.900	0.174	30.77	
	2	6.4	3.200	0.192	33.95	
0.5	4	11.8	2.950	0.177	31.30	31.97
	6	17.8	2.967	0.178	31.48	
	8	24	3.000	0.180	31.83	
	10	29.5	2.950	0.177	31.30	

iii. 22 h broth

- Cross-flow velocity of 0.27 m/s

Table A.36: Results of critical flux experiments using 22 h broth at cross-flow velocity of 0.27 m/s

TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	2	1.000	0.060	10.61	
	4	4.2	1.050	0.063	11.14	
0.1	6	6.8	1.133	0.068	12.02	11.17
	8	8.5	1.063	0.064	11.27	
	10	10.2	1.020	0.061	10.82	
	2	2	1.000	0.060	10.61	
	4	4	1.000	0.060	10.61	
0.2	6	6.5	1.083	0.065	11.49	10.79
	8	8	1.000	0.060	10.61	
	10	10	1.000	0.060	10.61	
	2	1.8	0.900	0.054	9.55	
	4	3.2	0.800	0.048	8.49	
0.3	6	5.5	0.917	0.055	9.73	9.19
	8	7	0.875	0.053	9.28	
	10	8.4	0.840	0.050	8.91	
	2	1.5	0.750	0.045	7.96	
	4	3	0.750	0.045	7.96	
0.4	6	5.8	0.967	0.058	10.26	8.74
	8	6.8	0.850	0.051	9.02	
	10	8	0.800	0.048	8.49	
	2	1.2	0.600	0.036	6.37	
0.5	4	2.8	0.700	0.042	7.43	8.02
	6	5.2	0.867	0.052	9.20	
	8	6.5	0.813	0.049	8.62	
	10	8	0.800	0.048	8.49	

- Cross-flow velocity of 0.50 m/s

Table A.37: Results of critical flux experiments using 22 h broth at cross-flow velocity of 0.50 m/s

TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	3	1.500	0.090	15.92	
	4	7.2	1.800	0.108	19.10	
0.1	6	10.5	1.750	0.105	18.57	18.30
	8	14.2	1.775	0.107	18.83	
	10	18	1.800	0.108	19.10	
	2	4.4	2.200	0.132	23.34	
	4	8	2.000	0.120	21.22	
0.2	6	11.8	1.967	0.118	20.87	21.47
	8	16	2.000	0.120	21.22	
	10	19.5	1.950	0.117	20.69	
	2	4.5	2.250	0.135	23.87	
	4	8.2	2.050	0.123	21.75	
0.3	6	12	2.000	0.120	21.22	21.86
	8	16	2.000	0.120	21.22	
	10	20	2.000	0.120	21.22	
	2	4	2.000	0.120	21.22	
	4	8.2	2.050	0.123	21.75	
0.4	6	12	2.000	0.120	21.22	21.33
	8	16	2.000	0.120	21.22	
	10	20	2.000	0.120	21.22	
	2	4	2.000	0.120	21.22	
0.5	4	8	2.000	0.120	21.22	21.18
	6	12	2.000	0.120	21.22	
	8	16	2.000	0.120	21.22	
	10	19.8	1.980	0.119	21.01	

- Cross-flow velocity of 0.81 m/s

Table A.38: Results of critical flux experiments using 22 h broth at cross-flow velocity of 0.81 m/s

TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Permeate flux, J (LMH)	Arithmetic mean of the 5 permeate flux readings (LMH)
	2	5.5	2.750	0.165	29.18	
	4	10	2.500	0.150	26.53	
0.1	6	14.2	2.367	0.142	25.11	26.17
	8	19	2.375	0.143	25.20	
	10	23.4	2.340	0.140	24.83	
	2	5.6	2.800	0.168	29.71	
	4	10	2.500	0.150	26.53	
0.2	6	15	2.500	0.150	26.53	27.16
	8	20	2.500	0.150	26.53	
	10	25	2.500	0.150	26.53	
	2	5.8	2.900	0.174	30.77	
	4	10.5	2.625	0.158	27.85	
0.3	6	15.6	2.600	0.156	27.59	28.28
	8	20.8	2.600	0.156	27.59	
	10	26	2.600	0.156	27.59	
	2	6	3.000	0.180	31.83	
	4	10.8	2.700	0.162	28.65	
0.4	6	16	2.667	0.160	28.29	28.88
	8	21	2.625	0.158	27.85	
	10	26.2	2.620	0.157	27.80	
	2	6	3.000	0.180	31.83	
0.5	4	10.6	2.650	0.159	28.12	28.66
	6	15.8	2.633	0.158	27.94	
	8	21	2.625	0.158	27.85	
	10	26	2.600	0.156	27.59	

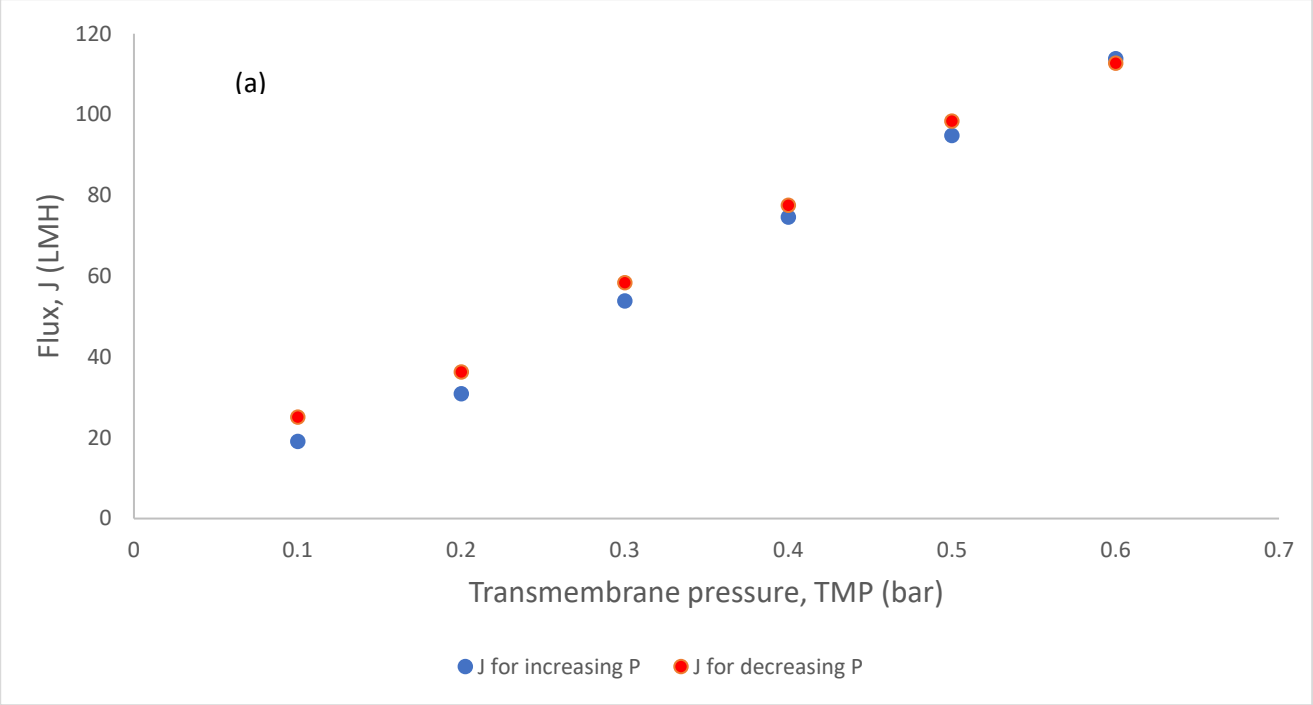
A.6 Pure water flux (PWF) experiments

Table A.39: Raw data generated for pure water flux (PWF) curve for 100 kDa ultrafiltration membrane to determine its initial performance

		Increasing pressure			Decreasing pressure		
TMP (bar)	Time (min)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flow rate (L/h)	Volume of permeate (mL)	Permeate flow rate (mL/min)	Permeate flowrate (L/h)
0.1	2	4.5	2.250	0.135	5	2.500	0.150
	4	7	1.750	0.105	9	2.250	0.135
	6	10	1.667	0.100	14	2.333	0.140
	8	13.2	1.650	0.099	19	2.375	0.143
	10	17	1.700	0.102	24	2.400	0.144
0.2	2	6.2	3.100	0.186	7.5	3.750	0.225
	4	11.5	2.875	0.173	14	3.500	0.210
	6	17	2.833	0.170	20	3.333	0.200
	8	23	2.875	0.173	26.5	3.313	0.199
	10	29	2.900	0.174	32	3.200	0.192
0.3	2	10	5.000	0.300	11	5.500	0.330
	4	20	5.000	0.300	22	5.500	0.330
	6	30.5	5.083	0.305	33	5.500	0.330
	8	41	5.125	0.308	44.2	5.525	0.332
	10	52	5.200	0.312	55	5.500	0.330
0.4	2	14	7.000	0.420	14.5	7.250	0.435
	4	28	7.000	0.420	29	7.250	0.435
	6	42	7.000	0.420	44	7.333	0.440
	8	56.5	7.063	0.424	59	7.375	0.443
	10	71	7.100	0.426	73.5	7.350	0.441
0.5	2	18	9.000	0.540	18	9.000	0.540
	4	35.5	8.875	0.533	36.5	9.125	0.548
	6	54	9.000	0.540	56.5	9.417	0.565
	8	71.5	8.938	0.536	75	9.375	0.563
	10	89	8.900	0.534	94.5	9.450	0.567
0.6	2	21	10.500	0.630	21	10.500	0.630
	4	42.5	10.625	0.638	42.5	10.625	0.638
	6	64.5	10.750	0.645	64	10.667	0.640
	8	86.5	10.813	0.649	86	10.750	0.645
	10	109.5	10.950	0.657	106	10.600	0.636

Table A.40: Processed data used for plotting pure water flux (PWF) curve for 100 kDa ultrafiltration membrane to determine its initial performance

TMP (bar)	Time (min)	J (LMH) FOR INCREASING PRESSURE	AVERAGE J (LMH) FOR INCREASING PRESSURE	J (LMH) FOR DECREASING PRESSURE	AVERAGE J (LMH) FOR DECREASING PRESSURE
0.1	2	23.87	19.13	26.53	25.16
	4	18.57		23.87	
	6	17.68		24.76	
	8	17.51		25.20	
	10	18.04		25.46	
0.2	12	32.89	30.95	39.79	36.28
	14	30.50		37.14	
	16	30.06		35.37	
	18	30.50		35.15	
	20	30.77		33.95	
0.3	22	53.05	53.92	58.36	58.41
	24	53.05		58.36	
	26	53.93		58.36	
	28	54.38		58.62	
	30	55.17		58.36	
0.4	32	74.27	74.62	76.92	77.58
	34	74.27		76.92	
	36	74.27		77.81	
	38	74.93		78.25	
	40	75.33		77.98	
0.5	42	95.49	94.88	95.49	98.39
	44	94.16		96.82	
	46	95.49		99.91	
	48	94.83		99.47	
	50	94.43		100.27	
0.6	52	111.41	113.82	111.41	112.77
	54	112.73		112.73	
	56	114.06		113.17	
	58	114.72		114.06	
	60	116.18		112.47	



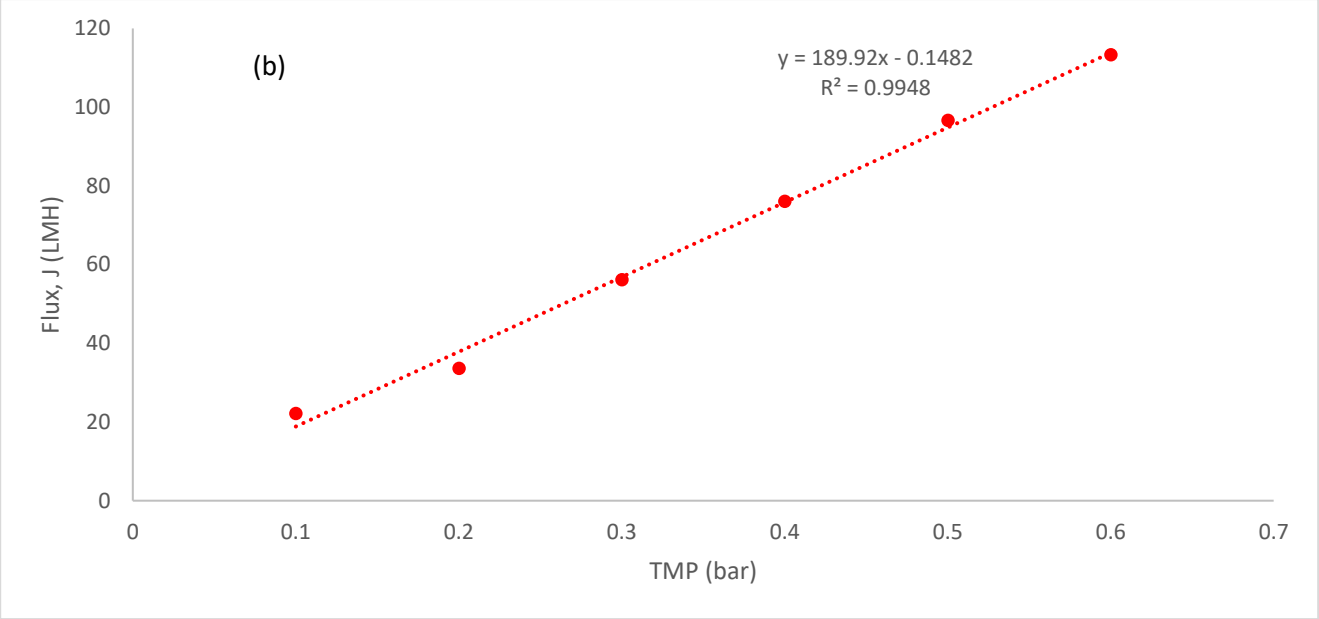


Figure A.2 (a) and (b): Pure water flux (PWF) curve for 100 kDa ultrafiltration membrane before use (served as a basis for membrane cleaning processes throughout the experimental runs)

(*The curve shown in Figure A.2b was generated by the average of flux for both the increasing P and decreasing P*)

Appendix B: Sample calculations

This section provides sample calculations for the various calculated parameters used in the present study.

B.1 Lactate yield (g/g)

For instance, at the end of batch fermentation run when all the glucose (of initial concentration of 30 g/L) had been consumed to produce lactate concentration of 22.58 g/L, then lactate yield on glucose consumed was calculated as:

$$\text{Lactate yield (g/g)} = \frac{\text{Lactate produced (g)}}{\text{Glucose consumed (g)}} = \frac{22.58\ \text{g}}{30\ \text{g}} = 0.75\ \text{g/g (equivalent to 75 \% yield).}$$

B.2 Dilution rate (h^{-1})

The working volume of the fermenter was 1.5 litres in the present study. The feed flow rate was adjusted to correspond to the permeate flow rate. Therefore, if the feed flow rate was 1.8 mL/min (i.e., 0.108 L/h) then,

$$\text{Dilution rate } (h^{-1}) = \frac{\text{Feed flow rate (L/h)}}{\text{Working volume of the fermenter (L)}} = \frac{0.108\ \text{L/h}}{1.5\ \text{L}} = 0.072\ h^{-1}$$

B.3 Lactate productivity [g/(L.h)]

- **For batch fermentation runs**

Lactate concentration after 15 h of batch fermentation was 25.50 g/L, therefore, the lactate productivity was calculated as:

$$\text{Lactate productivity [g/(L.h)]} = \frac{\text{Lactate concentration (g/L)}}{\text{Fermentation time (h)}} = \frac{25.50\ \text{g/L}}{15\ \text{h}} = 1.70\ \text{g/(L.h)}$$

At the end of a 24 h batch fermentation run, the lactate concentration was 22.58 g/L.

Therefore, overall lactate productivity = $\frac{22.58\ \text{g/L}}{24\ \text{h}}$ = 0.94 g/(L.h)

- **For continuous fermentation runs**

For instance, if the lactate concentration at 21 h of a continuous fermentation run was 47.91 g/L, and dilution rate was 0.072 h^{-1}, then:

Lactate productivity [g/(L.h)] = lactate concentration (g/L) × dilution rate (h^{-1}) = 47.91 × 0.072 = 3.449 g/(L.h)

B.4 Membrane surface area (m^2)

The membrane surface area used in the calculations of the permeate flux was calculated from the inner diameter of the membrane and its length. The ultrafiltration membrane had the following dimensions: internal diameter = 6 mm, external diameter = 10 mm, and length = 300 mm. Therefore,

$$\text{membrane surface area } (\text{m}^2) = \pi \times \text{inner diameter (m)} \times \text{length (m)} = \pi \times 6\ \text{mm} \times 300\ \text{mm} = 5655\ \text{mm}^2 = 0.005655\ \text{m}^2$$

B.5 Permeate flux, J (LMH)

A measuring cylinder and a stopwatch was used to determine the permeate flow rate. For instance, if the volume of permeate recorded for 2 minutes was 3.6 mL, then the permeate flow rate was 1.8 mL/min (i.e., 0.108 L/h). The permeate flux was calculated as follows:

Permeate flux, J (LMH) = $\frac{\text{Permeate flow rate (L/h)}}{\text{Membrane surface area } (\text{m}^2)}$ = $\frac{0.108\ \text{L/h}}{0.005655\ \text{m}^2}$ = 19.10 L/m^2.h

L/m^2.h was written as LMH

B.6 Cross-flow velocity (m/s)

The cross-flow velocity was calculated based on the flow rate of fluid through the membrane module which was varied by changing the pump setting positions. The inner cross-sectional area of the membrane module was calculated using the inner diameter (6 mm) of the membrane as follows:

Inner cross-sectional area of the membrane module = $\frac{\pi \text{ x } 6 \text{ x } 6}{4}$ = 28.27 mm^2 = 2.827 x 10^{-5} m^2

For instance, at pump setting position 2, the pump flow rate was 39.0 L/h (i.e., 1.0833 x 10^{-5} m^3/s). Therefore, cross-flow velocity (m/s) =

$$\frac{\text{Volumetric flow rate of fluid through the membrane module } (m^3/s)}{\text{inner cross-sectional area of the membrane module } (m^2)} = \frac{1.0833 \text{ x } 10\text{^}-5}{2.827 \text{ x } 10\text{^}-5} = 0.38 \text{ m/s}$$

Appendix C: Additional information

C.1 pH control system: calibration procedure, cleaning and storage of the pH probes

i. Procedure for the calibration of the pH controller

The following buffer solutions were used for the calibration purposes (as per the instruction manual from the supplier of the pH controller):

- HI 7004 M/L pH 4.01 buffer solution
- HI 7007 M/L pH 7.01 buffer solution

To calibrate the pH controller, it was first set in measurement mode. The pH probe was then immersed in pH 7.01 buffer solution. After the pH reading had stabilized, the OFFSET trimmer was adjusted until pH 7.01 could appear on the screen. Thereafter, the pH probe was rinsed with reverse osmosis water and immersed in pH 4.01 buffer solution. The SLOPE trimmer was then adjusted until pH 4.01 could appear on the screen.

ii. Cleaning and storage of pH probes

- For cleaning purposes, the pH probe was soaked in HI 7061M/L solution for 30 minutes and then rinsed with reverse osmosis water.
- The pH probes were stored in HI 70300 M/L solution when not in use.

C.2 Permit for the importation of bacteria

agriculture, forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

Directorate Plant Health

Permit No. **P0098365**

PERMIT FOR THE IMPORTATION OF CONTROLLED GOODS

In terms of the provisions of section 3(1) of the Agricultural Pests Act, 1983 (Act 36 of 1983) and subject to the conditions stated here under, authorisation is hereby granted to-

TOBIAS OBONDO **Tel No:**

PROCESS ENGINEERING DPT
BANGHOEK STREET
STELLENBOSCH
7600

to import into the Republic the following controlled goods **ORGANISMS FOR LABORATORY RESEARCH**

AS PER ATTACHED CONDITIONS **2** **SAMPLE (S)**

Name and address of foreign supplier **UNITED KINGDOM**

Conditions **1. AS ATTACHED**

Port of Entry : **CAPE TOWN INTERNATIONAL AIRPORT**

Import authorized from **2019/12/03** TO **2020/12/03**

IMPORTANT : This permit does not exempt the holder from the provisions of any other Act, ordinance or agreement

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
DIRECTORATE PLANT HEALTH
2019 -12- 03
DIRECTORATE PLANT HEALTH
PO BOX 40024 ARCADIA 0007

Date

Executive Officer

Reference Number **9/19/176**

INQUIRIES : TEL.: (012)319 6102 (Christina Makgoba) **FAX: (012)319 6370**

agriculture,
forestry & fisheries

Department:
Agriculture, Forestry and Fisheries
REPUBLIC OF SOUTH AFRICA

PHYTOSANITARY REQUIREMENTS FOR THE IMPORTATION OF ORGANISM (S) FOR LABORATORY RESEARCH

1. The consignment must be inspected at the point of entry and found free from contaminants.

2. Quarantine label to be affixed to the parcel;

3. The imported ***Lactobacillus delbrueckii*** **subsp.** ***delbrueckii*** **and** ***L. casei*** **subsp.** ***casei*** sample (s), shall only be handled in the at **Stellenbosch University (Faculty of Engineering: Department of Process Engineering)** laboratory facility

4. Import sample(s) in a sealed container(s) shall be addressed to **Tobias Obondo**

   Name of institution / company: **Stellenbosch University**

   Postal address: **Faculty of Engineering**
   Department of process Engineering
   Banghoek road
   Stellenbosch
   7600

5. The importer shall be responsible for custom clearance;

6. The container(s) shall be opened and the material handled in the facility at

   Name of Laboratory facility: **Stellenbosch University (Faculty of Engineering: Department of Process Engineering)**

   Physical address: **Banghoek road**
   Stellenbosch

   Responsible laboratory technician: **Tobias Obondo/ Prof. Lingam Pillay/ Dr. Robbie Pott**

   Tel nr: **021 808 4485**

7. Destroy all packing material and wrapping by incineration or autoclaving.

8. Due to the foreign status of the sample(s) all experimental material shall be marked as potentially dangerous to the South African agricultural industry.

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
PLANT HEALTH
2019 -12- 03
DIRECTORATE PLANT HEALTH
P.O. BOX 40024 ARCADIA 0007

9. Take precautions at all times to prevent the escape and introduction of any pest(s), which may be present in/on the samples into the RSA.

10. No cultures, sub-cultures or specimens of the imported sample(s) may be given to any other person or be used for any work outside the facility mentioned in 3. of this condition for any reason whatsoever, without the written consent of the Director

11. **All imported sample(s) and organisms isolated from the samples must be destroyed by autoclaving/incineration after completion of the laboratory analysis and notify the Manager, (for attention: Ms Rorisang Mahlakoana: Fax 012 319 6101) immediately thereof in writing.**

12. If any of the above-mentioned conditions are not complied with or are violated, the material shall be destroyed, at the importer's expense.

THE FOLLOWING UNDERTAKING TO BE COMPLETED BY IMPORTER OR HIS AUTHORISED AGENT:

I, the undersigned,

_____

I D No: _____

am fully aware of the above-mentioned conditions and understand that should I contravene or fail to comply with any of the above conditions I shall be guilty of an offence and be liable for prosecution under the Agricultural Pests Act, 1983 (Act No. 36 of 1983).

SIGNED:_____ DATE: _____

PERMIT NO.: _____

DEPARTMENT OF AGRICULTURE FORESTRY AND FISHERIES
DIRECTORATE PLANT HEALTH
2019 -12- 03
DIRECTORATE PLANT HEALTH
P.O. BOX 40024 ARCADIA 0007

C.3 Reviving freeze-dried bacterial cultures – Instructional guide

ATCC®

Reviving Freeze-Dried Microorganisms

Instructional Guide

Check each culture thoroughly upon receipt. If you received a double glass vial, inspect the blue crystal desiccant (silica beads) in the bottom ofthe outer vial. If the desiccant is clear or pink, the vacuum seal may have been compromised and the material may not be viable. If a culture is unsatisfactory, notify ATCC so that the strain in question can be investigated. Store freeze-dried cultures at 2°C to 8°C or lower if they are not immediately rehydrated (except plant viruses, which should be stored at -20°C). Use the medium and incubation conditions specified on the product sheet when first reviving strains to ensure optimal conditions for recovery. Product sheets are available online at www.atcc.org.

BACTERIA AND ALGAE

The preferred method for long-term preservation of bacteria and algae is freeze-drying; however, some bacteria do not survive freeze-drying well andare frozen instead. For freeze-dried cultures, using a single tube of the recommended media (5 to 6 mL), withdraw approximately 0.5 to 1.0 mL witha Pasteur or 1.0 mL pipette. Use this to rehydrate the entire pellet and transfer the entire suspension back into the broth tube and mix well. The lastfew drops of this suspension may also be transferred to an agar slant. Alternatively, algal cultures must be initiated on agar plates. Please note thatanaerobic bacterial cultures must be rehydrated in an anaerobic environment; the viability of the cells decrease rapidly if the vial is rehydrated in anoxygenic environment.

Incubate cultures under the appropriate conditions. Given proper treatment and conditions, most freeze-dried cultures will grow out in a few days.However, some may exhibit a prolonged lag period and should be given twice the normal incubation time before discarding as nonviable.

BACTERIOPHAGES

Prior to rehydrating the phage, prepare an actively growing broth culture of the bacterial host. Rehydrate the freeze-dried phage specimen aseptically with 1.0 mL of appropriate broth (refer to the product sheet) and mix well. Use 0.1 mL of this mixture for the preparation of a new high-titer phagesuspension. Preserve the remaining mixture in a sterile screw-capped vial at 2°C to 10°C. Refer to the product sheet for specific information on howto propagate the phage.

FILAMENTOUS FUNGI AND YEAST

Prior to rehydrating your fungi, refer to the product sheet for any specific instructions regarding the handling of your culture. For freeze-dried fungi,use a Pasteur pipette to add approximately 0.5 to 1.0 mL sterile water to the inner vial of a double vial or to a serum vial (Preceptrol®). Then, drawup the entire contents into the pipette and transfer to a test tube with about 5 to 6 mL sterile water. Let the yeast or fungus rehydrate for at least acouple of hours before transferring to broth or solid agar; longer rehydration (e.g., overnight) might increase the viability of some fungi. Incubate atthe recommended temperature. Keep in mind that some cultures may exhibit a prolonged lag period and should be given twice the normal incubationtime before discarding as nonviable. Save the mixture of lyophilized material and water until you know you have growth.

C.4 Photo of the membrane bioreactor system used in the present study