EFFECT OF pH AND IONIC STRENGTH ON MEMBRANE FLUX IN THE SEPARATION OF *Escherichia coli* BY USING CROSS-FLOW MICROFILTRATION

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EFFECT OF pH AND IONIC STRENGTH ON MEMBRANE FLUX IN THE SEPARATION OF *Escherichia coli* BY USING CROSS-FLOW MICROFILTRATION

NADIRA BINTI AB AZIZ

A dissertation submitted in partial fulfillment of the requirements for the award of the degree of Bachelor of Chemical Engineering (Biotechnology)

Faculty of Chemical and Natural Resources Engineering
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APRIL 2010

I declare that this dissertation entitled “Effect of pH and Ionic Strength on Membrane Flux in the Separation of *Escherichia coli* by Using Cross-Flow Microfiltration” is the result of my own research except as cited in the references. The dissertation has not been accepted for any degree and is not concurrently submitted in candidature of any other degree.

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Special Dedication to:

My mom, Puan Nor'Ashikin binti Hussin,
My dad, Encik Ab Aziz bin Ab Rani,
My family members,
My fellow lecturers,
My friends and
My fellow colleagues

For all your care, support and believe in me.

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ABSTRACT

Recently, cross-flow microfiltration has been used to separate cells in continuous fermentation processes. The main objective of this study is to investigate the effect of pH and ionic strength on membrane flux during the separation of *Escherichia coli*. In order to achieve the objective of the study, hollow fibre membrane with molecular pore size of 0.2µm and surface area of 110 cm^2 was used as the filter media. The experiment was carried out at a constant transmembrane pressure of 0.8 bar by varying five different pH values, ranging from pH 4.5 to 8.5 and five different concentration of salt, ranging from 0.1M to 0.5M. From this study, it is found that pH 6.5 with low concentration of salt was the best condition for the separation of *E.coli*. Increasing in pH increased the permeate flux, while high concentration of salt decreased the permeate flux. As a conclusion, the membrane used in this experiment can be used in the fermentation of *E.coli* for cell recycling because pH 6.5 is the optimum condition for fermentation of *E.coli* in its fermentation broth.

ABSTRAK

Sejak kebelakangan ini, penapis mikro telah digunakan untuk memisahkan sel di dalam proses penapaian secara berterusan. Tujuan utama kajian ini dijalankan adalah untuk mengkaji kesan pH dan kekuatan ionik ke atas arus membran di dalam pemisahan *Escherichia coli.* Dalam mencapai objektif kajian, membran jenis fiber berongga dengan saiz liang molekul 0.2µm dan luas permukaan 110 cm^2 telah digunakan sebagai media penapis. Eksperimen ini telah dijalankan pada tekanan yang tetap, iaitu 0.8 bar dengan mempelbagaikan lima nilai pH yang berbeza, iaitu di dalam julat 4.5 hingga 8.5 dan lima kepekatan garam yang berbeza, iaitu di dalam julat 0.1 molar hingga 0.5 molar. Kajian ini menunjukkan bahawa pemisahan *E.coli* yang terbaik berlaku pada pH 6.5 dengan kepekatan garam yang rendah. Arus resapan meningkat dengan peningkatan pH, manakala kepekatan garam yang tinggi mengurangkan arus resapan. Kesimpulannya, membran yang telah digunakan di dalam eksperimen ini boleh digunakan di dalam penapaian *E.coli* untuk sel dikitar semula kerana pH 6.5 adalah keadaan optimum untuk menapai *E.coli* di dalam medium penapaiannya.

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LIST OF SYMBOLS/ABBREVIATIONS

E. coli	-	*Escherichia coli species*
µm	-	micrometer
cm	-	centimeter
LPG	-	liquefied petroleum gas
M	-	molar
NaCl	-	sodium chloride
TCA	-	tricarboxylic acid
CO_2	-	carbon dioxide
LB	-	Luria Bertani
PBS	-	polybutyrate succinate
NaOH	-	sodium hydroxide
PHA	-	polyhydroxyalkanoate
°C	-	degree Celcius
DNA	-	deoxyribonucleic acid
pH	-	potential for hydrogen ion concentration
MF	-	microfiltration
rpm	-	revolution per minute
TMP/ΔP	-	transmembrane pressure
UF	-	ultrafiltration
NF	-	nanofiltration
mL	-	mililitres
min	-	minute
pI	-	isoelectric point

CHAPTER 1

INTRODUCTION

1.1 Background of Study

Succinic acid is a dicarboxylic acid having the molecular formula of $C_4H_6O_4$. It has been produced by microbial fermentation for the use in agricultural, food and pharmaceuticals industries. Currently, most of commercially available succinic acid is produced by chemical processes, in which liquefied petroleum gas (LPG) or petroleum oil is used as a starting material. However, the assessment of raw material cost and the estimation of the potential market size clearly indicate that the current petroleum-based succinic acid process will be replaced by the fermentative succinic acid production system in the foreseeable future (Song and Lee, 2006).

Many different microorganisms have been screened and studied for for succinic acid production from various carbon sources. *Anaerobiospirillum succiniciproducens*, *Actinobacillus succinogenes* and *Mannheimia succiniciproducens* MBEL55E are among the bacterias found to be used in the fermentation of succinic acid due to their ability to produce a relatively large amount of succinic acid (Song and Lee, 2006). However, there

has been much effort in developing recombinant *Escherichia coli* strains which are capable of enhanced succinic acid production whether under aerobic or anaerobic conditions.

A typical process for the production of a bioproduct like succinic acid by microbial fermentation consists of seed cultivation, fermentation, product recovery, concentration and purification (Song and Lee, 2006). The separation of cells is the first separation step after fermentation either to recover extracellular products, to concentrate and wash cells before product recovery, or for cell recycle. Usually, the most common methods for this separation are centrifugation and plate and frame filters. However, these methods have its own limitations. The only other technique which has been suggested for cell separation is sedimentation. Nevertheless, this technique requires long residence times and always requires another separation step (Warren *et al.*, 1991).

Recently, cross-flow microfiltration has been used to separate cells in continuous fermentation processes. A successful succinic acid recovery approach in continuous fermentation is in a cell-recycled reactor where the cells are separated by a filtration unit and returned to the fermenter while the product is removed in the permeate (Li *et al.*, 2006).

The use of synthetic dead-end membrane filters has now become standard in any biological laboratory. However, for industrial cell separations, where a continuous high throughput stream is required, this design is inadequate because of the huge resistance created by cell build-up on the upstream side of the membrane. Instead, a cross-flow or tangential flow system, in which the membrane surface is parallel to the inlet flow has been suggested. This allows much of the cellular fouling on the membrane surface to be eliminated by the cells being swept away by the tangential flow, so a steady state exists where the rate of deposition due to the filtrate flow is balanced by the rate of removal by

the cross-flow. The filter can then be operated for considerably longer time periods and with higher fluxes than dead-ended filtration (Warren *et al.*, 1991).

The efficiency of cross-flow microfiltration is primarily a function of the operating parameters, and is measured by the filtrate flow rate (flux) and its quality. Cross-flow velocity, transmembrane pressure, temperature, pore size of the membrane and concentration of suspended solids in the feed were reported to affect the performance of cross-flow microfiltration (Al-Malack *et al.*, 2004).

In this study, cross-flow microfiltration system with hollow fibre membrane as the filter media was implemented to separate *E.coli* cells from its fermentation broth. *E.coli* is essential in the production of succinic acid. In industrial cell separations, hollow fibre membrane is located in the fermenter. It is functioning to retain the cells in the fermenter for cell-recycling, while the product which is succinic acid is removed in the permeate. Cell-recycling can reduce the production cost of succinic acid. Hollow fibre membrane is used in this filtration system instead of other types of membrane such as flat sheet due to its minimum-required space. The production cost of succinic acid can be reduced without spending much money on larger equipments which also requires larger space. This study investigates the effect of pH and ionic strength on the membrane flux to determine the optimum condition for the separation of *E.coli*. The most suitable pH and ionic strength in the separation of *E.coli* will enhance high flux and high rejection of cells.

1.2 Problem Statement

Recently, increasing interest has been generated in the separation of cells by cross-flow filtration. This technique is usually used as the first separation step after fermentation either to recover extracellular products, to concentrate and/or wash cells before product recovery, or for cell recycle. Presently, the most common methods for this separation are centrifugation and plate and frame filters. However, centrifuges (i) have a high complexity and cost, (ii) often leave turbid supernatants, (iii) require high gravitational forces and (iv) can create aerosols; while plate and frame filters (i) are labour intensive, (ii) have fluxes which decrease with time, (iii) waste significant amounts of product, (iv) require filtering aids and (v) have problems with hygiene. The only other technique which has been suggested for cell separation is sedimentation. Although inexpensive, this technique requires long residence times and, because it produces imperfect separations, sedimentation usually requires another cell separation step (Warren *et al.*, 1991).

Cross-flow microfiltration can avoid those limitations. Nevertheless, membrane-based separation often faced with membrane fouling. The long term performance of membrane units at high cell densities is always affected by the fouling of filtration membranes, which require extensive cleaning protocols (Li *et al.*, 2006).

1.3 Rationale and Significance

To avoid limitations mentioned in the previous sub-chapter, cross-flow microfiltration technique was chosen as the separation method because it allows continuous and complete separations which are not dependent upon the density

difference or particle size and can be performed in a contained system which is both sterile and aerosol-free (Warren *et al.*, 1991). In cross-flow or tangential microfiltration, the particles deposited on the filter medium are swept away by the cross-flow velocity action, which produces shear and lift forces on the particles as they become attached to the filter medium (Al-Malack *et al.*, 2004). Membrane with higher flux and rejection of cells is preferred to separate *E.coli* from its fermentation broth.

Membrane fouling, which results in loss of productivity, is one of the major operational concerns of membrane processes (Tansel *et al.*, 2000). Further study on membrane fouling has been done to increase the flux, hence reduce fouling and increase the life-span of the membrane. Extensive cleaning protocols are essential in order to increase the life-span of the membrane.

1.4 Objective

This main objective of this study was to investigate the effect of pH and ionic strength on membrane flux during the separation of *E.coli*.

1.5 Scope of Study

In order to achieve the objective of the study, the following scopes have been identified:

1) The value of pH was varied between pH 4.5 to pH 8.5.
2) The concentration of sodium chloride (NaCl) was varied from 0.1M to 0.5M.
3) Hollow fibre membrane with molecular pore size of 0.2 µm and surface area of 110 cm^2 were used.
4) Constant transmembrane pressure of 0.8 bar was implemented.

CHAPTER 2

LITERATURE REVIEW

2.1 Succinic Acid

Succinic acid, also known as amber acid or butanedioic acid, is a dicarboxylic acid having the molecular formula of $C_4H_6O_4$. After its first purification of succinic acid from amber by Georgius Agricola in 1546, it has been produced by microbial fermentation for the use in agricultural, food and pharmaceutical industries (Song and Lee, 2006). Traditionally, succinic acid is produced by chemical synthesis from petroleum feedstocks which are not renewable. Succinic acid is an intermediate metabolite in the tricarboxylic acid (TCA) cycle, and can be produced by some obligate or facultative anaerobes. It is considered one of the most possible commercial products obtained from alternative feedstocks. Therefore, production of succinic acid from renewable substrates has been investigated in recent years for sustainable development (Wu *et al.*, 2009).

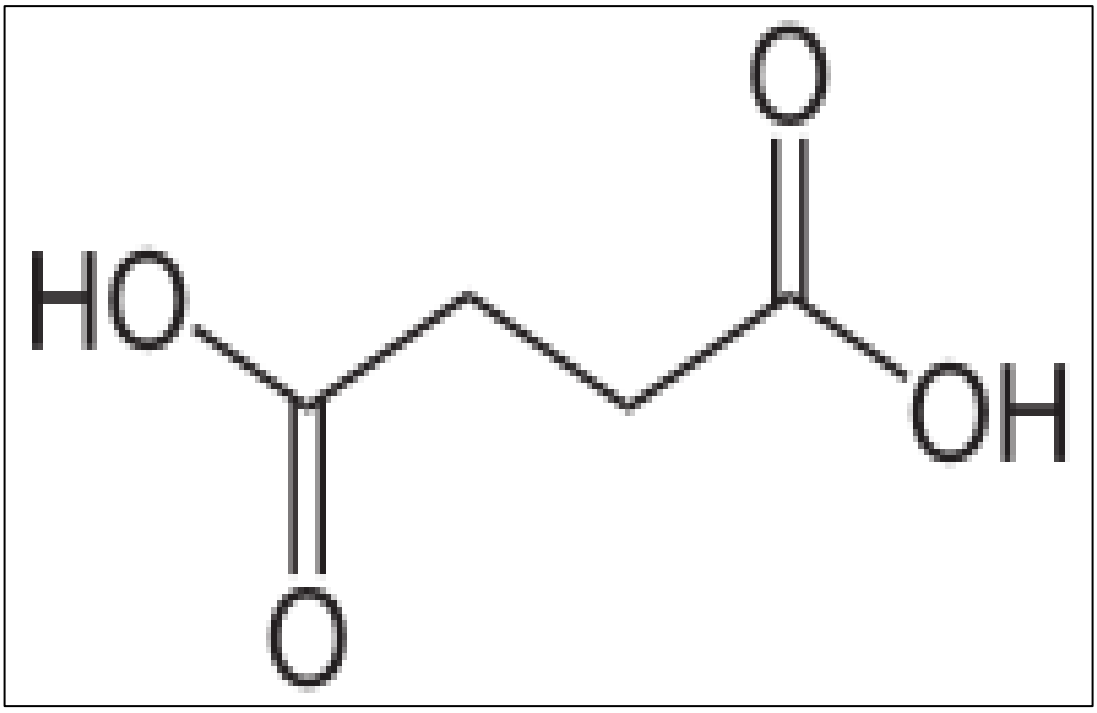

Figure 2.1 Molecular structure of succinic acid

2.1.1 Succinic Acid Production

Succinic acid is currently chemically produced by hydrolyzing petroleum products, which is associated with certain environmental hazards leading scientists to develop biological processes for its continuous production. This is because it is a common intermediate in the metabolic pathway of several anaerobic microorganisms. Efforts are being made worldwide to develop low-cost fermentation processes using renewable resources such as agricultural, dairy, and industrial waste products, so as to replace current processes using petroleum as a feedstock (Agarwal *et al*., 2007).

As the importance of succinic acid for use as a biodegradable polymer has increased, the biological production by fermentation has been focused on as the alternative to the petrochemical-based process (Huh *et al.*, 2006). Many different

microorganisms have been screened and studied for succinic acid production from various carbon sources. Among them, *Anaerobiospirillum succiniciproducens* and *Actinobacillus succinogenes* have been most intensively studied due to their ability to produce a relatively large amount of succinic acid. More recently, a new succinic acid producing bacterium *Mannheimia succiniciproducens* MBEL55E was isolated from bovine rumen. Also, there has been much effort in developing recombinant *Escherichia coli* strains which are capable of enhanced succinic acid production under aerobic and anaerobic conditions (Song and Lee, 2006). Table 2.1 shows various kinds of microorganism that can produce succinic acid.

Table 2.1 Succinic acid production from various microorganisms

Microorganism	Description	Reference
Actinobacillus succinogenes	Fermentative production of succinic acid from straw hydrolysate.	Zheng *et al.*, 2009
Mannheimia succiniciproducens	Produces succinic acid as a major product, acetic and formic acids as the second major ones from various carbon sources under 100% CO_2 condition at pH of 6.0 to 7.5.	Huh *et al*., 2006
Anaerobiospirillum succiniciproducens	Produces succinic and acetic acids as major fermentation products and ethanol and lactic acid as minor ones under strictly anaerobic condition.	Lee *et al*., 1999
Recombinant Escherichia coli	Ferments glucose to ethanol, formic, acetic and lactic acids with only detectable amounts of succinic acid under anaerobic condition.	Song and Lee, 2006
Bacteroides fragilis	Produces a polysaccharide capsule high in succinic acid.	Isar *et al*., 2006

2.1.2 Application of Succinic Acid

Succinic acid can be used as a precursor of many industrially important chemicals including adipic acid, 1,4-butanediol, tetrahydrofuran, *N*-methyl pyrrolidinone, 2-pyrrolidinone, succinate salts and gamma-butyrolactone. Furthermore, the increasing demand for succinic acid is expected as its use is extended to the synthesis of biodegradable polymers such as polybutyrate succinate (PBS) and polyamides, and various green solvents (Song and Lee, 2006). Figure 2.2 shows various chemicals and products that can be synthesized from succinic acid.

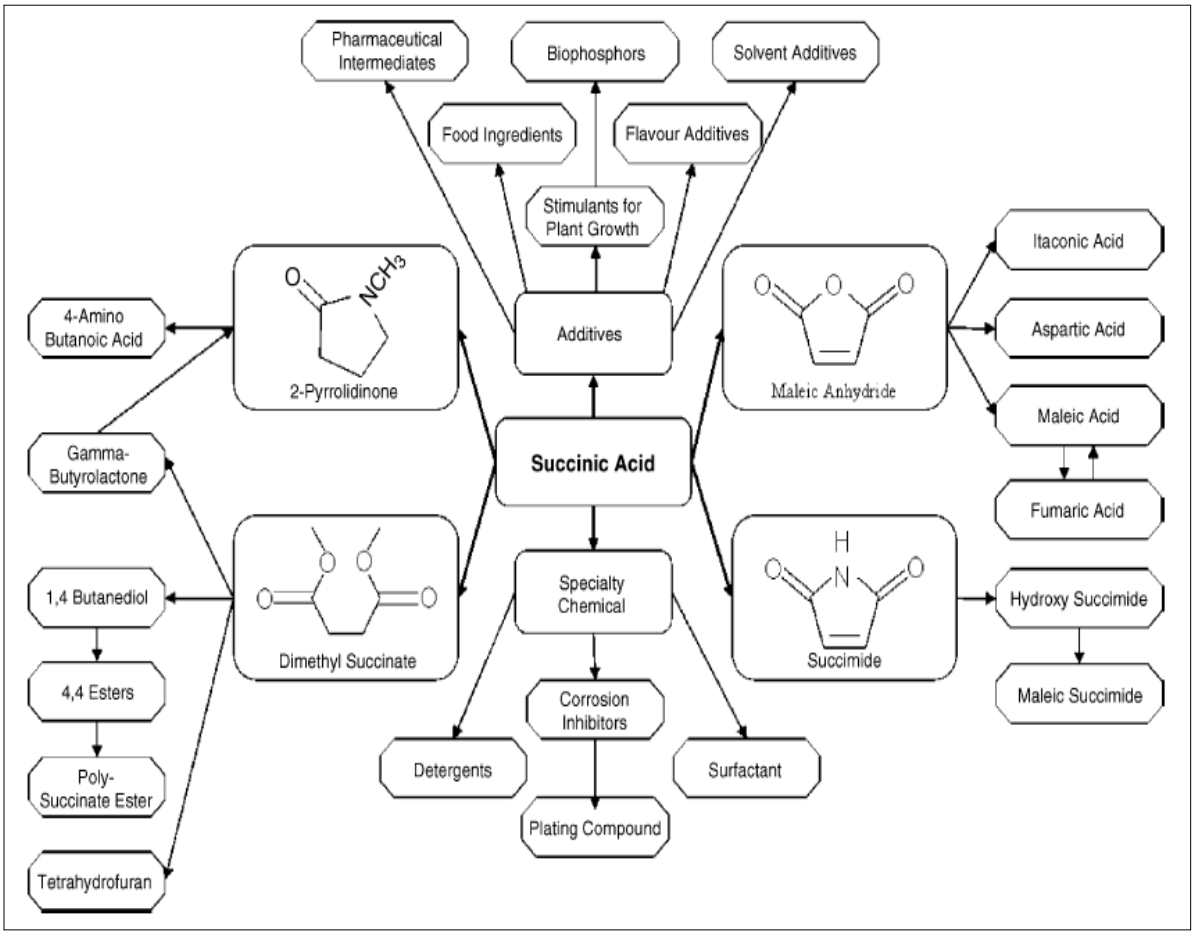

Figure 2.2 Various chemicals and products that can be synthesized from succinic acid

2.2 *Escherichia coli sp.*

Escherichia coli was first described and isolated by Theodore Escherich in1885. *E.coli* strains K-12 and B are apparently both derived from normal commensals of the human gut, and their many derivatives have been in laboratory since 1922 and before 1918, respectively (Jeong, *et al.*, 2009). *E.coli* is a gram-negative, facultative anaerobic and non-sporulating cell. It is typically rod-shaped and is about 2 micrometres (µm) long and 0.5 µm in diameter. It has a cell volume of 0.6 to 0.7 μm^3 (Kubitschek, 1990). It can live on a variety of substrates. *E.coli* uses mixed-acid fermentation in anaerobic conditions to produce lactate, succinate, ethanol, acetate and carbon dioxide.

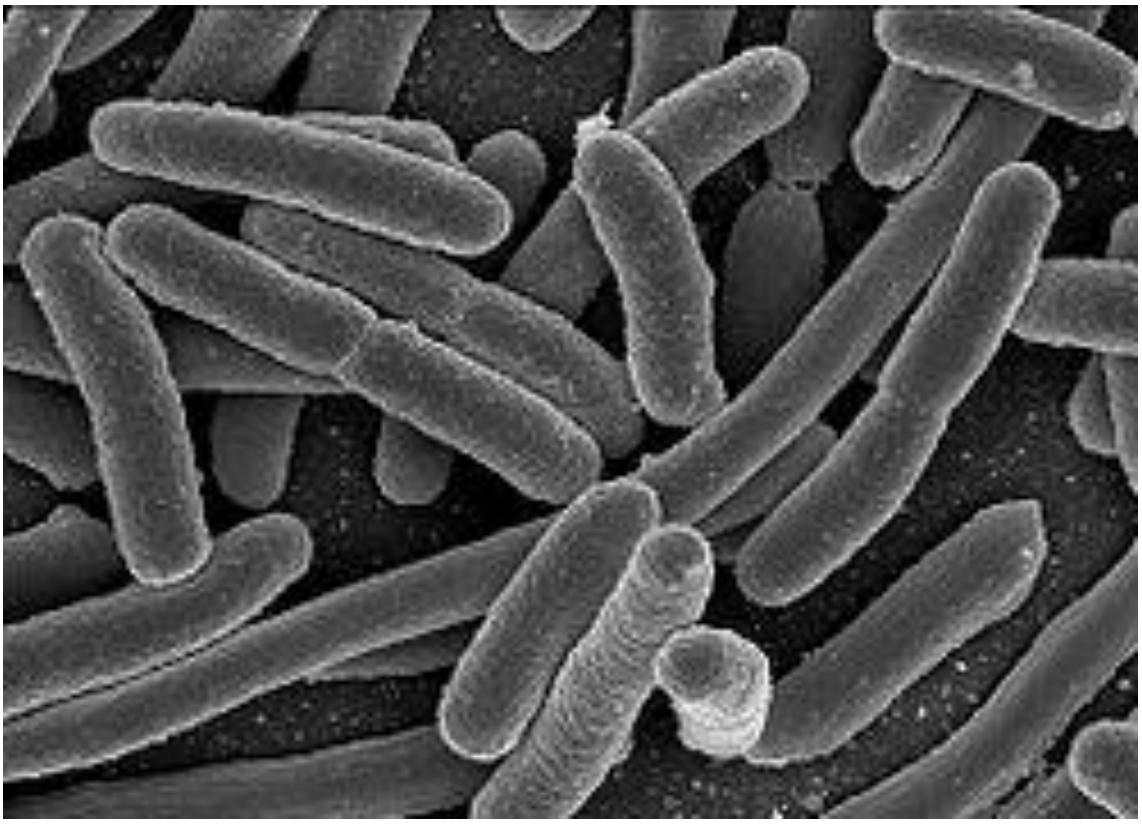

Figure 2.3 *Escherichia coli sp.*

2.2.1 Industrial Application of *Escherichia coli*

Current commercial products obtained from *E.coli* cultures include mainly recombinant proteins from prokaryotic and eukaryotic sources which are considered to be low-volume-high-value products. In addition, recent advancements in metabolic engineering made it possible to use *E.coli* as a platform to produce high-volume-low-value-products such as polyhydroxybutyrate, succinic acid, octanoic acid, aromatic compounds, ethanol, acetone and styrene oxide (Shiloach and Fass, 2005). Table 2.2 shows some applications of *E.coli* in production of bioproducts.

Table 2.2 Application of *E.coli* in production of bioproducts

Application	Description	Reference
Production of Penicillin Acylase (PAC)	An important industrial enzyme for the production of many β-lactam antibiotics.	Chou *et al.*, 1999
Production of L-aspartic acid	Immobilization of *Escherichia coli* cells using polyethyleneimine-coated porous support particles for L-aspartic acid production.	Huang *et al.*, 2009
Production of L(-)-Carnitine	L(-)-Carnitine production with immobilized *Escherichia coli* cells in continuous reactors.	Obon *et al.*, 1997
Production of ethanol from xylose	*E. coli* FBR5 is developed to produce ethanol in high yields from corn fibre hydrolysate and other agricultural residues.	Quresh *et al.*, 2006
Industrial production of polyhydroxyalkanoates	*E. coli* is used due to higher growth rates and PHA production levels, nutrient limitation is not required, and easier PHA recovery due to cell fragility and larger granule size	Wegen *et al.*, 1998

2.2.2 Production of Succinic Acid from *Escherichia coli*

A number of metabolic engineering strategies have been developed to enhance succinic acid production by *E.coli*. The approaches can be broadly categorized to the inactivation of enzymes participating in the reactions which compete with succinic acid pathways, the amplification of enzymes involved in succinic acid pathways, and the introduction of heterologous enzymes catalyzing reactions towards increased succinic acid formation (Song and Lee, 2006).

2.3 Cultivation of *Escherichia coli*

Microbiological cultures utilize petri dishes of differing sizes that have a thin layer of agar based growth medium in them. Once the growth medium in the petri dish is inoculated with the desired bacteria, the plates are incubated in an oven usually set at 37 degrees Celsius (°C). Another method of bacterial culture is liquid culture, in which case desired bacteria are suspended in liquid broth, a nutrient medium. These are ideal for preparation of an antimicrobial assay. The experimenter would inoculate liquid broth with bacteria and let it grow overnight in a shaker for uniform growth, then take aliquots of the sample to test for the antimicrobial activity of a specific drug or protein.

2.3.1 Aseptic Technique

Aseptic techniques must be used to reduce the likelihood of bacterial contamination. This usually involves disinfection of working areas, minimizing possible access by bacteria from the air to exposed media, and use of flames to kill bacteria

which might enter vessels as they are opened. *E.coli* may be introduced to the media (inoculated) by various means. Usually, *E.coli* from a drop in a heat-sterilized loop is spread on the surface of agar. A similar technique is used with broth cultures.

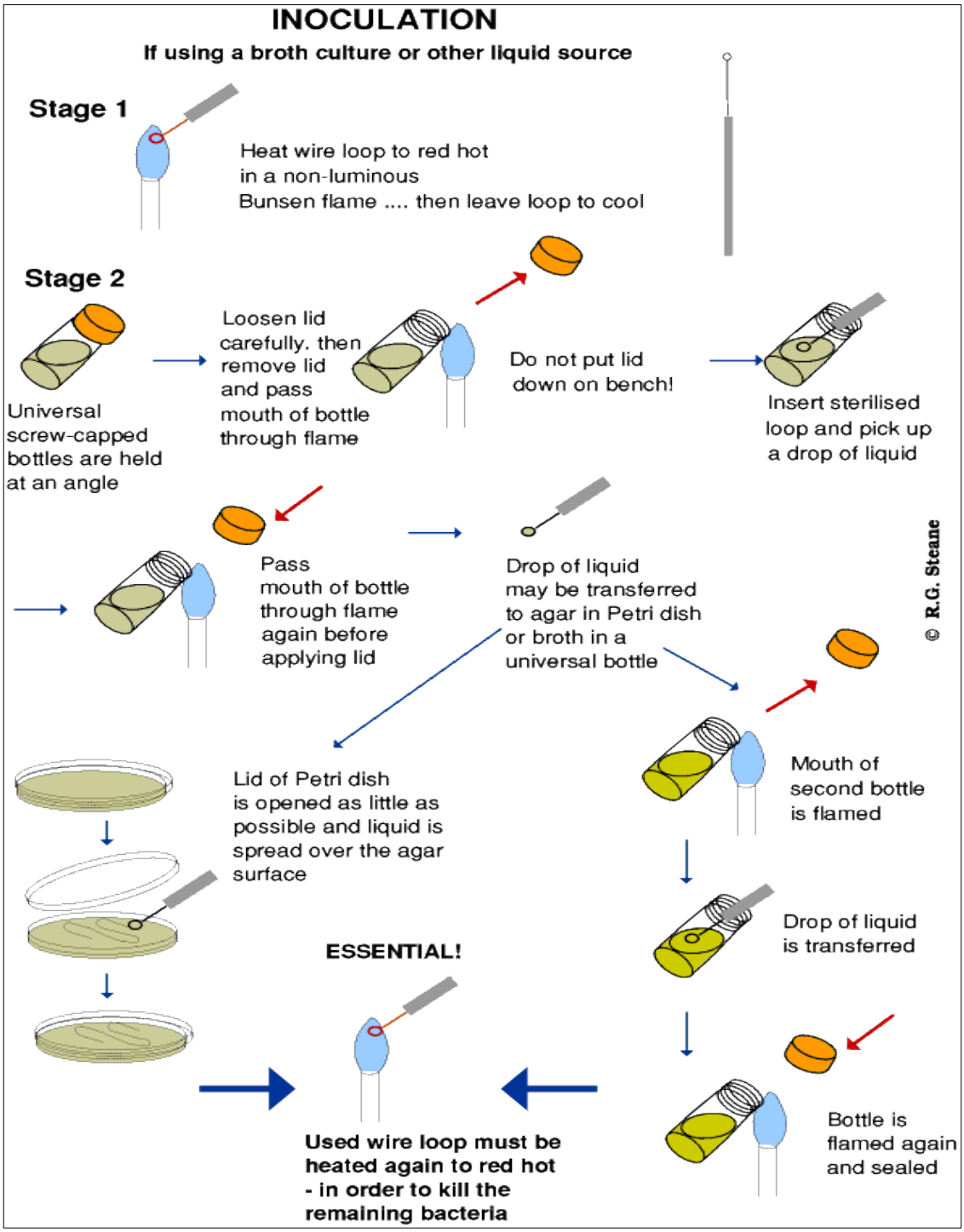

Figure 2.4 Inoculation techniques for *Escherichia coli*

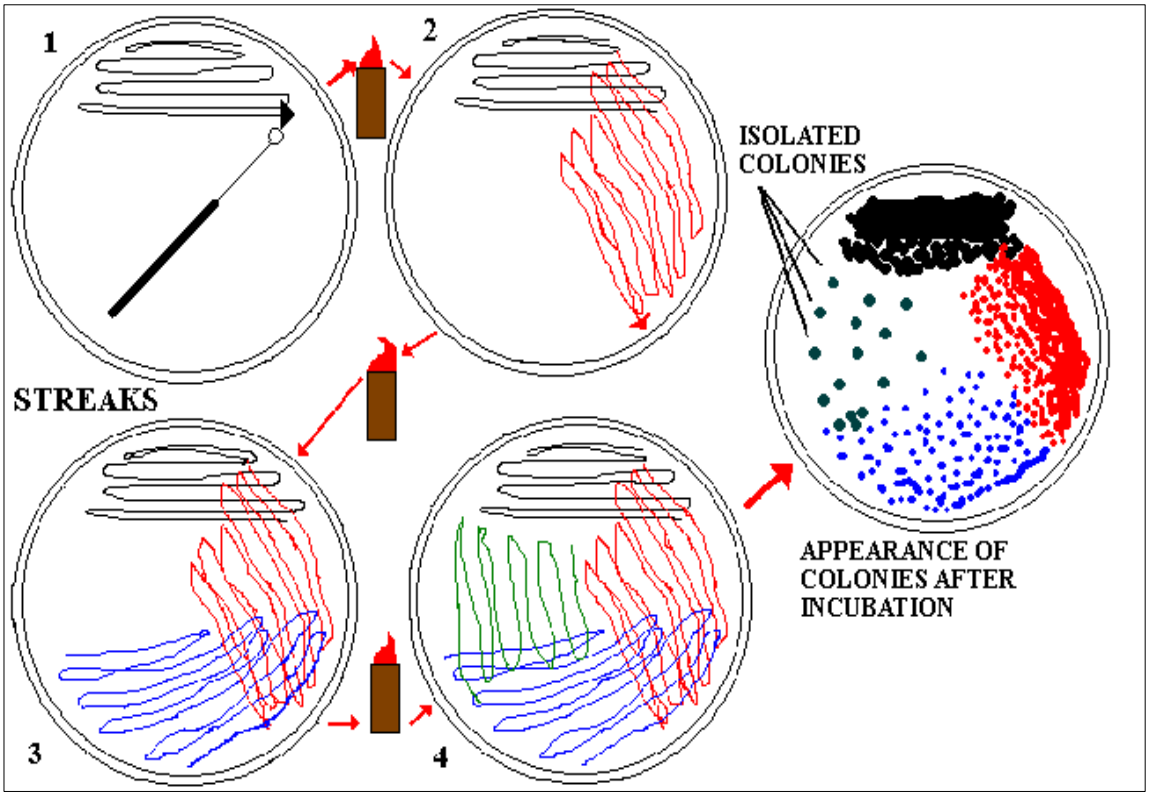

Figure 2.5 Streak plate methods

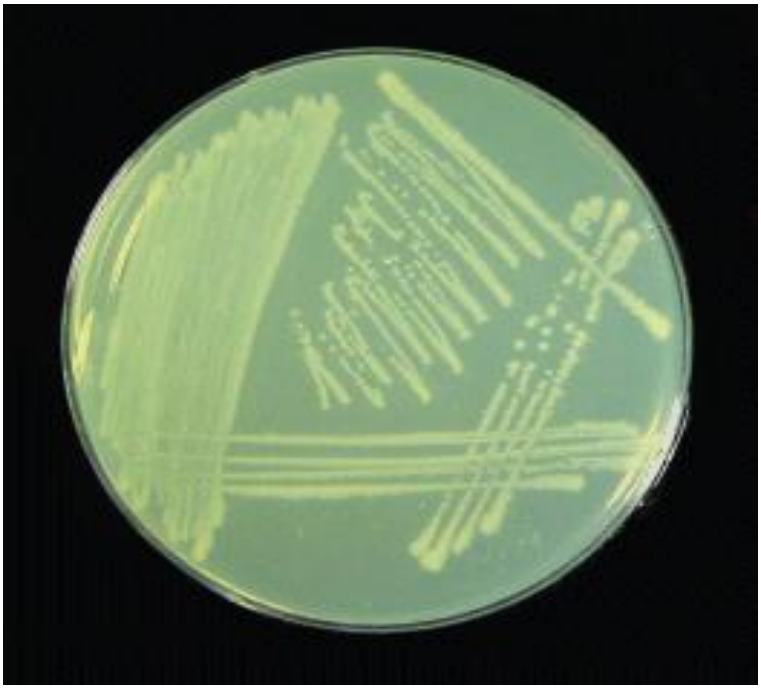

Figure 2.6 *E.coli* on agar plate after incubation

2.3.2 Luria Bertani Broth

The widely used rich medium called Luria Bertani (LB) broth is popular with bacteriologists because it permits fast growth and good growth yields for many species. LB media formulations have been an industry standard for the cultivation of *E.coli* as far back as the 1950s. These media have been widely used in molecular microbiology applications for the preparation of plasmid DNA and recombinant proteins. It continues to be one of the most common media used for maintaining and cultivating recombinant strains of *E.coli*. (Sezonov *et al.*, 2007).

There are several common formulations of LB. Although they are different, they generally share a somewhat similar composition of ingredients used to promote growth, including peptides and casein peptones, vitamins (including B vitamins), trace elements (nitrogen, sulphur, magnesium) and minerals. Peptides and peptones are provided by tryptone. Vitamins and certain trace elements are provided by yeast extract. Sodium ions for transport and osmotic balance are provided by sodium chloride. Bacto-tryptone is used to provide essential amino acids to the growing bacteria, while the bacto-yeast extract is used to provide a plethora of organic compounds helpful for bacterial growth. The tryptone used is a pancreatic digest of casein from cow's milk, and the yeast extract used is an autodigest of *Saccharomyces cerevisiae* (Sezonov *et al.*, 2007).

Figure 2.7 Luria Bertani broth

2.4 Separation of Cells

Separation technique is usually the first step after fermentation either to recover extracellular products, to concentrate and/or wash cells before product recovery, or for cell recycle (Warren *et al*., 1991). The processes of bioproducts production include two key stages which are (a) fermentation and (b) product recovery. The biggest challenge in the production lies in the recovery and not in the fermentation step. Usually, most of the

separation of microorganisms from fermentation broth is performed by centrifugation (Li *et al*., 2006). Table 2.3 shows the separation of cells from various techniques.

Table 2.3 Separation of cells from various techniques

Microorganism/Cell	Separation Technique	Reference
Saccharomyces cerevisiae	Cross-flow microfiltration	Warren *et al*., 1991
Protein and cells from cheese whey	Cross-flow ultrafiltration	Li *et al*., 2006
***Salmonella typhimurium* LT2**	Free flow electrophoresis (FFE)	Akiba *et al*., 1995
***E.coli* expressing functional cell-wall bound antibody fragments**	Fluorescence assisted cell sorting (FACS).	Fuch *et al*., 1996
Bacterial cell lysates	Cross-flow microfiltration	Parnham and Davis, 1996)

2.5 Membrane Technology

Membrane is a thin barrier which allows passage of particle with a certain size, particular physical or chemical properties (Ghosh, 2003). A membrane is a thin semi-permeable barrier which can be used for particle-liquid separation, particle-solute separation, solute-solvent separation and solute-solute separation (Ghosh, 2006). Membrane separation of mixtures is widely used in industry, medicine, households, and other areas due to their high efficiency and low power capacity (Borshchev *et al*., 1994).

A membrane may be made from organic polymers or inorganic material such as glass, metals and ceramics, or even liquids. Examples of organic membrane are those that are made from cellulose, cellulose acetate (CA), polysulfone (PS), polyethersulfone (PES), polyamides (PA), polyvinylidine fluoride (PVDF), polyacrylonitrile (PAN). The factors that utilized in membrane-based separation are solute size, electrostatic charge, diffusivity, and solute shape (Ghosh, 2006).

2.6 Type of Membrane

From a structural point of view, membranes are broadly divided into symmetric (isotropic) and asymmetric (anisotropic). A symmetric membrane has similar structural composition and morphology at all positions within it. An asymmetric membrane is composed of two or more structural planes of non-identical composition or morphology (Ghosh, 2006).

From a morphological point of view, membranes can be classified into porous or dense. Porous membranes have tiny pores or pore networks. On the other hand, dense membranes do not have any pores and solute or solvent transport through these membranes take place by a partition-diffusion-partition mechanism (Ghosh, 2006).

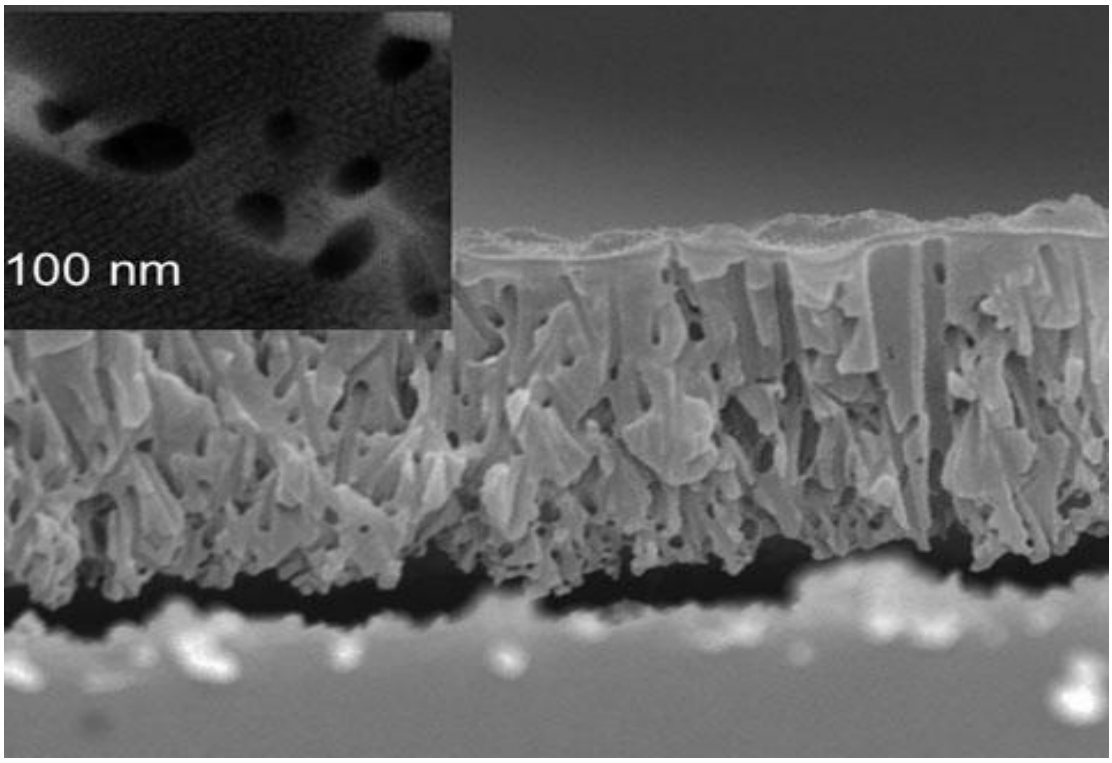

Figure 2.8 Porous membrane

Membranes are available in three basic forms which are flat sheet membrane, tubular membrane and hollow fibre membrane.

2.6.1 Flat Sheet Membrane

Flat sheet membranes look like filter paper. They are available in the form of filter disc or rectangular sheets (Ghosh, 2006).

Figure 2.9 Flat sheet membranes

2.6.2 Tubular Membrane

The most common type of tubular membrane looks like a single hollow tube of circular cross-section, where the wall of the tube functioning as the membrane. There are also tubular membranes with square and other types of cross-section. Monolith tubular membranes look like cylindrical blocks with large numbers of parallel tubes within

them. These tubes typically have diameter in the range of 0.5 cm to 2 cm (Ghosh, 2006). Figure 2.10 shows how a tubular membrane is used.

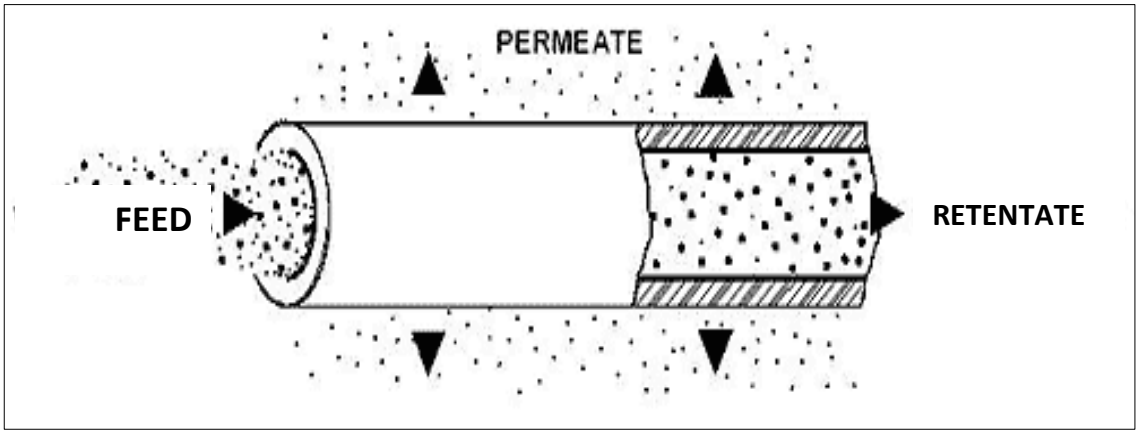

Figure 2.10 Tubular membrane cut-away

Figure 2.11 Tubular membrane

2.6.3 Hollow Fibre Membrane

Hollow fibres are also tube-like in appearance. However, these membranes have much smaller diameters than tubular membranes. Typical fibre diameter is of the order or 1 mm. If the inner wall of hollow fibre acts as the membrane, it is of the inside-out type whereas when the outer wall acts as the membrane, it is referred to as the outside-in type. There are also double skinned hollow fibre membranes which can function both as inside-out and outside-in membranes (Ghosh, 2006).

(a)

(b)

Figure 2.12 Hollow fibre membranes

2.7 Classification of Membrane Processes

Pressure driven membrane based bioseparation processes can be classified ito four types based on the size of the permeable species. A fifth type called dialysis allows solutes similar those in nanofiltration to pass through. However, unlike nanofiltration, which is a pressure driven process, dialysis is a concentration gradient or diffusion driven process (Ghosh, 2006).

Table 2.4 Characteristics of various membrane separation processes

Membrane Separation	Description	Initial or Feed Phase	Membrane Structure	Industrial Application	Driving Forces	Permeates	Concentrates
Dialysis	Process for selective removing of low molecular weight solids from a solution	Liquid	Porous membrane	Hemodialysis (removal of waste metabolites, excess body water, and restoration of electrolyte balance in blood)	Pressure/ Concentration Difference	Blood	Waste metabolites, etc.
Gas Separation	Component(s) of a gas are removed through a pressure gradient	Vapor of Gas	Nonporous membrane	Separation of air	Pressure/ Concentration Difference	Oxygen	Nitrogen
Liquid Membrane	Extraction of a solute	Vapor and/or Liquid	Liquid membrane	Recovery of zinc from wastewater	Pressure Difference	Wastewater	Zinc
Microfiltration	Separation of organic and polymeric compounds with micro pore ranges of 50-500 angstrom	Liquid or Gas	Microporous membrane	Separation of suspended solids from liquid mixture	Pressure Difference	Water, Solvents	Suspended solids
Pervaporation	Component(s) of a mixture diffuse through, evaporate under a low pressure, and are removed by a vacuum	Liquid	Nonporous membrane	Dehydration of ethanol- water azeotrope	Pressure Difference	Ethanol	Water
Reverse Osmosis	Passage of a solvent through a dense membrane that is permeable to solvent but not solute(s)	Liquid	Nonporous membrane	Separation of Ions	Pressure Difference	Water	Salt Ions
Ultrafiltration	Separation of polymeric compounds from aqueous solution	Liquid	Microporous membrane	Separation of polymeric compounds from aqueous solution	Pressure Difference	Water, Low molecular weight component	Colloid, High molecular weight component

2.7.1 Microfiltration

Microfiltration (MF) separates micron-sized particles from fluids. The transmembrane pressure (TMP) ranges from 1 to 50 psig. MF membranes are microporous and retain particles by a purely sieving mechanism. Typical permeate flux values are higher than in ultrafiltration processes even though MF is operated at much lower TMP. MF is most commonly used for clarification, sterilization and slurry concentration. A microfiltration process can be operated either in a dead-end mode or cross-flow mode (Ghosh, 2006). Table 2.5 shows various separation processes by using microfiltration.

Table 2.5 Separation processes by microfiltration

Process	Type of Filter	Reference
Cross-flow microfiltration of *Saccharomyces cerevisiae*	Polysulfone hollow fibre	Warren *et al*., 1991
Protein recovery from bacterial cell debris	Flat plate	Parnham and Davis, 1996
Cross-flow microfiltration of electrocoagulated kaolin suspension	Polyester tubular	Al-Malack *et al*., 2004
Microfiltration of colloids and natural organic matter	Hydrophilicm (GVWP) and hydrophobic (GVHP)	Schäfer *et al*., 2000

2.7.2 Ultrafiltration

Ultrafiltration (UF) separates solutes in the molecular weight range of 5 kDa to 500 kDa. UF membranes have pore ranging from 1 to 2 nm in diameter. Most UF membranes are anisotropic, with a thin "skin layer", typically around 10 μm thick fused on top of a microporous backing layer. The skin layer confers selectivity to the membrane while the microporous backing layer provides mechanical support. The ability of an UF membrane can retain macromolecules is traditionally specified in terms of its molecular cut-off (MWCO). UF is usually operated in the cross-flow mode (Ghosh, 2006). Table 2.6 shows various separation processes by using ultrafiltration.

Table 2.6 Separation processes by ultrafiltration

Process	Type of Filter	Reference
Separation of cells and proteins from fermentation broth	Polyethersulfone (PES) and polysulfone (PS)	Li *et al.*, 2006
Ultrafiltration of humic substances solutions	Cellulose acetate asymmetric membrane	Costa and Pinho, 2005
Membrane filtration of natural organic matter (NOM)	Tubular ceramic	Rubia *et al*., 2006
Dead-end ultrafiltration of *Bacillus subtilis* fermentation broths	Stirred cell	Juang *et al*., 2008
Cross-flow ultrafiltration of mosambi (*Citrus sinensis* (L.) Osbeck) juice	Polyethersulfone (PES)	Sarkar *et al*., 2009

2.7.3 Nanofiltration

Nanofiltration (NF) membranes allow salts and other small molecules to pass through but retain larger molecules such as peptides, hormones and sugars. The TMP in NF ranges from 40 to 200 psig. Most NF membranes are composite (Ghosh, 2006). NF is a relatively new process concept among the pressure driven membrane separation processes and it offers higher fluxes than reverse osmosis and significantly better retentions than ultrafiltration for lower molar mass molecules such as sugars, natural organic matter (NOM) and even ions (Manttari *et al.*, 2006). Table 2.7 shows various separation processes by using nanofiltration.

Table 2.7 Separation processes by nanofiltration

Process	Type of Filter	Reference
Nanofiltration of naturally-occurring dissolved organic matter	Polyamide thin film composite	Kilduff *et al*., 2004
Rejection of Gd(III) by nanofiltration assisted by complexation on charged organic membrane	Flat sheet tangential flow	Sorin *et al.*, 2005
Separation of Cr(III) from acid solutions	Flat sheet	Gomes *et al.*, 2010
Separation of indium	Flat sheet	Wu *et al.*, 2004
Nanofiltration of sweet whey	Flat and spiral wound	Cuartas-Uribe *et al.*, 2007

2.7.4 Reverse Osmosis

Reverse osmosis is a physical process in which a proportion of water from a pressurized supply (feed water) is forced through a semi-permeable membrane (permeator) to become product water (permeate) leaving behind almost all of the impurities in the remaining water (concentrate) (Khan, 2009). Reverse osmosis (RO) membranes allow water to go through but retain all dissolved species present in the feed. In osmosis, water travels from the lower solute concentration side to the higher solute concentration side of the membrane. In RO, the reverse takes place due to the application of TMP. The normal TMP pressure range in RO is 200 to 300 psig. Recently, developed membranes allow flow of water at as low as 125 psig TMP (Ghosh, 2006).

2.8 Mode of Filtration

2.8.1 Cross-Flow Filtration

A cross-flow filtration allows much of the cellular fouling on the membrane surface to be eliminated by the cells being swept away by the tangential flow. Hence, a steady state exists where the rate of deposition due to the filtrate flow is balanced by the rate of removal by the cross-flow. The filter can then be operated for considerably longer time periods and with higher fluxes than dead-ended filtration. For cross-flow systems, the filtrate flux decreases with time until a pseudo steady-state value is reached and it is this flux which is important for long-term operation. At a constant temperature, the variables which affect the steady-state flux are the pressure difference across the membrane, the shear rate and the cell concentration (Warren *et al*., 1991).

2.8.2 Dead-End Filtration

The dead-end filtration consists in gradually increasing the cake thickness up to a level determined by pressure drop or flow velocity. In the end, a cake and a clarified filtrate are formed. Dead-end filtration is often used as a method to estimate the specific cake resistance for cross-flow filtration and usually gives reasonable data for spherical and ellipsoidal-shaped cells (Mota *et al.*, 2002).

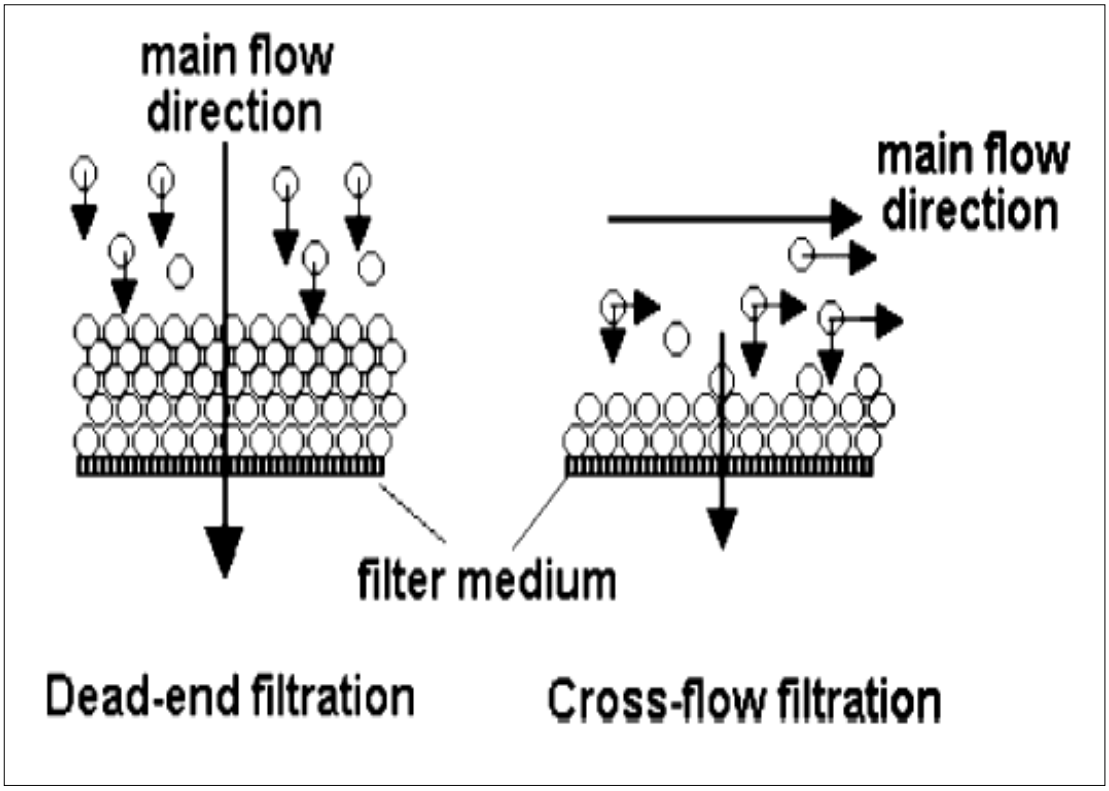

Figure 2.14 Sketch of dead-end and cross-flow filtration

CHAPTER 3

METHODOLOGY

3.1 Introduction

The purpose of this study is to analyze and study the effect of pH and ionic strength on the membrane flux and rejection in the separation of *E.coli*. The experiment was conducted starting from the preparation of Luria Bertani (LB) broth for the cultivation of *E.coli* by dissolving yeast extract, tryptone and sodium chloride (NaCl) in distilled water. The broth was then stored in the freezer. The next step was the preparation of buffer and NaCl solution. Acetate buffer and phosphate buffer solution were prepared at five different pH values, while NaCl solution was prepared in five different concentrations. This step was followed by the preparation of cell culture. The *E.coli* cell was first pre-cultured on agar plate by using streaking method. After 24 hours of incubation, the cell was transferred into 250 mL of LB medium and incubated again for 24 hours. To prepare the sample solution, the cell culture was centrifuged at 4°C and 10 000 rpm. The cell was then washed with deionized water and the cell precipitate was dissolved in 2L buffer solution. To investigate the effect of ionic strength, the sample solution was added with NaCl solution. This study was continued with the experiment to investigate the effect of pH and ionic strength on membrane flux by using cross-flow

filtration system of hollow fibre membrane. Finally, permeate of the membrane were analyzed by measuring the volume of permeate in order to determine the membrane flux. Cleaning process of the membrane was done after each experiment was completed. Figure 3.1 shows overall process flow of this experiment.

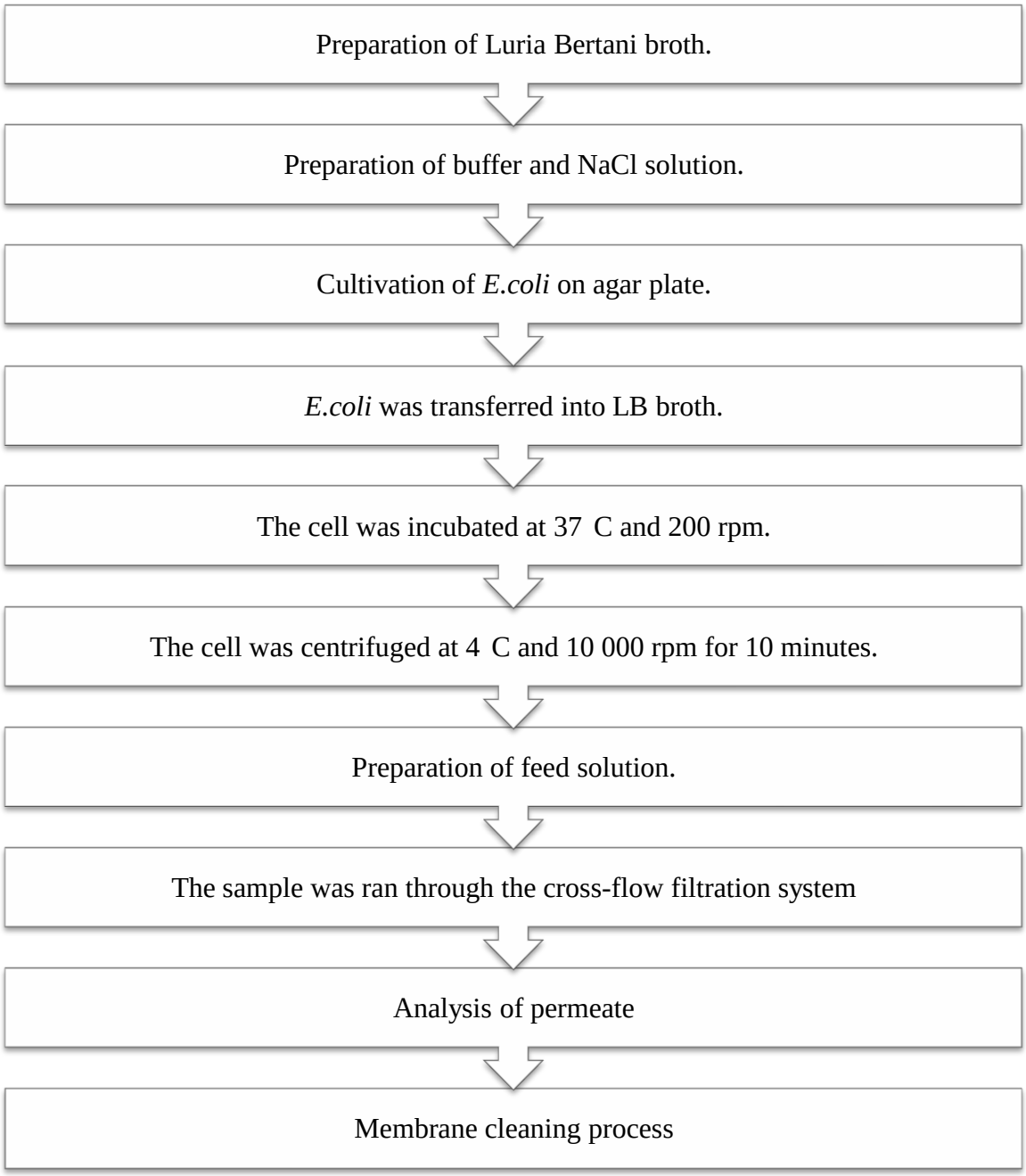

Figure 3.1 Overall process flow of experiment

3.2 Equipments/Apparatus

There were some equipments required in completing this study. They were:

1. Electrical balance (Shimadzu A W220)
2. Autoclave (Hirayama)
3. pH meter 827 (Metrohm)
4. Laminar air flow
5. Microbiological incubator (Memmert)
6. Shaker incubator (Infors HT Ecotron)
7. Refrigerated centrifuge (Eppendorf 5810 R)
8. Vortex mixer (VELP Scientifica)
9. Cross-flow filtration system
10. Weighing boat
11. Spatula
12. Beaker
13. Measuring cylinder
14. Schott bottle
15. Glass rod
16. Petri dish
17. Inoculating loop
18. Conical flask
19. Centrifuge tube 50 mL
20. Volumetric flask
21. Sample bottle 9 mL

3.3 Materials

There were some chemicals or reagents required in completing this study. They were:

1. 0.2µm hollow fibre membrane
2. Yeast extract
3. Tryptone
4. Sodium chloride (NaCl)
5. Sodium hydroxide (NaOH)
6. Potassium dihydrogen phosphate (KH_2PO_4)
7. *Escherichia coli* Strain B
8. Deionized water
9. Distilled water

3.4 Preparation of Luria Bertani (LB) Broth

Luria Bertani broth is the most common liquid medium used to grow *E.coli*. It permits fast growth and good growth yield for the cell. Five grams of yeast extract, 10 g of tryptone and 10 g of NaCl were suspended in 800 mL of distilled water. Further distilled water was then added to make a total of 1 L of LB broth. Next, the broth was autoclaved at 121°C and the bottle was swirled after cooling to ensure mixing. LB broth is a rich medium, containing all the nutrients needed for bacteria to proliferate. Yeast extract and tryptone provide vitamins and amino acids, while NaCl is added to keep the broth at a certain ionic strength (Sezonov *et al*., 2007).

Figure 3.2 Luria Bertani broth

3.5 Preparation of Buffer Solution

Buffer solution is used as a means of keeping pH at a nearly constant value in the fermentation broth. There were two types of buffer solution prepared in this study. Acetate buffer was prepared for pH 4.5, while phosphate buffer was prepared for pH values of 5.5, 6.5, 7.5 and 8.5. 0.1M of glacial acetic acid and 0.1M of sodium acetate (tri-hydrate) solution were required to prepare acetate buffer at pH 4.5. Both solutions were mixed together until the required pH was achieved. To prepare phosphate buffer, 0.1M NaOH solution was mixed together with 0.1M KH_2PO_4 solution until the required pH were achieved.

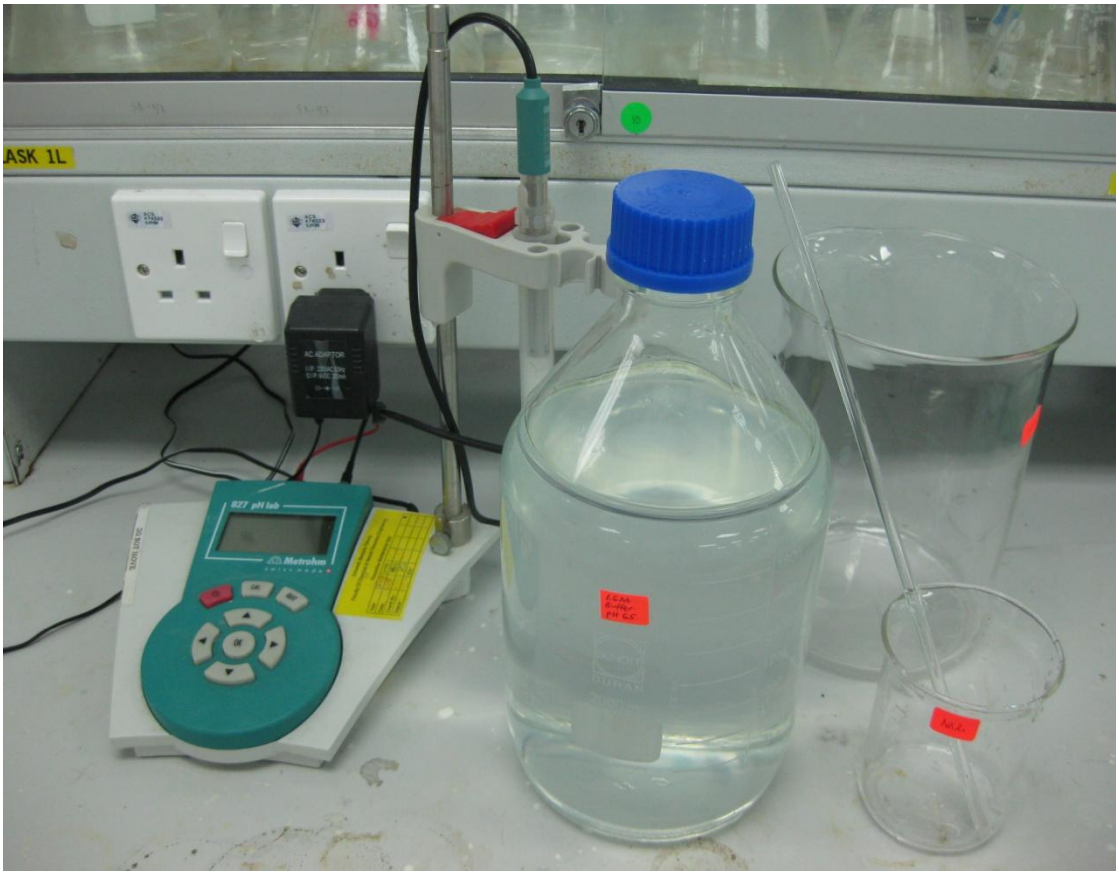

Figure 3.3 Buffer solution

3.6 Preparation of NaCl Solution

NaCl solution is used to keep the broth at a certain ionic strength. In this study, five different concentrations of NaCl solution were prepared. Solid NaCl were dissolved in deionized water to produce NaCl solutions with concentrations of 0.1M, 0.2M, 0.3M, 0.4M and 0.5M.

3.7 Preparation of *Escherichia coli* Cell

In this study, it is important to multiply the amount of *E.coli* cells. The cell was first pre-cultured on agar plate from its original agar medium by using streaking method. Sterile inoculating loop was used to streak the cells. The agar plate was then incubated in microbiological incubator for 24 hours at 37°C. Next, a loop of cells from the agar plate was transferred into a conical flask containing 10 mL of LB medium. The cell in the conical flask was then incubated in shaker incubator for 24 hours at 37°C and 200 rpm. Finally, the 10 mL cells was transferred into 250 mL of LB medium and incubated again at the same condition (Sezonov *et al*., 2007).

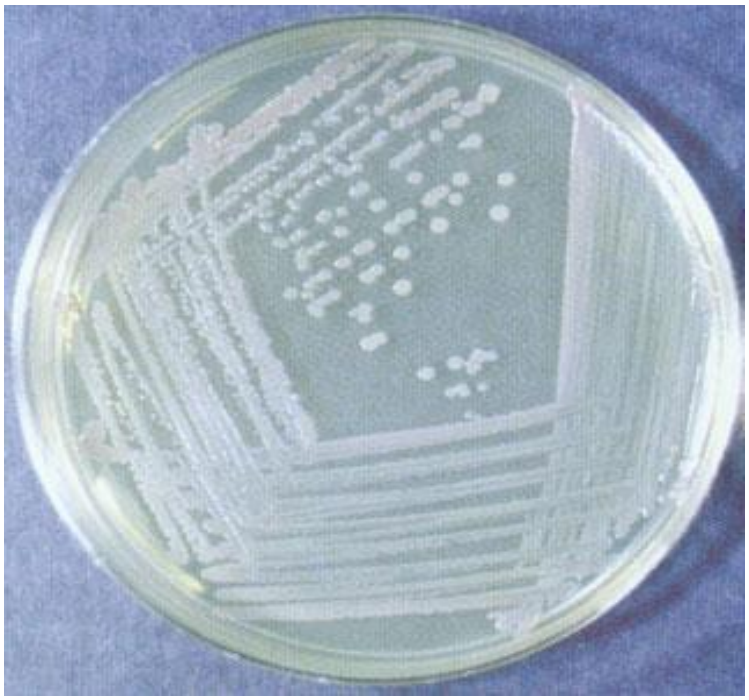

Figure 3.4 *E.coli* on agar plate

3.8 Preparation of Feed Solution

3.8.1 Preparation of Feed Solution for the Effect of pH

200 mL from 250 mL of *E.coli* cells incubated in the previous procedure was centrifuged at a condition of 4°C at 10 000 rpm for 10 minutes. The cell precipitate was then washed with deionized water and re-centrifuged again at the same condition. Next, the cell precipitate was dissolved in 2L buffer solution.

3.8.2 Preparation of Feed Solution for the Effect of Ionic Strength

The sample was prepared at pH 6.5. 200 mL from 250 mL of *E.coli* cells in the conical flask was centrifuged at a condition of 4°C at 10 000 rpm for 10 minutes. The cell precipitate was then washed with deionized water and re-centrifuged again at the same condition. Next, the cell precipitate was dissolved in buffer solution of pH 6.5 and NaCl solution until the total volume of the sample solution was 2L.

Figure 3.5 Feed solution

3.9 Separation of *Escherichia coli* Experiment

The experimental procedures for both parameters were similar. Cross-flow filtration system was used in this separation process and hollow fibre membrane with molecular pore size of 0.2µm and filter area of 110 cm^2 was used as the filter media. The experiment was run at a constant transmembrane pressure, ΔP of 0.8 bars. To ensure ΔP is always constant, the system was controlled by peristaltic pump and valves. Permeate was collected for every 5 minutes intervals until the volume of permeate showed constant readings, while retentate was recycled back into the feed tank. Permeate flux can be determined from the readings by using the following equation:

$$Permeate\ flux = \frac{Volume\ of\ permeate}{Time\ \times Surface\ area}$$

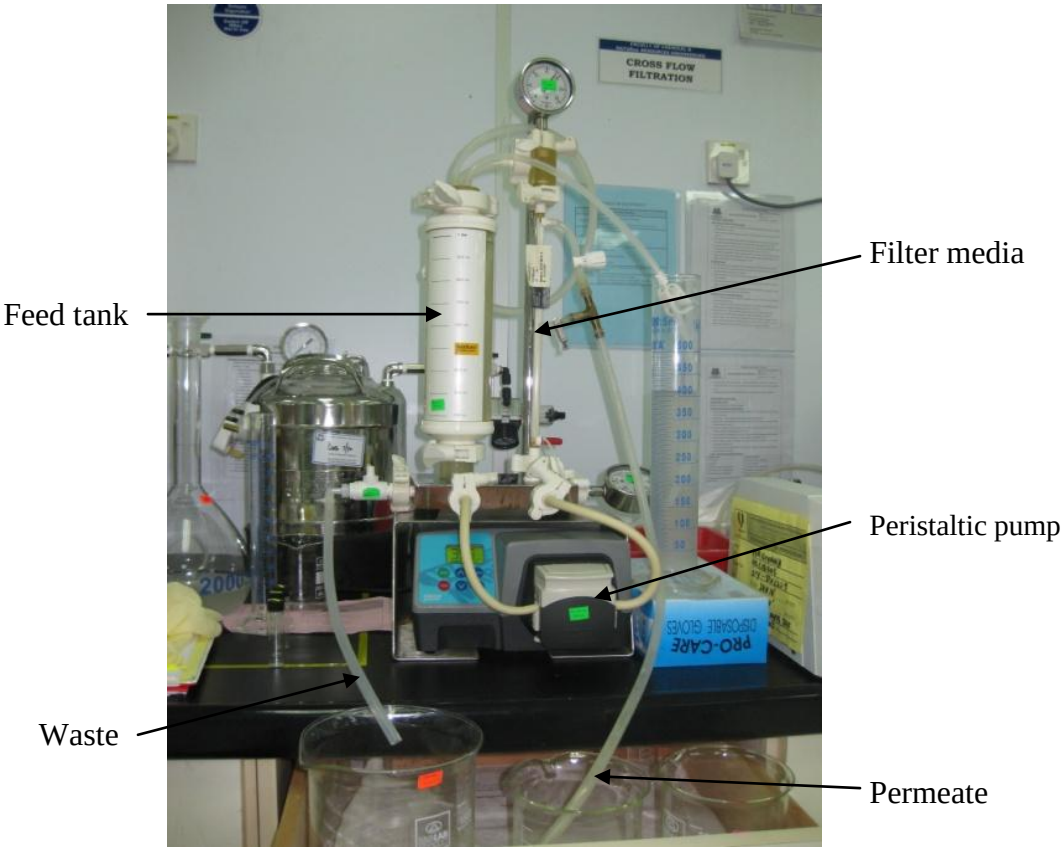

Figure 3.6 Cross-flow filtration system

Figure 3.7 Hollow fibre membranes

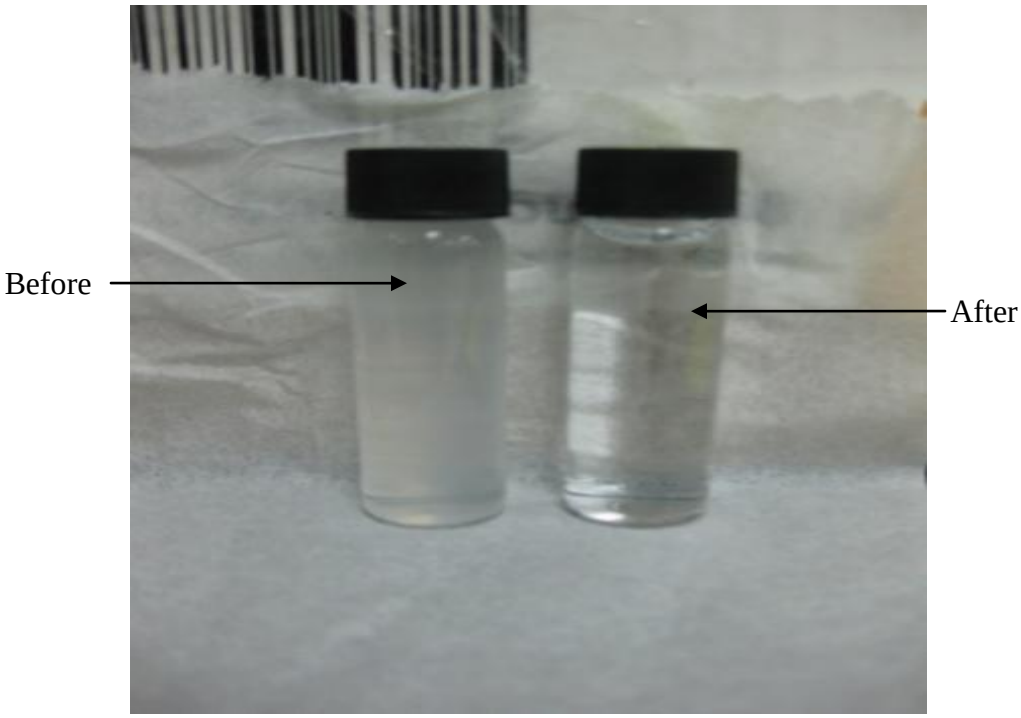

Figure 3.8 Samples before and after filtration

3.10 Membrane Cleaning Process

Cleaning process must be done in order to increase the life span of a membrane. Membrane is washed to remove particles that remain in its pores. In this study, 0.1M NaOH solution was circulated through the filtration system for about 60 minutes followed by deionized water for about 50 minutes after each experiment was completed.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Effect of pH

In order to investigate the effect of pH in the separation of *E.coli*, a series of microfiltration experiment was carried out with five different values of pH, starting with pH 4.5 and followed by 5.5, 6.5, 7.5, and lastly pH 8.5. Two litres of sample mixture of *E.coli* and buffer were circulated in the cross-flow filtration system at a constant transmembrane pressure (TMP) of 0.8 bar. The membrane used in this system was hollow fibre with molecular pore size of 0.2 μm and surface area of 110 cm^2.

The permeate flux was determined for every 5 minutes intervals until the readings went constant by collecting the permeate volume for each intervals. The permeate flux was calculated as follow:

$$Permeate\ flux\ = \frac{Volume\ of\ permeate}{Time\ \times Surface\ area}$$

4.1.1 Effect of pH 4.5

Table 4.1 Analysis data for pH 4.5

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	53.0	0.096363636
10	43.0	0.078181818
15	44.0	0.080000000
20	42.0	0.076363636
25	42.0	0.076363636
30	41.0	0.074545455
35	40.0	0.072727273
40	39.0	0.070909091
45	38.0	0.069090909
50	38.0	0.069090909
55	39.0	0.070909091
60	37.0	0.067272727
65	38.0	0.069090909
70	38.0	0.069090909
75	38.0	0.069090909
80	38.0	0.069090909

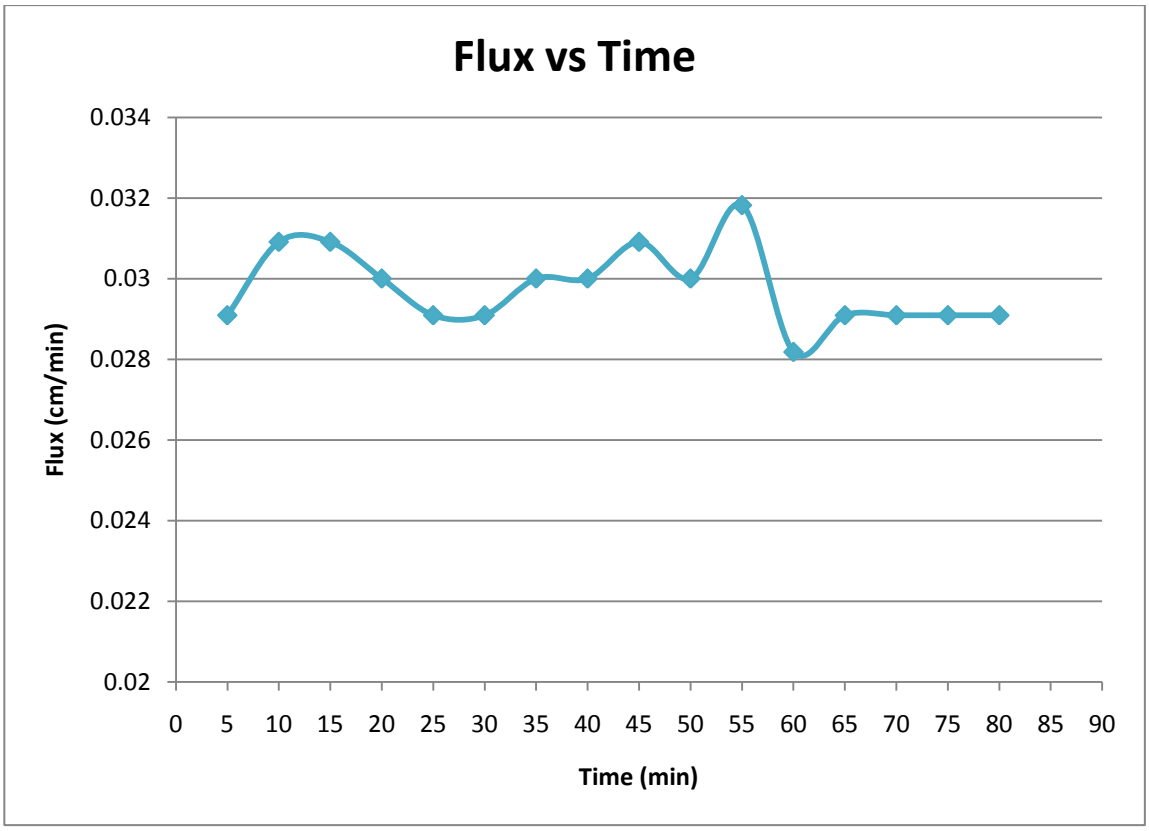

Figure 4.1 Graph of flux versus time for pH 4.5

Table 4.1 and Figure 4.1 show the relationship between the permeate flux and the separation time at pH 8.5. The flux increased after 10 minutes of separation and then decreased with time until the minute of 25. However, it started to fluctuate at the 35th minute until it became constant at the 65th minute. The maximum flux obtained was 0.032 cm/min at the minute of 55, while the minimum flux of 0.028 cm/min occurred at the 60th minute. From the graph, it can be shown that the greatest flux decline was obtained between 55th and 60th minute.

4.1.2 Effect of pH 5.5

Table 4.2 Analysis data for pH 5.5

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	20.0	0.036363636
10	25.0	0.045454545
15	24.5	0.044545455
20	24.5	0.044545455
25	23.0	0.041818182
30	22.0	0.040000000
35	21.5	0.039090909
40	23.0	0.041818182
45	23.5	0.042727273
50	23.0	0.041818182
55	24.0	0.043636364
60	22.5	0.040909091
65	25.0	0.045454545
70	24.0	0.043636364
75	24.0	0.043636364
80	24.0	0.043636364

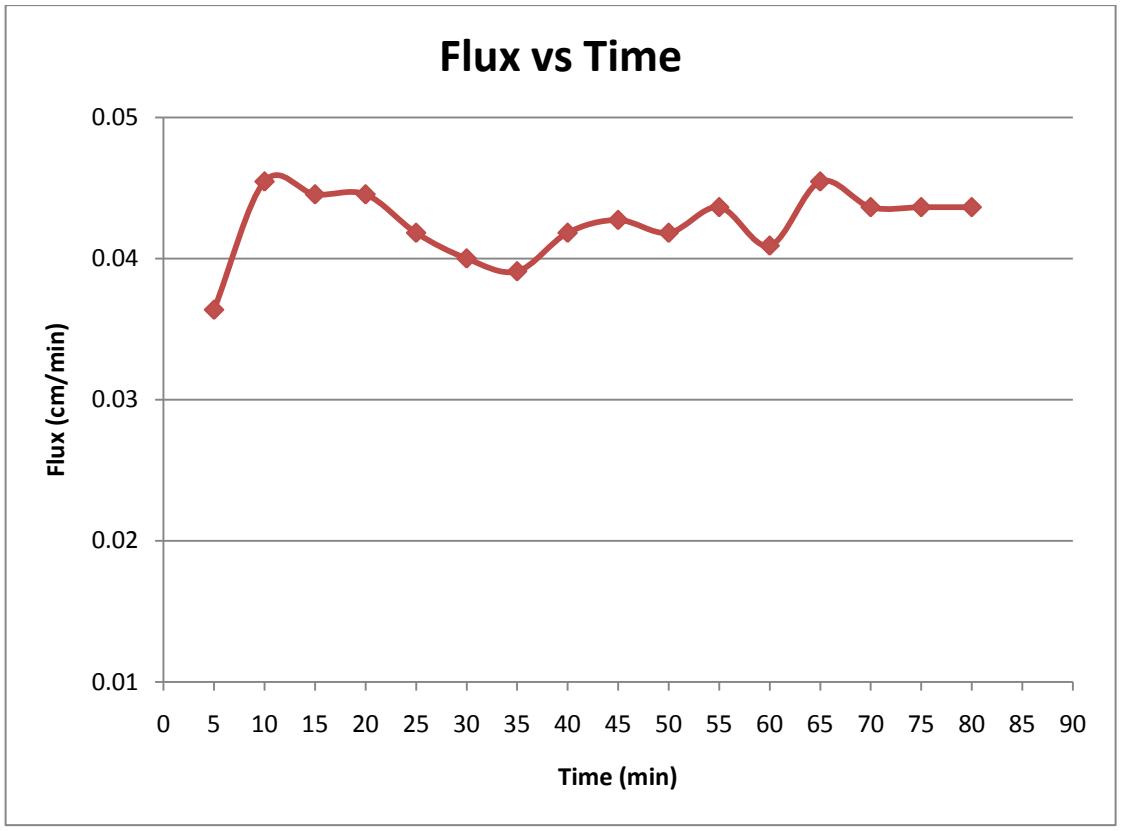

Figure 4.2 Graph of flux versus time for pH 5.5

Table 4.2 and Figure 4.2 show the relationship between the permeate flux and the separation time at pH 5.5. The flux increased after 10 minutes of separation and then decreased with time until the minute of 35. However, it started to fluctuate at the 40th minute until it became constant at the 70th minute. The maximum flux obtained was 0.045 cm/min at the minute of 10, while minimum flux was obtained after 5 minutes of separation which was 0.036 cm/min. From the graph, it can be shown that the greatest flux decline was obtained between 55th and 60th minute.

4.1.3 Effect of pH 6.5

Table 4.3 Analysis data for pH 6.5

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	54.5	0.099090909
10	47.5	0.086363636
15	51.0	0.092727273
20	43.0	0.078181818
25	46.5	0.084545455
30	45.5	0.082727273
35	45.5	0.082727273
40	45.0	0.081818182
45	46.0	0.083636364
50	43.0	0.078181818
55	45.0	0.081818182
60	43.5	0.079090909
65	44.0	0.080000000
70	43.5	0.079090909
75	43.5	0.079090909
80	43.5	0.079090909

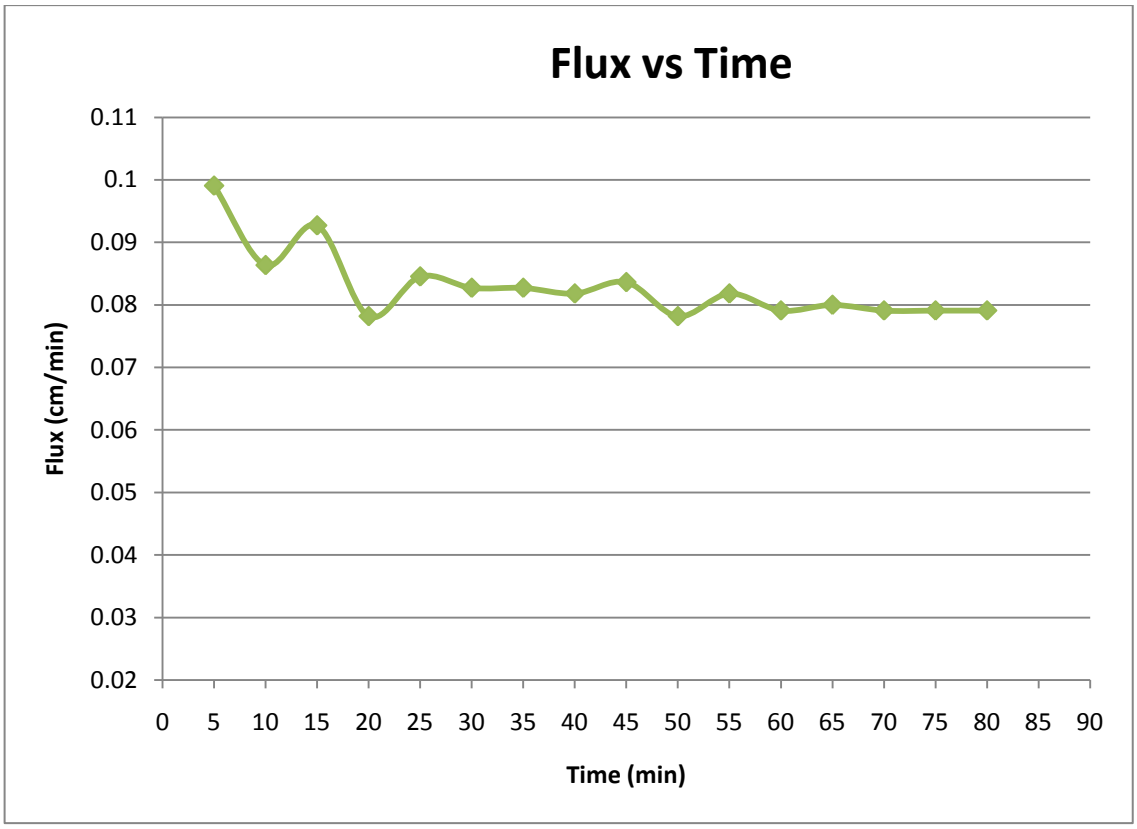

Figure 4.3 Graph of flux versus time at pH 6.5

Table 4.3 and Figure 4.3 show the relationship between permeate flux and time for separation at pH 6.5. At the beginning of the experiment, permeate flux was high but then decreased with time until it became constant. However, there were some increments of flux at the 15th, 25th, 45th and 55th minute. The flux was constant at 70th minute. The maximum flux obtained was 0.099 cm/min after 5 minutes of separation, while the minimum flux of 0.078 cm/min occurred at 20th and 50th minute. From the graph, it is shown that the greatest flux decline was occurred between 15th and 20th minute.

4.1.4 Effect of pH 7.5

Table 4.4 Analysis data for pH 7.5

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	31.0	0.056363636
10	38.0	0.069090909
15	30.0	0.054545455
20	31.0	0.056363636
25	28.0	0.050909091
30	26.5	0.048181818
35	24.5	0.044545455
40	22.0	0.040000000
45	20.0	0.036363636
50	17.5	0.031818182
55	18.5	0.033636364
60	18.0	0.032727273
65	17.0	0.030909091
70	16.0	0.029090909
75	16.0	0.029090909
80	16.0	0.029090909

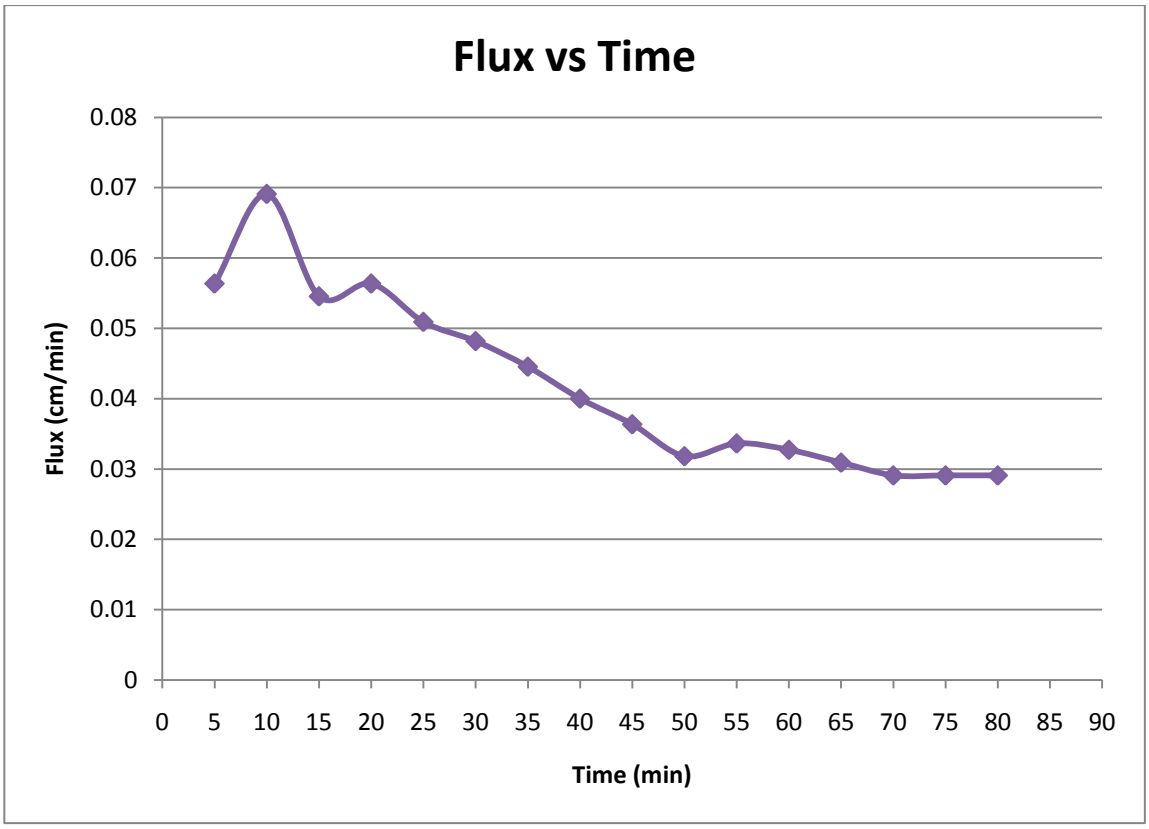

Figure 4.4: Graph of flux versus time at pH 7.5

Table 4.4 and Figure 4.4 show the relationship between the permeate flux and the separation time at pH 7.5. The flux increased after 10 minutes of separation and then decreased with time until the flux was constant at 70th minute. However, there were some increments of flux at 10th, 20th and 55th minute. The maximum flux obtained was 0.069 cm/min at 10th minute, while the minimum flux of 0.029 cm/min occurred at 70th minute and it remained constant. From the graph, it is shown that the greatest flux decline was occurred between 10th and 15th minute.

4.1.5 Effect of pH 8.5

Table 4.5 Analysis data for pH 8.5

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	53.0	0.096363636
10	43.0	0.078181818
15	44.0	0.080000000
20	42.0	0.076363636
25	42.0	0.076363636
30	41.0	0.074545455
35	40.0	0.072727273
40	39.0	0.070909091
45	38.0	0.069090909
50	38.0	0.069090909
55	39.0	0.070909091
60	37.0	0.067272727
65	38.0	0.069090909
70	38.0	0.069090909
75	38.0	0.069090909
80	38.0	0.069090909

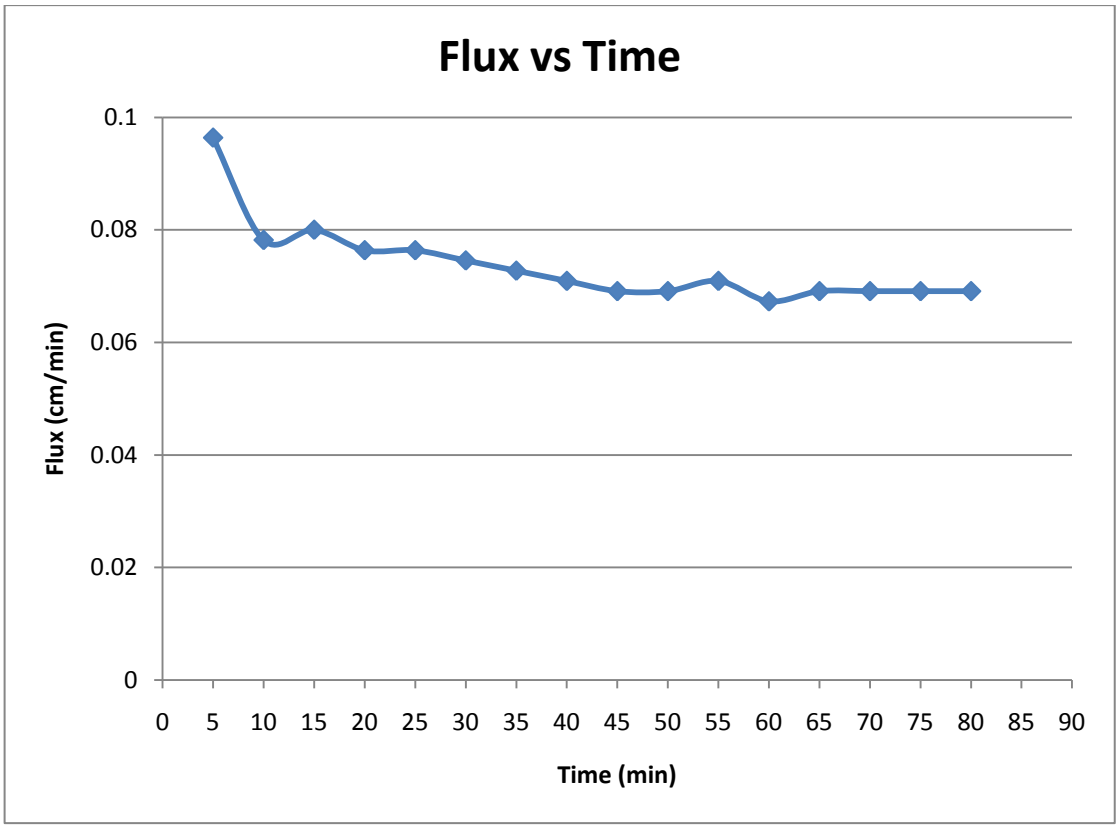

Figure 4.5 Graph of flux versus time at pH 8.5

Table 4.5 and Figure 4.5 show the relationship between permeate flux in pH 4.5 and the separation time. The flux decreased with time. At the beginning of the experiment, permeate flux was high but then decreased with time until it became constant. The flux was constant at the 65th minute. The maximum flux obtained was 0.096 cm/min at the 5th minute, while the minimum flux was 0.067 cm/min at the 60th minute. The greatest flux decline was obtained between the 5th minute and 10th minute.

4.1.6 Overall Effect of pH

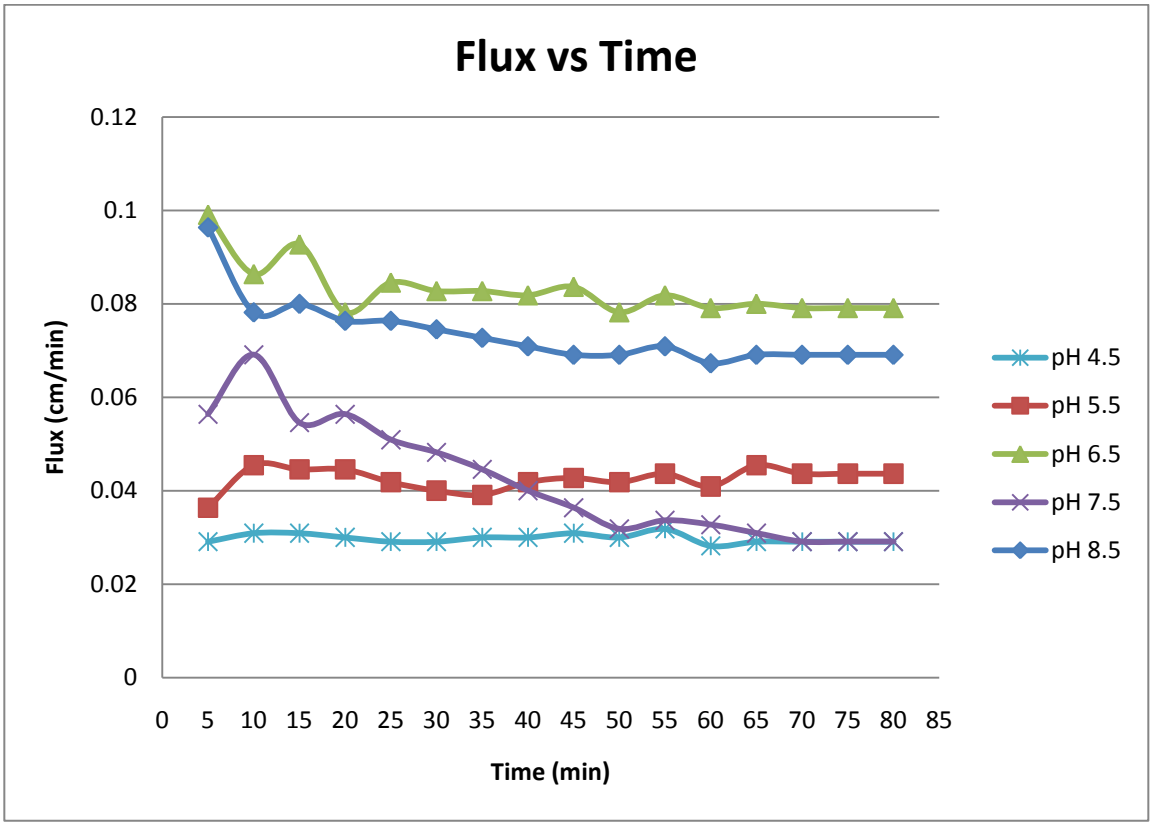

Figure 4.6 Graph of permeate flux versus time at different pH

Figure 4.6 shows the relationship between permeate flux and time at five different pH values. It is shown that the flux decreased with time. By comparing with the five different values of pH, it is shown that the highest permeate flux exited from pH 6.5. This pH was similar to the optimum condition for fermentation of *E.coli* to produce succinic acid. It also showed that the type of membrane used in this experiment can be used in the fermentation of *E.coli.* At this pH, the cells would 100% retained in the fermenter for cell recycling, while succinic acid would be removed in permeate as products.

The lowest permeate flux was exited from the pH 4.5 solution. This is due to the electrostatic repulsion between *E.coli* and the membrane surface. The pH 4.5 was near to the isoelectric point (pI) of *E.coli* which is around 4, causing it to be less negatively charged in the solution. Morphology of the polymer membrane also affected the repulsion. The polymer chain of the membrane was negatively charged. There was an opposite charge between *E.coli* cells and the membrane surface. The cells were attracted to the surface and remained in the pore, hence increased the retention of the flux. The cells also tend to form macromolecules when it was in the pI and this causing low flux transmission.

At higher pH, the membrane matrix would be in a more expanded state due to greater intramembrane electrostatic repulsion. Membrane pore size was slightly larger, causing lower retention of flux. On the whole, higher pH resulted in higher permeate flux.

4.2 Effect of Ionic Strength

In order to investigate the effect of ionic strength in the separation of *E.coli*, a series of microfiltration experiment was carried out with five different concentration of salt, starting with 0.1M and followed by 0.2M, 0.3M, 0.4M and lastly 0.5M. These experiments were carried out at pH 6.5 because this pH resulted in higher permeate flux in the experiment to investigate the effect of pH. *E.coli* was dissolved in 6.5 buffer solution and added with salt solution of the mentioned concentration to two litres of sample solution. Similar experimental procedures for the effect of pH were carried out to investigate the effect of ionic strength on the membrane flux.

4.2.1 Effect of 0.1M Ionic Strength

Table 4.6 Analysis data for 0.1M ionic strength

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	29.0	0.052727273
10	29.0	0.052727273
15	30.0	0.054545455
20	28.0	0.050909091
25	30.0	0.054545455
30	31.5	0.057272727
35	32.5	0.059090909
40	31.5	0.057272727
45	32.0	0.058181818
50	33.5	0.060909091
55	32.0	0.058181818
60	33.0	0.060000000
65	33.0	0.060000000
70	33.0	0.060000000

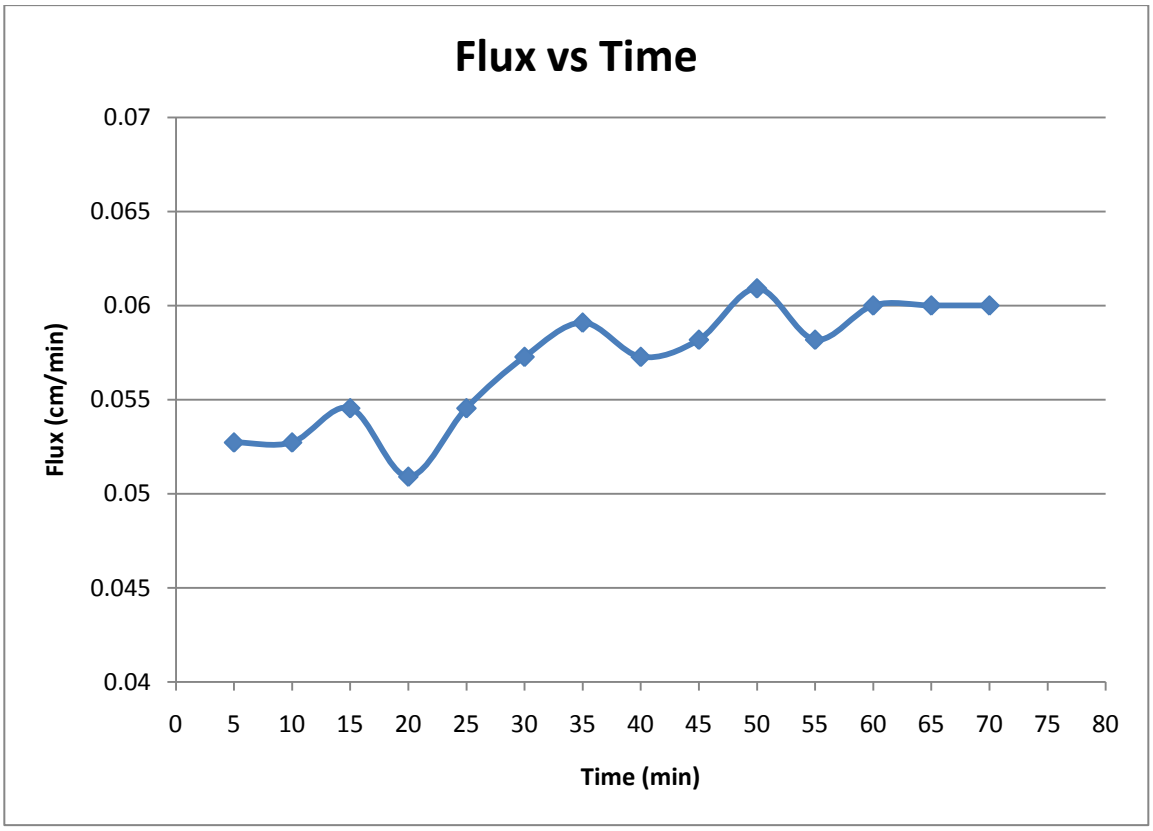

Figure 4.7 Graph of flux versus time at 0.1M ionic strength

Table 4.6 and Figure 4.7 show the relationship between the permeate flux and the separation time at 0.1M ionic strength. The flux increased after 15 minutes of separation and then decreased at 20th minute. However, it started to fluctuate at the 25th minute until it became constant at the 60th minute. The maximum flux obtained was 0.061 cm/min at the minute of 50, while the minimum flux of 0.051 cm/min occurred at the 20th minute. From the graph, it can be shown that the greatest flux decline was obtained between 15th and 20th minute.

4.2.2 Effect of 0.2M Ionic Strength

Table 4.7 Analysis data for 0.2M ionic strength

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	35.5	0.064545455
10	39.0	0.070909091
15	37.0	0.067272727
20	38.0	0.069090909
25	37.0	0.067272727
30	36.5	0.066363636
35	37.0	0.067272727
40	37.0	0.067272727
45	37.0	0.067272727
50	37.0	0.067272727
55	37.0	0.067272727
60	37.0	0.067272727
65	37.0	0.067272727
70	37.0	0.067272727

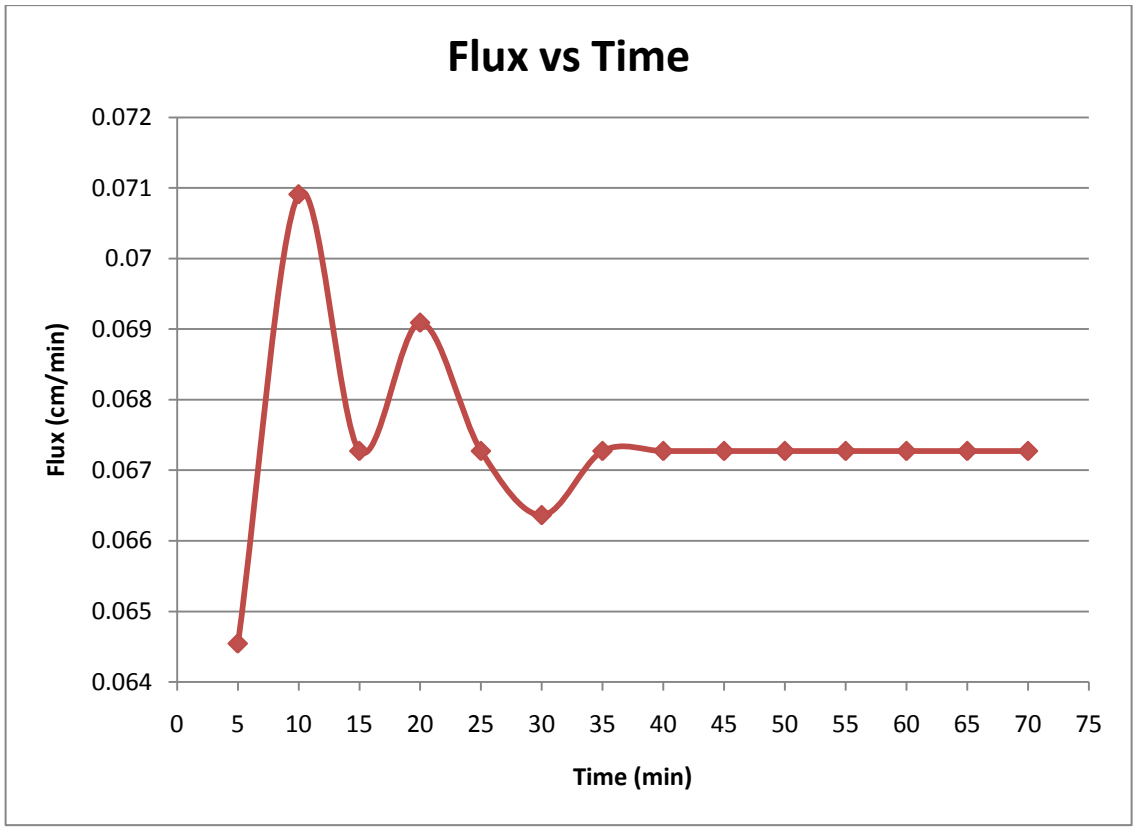

Figure 4.8 Graph of flux versus time at 0.2M ionic strength

Table 4.7 and Figure 4.8 show the relationship between permeate flux and time for separation at 0.2M ionic strength. The flux increased after 10 minutes of separation and then fluctuated until it became constant at the 35th minute. The maximum flux obtained was 0.071 cm/min at the minute of 10, while the minimum flux of 0.065 cm/min occurred at the 5th minute. From the graph, it can be shown that the greatest flux decline was obtained between 10th and 15th minute.

4.2.3 Effect of 0.3M Ionic Strength

Table 4.8 Analysis data for 0.3M ionic strength

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	36.5	0.066363636
10	38.5	0.070000000
15	38.0	0.069090909
20	38.0	0.069090909
25	36.0	0.065454545
30	36.0	0.065454545
35	36.5	0.066363636
40	35.5	0.064545455
45	35.0	0.063636364
50	35.0	0.063636364
55	35.0	0.063636364
60	35.0	0.063636364
65	35.0	0.063636364
70	35.0	0.063636364

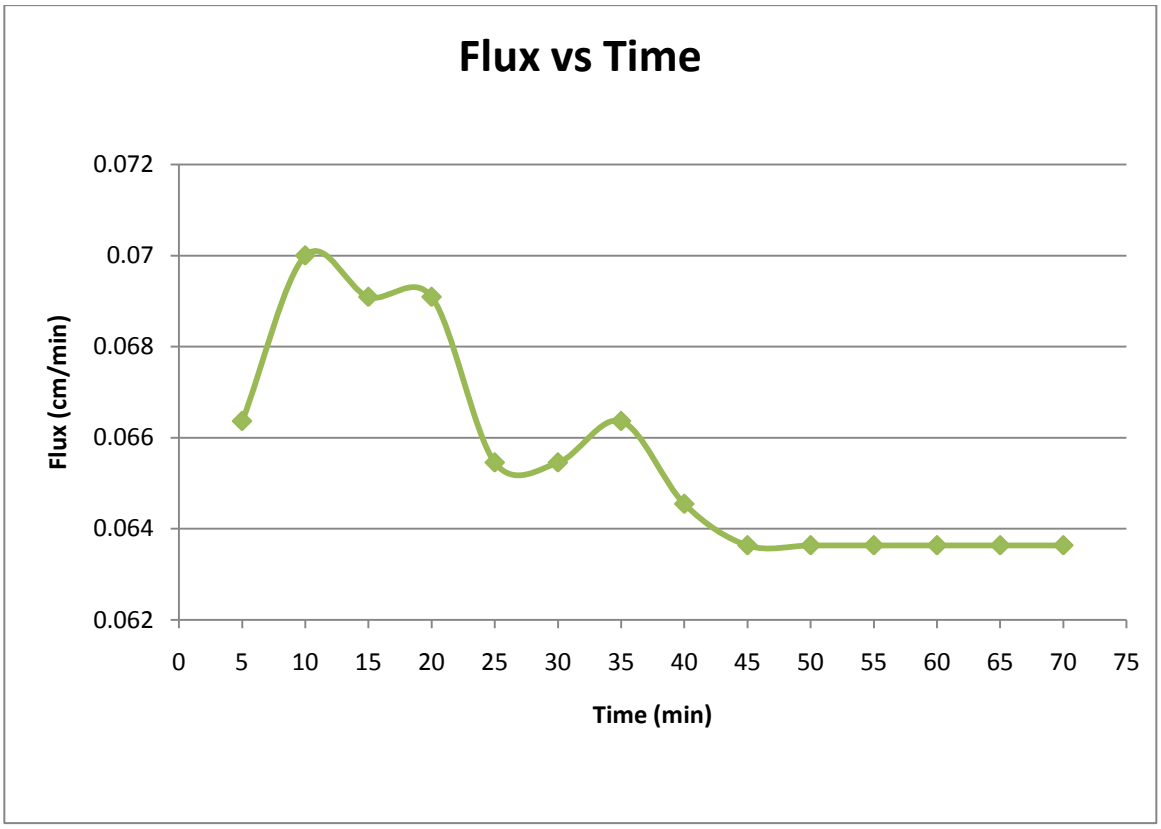

Figure 4.9 Graph of flux versus time at 0.3M ionic strength

Table 4.8 and Figure 4.9 show the relationship between permeate flux and time for separation at 0.3M ionic strength. The flux increased after 10 minutes of separation and then decreased with time until the flux was constant at 45th minute. However, there were some increments of flux at 20th and 35th minute. The maximum flux obtained was 0.07 cm/min after 10 minutes of separation, while the minimum flux of 0.064 cm/min occurred at 45th minute and it remained constant. From the graph, it is shown that the greatest flux decline was occurred between 20th and 25th minute.

4.2.4 Effect of 0.4M Ionic Strength

Table 4.9 Analysis data for 0.4M ionic strength

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	42.5	0.077272727
10	45.0	0.081818182
15	37.0	0.067272727
20	38.0	0.069090909
25	37.5	0.068181818
30	37.0	0.067272727
35	37.5	0.068181818
40	37.0	0.067272727
45	37.0	0.067272727
50	37.5	0.068181818
55	37.5	0.068181818
60	36.0	0.065454545
65	36.0	0.065454545
70	36.0	0.065454545

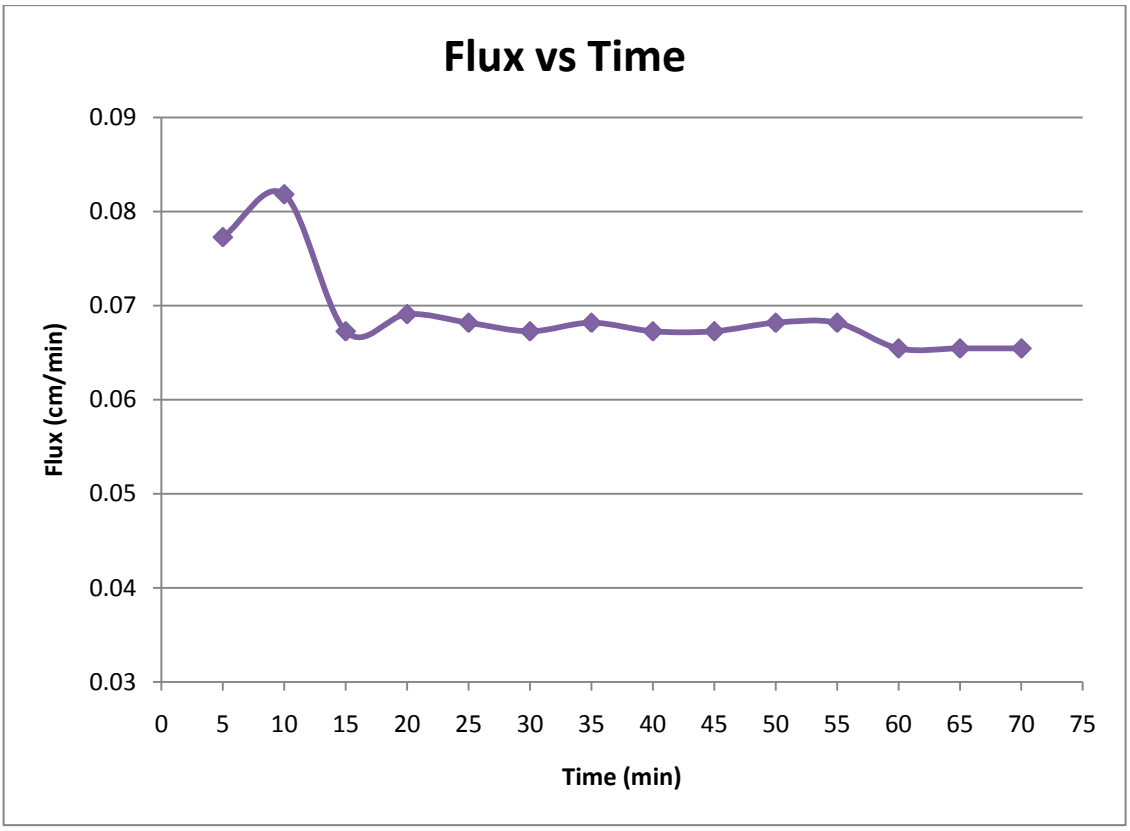

Figure 4.10 Graph of flux versus time at 0.4M ionic strength

Table 4.9 and Figure 4.10 show the relationship between the permeate flux and the separation time at 0.4M ionic strength. The flux increased after 10 minutes of separation and then decreased with time until the flux was constant at 60th minute. However, there were some increments of flux at 20th, 35th and 50th minute. The maximum flux obtained was 0.081 cm/min at 10th minute, while the minimum flux of 0.065 cm/min occurred at 60th minute and it remained constant. From the graph, it is shown that the greatest flux decline was occurred between 10th and 15th minute.

4.2.5 Effect of 0.5M Ionic Strength

Table 4.10 Analysis data for 0.5M ionic strength

Time (min)	**Volume of Permeate (cm^3)**	**Permeate Flux (cm/min)**
5	15.0	0.027272727
10	15.0	0.027272727
15	13.0	0.023636364
20	13.0	0.023636364
25	13.5	0.024545455
30	14.0	0.025454545
35	13.0	0.023636364
40	13.0	0.023636364
45	12.5	0.022727273
50	12.5	0.022727273
55	12.5	0.022727273
60	12.5	0.022727273
65	12.5	0.022727273
70	12.5	0.022727273

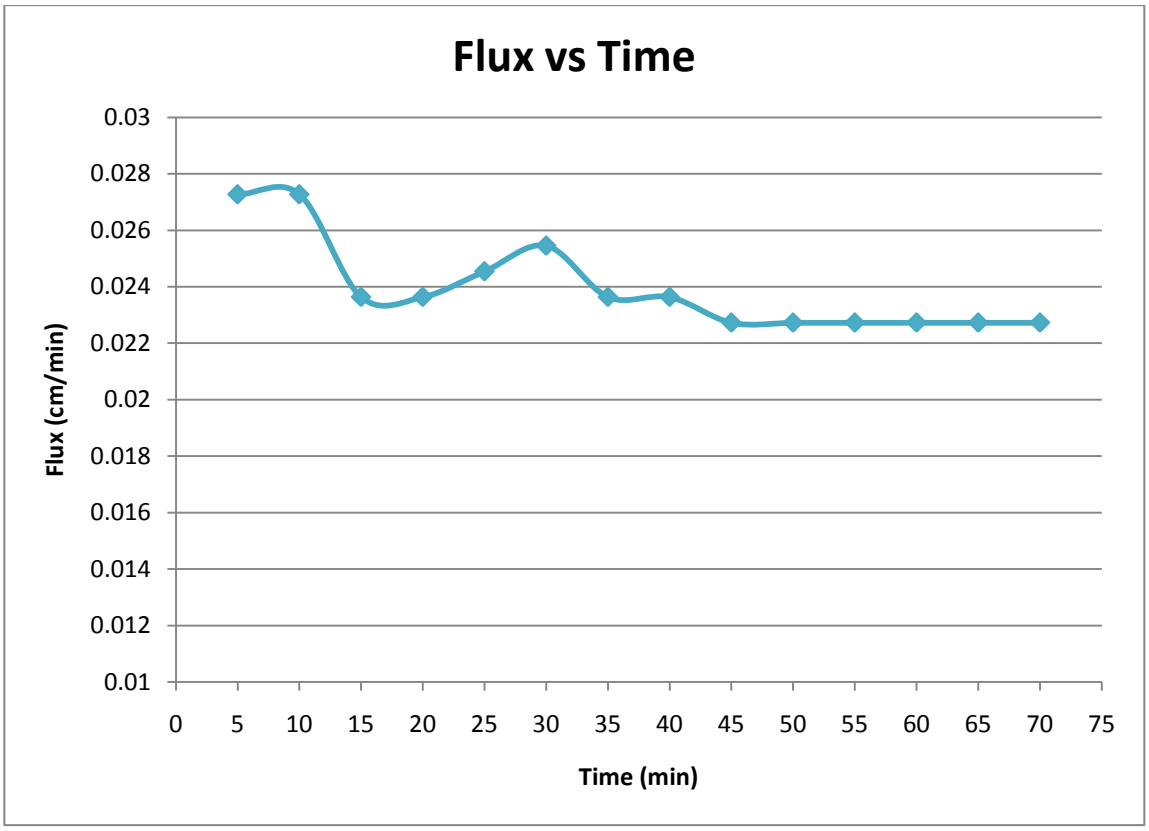

Figure 4.11 Graph of flux versus time at 0.5M ionic strength

Table 4.10 and Figure 4.11 show the relationship between the permeate flux and the separation time at 0.5M ionic strength. The flux increased after 10 minutes of separation and then decreased with time until the flux was constant at 45th minute. However, there were some increments of flux at 20th to 30th minute and at 40th minute. The maximum flux obtained was 0.027 cm/min at 10th minute, while the minimum flux of 0.023 cm/min occurred at 45th minute and it remained constant. From the graph, it is shown that the greatest flux decline was occurred between 10th and 15th minute.

4.2.6 Overall Effect of Ionic Strength

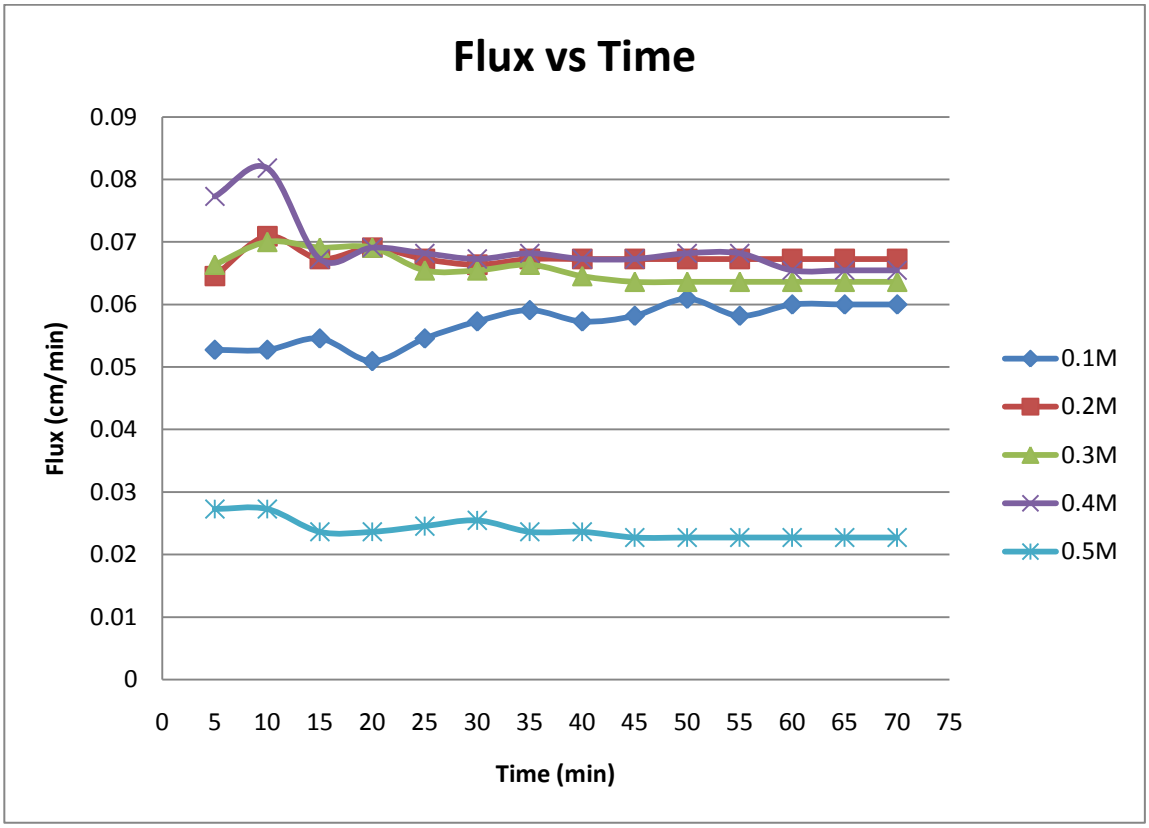

Figure 4.12 Graph of permeate flux versus time at different ionic strength

Figure 4.12 shows the overall flux analysis for the effect of ionic strength at pH 6.5. It is shown that the flux decreased with time. By comparing with the five different concentrations of salt, it is shown that the highest permeate flux occurred at 0.2M and the lowest flux was at 0.5M ionic strength.

At low concentration of salt, less *E.coli* adsorbed on the membrane surface. Similar charge between the cell and the membrane surface caused an electrostatic repulsion. The cells repelled and hence decreased the retention of flux.

However, higher concentration of salt resulted in lower permeate flux. This is due to the compaction of the membrane which decreased the pore size and hence, increased the retention of flux. On the whole, the flux increased when the concentration of salt decreased.

CHAPTER 5

CONCLUSION AND RECOMMENDATION

5.1 Conclusion

The study on the effect of pH and ionic strength in the separation of *E.coli* by cross-flow filtration has been successfully done. From the result obtained and the analysis that have been done, it can be conclude that the objective of this study has been achieved. This study was to investigate the effect of electrokinetic parameters on the membrane flux in the separation of *E.coli* by using cross-flow microfiltration system.

Recently, increasing interest has been generated in the separation of cells by cross-flow filtration. This technique is usually used as the first separation step after fermentation either to recover extracellular products, to concentrate and/or wash cells before product recovery, or for cell recycle (Warren *et al.*, 1991). A successful succinic acid recovery approach in continuous fermentation is in a cell-recycled reactor where the cells are separated by a filtration unit and returned to the fermenter while the product is removed in the permeate (Li *et al.*, 2006).

The usage of cross-flow microfiltration system could decrease the production cost of succinic acid. Hollow fibre membrane functioned to retain the cells in the fermenter for cell-recycling, while the product which is succinic acid is removed in the permeate. Cell-recycling can reduce the production cost of succinic acid. Hollow fibre membrane is used in this filtration system instead of other types of membrane such as flat sheet due to its minimum-required space, and can be put in the fermenter. The production cost of succinic acid can be reduced without spending much money on larger equipments which also requires larger space. Moreover, this study showed that this type of membrane can be used in the fermentation of *E.coli* because the optimum condition for both fermentation and separation process is at pH 6.5.

Based on the results obtained, the flux increased with increasing in pH and decreasing in concentration of salt. This study also showed that pH and ionic strength could affect the efficiency of the separation of solute. Addition of salt in the fermentation mixture could resulted in lower flux tranmission compared to the non-added salt mixture. This is due to the compaction of the membrane which decreased the pore size and hence, increased the retention of flux.

5.2 Recommendation

To ensure this study can be continued, some recommendations need to be considered. In this study, *E.coli* was separated from the buffer solution. This can be improved by separating *E.coli* from the actual fermentation broth in order to determine the actual optimum condition to separate *E.coli* in the production of succinic acid.

Further studies on this research can also be done by using different types of membrane to separate *E.coli.* Flat sheet membrane, tubular membrane and spiral wound membrane are among the membranes that can be used in this study. The most economical type of membrane with high separation efficiency for the production of succinic acid can be determined if types of membrane are varied.

Besides that, this study can be improved by analyzing permeate and retentate concentration using Total Organic Carbon Analyzer (TOC) to ensure that 100% cells are rejected. If 100% cells are rejected, TOC Analyzer will show that the final concentration of permeate is zero, meaning that no cells have passed through the membrane.

Transmission Electron Microscopy (TEM) can also be used for characterization of fouled membranes. The membranes fouling during recycling of *E.coli* broth will be immediately cooled to -170°C after the experiment and water vapour will be removed from the membrane by sublimation (Gatenholm *et al*., 1988).

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APPENDIX

Pictures during Experiment

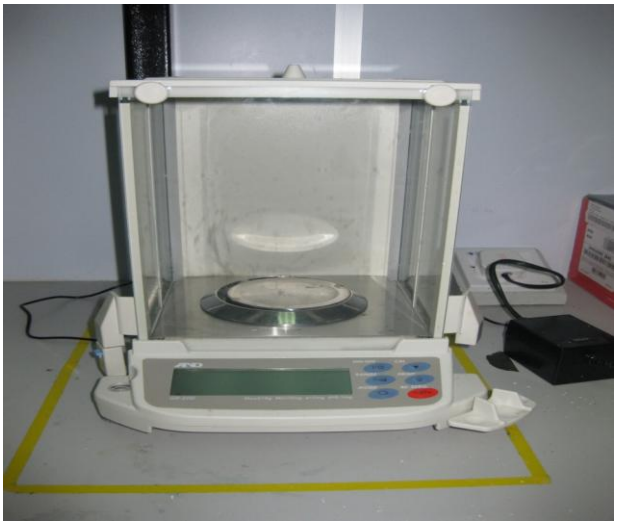

Figure 1 Electric balance

Figure 2 pH meter

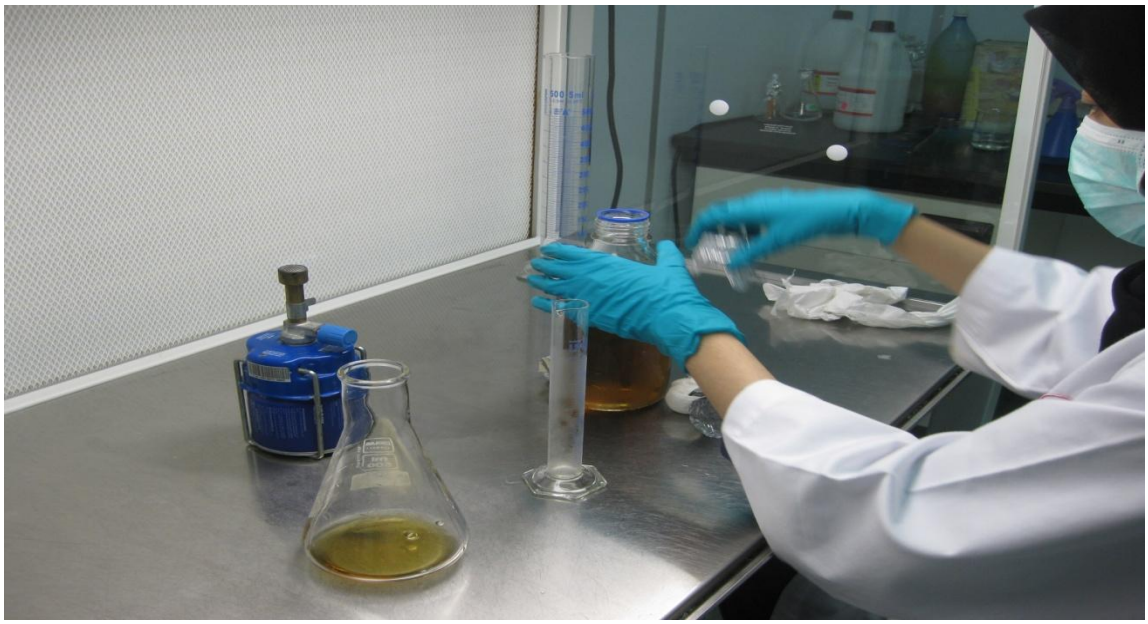

Figure 3 Cultivation of cell

Figure 4 Shaker incubator

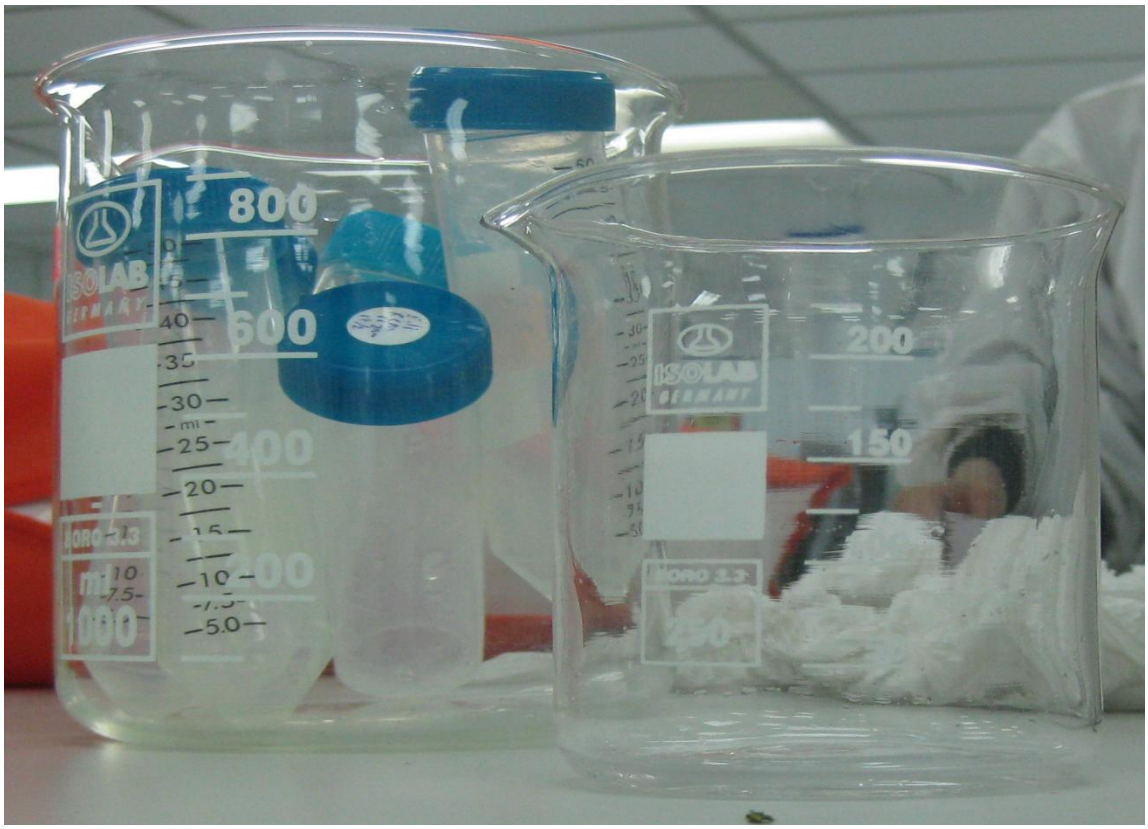

Figure 5 Centrifuge tubes and beaker

Figure 6 Autoclave

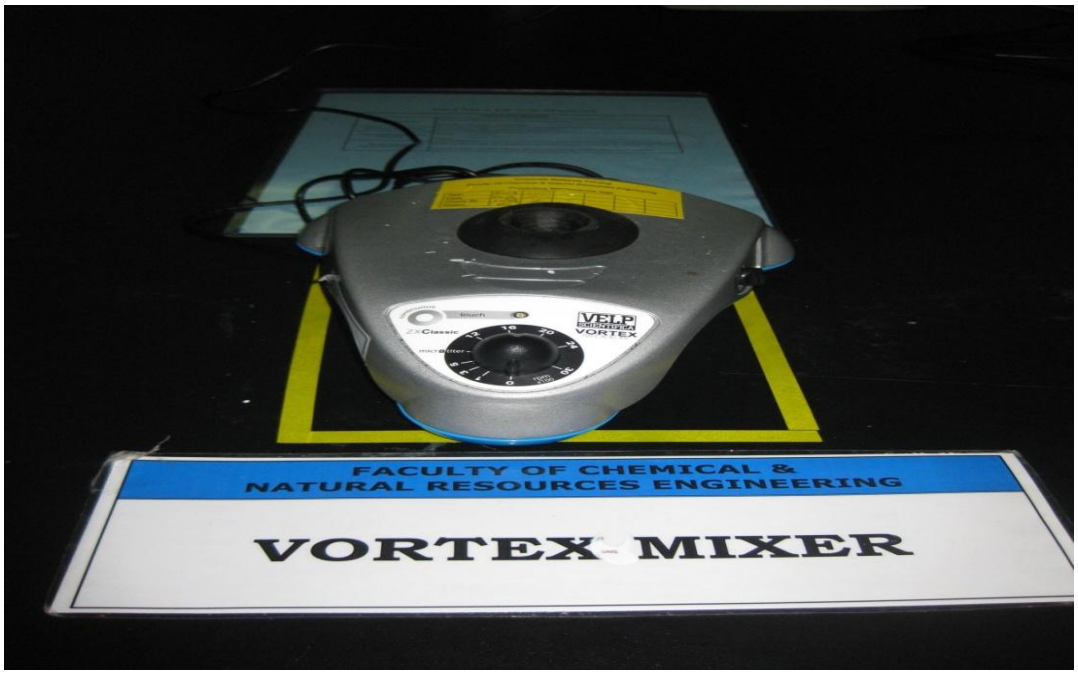

Figure 7 Vortex mixer

Figure 8 Refrigerated Centrifuge

Figure 9 Laminar flow

Figure 10 Microbiological incubator