SEPARATION AND PURIFICATION OF ORGANIC ACID FROM FERMENTATION BROTH BY MEMBRANE PROCESS: NANOFILTRATION

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Suranaree University of Technology has approved this thesis submitted in partial fulfillment of the requirements for the Degree of Doctor of Philosophy

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งานวิจัยนี้ศึกษาการแยกและทำบริสุทธิ์กรดแลคติก เช่น การกำจัดน้ำตาลกูลโคสจากน้ำหมักกรดแลคติกโดยใช้กระบวนการกรองแบบนาโนฟิวเตรชั่น แผ่นเยื่อบางที่เลือกศึกษาคือ Desal 5DK การทดลองจะถูกแบ่งเป็น 4 ส่วนตามชนิดของสารละลายที่ใช้นั่นคือ สารละลายสังเคราะห์ได้แก่ สารละลายที่มีตัวถูกละลายหนึ่งชนิด สองชนิด และสามชนิด และน้ำหมักจริง ตามลำดับ การทดลองส่วนแรก ศึกษากลไกการถ่ายเทมวลสารผ่านแผ่นเยื่อบางโดยใช้สารละลายที่มีตัวถูกละลายหนึ่งชนิด ได้แก่ กูลโคส NaLac (โซเดียมแลคเตท) NaCl และ Na_2SO_4 พบว่าที่ความเข้มข้นของแลคเตทสูง ๆ ค่ารีเทนชั่นของกูลโคส (ไม่ขึ้นกับความเข้มข้นของกูลโคส) มีค่าสูงกว่าค่ารีเทนชั่นของแลคเตทมาก (ลดลงตามการเพิ่มขึ้นของความเข้มข้นของแลคเตท) แสดงให้เห็นถึงความเป็นไปได้ในแยกกูลโคสออกจากแลคเตทเมื่อตัวถูกละลายทั้งสองผสมอยู่ด้วยกัน ในการทดลองส่วนที่สองศึกษาแรงกระทำระหว่างตัวถูกละลายที่ไม่มีขั้วกับตัวถูกละลายมีขั้ว และตัวถูกละลายที่มีขั้วด้วยกันเอง โดยศึกษาในสารละลายที่มีตัวถูกละลายสองชนิด พบว่า เมื่อความเข้มข้นของแลคเตทเพิ่มขึ้นไม่เพียงแค่ค่ารีเทนชั่นของแลคเตทมีค่าลดลงเท่านั้น แต่ค่ารีเทนชั่นของกูลโคสก็ลดลงด้วยเช่นกัน ค่ารีเทนชั่นของตัวถูกละลายทั้งสองจะลดลงตามการเพิ่มขึ้นของความเข้มข้นของ Cl^- แต่อย่างไรก็ตามค่ารีเทนชั่นของแลคเตทจะลดลงมากกว่า นอกจากนี้ค่ารีเทนชั่นของกูลโคสจะไม่ขึ้นกับความเข้มข้นของ SO_4^{2-} ขณะที่ค่ารีเทนชั่นของแลคเตทจะลดลงอย่างมากเมื่อเพิ่มความเข้มข้นของ SO_4^{2-} ในสารละลาย ปรากฏการณ์ที่เกิดขึ้นนี้จะถูกอธิบายไว้ในวิทยานิพนธ์นี้ การแยกกูลโคสออกจากแลคเตทจะสามารถเกิดขึ้นได้ ถ้ารักษาให้ค่าฟลักซ์การไหลต่ำและมีความเข้มข้นของแลคเตทสูง การมีอยู่ของตัวถูกละลายที่มีขั้ว เช่น Cl^- และ SO_4^{2-}ส่งผลกระทบต่อค่ารีเทนชั่นของกูลโคสและแลคเตทลดลงแตกต่างกัน ลักษณะเช่นนี้ทำให้เห็นถึงความเป็นไปได้ในการเพิ่มความสามารถในการแยกแลคเตทกับกูลโคสด้วยการเติมเกลือ NaCl หรือ Na_2SO_4 ลงไปในสารละลายที่มีกูลโคสและแลคเตทผสมอยู่ ในการทดลองส่วนที่สาม ศึกษาอิทธิพลของการเติมเกลือต่อความสามารถในการแยกแลคเตทและกูลโคส เกลือจะถูกเติมลงไปในสารละลายที่มีกูลโคสและแลคเตทผสมอยู่กลายเป็นสารละลายที่มีตัวถูกละลายสามชนิด พบว่า การเติมเกลือ NaCl ช่วยให้การแยกเพิ่มขึ้นเล็กน้อยขณะที่การเติมเกลือ Na_2SO_4 ช่วยให้การแยกเพิ่มสูงขึ้นมาก นอกจากนี้ยังได้มีการศึกษาประสิทธิภาพของแผ่นเยื่อบาง ภายใต้การทดลองภายใต้ระบบเพิ่มความเข้มข้นของ

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ลายมือชื่อนักศึกษา_____
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CHAKKRIT UMPUCH : SEPARATION AND PURIFICATION OF ORGANIC ACID FROM FERMENTATION BROTH BY MEMBRANE PROCESS: NANOFILTRATION. THESIS ADVISOR AT SURANAREE UNIVERSITY OF TECHNOLOGY : ASST. PROF. SUNTHORN KANCHANATAWEE, Ph.D., THESIS ADVISOR AT UNIVERSITÉ PAUL SABATIER : HÉLÈNE ROUX-DE BALMANN, Ph.D., 190 PP.

LACTIC ACID/NANOFILTRATION/RETENTION COEFFICIENT/SEPARATION FACTOR /PERCENT PURITY/PERCENT YIELD

The aim of this study was to investigate the separation and purification of lactic acid, *i.e.* sugar removal, from fermentation broth containing lactate using nanofiltration. The experiments were carried out with the Desal 5 DK membrane; model solutions of glucose, sodium lactate, NaCl and Na_2SO_4 were investigated in single-, binary- and ternary-solute solutions. A real fermentation broth containing lactate was also performed. There were four parts of experiments in this work depending on the solutions used. Firstly, the mass transfer mechanisms of solute across the membrane were determined using single-solute solutions. It was found that the retention of glucose was quite independent of its concentrations whereas the retention of lactate strongly decreased when lactate concentration increased. The separation between glucose and lactate was expected to be feasible, when both solutes were together in a binary-solute solution, since the retention of glucoses was much higher than that of lactate at high lactate concentration. Secondly, the interaction

between neutral solute/electrolyte and electrolyte/electrolyte was investigated using binary-solute solutions. The presence of sodium lactate or NaCl showed that the glucose retention was lower than that in single-solute solution, however, the presence of Na_2SO_4 did not affect the glucose retention. Moreover, the separation between glucose and sodium lactate is achievable, as expected, at certain conditions; *i.e.* maintaining low permeate flux and using high sodium lactate concentration. Thirdly, the effect of addition of NaCl or Na_2SO_4 on the separation between glucose and lactate was investigated with ternary-solute solutions. The separation was slightly improved with the addition of Cl^- and it was significantly improved with the addition of SO_4^{2-}, however, maintaining low permeate flux and high concentration ratio of higher retained/less retained solute such as SO_4^{2-}/lactate was required. Furthermore, the experiments were carried out in the concentration mode in order to investigate the performance of NF which showed 64% of the highest purity and 80% of the maximum yield. Finally, the separation performance and influence of added Na_2SO_4 on the separation in real fermentation broth were performed and the results were comparable to those observed with model solutions.

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Academic Year 2010

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Co-advisor's Signature_____

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LIST OF ABBREVIATIONS

J	=	Flux ($m^3.s^{-1}.m^{-2}$)
J_v	=	Permeate flux ($m^3.s^{-1}.m^{-2}$)
J_w	=	Water permeate flux ($m^3.s^{-1}.m^{-2}$)
J_{lim}	=	Limiting flux ($m^3.s^{-1}.m^{-2}$)
$R_{obs,i}$	=	Observed retention coefficient of component i
$R_{int,i}$	=	Intrinsic retention coefficient of component i
$C_{p,i}$	=	Concentration of component *i* in permeate (M)
$C_{f,i}$	=	Concentration of component *i* in feed (M)
$C_{m,i}$	=	Concentration of component *i* at membrane surface (M)
C_b	=	Bulk concentration (M)
C_s	=	Saturation concentration (M)
k	=	Mass transfer coefficient ($m.s^{-1}$)
A	=	Area (m^2)

LIST OF ABBREVIATIONS (continued)

δ_b	=	Concentration polarization thickness (nm)
d_h	=	Hydrulic diameter (nm)
D	=	Diffusivity ($m^2.s^{-1}$)
NaLac	=	Sodium lactate
$\alpha_{glucose}$	=	Extent of decrease of glucose retention in mixture
$\alpha_{lactate}$	=	Extent of decrease of lactate retention in mixture
$R_{glucose}$	=	Glucose retention in single-solute solution
$R'_{glucose}$	=	Glucose retention in mixed-solute solution
$R_{lactate}$	=	Lactate retention in single-solute solution
$R'_{lactate}$	=	Lactate retention in mixed-solute solution
V	=	Volume (m^3)
V_0	=	Initial feed volume (m^3)
V_R	=	Volume of retentate (m^3)

LIST OF ABBREVIATIONS (continued)

V_0/V_R	=	Volume reduction factor
t	=	Time (min)
L_{po}	=	Water permeability ($m^3.s^{-1}.m^{-2}.bar^{-1}$)
ΔP	=	Pressure difference (bar)
CP	=	Concentration polarization
d_h	=	Hydraulic diameter (nm)
D	=	Diffusivity ($m^2.s^{-1}$)
D_s	=	Diffusivity of the involved solute ($m^2.s^{-1}$)
D_w	=	Diffusivity of water ($m^2.s^{-1}$)
ρ	=	Solution density (kg m^3)
μ	=	Dynamic viscosity ($kg.s^{-1}.m^{-1}$)
L_{p0}	=	Water permeability coefficients ($m.s^{-1}.Pa^{-1}$)
u	=	velocity ($m.s^{-1}$)

CHAPTER I

INTRODUCTION

1.1 Background and signification of research problem

Lactic acid is widely used in the food, pharmaceutical, cosmetic, and chemical industries. It has gained more important recently to use as a monomer to produce biodegradable plastic (Poly-lactic acid; PLA). In a review by Wee, Kim and Ryu *et al*. (2006) reported that the global consumption of lactic acid could be estimated roughly to be 130,000 – 150,000 (metric) tones per year which is expected to increase up to 500,000 (metric) tones in 2010. Lactic acid can be produced by either microbial fermentation or chemical synthesis. Lactic acid has two optical isomers such as L(+)-lactic acid and D(-)-lactic acid. The purity of optical isomer is important to the physical properties of the biodegradable plastic because L(+)-lactic acid or D(-)-lactic acid can be polymerized to a high crystalline poly-lactic acid that is suitable for commercial uses (Lunt, 1998). In the fermentative route, selected strains are able to produce a pure isomer such as L(+)-lactic acid or D(-)-lactic acid, while the chemical synthesis produces a DL-racemix lactic acid by using limited petrochemical resources. According to the advantage of fermentation route, production of lactic acid by fermentation is preferred.

Moreover, it is possible to use renewable materials such as cassava, starch, rice, molasses, and whey as carbon source for lactic acid production, since these renewable materials are cheap, abundant, and available in Thailand. Therefore, the production of lactic acid from renewable materials is an alternative to those agricultural products consumptions. This results in higher added value of these materials.

The fermentation process is an important route converting substrate to meet high yield and high productivity of lactic acid by microorganisms such as lactic acid bacteria and fungi. However, fermentation broth does not only contain lactic acid but also other substances. Thus product recovery is an important step and needs more attention because it needs high cost to achieve the quality requirements for food grade lactic acid (Isabel González *et al*., 2008). In order to reduce the cost, numerous studies on lactic acid separation have been conducted using different separation techniques such as reactive extraction, membrane technology, ion exchange, electrodialysis (ED) and direct distillation (Joglekar *et al*., 2006). In a review by Huang *et al*. (2007), the solvent extraction is limited by undesirable distribution coefficients and using hazardous solvents. Adsorption process also has the disadvantages such as short lifetime of adsorbents, low capacity, and additional filtration. Direct distillation is an energy-intensive process, and it will cause product transformation, such as the polymerization of lactic acid. Membrane technologies such as Nanofiltration (NF) have been proving their advances in the fields of separation and purification. NF is a novel membrane process that is often more capital and energy efficient when compared with the chemical separation processes (Li *et al*., 2008). Therefore, this work will be focused on the lactic acid recovery by using NF process.

Because NF is a recent membrane process, the mass transfer mechanisms are rarely studied. For example, the results obtained from single-solute solutions in NF can not be directly used to predict those for mixed-solute solutions (Bargeman *et al*., 2005). Since the fermentation broth is a mixture containing a lot of components such as neutral and charged solutes; therefore, the study of the mixed-solute solution is needed. Bouchoux *et al*. (2006) reported that NF could achieve the purification of sodium lactate by removing divalent ion and disaccharide sugar such as lactose; however, the separation between lactate (monovalent ion) and glucose (monosaccharide) was found to be hardly achievable. In contradict; Kang *et al*. (2004) reported that the separation between glucose and lactate was achievable by using NF process. Because the interactions between different components and the membrane, and their effect on the separation characteristics of NF membranes are not yet sufficient understood, these phenomena will be investigated in this study.

1.2 Research objectives

The main task of this work is to investigate the separation and purification of lactic acid from fermentation broth on the basis of NF.

- To know the mass transfer mechanisms in NF and investigate the interaction between solute to solutes and solute to membrane.

- To obtain the high efficiency of NF in lactic acid recovery process.

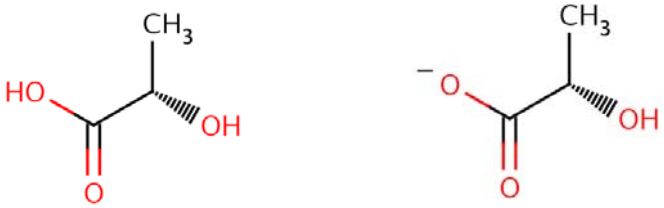
CHAPTER II

LITERATURE REVIEW

This chapter contains the literature review concerning lactic acid production process *via* fermentation as well as product recovery of lactic acid. The characteristic of lactic acid will be firstly introduced. Subsequently, publications that are related to the affecting factors on lactic acid production e.g. lactic acid producing microorganism, raw material for lactic acid production and final compositions of lactic acid/lactate fermentation broth from many related research articles will be reviewed. Fermentation approach to lactic acid production and downstream processing of lactic acid production will be then gone over. Furthermore, definition, characteristic and classification of membrane process and NF process will be introduced. The parameters that are often used to explain the mass transfer mechanisms in NF process e.g. permeate flux and retention coefficient will be discussed. Otherwise, concentration polarization that is usually observed in cross flow filtration will be discussed. The influence of concentration polarization on retention coefficient will be critiqued. The mass transfer mechanisms in NF process and the selectivity of NF membrane for the mixture containing neutral/neutral, electrolyte/electrolyte and neutral/electrolyte will be mentioned. Several parameters that indicate performance of membrane process, for example, separation factor, percent purity and percent yield will be discussed.

2.1 Lactic acid

Lactic acid or 2-hydroxypropanoic acid ($CH_3CHOHCOOH$) is also known in general as milk acid. It was first discovered in sour milk by a Swedish chemist, Carl Wihelm Scheele in 1780, who initially considered it as milk component. In 1857, however, Pasteur discovered that it was not a milk component, but a fermentation metabolite generated by certain microorganisms (Wee, Kim and Ryu *et al*., 2006). It is a simple typical compound that contains 2 functional groups as a hydroxyl group and a carboxylic group. In solution, the acidic group of lactic acid can lose a proton becoming dissociated form such as lactate ion (Isabel González *et al*., 2008) which depends on pH solution. It is miscible with water and ethanol. The molecular structures of lactic acid and lactate ion are shown in Fig. 2.1.



(a) (b)

Figure 2.1 The molecular structure of lactic acid (a) and lactate (b)

Biotechnological processes for the production of lactic acid usually include lactic acid fermentation and product recovery and/or purification. There have been numerous investigations on the development of biotechnological processes to lactic acid production, with the ultimate objectives to enable the process to be more efficient and economical. The biotechnological production, e.g. lactic acid fermentation and production recovery, of lactic acid will be further discussed.

2.1.1 Lactic acid production by fermentation

In review of Hofvendahl and Hahn-Hägerdal (2000), the production of lactic acid *via* fermentation depends on several parameters such as microorganism, carbon source, nitrogen source, fermentation mode, pH, and temperature. Several of which will be discussed below.

2.1.1.1 Lactic acid producing microorganism

Microorganism producing lactic acid can be divided into two groups such as lactic acid bacteria and filamentous fungi. Lactic acid bacteria, for example, *Lactobacillus (Lb) rhamnosus*, *Lb. helveticus*, *Lb. casei*, *Lb. plantarum* etc., are commonly used for the commercial production of lactic acid because they have high growth rate and high product yield. It is believed that most of lactic acid bacteria used belongs to genus *Lactobacillus*. Lactic acid bacteria can be classified into two groups, which are homofermentative and heterofermentative. The homofermentative lactic acid bacteria can convert glucose almost completely to lactic acid whereas the heterofermentative ones convert glucose into ethanol and CO_2 as well as lactic acid. Although lactic acid bacteria can provide high yield and productivity of lactic acid, lactic acid has complex nutrient requirements due to their limited ability to synthesize B-vitamins and amino acids (Wee *et al*., 2006).

On the other hand, filamentous fungi such as *Rhizopus oryzae* (Ruengruglikit *et al*., 2003) and *R. arrhizus* (Huang *et al*., 2005) can convert glucose aerobically to lactic acid. It can produce directly lactic acid from starch by using amylolytic enzyme; however, it has low production rate which is probably due to the low reaction rate caused by mass transfer limitation. There has been investigation to use both lactic acid bacteria and fungi to produce lactic acid in mixed culture; for example, Plessas *et al*. (2008) studied lactic acid fermentation using *Kluyveromyces marxianus* (yeast), *Lb. delbrueckii ssp. bulgaricus*, and *Lb. helveticus* from cheese

whey in both individual and mixed cultures. The mixed cultures increased lactic acid production in comparison to individual ones and the synergistic effects between the yeast and the two lactic acid bacteria were observed when all strains were used together.

2.1.1.2 Raw materials for lactic acid production

Raw materials for lactic acid production can be used as carbon and nitrogen sources. Carbon sources for lactic acid production are numerous which are pure sugars (e.g. glucose, xylose, and lactose etc.), renewable materials (e.g. starch and celluloses materials) and some industrial wastes (e.g. molasses and whey). The pure refined sugar provides high purity of lactic acid in the fermentation broth; however, the cost of pure fermentable sugars is high, which lactic acid is a cheap product, which is not economically favorable. Those renewable materials and some industrial wastes have been used instead of those of pure sugars because they are cheaper, abundant, and renewable. Starchy materials such as corn, cassava, potato, rice etc., are polysaccharides. They are hydrolyzed first to produce fermentable sugars which are done before doing fermentation. Tanaka *et al*. (2006) studied lactic acid production from rice bran containing glucose and disaccharides, by *Lb. delbrueckii*. Gao *et al*. (2006) also studied lactic acid production using starchy material with *Lb. rhamnosus*. Cellulosic materials have been used for lactic acid production in the same way as starchy materials. Marque *et al*. (2008) studied lactic acid production using recycled paper sludge as a raw material containing cellulosic and hemi-cellulosic fractions which can be completely converted to glucose and xylose by using enzymatic hydrolysis (Celluclast® 1.5L with Novozym® 188). The fermentation broth was obtained by using *Lb. rhamnosus* ATCC 7469. The maximum production of lactic acid could be performed simultaneously by means of hydrolysis and

fermentation. Some industrial wastes such as molasses, contains mainly sucrose, and cheese whey, contains mainly lactose, can be also used as substrate. For example, *Saccharomyces cerevisiae* OC-2T T165R was used for the production of lactic acid from molasses. This strain is metabolically engineered to produce optically pure L(+)-lactic acid in a high performance extractive fermentation process (Gao *et al*., 2009b).

There have been several attempts to add low cost nutrient into the fermentation broth such as corn steep liquor as nitrogen source in order to provide ability of bacteria to synthesis B-vitamin and amino acids (Yu *et al*., 2008). The effect of using corn steep liquor with glucose, molasses, Tween 80, and $MnSO_4$ on L(+)-lactic acid fermentation by *Lb. rhamnosus* CGMCC 1466 was studied. The corn steep liquor was preformed as a low cost nitrogen source instead of yeast extract; however, the using corn steep liquor provides lactic acid production 30.4% lower than that of yeast extract. There was an investigation of calcium-L-lactate (CaL_2) production *via* fermentation by using strain *Lb. rhamnosus* NBRC 3863 from pure glucose. The complete consumption of substrate could be achieved if yeast extract was added into the fermentation broth (Gao *et al*., 2009a). Lu *et al*. (2009) investigated D-lactic acid fermentation using unpolished rice as carbon source, wheat bran as nitrogen source, and yeast extract as growth factor by *Lb. delbrueckii* HG 106. The using unpolished rice as substrate does not provide only carbon source but also other important nutrients such as amino acids and B-vitamins. In comparison between fresh corn (their previous study) and polished rice, the D-lactic acid yields increased by 5.79% and 8.71%, and the raw material cost decreased by 65% and 52%, respectively, when the unpolished rice was used as a major nutrient source.

2.1.1.3 Lactic acid fermentation mode

Lactic acid is most commonly produced in the batch mode; however, there have been developed alternative mode such as fed-batch, repeated batch, and continuous fermentations. High lactic acid concentrations could be obtained in batch and fed-batch mode, while higher productivity may be achieved by the use of continuous cultures. Romaní *et al*. (2008) investigated lactic acid production using cellulosic biosludges generated in a kraft pulp mill as substrate by simultaneous saccharification and fermentation (SSF). Effects of operation mode (batch or fed batch) and nutrient supplementation MRS, which stands for de Man Rogosa and Shape, component or none were performed. Fed batch can improve lactic acid productivity keeping constant conversion yield. Ding and Tan (2006) attempted to produce lactic acid by fed-batch culture of *Lb. casei* using glucose as substrate. On the other hand, the lactic acid production *via* continuous mode using membrane provided higher productivity of lactic acid than that of batch mode (Kwon *et al*., 2001).

2.1.1.4 Effect of pH and temperature on lactic acid production

While lactic acid concentration is increasing in the fermentation broth, the pH of the broth is lowering. This can affect the productivity of microorganisms. The control pH could be improved the productivity of microorganism and lactic acid. Mussatto *et al*. (2008) studied the effects of medium supplementation and pH control on lactic acid production from brewer's spent grain by *Lb. dellobacillus*. Maintaining pH at 6 gave better productivity while pH dropped from 6.0 to 4.2 resulted in large amount of residual glucose was not consumed. The effect of temperature on the production of lactic acid has only been studied in few literatures which were not mentioned here.

2.1.1.5 Final composition of lactic acid fermentation broth

The final compositions of fermentation broths producing lactic acid which were produced by filamentous fungi genus *Rhizopus* are shown in Table 2.1. The concentrations of lactic acid are in the range of 10 – 96 $g.L^{-1}$ and average concentration is 37 $g. L^{-1}$. The several renewable materials and some industrial waste products were used as substrates such as glucose, xylose, arabinose, lactose, and sucrose for lactic acid production by using fungi. Several literatures mentioned the complete consumption of substrate such renewable materials and industrial wastes by using fungi showing in Table 1.

On the other hand, the detail of lactic acid that was produced by lactic acid bacteria is also given in Table 2.2. Lactic acid concentrations are in range 7-190 $g.L^{-1}$ and the average concentration is 70 $g.L^{-1}$. The variation of produced lactic acid depends on types and initial concentration of substrates. Most investigations of lactic acid production by using lactic acid bacteria were carried out with fermentable sugars such as glucose, maltose, lactose, and sucrose, and the yields ($Y_{p/s}$) are quite high. When renewable materials or some industrial waste products are used as substrate, some pretreatment are necessary prior to the fermentation. Some nutrients such as yeast extract and nitrogen source are also added during fermentation in order to increase lactic acid productivity. In these cases, the yields ($Y_{p/s}$) are in range of 43-111 %.

Table 2.1 Lactic acid production from various substrates by filamentous fungi

Organism	Substrate	pH/Temp	HLA*	Yield	P*	By-product	References
Rhizopus oryzae NRRL-395	76 g L^{-1} hemicellulosic hydrolysate + $CaCO_3$	7.2/32 °C	16.57g L^{-1}			3.36 g L^{-1} EtOH, + 22.29 g L^{-1} sugar	Woiciechowski *et al*., 1999
Rhizopus oryzae NRRL-395	5g (100ml corncobs)$^{-1}$ + $CaCO_3$	30 °C	299.4 g (kg corncob)$^{-1}$			none	Ruengruglikit *et al*., 2003
Rhizopus oryzae R 1021	100 g L^{-1} glucose + $CaCO_3$	5.5/30 °C	74.92 g L^{-1}	74%		none	Bai *et al*., 2003
Rhizopus oryzae R 1021	120 g L^{-1} corn starch + 40% NaOH	5.5/30 °C	79.04 g L^{-1}			completely consumed sugar	Bai *et al*., 2004
Rhizopus sp. MK-96-1196	50 g L^{-1} Corncob + liquid ammonia	6.5/37 °C	28 g L^{-1}	82%		5 g L^{-1} xylose	Miura, Arimura *et al*., 2004
Rhizopus oryzae	150 g L^{-1} glucose 50 g L^{-1} sucrose 400 g L^{-1} molasses 0.11 g L^{-1} carob pod + $CaCO_3$ powder	30 °C 30 °C 30 °C 30 °C	60 g L^{-1} 21 g L^{-1} 49 g L^{-1} 58 g L^{-1}			40-50% remained glucose none none none	Bulut *et al*., 2004
Rhizopus sp. MK-96-1196	120 g L^{-1} glucose + liquid ammonia	6.5/37 °C	96 g L^{-1}			none	Miura, Dwiarti *et al*., 2004

Table 2.1 (cont.) Lactic acid produ ction from various substrates by filamentous fungi

Organism	Substrate	pH/Temp	HLA*	Yield	P*	By-product	References
Rhizopus oryzae CBS 112.07	Wheat straw (10.3 g L^{-1} xylose + 19.2 g L^{-1} glucose)	6.5/37°C	6.8 g. L^{-1}			5.7 g L^{-1} EtOH, 1.5 g L^{-1} xylitol	Ronald *et al*., 2006
	100 g L^{-1} + 30 g L^{-1} xylose + Sodium phosphate	6.5/37°C	33.4 g. L^{-1}			2.1 g L^{-1} EtOH, 1.9 g L^{-1} glycerol + 0.5 g L^{-1} xylitol	
Rhizopus oryzae 2062	21.7 g L^{-1} potato starch wastewater	6/30 °C	10.31 g. L^{-1}	48%		completely consumed sugar	Huang *et al*., 2005
	20 g L^{-1} soluble starch medium	6/30 °C	15.69 g. L^{-1}	79%		completely consumed sugar	
R. aryarrhizus 36017	21.7 g L^{-1} potato starch wastewater	6/30 °C	19.74 g. L^{-1}	91%		completely consumed sugar	
	20 g L^{-1} soluble starch medium + 4 N NaOH	6/30 °C	12.31 g. L^{-1}	61%		completely consumed sugar	
Saccharomycess cerevisiae (Yeast)	200 g L^{-1} glucose + 10 mol L^{-1} KOH	3.6/28 °C	42 g. L^{-1}		0.429g $(L. h)^{-1}$	3 g L^{-1} glucose remain	Colombie *et al*., 2003

Remark: * HLA= lactic acid concentration, P=Productivity

Table 2.2 Lactic acid production from various substrates by lactic acid bacteria

Organism	Substrate	pH/Temp	HLA*	Yield	P*	By-product	References
E. casseliflavus	50 g L^{-1} xylose	6.8/30 °C	39 g L^{-1}			6.4 g L^{-1}acetic acid	Taniguchi *et al*.,
	50 g L^{-1} xylose + 100 g L^{-1} glucose	6.8/30 °C	76 g L^{-1}			47 g L^{-1} xylose	2004
L. vaccinostercus	50 g L^{-1} xylose	6.8/30 °C	26 g L^{-1}			17 g L^{-1} acetic acid	
	50 g L^{-1} xylose + 100 g L^{-1} glucose	6.8/30 °C	77 g L^{-1}			50 g L^{-1} xylose	
E. casseliflavus +	50 g L^{-1} xylose + 100 g L^{-1}glucose (SSF)	6.8/30 °C	75 g L^{-1}			28 g L^{-1} xylose	
L. vaccinostercus	50 g L^{-1} xylose + 100 g L^{-1} glucose + 4 N NaOH (2 stages)	6.8/30 °C	95 g L^{-1}			5.7 g L^{-1}acetic acid	
L. rhamnosus IFO 3863	200 g L^{-1} glucose + 10% aqueous ammonia solution	6/42°C	83 g L^{-1}	43%	1.05g (L h)$^{-1}$	completely consumed sugar	Min-tian *et al*., 2004
L.plantarum ATCC 21028	40 g L^{-1} lactose + 12N NaOH	5-6/37°C	41.2 g L^{-1}	103%		1 g L^{-1} glucose	Fu *et al*., 1999
L.sp. RKY2	125 g L^{-1} glucose (2.5L fermentor)	6/36°C	120.8 g L^{-1}	96%	1.3 g (L h)$^{-1}$	none	Wee, Yun, Park
KCTC 10353BP	125 g L^{-1} glucose (30L fermentor)	6/36°C	118.2 g L^{-1}	95%	1.2 g (L h)$^{-1}$	none	*et al*., 2006
	125 g L^{-1} glucose (30L fermentor) + 8N ammonia	6/36°C	115.1 g L^{-1}	92%	1.1 g (L h)$^{-1}$	none	
E. faecalis	Glucose in 50 g L^{-1} wood hydrolysate	7/38°C	49 g L^{-1}	97%	3.2 g (L h)$^{-1}$	none	Wee, Yun *et al*., 2004
RKY1	+ 10M NaOH						
E. faecalis	50 g l^{-1} glucose	38°C	48.6 g L^{-1}	97%	9.72 g (L h)$^{-1}$	none	Yun *et al*., 2003
RKY1	+ 10N NaOH						

Table 2.2 (cont.) Lactic acid production from various substrates by lactic acid bacteria

Organism	Substrate	pH/Temp	HLA*	Yield	P*	By-product	References
L. helveticus	4%w/v WPH + 4 M NaOH		29 g L^{-1}	96%		completely consumed sugar	Fitzpatrick *et al*., 2001
E. faecalis	130 g L^{-1} Sugar molasses (68 g L^{-1} glucose) + 10N NaOH	7/38°C	49 g L^{-1}	97%	3.2 g $(L\ h)^{-1}$	none	Wee, Kim *et al*., 2004
L. rhamnosus NBRC 3863	100 g L^{-1} glucose + 10% aqueous ammonia solution	6/42°C	87 g L^{-1}	97%	2.4 g $(L\ h)^{-1}$	completely consumed sugar	Gao *et al*., 2006b
L.sake 1.29 & *L. casei*1.6	Soybean stalk hydrolysate (15.7 g L^{-1} glucose, 4 g L^{-1} cellulose, 7 g L^{-1} xylose) + Sodium citrate	4.8/38°C	8.5 g L^{-1}	71%		3 g L^{-1} cellubiose 5 g L^{-1}xylose	Xu *et al*., 2007
L .casei LA-04-1	850 g L^{-1} glucose + 25 %w//w NH_4OH	6.5/42°C	210g L^{-1}	90.3%	2.14 g $(L\ h)^{-1}$	5 g L^{-1}glucose	Ding and Tan, 2006
L .rhamnosus NBRC 14710	60 g L^{-1} sugar (1.3%w/vGlucose, 0.4% w/vXylose, 0.1%w/v Arabinose)	37°C	19.0 g L^{-1}			completely consumed glucose	Shindo *et al*., 2004
Bacillus coagulans	70 g L^{-1} sucrose + 2-8N NH_3	6.1/32°C	55 g L^{-1}	92%		10 g L^{-1} sucrose, 0.5 g L^{-1} EtOH , 0.5 g L^{-1}acetate	Payot *et al*., 1999
L casei & *L lactis*	100 g L^{-1} lactose + 5 N NaOH	6/32°C	46 g L^{-1}	77%	1.91 g $(L\ h)^{-1}$	80% lactose utilized	Roukas *et al*., 1998

Table 2.2 (cont.) Lactic acid production from various substrates by lactic acid bacteria

Organism	Substrate	pH/Temp	HLA*	Yield	P*	By-product	References
L delbrueckii IFO 3203	100 g L^{-1} rice bran (36 g L^{-1} sugars soluble as glucose and disaccharides) + 4 N NaOH	5/37°C	28 g L^{-1}	78%		completely consumed sugar	Tanaka *et al*., 2006
L .casei	whey permeate (5%w/v MCN) + 10% liquid ammonia	5.4/38°C		90%		none	Liu *et al*., 2006
L helveticus 1999	54 g L^{-1} lactose + 8N NH_2OH	5.8/42°C	32 g L^{-1}		35 g $(L\ h)^{-1}$	completely consumed sugar	Kulozik and Wilde,
L .manihotivorans LMG 18011	200 g L^{-1} food wastes 50 g L^{-1} starch + 10N Sodium hydroxide	5/25°C 5/25°C	48.7 g L^{-1} 38.3 g L^{-1}	111% 82.8%		20.3 g L^{-1} residue sugar 1.61 g L^{-1} acetic acid	Ohkouchi *et al.,* 2006
L. rhamnosus B. coagulans	74 g L^{-1} total sugar 117 g L l^{-1} total sugar + 7% ammonia	6/30°C 6/30°C	61 g L^{-1} 86 g L^{-1}	97% 98%		14 g L^{-1} final sugar 31 g L^{-1} final sugar	Sakai *et al*., 2006
L. amylophilus GV6	15.2 g L^{-1} starch + 1N NaOH	37°C	13.5 g L^{-1}	92%		none	Altaf *et al*., 2007
L .rhamnosus NBRC 3863	100 g L^{-1} starch + 10% aqueous ammonia	6/42°C	87 g L^{-1}	97%	2.4 g $(L\ h)^{-1}$	none	Gao *et al*., 2006a
L. helveticuss	100 g L^{-1} lactose + 3N NaOH	5.5/42°C	51 g L^{-1}		0.05 g $(L\ h)^{-1}$	87% lactose utilization	Tango *et al*., 1999

Table 2.2 (cont.) Lactic acid production from various substrates by lactic acid bacteria

Organism	Substrate	pH/Temp	HLA*	Yield	P*	By-product	References
L. casei subsp. rhamnosus	60 g L^{-1} glucose + 5M NH_4OH	6/38°C	47 g L^{-1}			6.02 g l^{-1} residual glucose	Nancib *et al*., 2001
L .delbrueckii	250 g L^{-1} potato starch +Slurry $CaCO_3$	5.5/45°C	189 g L^{-1}	86%	1.87 g $(L\ h)^{-1}$	completely consumed sugar	Anuradha *et al*., 1999
L. casei ATCC 10863	50 g L^{-1} glucose + 5N NaOH	6/38°C	44 g L^{-1}	44%	1.22 g $(L\ h)^{-1}$	21 g L^{-1} residual sugar	Kurbanoglu *et al*., 2003
E. faecalis RKY1	200 g whole wheat flour + 10N NaOH	7/38°C	102 g L^{-1}	97%	4.87 g $(L\ h)^{-1}$	completely consumed sugar	Oh *et al*., 2005
E. faecalis RKY1	150g glucose 150g fructose 150g maltose + 6N $NaCO_3$	7/38°C 7/38°C 7/38°C	139 g L^{-1} 144 g L^{-1} 138 g L^{-1}		3.56 g $(L\ h)^{-1}$ 4.12 g $(L\ h)^{-1}$ 3.54 g $(L\ h)^{-1}$	completely consumed sugar	Yun *et al*., 2001
Streptococcus bovis	20 g raw starch + 5N NaOH	6/37°C	14.37 g L^{-1}	88%		16.74g L^{-1} sugar	Narita *et al*., 2004
L. rhamnosus	43.8 g L^{-1}monosaccharides + 5 M NaOH (22.8 g L^{-1} glucose, 14.8 g L^{-1} fructose, 2.5 g L^{-1} xylose + mannose + galactose, 2.8 g L^{-1} arabinose + rhamnose)	5.8/41.5°C	32.5 g L^{-1}	88%	5.41 g $(L\ h)^{-1}$	completely consumed sugar	Gullon *et al*., 2007
L. amylophilus GV6	9g of wheat bran (3.96 g of starch) + $CaCO_3$	6.5/37°C	3.5g			none	Naveena *et al*., 2004

Table 2.2 (cont.) Lactic acid production from various substrates by lactic acid bacteria

Organism	Substrate	pH/Temp	HLA*	Yield	P*	By-product	References
L. lactis ssp. lactis ATCC 19435	160 g L^{-1}final sugar (wheat flour hydrolysate)	6/30°C	106 g L^{-1}	88%	8 g $(L\ h)^{-1}$	8 g L^{-1} glucose 0.9 g L^{-1} maltose	Hofvendahl and Hähn-Hagerdal, 1997
L. lactis ssp. lactis AS 211	160 g L^{-1}final sugar (wheat flour hydrolysate)	6/30°C	107 g L^{-1}	91%	2.8 g $(L\ h)^{-1}$	2.8 g L^{-1} glucose 1.9 g L^{-1} maltose	
L. delbrueckii ssp. delbrueckii ATCC 9649	160 g L^{-1} final sugar (wheat flour hydrolysate)	6/37°C	109 g L^{-1}	91%	1.2 g $(L\ h)^{-1}$	1.2 g L^{-1} glucose 2.0 g L^{-1} maltose	
L. delbrueckii ssp. bulgaricus DSM 20081	160 g L^{-1} final sugar (wheat flour hydrolysate) + 20% w v^{-1} NaOH	6/45°C	26 g L^{-1}	18%	0.85 g $(L\ h)^{-1}$	67 g L^{-1} glucose 0.6 g L^{-1} maltose	
L. casei	55 g L^{-1} lactose (whey permeate) + 4M NaOH	5.4/38°C	46 g L^{-1}	90%		Completely consumed sugar	Pauli *et al*., 2002

Remark: * HLA= lactic acid concentration, P=Productivity

2.1.2 Product recovery of lactic acid

Product recovery is an important step in lactic acid production process that is related to separation and purification of lactic acid/lactate form fermentation broth. Lactic acid fermentation broth contains several impurities such as color, residual sugars and nutrients and other organic acids, a part of cell mass (Joglekar *et al*., 2006). These impurities need to be removed from the broth in order to obtain more purity of lactic acid. Firstly, the macro impurities, e.g. biomass, are calcified by microfiltration.

Inskeep *et al*., 1952 and Peckharm *et al*., 1944, mentioned that conventional process of lactic acid product recovery could be divided into two routes which are (i) the clarified fermentation liquor is concentrated to 32% well above crystallization point and acidified with sulphuric acid to get the crude lactic acid and (ii) the crude calcium salt which precipitates from the concentrated fermented liquor is crystallized, filtered, dissolved and subsequently acidified with sulphuric acid, which were written in Joglekar's review (2006). The latter route is more preferential because it can avoid the product inhibition which can be described by following 4 steps such as (i) fermentation and neutralization, (ii) hydrolysis with H_2SO_4, (iii) esterification, and (iv) hydrolysis by H_20, respectively. The first step, the clarified fermentation broth is added by alkali such as lime, sodium carbonate or ammonium hydroxide in order to maintain pH of the broth because of product inhibition. Lime is usually used because it is the cheapest of the alkalis. Since solubility of produced calcium lactate in water is low, it is possible to be separated by precipitation. The insoluble calcium sulphate is removed by filtration and thereby removes water-soluble impurities in this step. Second step, the lactate salt is then converted to lactic acid by hydrolysis with H_2SO_4. Unfortunately, this step produces lots of gypsum ($CaSO_4$) that

is environmental impact because it is hardly to depose of (Joglekar *et al*., 2006). Third step, in order to obtain high purity of lactic acid, esterification of crude lactic acid with alcohols and distillation of ester are carried out. The esterification is the only downstream process, which separates other organic acids from lactic acid. Finally, hydrolysis of the distillated lactate ester to yield the alcohol and lactic acid is further done. Those steps of downstream processing of lactic acid are described as followed:

Step 1 Fermentation and neutralization

$C_6H_6O_6 + Ca(OH)_2 \longrightarrow (2CH_3CHOHCOO^-)Ca^{2+} + 2H_2O$

Carbohydrate Calcium hydroxide Calcium lactate

Step 2 Hydrolysis by H_2SO_4

$(2CH_3CHOHCOO^-)Ca^{2+} + H_2SO_4 \longrightarrow 2CH_3CHOHCOOH + CaSO_4$

Calcium lactate sulphuric acid lactic acid Calcium sulphate

Step 3 Esterification

$CH_3CHOHCOOH + CH_3OH \longrightarrow CH_3CHOHCOOCH_3 + H_2O$

Lactic acid Methanol Methyl lactate

Step 4 Hydrolysis by H_2O

$CH_2CHOHCOOCH_3 + H_2O \longrightarrow CH_3CHOHCOOH + CH_3OH$

Methyl lactate Lactic acid Methanol

2.2 Nanofiltration

First of all, background of membrane process will be introduced in order to draw out the general filtration processes. After a brief introduction into the

fundamentals of membranes and membrane process, the NF process will be mentioned. The mass transfer mechanisms of NF process will be discussed. Finally, the performance of NF process will be gone over.

2.2.1 General concerns

Membrane processes are rather new separation method, since it was not interested as expected in past time. At present, the membrane processes are used in a wide range of applications and number of them is still increasing. A complete definition of membrane which covers all its view is quite difficult; however, a general definition could be a selective barrier between two phases. A membrane can be thick or thin. The membrane thickness may vary between less than 1 nm to more than a centimeter. A schematic diagram of membrane process is depicted in Fig. 2.2. Phase 1 is usually considered as the feed or upstream side phase while phase 2 is considered as permeate or downstream side. The membrane can be either solid or liquid. It may be neutral or carry positive or negative charges, or may be bipolar. Mass transport can be result of convection or diffusion of individual molecules, induced by an electric field, or a concentration, pressure or temperature gradient. In addition, membrane can be natural or synthetic (Mulder, 1997). Separation is achieved because the membrane has the ability to transport one component from the feed mixture more readily than any other component or components.

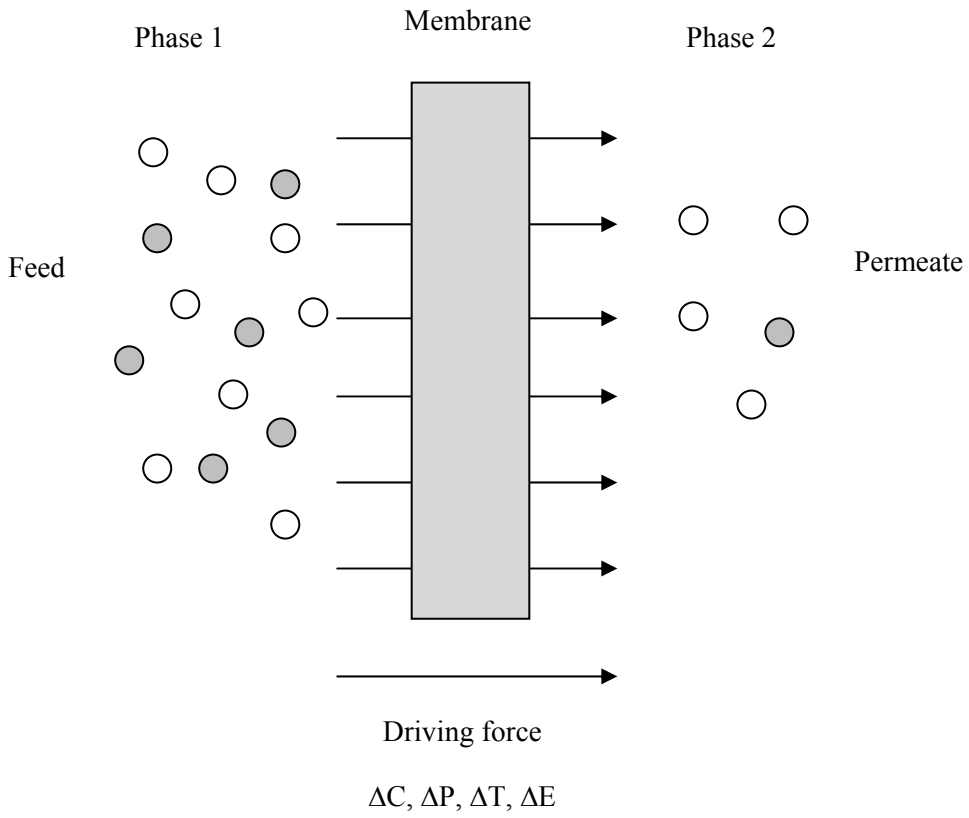


Figure 2.2 Schematic representation of a two-phase system separated by a membrane (Mulder, 1997).

Membrane filtration can be categorized into four major pressure-driven membrane processes: microfiltration (MF), ultrafiltration (UF), nanofiltration (NF), reverse osmosis (RO). MF is principally used for separation of micron-sized species that are usually particles or large macromolecules. UF is used for the separation of macromolecules (e.g. proteins) and NF and RO are used for low-molecular weight solutes (e.g. salts).

The separation mechanism for membrane processes is based upon either size exclusion or solute diffusion. To know which mechanism is dominant can be identified by the pore size and the structure of the membrane. MF and UF membranes have well-defined pores and the separation is mainly based upon size exclusion. If the size of a species *i* is larger than that of the membrane pores, it cannot

pass through the membrane. For NF and RO membranes, which have smaller pores, there still exists some debate concerning the separation mechanism. The suggested mechanisms are size exclusion similar to MF and UF, or solution diffusion. In the solution-diffusion mechanism, species are absorbed into the membrane, which diffuses through the membrane structure, and are then desorbed. The relative rates of the adsorption desorption, and diffusion of the species controls the separation (Sablani *et al.*, 2007). Moreover, the electrostatic repulsion interaction between the charged solute and the fixed charge on membrane surface was also purposed (Wang *et al*., 1997). The separation mechanism of NF membrane will be more described in section 2.2.3.

NF is a pressure driven membrane separation process. The driving force is pressure difference between the feed (retentate) and the filtrate (permeate) sides at the separation layer of the membrane (Wang *et al*., 2002). NF membranes are characterized by effective pore diameters ranging about 1 to a few nanometers, and by molecular weight cut-off between reverse osmosis membranes (dense structure) and ultrafiltration membranes (porous structure). Because of its selectivity, one or several components of a dissolved mixture are retained by the membrane despite the driving force, while water and substances with a molecular weight about 200 - 2000 Daltons are able to pass through. Because NF membranes also have selectivity with respect to the charge of the dissolved components, monovalent ions will pass the membrane and divalent and multivalent ions will be rejected (Schäfer *et al*., 2005).

2.2.2 Process characteristic parameters

Before going further into the mass transfer mechanisms of NF, two parameters need to be introduced here which are permeate flux and retention coefficient. Concentration polarization is a phenomenon that is often observed in

cross-flow nanofiltration will be pointed out. Moreover, the efficient of nanofiltration process is expressed in several parameters e.g. separation factor, percent purity, and percent product recovery will be mentioned.

2.2.2.1 Permeate flux

In membrane processes, the pressure applied across the membrane which is a driving force pushing solute toward the membrane to another side is called "transmembrane pressure". The portion of the feed that passes through the membrane is termed the filtrate or permeate. The volumetric flux rate of permeate per unit area of membrane is the *permeate flux*, usually denoted by J_V,

$$J_V = \frac{dV}{Adt} \tag{2.1}$$

where V is the total volume that has permeated through the membrane at time t, and A is the area of the membrane. The SI units of flux are $m^3.s^{-1}.m^{-2}$; however, flux is often stated in $L.m^{-2}.h^{-1}$. The permeate flux (J_v) is calculated by measuring the quantity of permeate collected during a certain time and dividing it by the effective membrane area for filtration.

Furthermore, other parameter that is usually determined before doing each experiment is the water flux (J_w). It can be used to calculate the membrane permeability, L_{p0}, which is an image of the membrane.

$$L_{p0} = \frac{J_W}{\Delta P} \tag{2.2}$$

Where L_{po} is the water permeability ($m^3.s^{-1}.m^{-2}.bar^{-1}$) and ΔP is the pressure difference (bar).

2.2.2.2 Retention coefficient

A measure of the solute transmission across the membrane is conventionally expressed in term of "retention coefficient". The experimentally measured value of retention is the so-called observed retention, R_{obs}, is a measure of membrane selectivity towards a solute as shown below:

$$R_{obs,i} = 1 - \frac{c_{p,i}}{c_{f,i}} \tag{2.3}$$

Where R_{obs} is the observed retention of component i, $c_{p,i}$ is the concentration of component i in permeate (M), and $c_{f,i}$ is the concentration of component i in feed (M).

R_{obs} is dependent on hydrodynamic conditions unlike the intrinsic retention coefficient (R_{int}) which directly deals with the concentration at the membrane surface ($c_{m,i}$) as defined follows:

$$R_{int,i} = 1 - \frac{c_{p,i}}{c_{m,i}} \tag{2.4}$$

Retention coefficient equal to 1 means that the solute i is completely retained by the membrane while a retention coefficient equal to 0 means that the solute i is fully permeated across the membrane.

2.2.2.3 Separation factor

An alternative parameter that is used to express the separation efficiency of NF membrane beside retention coefficient is separation factor. The lactate separation factor is a measure of lactate purification from glucose. This factor indicates the variation of lactate and glucose composition in the permeate compared with those in the feed. The separation is achieved if the separation factor differs from unity. A value greater than one indicates lactate enrichment in the permeate:

$$Separation\ factor = \frac{C_{p,lactate}/C_{p,glucose}}{C_{f,lactate}/C_{f,glucose}} \quad (2.5)$$

2.2.2.4 Percent purity and Percent recovery

Furthermore, other parameters can be used to characterize the membrane separation efficiency such as the percent of purity and percent of recovery obtained by the following relationships:

The purity is defined as:

$$\%\ purity = \frac{C_{p,lactate}}{C_{p,lactate}+C_{p,glucose}} \times 100\% \quad (2.6)$$

The percent of lactate recovery is defined as:

$$\%\ recovery = \frac{C_{p,lactate}}{C_{f,lactate}} \times 100\% \quad (2.7)$$

2.2.3 Mass transfer mechanisms

The retention of uncharged solutes, e.g. lactic acid, glucose and lactose etc., through NF can be explained by size effect which is governed by solute and membrane pore size (Wang *et al*., 1997). NF membrane allows the component which its size is not larger than pore size, passing through the membrane. The large solute molecule is retained or held in feed side (retentate) while the smaller one passes through the membrane to filtrate side (permeate) easier as shown in Fig. 2.3(a). The smaller solutes predominantly transport across NF membrane by convection (mass is carried along with fluids) in the membrane pores due to pressure difference and the bigger solutes predominantly transport across NF membrane by diffusion (mass spreads from area of high concentration, to areas of low concentration) due to concentration gradient across the membrane. On the other hand, the both solutes can

pass through the membrane more easily when they are filtrated with the more open membrane (wider membrane pore size) as shown in Fig. 2.3(b). For instance, Pontalier *et al*. (1997) studied solute transfer mechanisms of glucose and lactose with 400Da membrane in single-solute solutions. It was reported that percent retention of glucose was 68%, while that of lactose was 99%. Wang *et al*. (2002) studied the mass transfer mechanism of glucose and sucrose in single-solute solutions with 300Da NF membrane. They found that percent retention of glucose was 80% and sucrose retention was almost 100%. It can be explained that the size of lactose is bigger than that of glucose so that the glucose pass through the membrane easier than lactose.

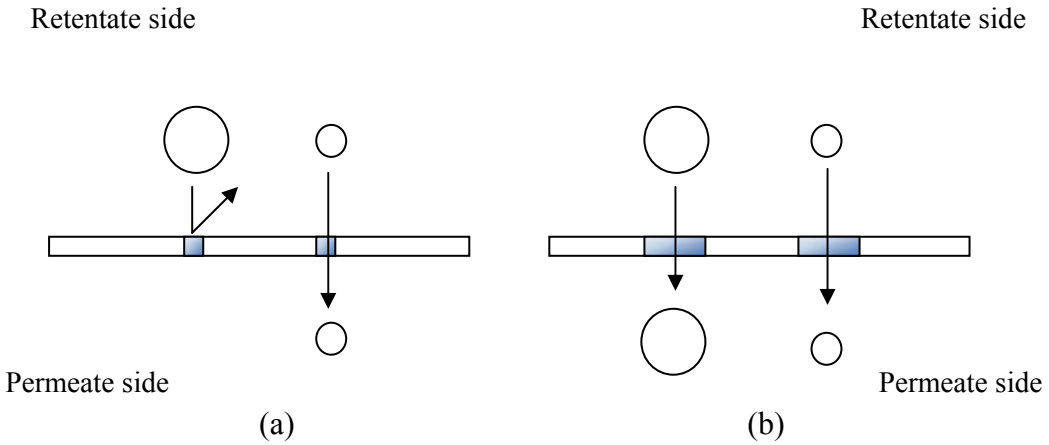


Figure 2.3 Mass transfer mechanisms of two different size solutes through a NF membrane (a) "tight membrane" (b) "open membrane" (Schäfer *et al*., 2005).

Mass transfer mechanism of charged solutes e.g. lactate ion, magnesium ion, and chloride ion etc., can be explained by combination of size effect and charged effect. Indeed Schaep *et al*. (1998) fell describing the mass transfer mechanisms of charged solute by charge effect only. The charged effect is an influence of electrostatic interaction (repulsion between same sign of charges and attraction between opposite sign of charges) between the solutes and the fixed charges on the membrane surface. The co-ions (same sign of charge as fixed membrane charges) are repelled by membrane surface, while the counter-ion (opposite sign of

charge to the fixed charge of membrane) can pass through it. However, the co-ions can pass through the membrane as well as the counter-ion because of electroneutrality (charges in both retentate and permeate have to be balanced). In the same way co-ions are retained in retentate, counter-ions need to also stay in retentate to neutralize repelled co-ions and thus salt retention occurs.

Variation of feed concentration can affect the retention of monovalent ion whereas it is less effective on retention of divalent ions. This can be explained by considering the mass transfer mechanism of ionic solutes permeation through NF membrane as shown in Fig. 2.4. Fig. 2.4(a) shows ionic solutes cannot be permeated through NF membrane at low feed concentration. As the monovalent counter-ions and co-ions always accompany to each other, they cannot pass through the membrane separately. If co-ions molecule is repelled by fixed charges of membrane, both cannot pass through the membrane. Divalent ions are fully retained because its size is larger than that of pore and/or stronger electrostatic repulsion effect e.g. Na_2SO_4 and $MgCl_2$ are fully retained by NF40 membrane with all feed concentration (Schaep *et al*. 1998). Fig. 2.4(b) illustrates ionic solutes at high salt feed concentration can be passed through the membrane, which is more easily than those at low feed concentration. The retention of monovalent salts such as NaCl, KCl and sodium lactate, generally decreases with increasing salt concentration (Bargeman *et al*. 2005). For instance, the sodium lactate retention decreases from 80% to 25% for increasing sodium lactate concentration from 0.1M to 1M, respectively (Bouchoux *et al*. 2005). The decrease of salt retention in presence of high feed concentration can be explained as followed. At the low salt concentrations, electrostatic repulsions are predominant so that high salt retentions are obtained. As the feed concentration increases, electrostatic interactions become weaker and thus the retention decreases. This can be explained by the ‘screening effect’ (Kang *et al*. 2004) that is membrane

surface is screened up by counter-ions and thus the repulsion between co-ions and fixed charges on membrane surface becomes smaller causing the retention decline as shown in Fig. 2.4(b). An increase of salt concentration causes stronger driving force that is difference between solute concentration in feed and that in permeate, as consequence of higher salt diffusion. Thus, decrease in retention is observed (Schaep *et al*. 1998). Ideally, at a sufficient salt concentration, electrostatic interactions are negligible so that charged solutes are only retained *via* size effects. This point is important when high purity of lactic acid is desired.

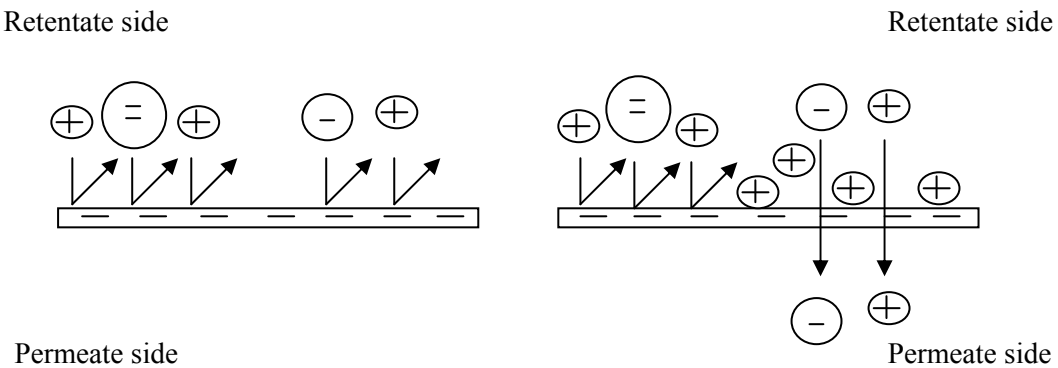


Figure 2.4 Mass transfer mechanisms of charge solute in NF a) low concentration b) high concentration (Schäfer *et al*., 2005).

2.2.3.1 Concentration polarization

Concentration polarization is a phenomenon in which the solute or particle concentration in the vicinity of the membrane surface is higher than that in the bulk. This phenomenon, inherent to all cross-flow filtration processes, occurs as long as the membrane shows different permeability for the various components of the solution or suspension. Concentration polarization is a natural consequence of preferential transport of some solutes through the membrane. The applied pressure across the membrane causes convection of solutes towards the membrane. The

concentration of rejected solutes increases at the membrane surface. This creates a concentration gradient leading to back-transport of the rejected solutes to the bulk. These solutes generate significant osmotic pressure at the membrane wall reducing the effective applied pressure across the membrane. A reduction in the effective applied pressure cuases a reduction in the permeate flux. Concentration polarization is of considerable interest since a high permeate rate is most desirable in filtration processes (Song and Elimelech, 1995). As a result, much research effort has been expended in this area.

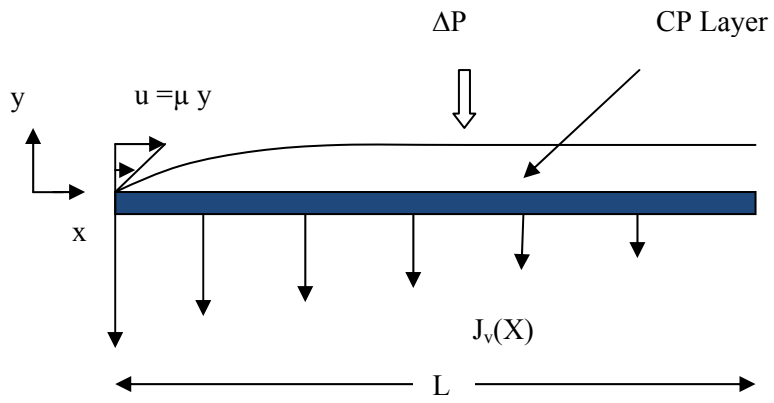


Fig. 2.5 Schematic description of concentration polarization over a membrane surface in cross-flow filtration; there is a concentration gradient filtration (Song and Elimelech, 1995).

Fig. 2.5 shows schematic representation of a cross-flow membrane filtration channel of length L depicting a steady state concentration polarization (CP) layer on the membrane surface. The axial (cross-flow) velocity *u* varies with the transverse (y direction) distance from the membrane surface. Within the confines of the polarized layer thickness, this velocity profile is assumed to be linear. The thickness of the CP layer increases, while the local permeate flux J_v decreases, with the axial (x direction) distance.

In order to link concentration polarization to the permeate flux; the film mass-transfer equation is required. The equation is based on a mass balance in the mass-transfer boundary layer. The concentration profile of solute molecules along the membrane surface is shown in Fig. 2.6.

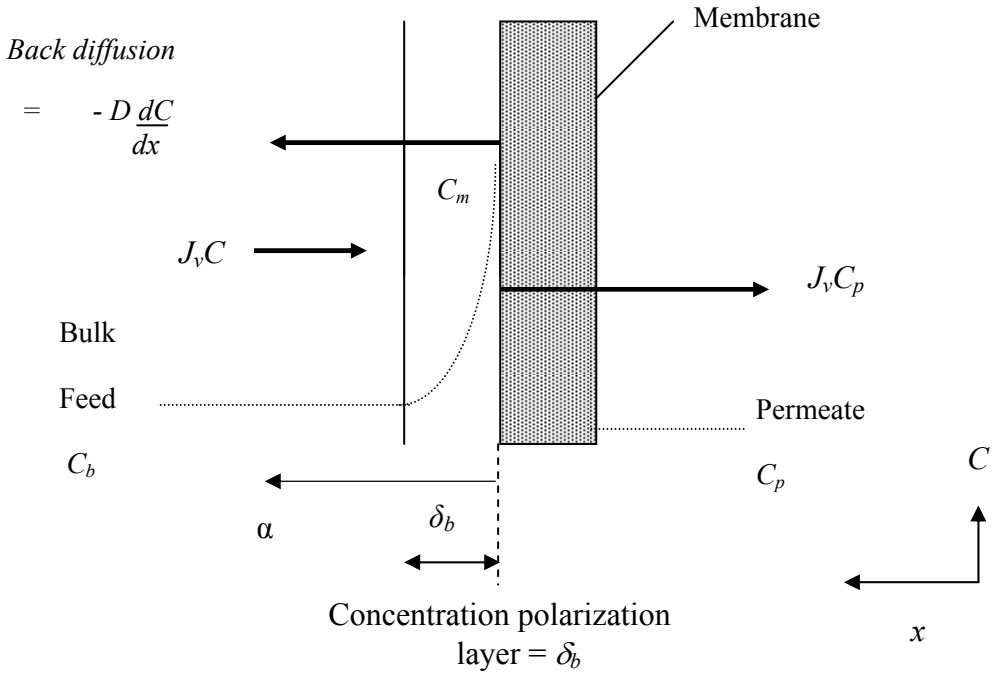


Fig. 2.6 Concentration profile of concentration polarization in cross-flow filtration (Song and Elimelech, 1995).

At steady state, the convective flux of solutes to the membrane must be balanced by a diffusive flux of species back to the bulk and a permeation of solutes through the membrane. A solute balance in a control volume within the concentration polarization layer yields the following differential equation:

$$J_V C - J_V C_p + D\frac{dC}{dx} = 0 \qquad (2.7)$$

Integrating this with boundary conditions ($C = C_m$ at $x = 0$; $C = C_b$ at $x = \delta_b$), we get

$$J_V = k \ln\left(\frac{C_m - C_p}{C_b - C_p}\right) \tag{2.8}$$

k (mass transfer coefficient) = D / δ_b

This equation is known as the concentration polarization equation for partially rejected solutes. For total solute rejection, *i.e.*, when C_p (permeate concentration) = 0, the equation reduces to

$$J_V = k \ln\left(\frac{C_m}{C_b}\right) \tag{2.9}$$

When the solute concentration at the membrane surface reaches the saturation concentration for the solute (C_s), or the gelation concentration of the macromolecule (C_g), there can be no further increase in C_m. Thus

$$J_V = k \ln\left(\frac{C_s}{C_b}\right) = k \ln\left(\frac{C_g}{C_b}\right) \tag{2.10}$$

This is referred to as the gel polarization equation. It indicates that when C_m equals C_s (or C_g) the permeate flux is independent of the transmembrane pressure. In the pressure independent region, the permeate flux for a given feed solution only depends on the mass transfer coefficient. For a particular mass transfer coefficient the pressure independent permeate flux value is referred to the limiting flux (J_{lim}). According to the gel polarization model, the existence of this limiting flux is a consequence of gelation of the solute at the membrane-solution interface.

2.2.3.2 Effect of concentration polarization on retention coefficient

The concentration polarization, being negligible at a low flux, is very severe at a high flux, which can cause a great decrease in the retention. Theoretically, concentration polarization increases exponentially with the flux. The

concentration contributions of the increased flux and increased concentration polarization at a high pressure are considered to cause the retention to level off (Kang *et al*., 2005). This is an example of the effect of concentration polarization on the retention. In order to avoid the concentration polarization effect, the intrinsic retention (R_{int}) can be used instead of the observed retention (R_{obs}).

The R_{int} can be obtained by calculating from the R_{obs} using "velocity variation method" is generally recognized as the most appropriate (Van den Berg *et al*., 1989). This method based on the description of the concentration polarization phenomena by the film theory, which gives:

$$\ln\left(\frac{1-R_{obs}}{R_{obs}}\right)_v = \ln\left(\frac{1-R_{int}}{R_{int}}\right) + \frac{J_V}{k} \tag{2.11}$$

The mass transfer coefficient k can generally be calculated from Sherwood's relations of the type:

$$Sh = k\frac{d_h}{D} = aRe^b Sc^{0.25} \tag{2.12}$$

With d_h as the hydraulic diameter and D the diffusivity of the involved solute. The adjustable parameters a and b are equal to 0.023 and 0.875, respectively, for turbulent conditions (Deissler expression).

This relation shows dependence between the mass transfer coefficient k and the cross-flow velocity v that can be expressed as:

$$k = \frac{1}{c}v^b \tag{2.13}$$

With

$$C = \frac{1}{a} d_h^{(1-b)} D^{-0.75} \rho^{(0.25-b)} \mu^{(b-0.25)} \quad (2.14)$$

where ρ and μ are the solution density and the dynamic viscosity, respectively. As variations of properties in the polarization layer exist (increase of viscosity and changes in diffusivity and density as a result of increasing concentration near the membrane), these values correspond to the mean concentration in the polarization layer. Combining Eqs. (2.9) and (2.12), we obtain:

$$\ln\left(\frac{1-R_{obs}}{R_{obs}}\right) = \ln\left(\frac{1-R_{int}}{R_{int}}\right) + c\frac{J_V}{V^{0.975}} \quad (2.15)$$

It is assumed that *c* is independent of the polarization level and can be considered as independent of the cross-flow velocity and permeation flux.

2.2.3.3 Negative retention coefficient in NF

It was reported by many research groups that numerous NF membrane show lower retention of monovalent anion such as chloride ion, formate, acetate and lactate than divalent anions such sulfate, succinate (Pontalier *et al*., 1997). Especially, in case of mixed-salt solutions with mono- and divalent anions, nanofiltration shows negative retention to monovalent anions (Garcia-Aleman *et al.*, 2004). Negative retention is well documented. Kang *et al*. (2005) observed the negative retention of monovalent anion in binary-solute solution containing succinate and lactate in flux range test. The term “negative retention” refers to the situation where a solute in the permeate of a membrane filtration is recorded at a higher concentration than that observed in the bulk feed.

Fig. 2.7 shows schematic diagram of solute transport though NF membrane with negative retention. Negative retention is common in systems such

as two salt mixtures sharing a common cation (e.g. Na_2SO_4 and NaCl), are filtrated. In these circumstances a large ion can be excluded from transport by a like charged membrane (e.g. SO_4^{2-}) this results in a higher than normal concentrations of counter-ions (Na^+) in the downstream permeate. Consequently the smaller membrane-permeable co-ions (Cl^-) are drawn across the membrane to neutralize the charge imbalance. At this stage the concentration of these smaller ions can be greater than in the feed giving negative retention (Mandal and Jones, 2008). Richard Bowen and Mukhtar (1996) carried out such experiments when evaluating NF transport models, clearly illustrating the negative retention of the chloride ion.

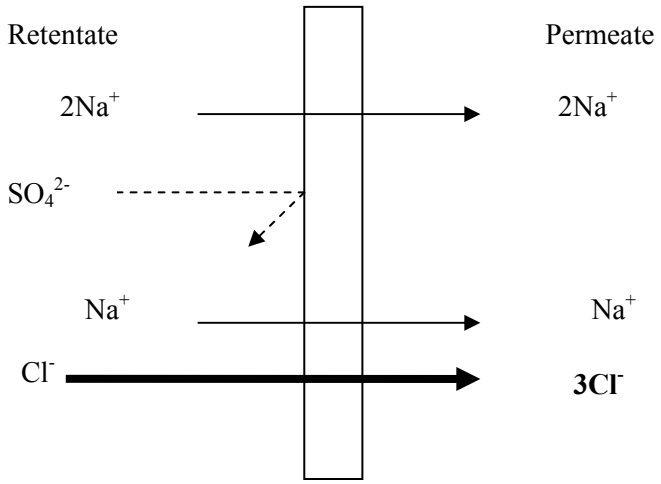


Fig. 2.7 Schematic diagram of two salts transport through a NF membrane.

2.2.4 Selectivity for NF

NF membrane presents the possibility to separate monosaccharide and disaccharide. The difference of monosaccharide and disaccharide retentions causes of their selectivity. Goulas *et al*. (2002) found that DS-5DL membrane presented the 77% retention of fructose and 99% retention of sucrose in the binary mixture. The difference between monosaccharide and disaccharide retentions causes of possible separation. Therefore, it might notice here that

separation between monosaccharide and disaccharide solutes can be achieved by using NF process. Moreover, NF presents also the possibility of separation between monosaccharide and disaccharide when they are in mixture.

NF membrane presents the possibility of separation between monovalent ions and divalent ions. Low retention of monovalent ions (at high feed concentration) and very high retention of divalent ions in a mixture can be obtained with NF. Pontalier *et al*. (1997) studied selective retention of $NaCl/Na_2SO_4$ in mixture with a 400Da membrane. The both salts contain the same counter-ion and different co-ions. The retention of Cl^- decreases with increasing the salt concentration (either NaCl or Na_2SO_4). SO_4^{2-} are almost completely retained with all salt concentrations while Na^+ can pass through the membrane easily, resulting an excess of positive charge on the permeate side. This excess generates an electrostatic force which increases anion transfer, particularly of Cl^-, because SO_4^{2-} cannot cross the membrane. However, Na^+, which is the counter-ions, is retained because anions and cations cannot permeate independently, but permeate the membrane while maintaining electroneutrality (Wang *et al*., 2002). The difference of mass transfer mechanism between divalent co-ions, which are completely retained, and monovalent co-ions, which are allowed passing the membrane partly, contributes the selectivity. Therefore, it can be concluded that the possibility of separation monovalent ion from divalent ion in mixture with NF membrane can be obtained.

The possibility of NF process for monovalent ion and disaccharide sugar separation was investigated (Li and Shahbazi, 2006). The retention of disaccharides sugar is much higher (about 99%) than that of sodium lactate (about 25%) at high salt concentration. Thus, the purification was expected to be feasible. It

could be mentioned that it is possible to separate sodium lactate and disaccharides sugar.

Most recently Bouchoux and co-workers (Bouchoux *et al*., 2005) investigated the separation between glucose amd sodium lactate. This represented the rarely studied system of a combined electrolyte/non-electrolyte solution. The retention of glucose and sodium lactate is carried out. They revealed that glucose retention strongly decreased with increasing sodium lactate concentration and thus the both solutes retention became very close to each other. This results in unachievable purification. Several hypotheses have been postulated to explain this phenomenon. The first one is that the retention lowering may be a result from an increase in the average pore size due to the repulsive interaction between the counter-ions inside the pores. This phenomenon is usually referred to pore swelling (Wang *et al*., 2002 and Bargeman *et al*., 2005). The second hypothesis is that ions may decrease the effective size of the neutral species because water preferentially solvate ions (this would be a kind of salting out effect as first observed by Hofmeister ("salting-out" effect see Hofmeister's works recently translated by Kunz *et al*., 2002) in his work on the influence of the nature of the background salt on the precipitation of hen-egg-white protein. Moreover, Bouchoux *et al*. (2005) also reported that the presence of glucose does not affect the retention of sodium lactate. NF cannot separate monosaccharide from lactate monovalent ion in these conditions. Therefore, it can be noted that the separation of monovalent ion and monosaccharide with NF is unachievable.

CHAPTER III

MATERIALS AND METHODS

3.1 Membrane and chemicals

NF membrane used in this work was Desal 5DK membrane, supplied by Osmonics. It is a flat sheet composite membrane, which includes three layers such as polyamide, polysulfone, and 'Osmonics proprietary layer'. It is negatively charged at pH higher than 4 (Tanninen *et al*., 2006). Average molecular weight cut-off of this membrane is about 150-300 g. mol^{-1}. It shows 98% retention of Mg_2SO_4 (at [Mg_2SO_4] = 2 g.L^{-1} and Δp = 6.9 bar), and hydraulic permeability as approximately 5.5 L.h^{-1}.m^{-2}.bar^{-1}. Maximum temperature that the membrane can be operated is 70ºC. These characteristics of the membrane were provided by the supplier. The average pore size of the membrane is in the range of 0.4 - 0.46 nm (Bargeman *et al*., 2005; Wang *et al*., 1997; Straatsma *et al*., 2002) and the average thickness of the active layer of membrane is 2.6 µm (Bargeman *et al*., 2005).

A neutral solute used was glucose (Acros Organics). Three types of sodium salts used were sodium lactate (NaLac), sodium chloride (NaCl) and sodium sulfate (Na_2SO_4) (Prolabo-Merck Eurolab). All feed solutions were prepared with ultra-pure water.

Table 3.1 Feed compositions of single- and mixed-solute solutions used for constant feed concentration experiments

Solutions	**Compositions**	**Analyzer**
Single-solute solutions	0.05 and 0.1M glucose, 0.1 to 1M NaLac, 0.1 to 1M NaCl,	Refractometer
	and 0.05 to 0.5M Na_2SO_4	Refractometer
Binary-solute solutions	(0.1M glucose) + 0.1 to 1M NaLac, (0.1M glucose) + 0.1 to 1M Na_2SO_4	HPLC (shodex)
	(0.1M glucose) + 0.1 to 1M NaCl	Refractometer and Conductometer
	(0.1M NaLac) + 0.1 to 1M NaCl, (0.1M NaLac) + 0.1 to 1M Na_2SO_4	HPLC (shodex) and IC
Ternary-solute solutions	[0.1M glucose + 0.1M NaLac] + 0.1 to 1M NaCl	HPLC (shodex) and IC
	[0.1M glucose + 0.5M NaLac] + 0.1 to 1M NaCl	HPLC (shodex) and IC
	[0.1M glucose + 0.1M NaLac] + 0.05 to 0.5M Na_2SO_4	HPLC (shodex)

Table 3.2 Feed compositions of binary- and ternary-solute solutions used for experiments in concentration mode

Solutions	Compositions	Analyzer
Binary-solute solutions	0.1M glucose + 0.1 M NaLac	Refractometer and Conductometer
Ternary-solute solutions	0.1M glucose + 0.1M NaLac + 0.125M Na_2SO_4	HPLC
	0.1M glucose + 0.2M NaLac + 0.125M Na_2SO_4	HPLC

Table 3.3 Feed compositions of the fermentation broths, used in this study

Solutions	**Compositions (M)**					**Analyzer**
	Glucose	**Lactate**	**Cl^-**	**SO_4^{2-}**	**Na^+**	
Raw fermentation broth	9.87×10^{-4}	0.989	3.24×10^{-2}	7.41×10^{-3}	1.165	
	(0.178)[1]	(11.5)[1]	(26.8)[1]	(88.0)[1]	(0.711)[1]	
1) Fermentation broth used	6.9×10^{-4}	0.7	3.24×10^{-2}	5.19×10^{-3}	-	HPLC
2) Modified fermentation broth	0.1	0.1	5.21×10^{-2}	-	-	HPLC
3) Modified fermentation broth with adding Na_2SO_4	0.1	0.1	5.20×10^{-4}	0.125	-	HPLC

Remark: [1] concentration in $g.L^{-1}$. All solute concentrations were determined by HPLC (Dionex).

The feed concentrations of glucose and NaLac used were determined in accordance with the final compositions of sodium lactate fermentation broth. The compositions of feed solutions in single- and mixed- solute solutions at constant feed concentration are listed in Table 3.1. The compositions of feed solutions in binary- and ternary-solute solutions under concentration mode are listed in Table 3.2. There were three experiments concerning fermentation broth in this work which were the fermentation broth, modified fermentation broth and modified fermentation with adding Na_2SO_4. The three broth compositions are shown in Table 3.3. The relevant characteristics of these different solutes used are listed in Table 3.4.

Table 3.4 Relevant characteristics of the solutes used

Solutes	**Molecular weight** ($g.mol^{-1}$)	**Diffusion coefficient** [a] (10^{-10} $m^2.s^{-1}$)	**Stokes radius** (nm)
Glucose	180.16	6.9	0.365[c]
Na^+	22.99	13.3	0.184[c]
Cl^-	35.45	20.3	0.121[c]
Lactate	89.07	10.6	0.23[b]
SO_4^{2-}	96.1	10.6	0.23[c]

Remark: [a] see (Weast *et al*., 1986). [b] Calculated from the stokes-Einstein relation $r_s=k_BT/6\pi\mu_0D$, with $k_B=1.3807 \times 10^{-23}$ $J.K^{-1}$, $\mu_0 = 8.9 \times 10^{-4}$ Pa.s, T=298.15K. [c] See (Nightingal, 1959).

3.2 Analytical methods

The analyzers used in this work are shown in Table 3.1. They were a refractometer (Atago RX-5000, Tokyo, Japan), a conductometer (LF318 WTW, Germany), two HPLCs (high-performance liquid chromatography) such as HPLC Shodex (Showa Denko,Kawasaki, Japan) and HPLC Dionex (sunny vale, CA, USA) , and a IC (Ion chromatography; sunny vale, CA, USA).

HPLC (Shodex) was used to determine solute concentrations at feed constant concentration. It could be used to analyze both neutral and charged solutes with a Shodex SUGAR SH1011 and a refractive index detector. The column was operated at 50 °C using 0.01N H_2SO_4 as mobile phase with a flow rate of 1 $mL.min^{-1}$. The injection volume of sample was 20 μL. Those samples where diluted to 20 folds before injection.

HPLC (Dionex) was also used for analyzing solute concentration in the solution under concentration mode and fermentation broth. It could be analyzed both neutral and charged solute concentrations by using different columns. Glucose concentration was determined by using a CarboPac^{TM} PA1 column and an electrochemical ED40 detector. The mobile phase was 150 mM NaOH at a flow rate of 1 $mL.min^{-1}$. The column temperature was set to 30 °C. The samples were diluted about 10,000 folds before injection. The anion concentrations were determined with using an Ionpac AS11 column. The mobile phase was 5 mM NaOH at a flow rate of 1 $mL.min^{-1}$ and cations concentration were determined with using an Ionpac CS12 column and using a CD20 conductivity detector. The mobile phase was 20 mM $CH_4O_3SO_4$ at 1 $mL.min^{-1}$

Dionex IC (ion chromatography system) was used for analyzing ions concentration at constant feed concentration. The anion concentrations were determined with using an Ionpac AS11 column and a CD20 conductivity detector.

The column temperature was kept constant at 30 °C by using 14.5 mM NaOH as mobile phase with a flow rate at 1 $mL.min^{-1}$. The injection volume was 20 µL. The samples were diluted about 200 folds by ultra-pure water before injection. The cation (Na^+) concentrations was determined using an Io npac CS12A column with 20mM CH_4O_3S as eluent.

In single-solute solution, glucose, NaLac, NaCl and Na_2SO_4 concentrations were determined by using refractometry.

In binary-solute solution, there were four different analytical methods which were classified by used analyzers to determine the solute concentrations. Firstly, the solute concentrations of binary-mixtures containing glucose/NaLac and glucose/Na_2SO_4 were determined by HPLC using a Shodex SUGAR SH1011. Secondly, the glucose and NaCl concentration of binary-mixtures containing glucose/NaCl was determined by using refractometer and conductometer, respectively. Thirdly, the ions concentrations in the binary mixtures containing NaLac/Na_2SO_4 were measured by using the HPLC with Shodex SUGAR SH1011. Finally, the ions concentrations in the binary mixture containing NaLac/NaCl were determined by using IC (Dionex). In this work, the Na^+ concentrations was not only determined by IC but also calculated from mass balance according to electroneutrality. Both obtained Na^+ concentrations were compared, for example, the comparison between Na^+ retentions obtained from the IC analysis with cationic column and those calculated from mass balance The comparison of Na+ retention obtained from both methods are shown in Fig. 4.18 showing the difference is less than 0.1%.

In ternary-solute solutions, the solute concentrations were mostly determined by using only the HPLC (Dionex) except for Cl^- concentration was determined by IC.

For fermentation broth, the solute concentrations were only determined by using Dionex HPLC (sunny vale, CA, USA). Moreover, the solute concentrations in concentration mode were only determined by using DIONEX HPLC system (sunny vale, CA, USA).

Table 3.5 Global mass balance of all solute of the initial feed solution containing 0.1M glucose, 0.1M lactate and 0.125M Na_2SO_4 at V_0/V_R = 2.5 in a concentration mode

Solute	**$C_R \times V_R$ (Mole)**	**$C_P \times V_P$ (Mole)**	**$[C_R \times V_R + C_P \times V_P]$ (Mole)**	**$C_0 \times V_0$ (Mole)**	**ERROR (%)**
SO_4^{2-}	0.624×2	0.0235×3	0.659	0.625	5%
Glucose	0.381×2	0.0392×3	0.472	0.493	4.3%
Lactate	0.306×2	0.197×3	0.481	0.5	3.8%

Remark: C_0 is initial concentration (M), C_R is retentate concentration (M), C_P is permeate concentration (M), V_0 is initial feed volume (L), V_R is retentate volume (L) and V_p is accumulatd permeate volume.

3.3 Nanofiltration experiments

3.3.1 Experimental set-up

The NF membrane sheet was placed in the Osmonics Sepa CF II cell as depicted in Fig 3.1. Inside the membrane cell, a permeate carrier was placed on top of the membrane, feed spacer below the membrane, and O-ring was used to seal the cell. The active side of the membrane faced the feed spacer. The permeate carrier and

the feed spacer were pre-wetted with ultra-pure water and placed in the cell body. The surface area of the flat sheet membrane was 138 cm^2.

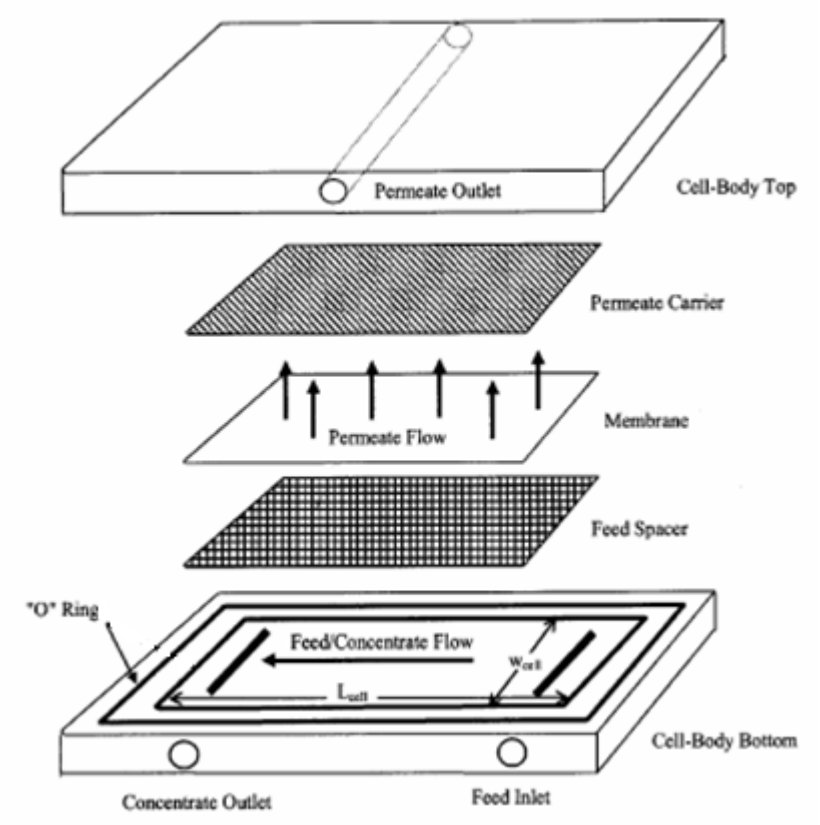


Figure 3.1 Schematic diagram of the membrane cell set-up (Falls, 2002)

3.3.2 Experimental procedure

The experimental bench used for the NF experiments is depicted in Fig 3.2. Feed solution was contained in a 5 L feed vessel. Temperature of the system was maintained at 25 ± 0.5 °C by using a circulating thermostatic bath. A high-pressure pump was used to pull feed solution into the membrane cell and flows tangentially along the membrane surface. Transmembrane pressure was controlled by a back pressure valve (stainless steel control valve), which was installed on the retentate outlet. The pressures were monitored through two digital manometers located on the inlet and outlet of the cell. The retentate and permeate streams were recycled back into the feed vessel in order to maintain a constant feed concentration for experiments

at constant feed concentration while only retentate streams was recycled back into the feed vessel for experiments with concentration mode. Permeate samples were taken through a by-pass mounted on the permeate outlet.

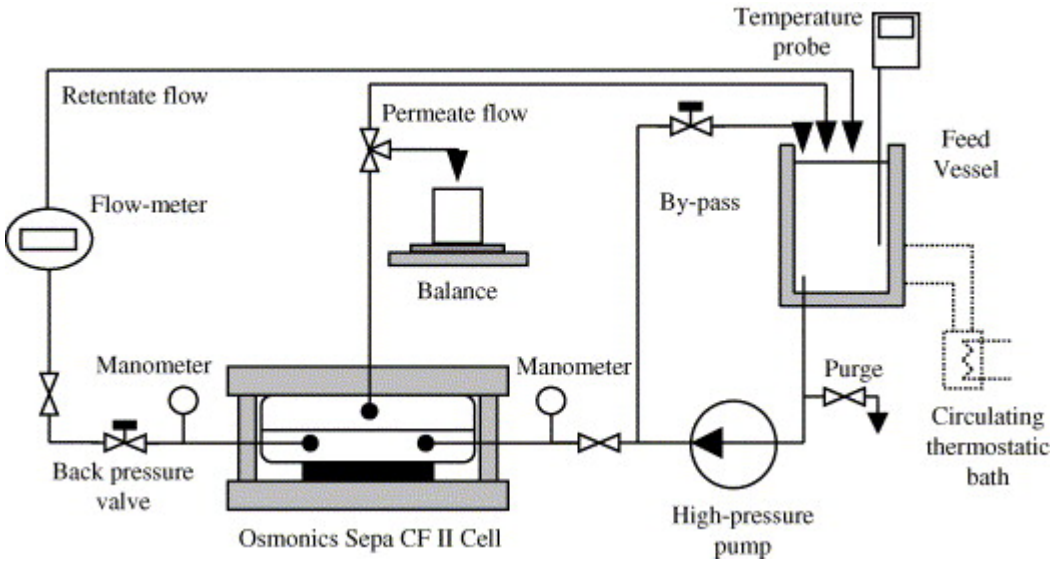


Figure 3.2 Flow schematic diagram of the experimental set-up (Bouchoux *et al*., 2005).

3.3.2.1 Retention measurement at constant feed concentration

Experiments at constant feed concentration (permeate and retentate are re-circulated back to the feed tank) were carried out. The circulation of 2 L feed solution was operated at the flow rate of 400 $L.h^{-1}$ (64% pump frequency) and at applied pressure of 2 bar for 30 min. The applied pressures are varided as 2, 4, 6, 10, 14, and 18 bars, respectively, which are adjusted by the stainless steel control valve. The pump frequency is adjusted again if the flow rate moves away from 400 $L.h^{-1}$. After each pressure adjustment, the sample is collected when permeate volume comes out greater than 20 mL. All solutes were weighed to determine the permeation flux. Moreover, conductivity and pH of samples containing charged solute are measured.

3.3.2.2 Retention measurement in a concentration mode

The binary mixtures containing glucose/NaLac and the ternary mixtures containing glucose/NaLac/Na_2SO_4 were carried out under concentration mode (only retentate is recycled back to the feed tank whereas the permeate is collected in the permeate tank). The volumes of feed solutions were 3 and 5 L. The experiment was performed at flow rate of 400 $L.h^{-1}$. The broth was first circulated for 30 min and then the pressure is fixed to the designed values. The samples were collected every 1 h. Determining permeate flux and solute concentrations were performed like those at feed concentration experiment. Moreover, conductivity and pH of samples containing charged solute are measured.

3.3.3 Membrane characterization

3.3.3.1 Membrane pretreatment and cleaning

The membrane pretreatment and cleaning procedures are crucial steps to obtain repeatable results. In this work, the virgin membrane was pre-compacted by circulating ultra-pure water at 20 bar, until the water permeation flux (J_v) was constant (usually within 1 h). At the end of each experiment, the membrane was cleaned by circulating twice with RO water (filtrated by reverse osmosis) and then ultra-pure water until the conductivity of water in the vessel is below 5 $\mu S.cm^{-1}$. The circulation steps were operated at 25 ± 0.5°C, 10 bars, and 150 $L.h^{-1}$.

3.3.3.2 Water permeability

The water permeability is always measured prior any experiment by circulating ultra-pure water in order to check whether the membrane performance has changed during the previous experiment. The mean hydraulic permeability L_{p0} is then calculated from the slope of the plot of J_V versus ΔP, which is

in accordance with equation 2.10 (calculation subject to a maximum standard deviation of ±5%). The water flux was measured at applied pressures such as 4, 8, 12, 16 and 20 bars. The applied pressure was firstly interval increased from 2 to 20 bars and then interval decreased from 20 to 2 bars. The collected permeate samples were weighed in order to determine the permeation flux. A membrane sample presenting a visible mechanical damage or an abnormally high water flux (more than 20% difference between two consecutive permeability measurements) was replaced by a new one.

Table 3.6 shows the initial hydraulic permeabilities (L_{p0} that was determined before doing the first experiment) of each membrane used in this work. We can state that the water permeability varies about 0.096 to 0.144× 10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$ and has average value as 0.133 ± 0.015 $m^3.s^{-1}.m^{-2}.bar^{-1}$. In addition, R-square values obtained from the linear plot J_V versus ΔP were higher than 0.9899 and had average values as 0.9967 ± 0.0034. This implies that there is no fouling on the membrane during permeability measurement (Isable Gonzàlez *et al*., 2008). The slight deviation of the initial water permeabilities of each membrane confirms that membranes used were in the same water permeability condition. This shows a repeatability of used membrane.

Table 3.6 Initial pure water permeabilities of the Desal 5DK in this work (25°C and 2 - 20 bars)

Number of membrane	**Pure water permeability** L_{p0} **(10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)**	**R-squared**
1	0.136	0.9974
2	0.138	0.9899
3	0.136	0.9928
4	0.137	0.9983
5	0.139	0.9990
6	0.144	0.9990
7	0.140	0.9988
8	0.096	0.9980
Average	0.133 ± 0.015	0.9967 ± 0.0034

Table 3.7 Pure water permeabilities reported for Desal 5DK from the literature (25°C)

Pure water permeability L_{p0} **(10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)**	**References**
0.138	This work
0.147	Bouchoux *et al*. (2005)
0.131	Meighong *et al*. (2008)
0.196	Mazzoni and Bandini (2006)
0.22 (first batch)	Hagmeyer and Gimbel (1998)
0.17 (second batch)	Hagmeyer and Gimbel (1998)
0.14	Bowen and Mohammad. (1997)
0.13	Straatsma *et al*. (2002)
0.15	Bargeman *et al*. (2002)

The average water permeability value is compared to those reported by the other studies as listed in Table 3.7. The pure water permeability value in this work is reasonably correspondence with those reported by Bowen and Mohammad (1997), Straatsma *et al*. (2002), Bouchoux et al. (2005), Bargeman *et al*. (2005), Hagmeyer and Gimbel (2^{nd} batch; 1999) and Meihong *et al*. (2008). It is lower than those reported by Nazzoni & Bandini (2006) and Hagmeyer & Gimbel (1^{st} batch; 1999). Such differences are probably due to different pre-compaction procedures (not always presented by the various authors) and to the fact that measurement of the pure

water permeability value is generally obtained by the permeate flux measurement at only one pressure in other studies.

3.3.3.3 Membrane characterization using uncharged solute

The retention of an uncharged solute are measured in order to check the membrane characteristics. This was systematically performed after the membrane pretreatment step for any new membrane and then randomly once a month. 2 L of 0.1M glucose solution was circulated at 2 bar and at the flow rate of 150 $L.h^{-1}$ for 30 min in order to homogenize the feed solution. Then, the flow rate was fixed at 100 $L.h^{-1}$. The applied pressures were increased from 2 to 4, 6, 10, 14, and 18 bars, respectively, by using the stainless steel control valve. For each pressure, a permeate sample was collected when permeate volume comes out greater than 20 mL. It was weighed in order to determine the permeation flux and analyzed to get the glucose concentration in the permeate. The experiment was also carried out at other flow rates such as 200, 300, and 400 $L.h^{-1}$, respectively.

Fig. 3.3 shows the values of the retention of glucose as function of permeate flux. The glucose retention increases with increasing permeate flux to level off. The observed retention of glucose slightly increases and approaches to the respective limit value with increasing in the tangential flow rate from 100 to 200 $L.h^{-1}$. At flow rate higher than 200 $L.h^{-1}$, the retention of glucose approaches the maximum value and is independent of the flow rate. In cross-flow nanofiltration, the concentration at the membrane surface is generally higher than that in the bulk as a result of concentration polarization which causes decreasing in observed retention.

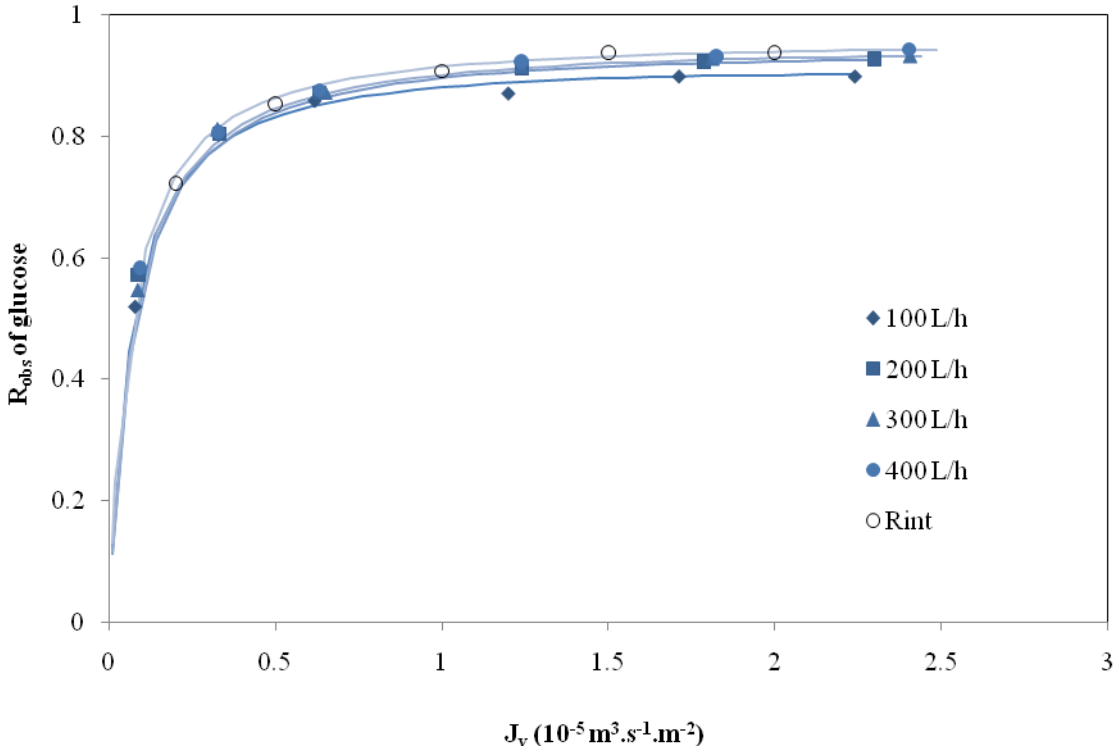


Figure 3.3 Intrinsic and observed retention of glucose as function of the permeation flux – influence of tangential flow rates (see legends) - 0.1M glucose solution

On basis of the film model theory (Straatsma *et al.*, 2002), the thickness of concentration polarization strongly depends on flow rate along the membrane. Consequently, the concentration polarization is strong at low tangential flow rates while it is weak at the high tangential flow rates. However, the retention increased slightly with increasing tangential flow rates from 100 to 400 $L.h^{-1}$ was observed in this work; therefore, it can be assumed that concentration polarization in these conditions can be neglected. It is interesting that the retention increased at higher flux, although there is no concentration polarization. This depends probably on the solution-diffusion in the nanofiltration membranes which limited more the free of solute molecules than that of the solvent. At the limiting retention, the membrane was packed with solute and the retention was limited by solute sorption and desorption. In ultrafiltration membranes, where the solute flux is controlled mostly by convection, retention stayed more or less the same, independent of flux (Nyström *et al.*, 2004).

A membrane sample presenting abnormally low glucose retention (less than 85% glucose retention at 18 bar) was replaced by a new one. The average of glucose retention in this work obtained at 18 bar is about 0.92 ± 0.04. The range of glucose retention at the applied pressure of 18 bar is 0.98 - 0.86. This also confirms the repeatability of the membrane used. There were 2 membranes showing the glucose retention was lower than 30% at 18 bar although they showed good initial water permeability (L_{p0}) of 0.124 and 0.13× 10^{-5} $m^3.m^{-2}.s^{-1}.bar^{-1}$. They were not further used. This means that the glucose retention measurement is better to check the state of a NF membrane than that of the water permeability only.

CHAPTER IV

RESULTS AND DISCUSSIONS

All experimental results and discussions in this work are included in this chapter. Experiments are classified into 5 sections. Section 4.1 deals with mass transfer mechanisms of neutral and charged solutes in single-solute solutions at constant feed concentration. Section 4.2 studies the influence of ionic composition on mass transfer mechanisms of glucose and lactate in binary-solute solutions at constant feed concentration. Section 4.3 looks into impact of the addition of an electrolyte on the separation between glucose and lactate at constant feed concentration. Section 4.4 examines impact of addition of an electrolyte on the separation between glucose and lactate under concentration mode. Section 4.5 investigates effect of addition salt on the separation between glucose and lactate in real fermentation broth. Each section, the experimental results and discussions are presented separately and the conclusion is located at the end. After the first section, the following experiments were designed by considering the experimental results from the previous one. The following discussions were not only compared to those from the other sections in this work, but also other authors.

4.1 Transfer mechanisms of neutral and charged solutes in single-solute solution

This section investigates the mass transfer mechanisms of solute across the membrane with single-solute solutions. The experimental procedures follow those in Bouchox's thesis (Bouchoux *et al*., 2005). The experiments are carried out with both neutral solute e.g. glucose and charged solutes e.g. NaLac, NaCl and Na_2SO_4. The retentions of glucose, Nalac and NaCl are first determined and compared to former ones obtained in previous studies. There are also used as reference for those further obtained in mixed-solute solutions. All the experiments were performed at constant feed concentration and constant feed flow rate (400 $L.h^{-1}$).

4.1.1 Influence of the feed concentration on the glucose retention

The variation of the observed retention coefficient of glucose as function of the permeate flux for different feed concentrations is shown in Fig. 4.1. One can observe that it is quite independent of the concentration. Generally, the retention of an uncharged solute results from the only steric-hindrance effect, which is fixed by the hydrodynamic radius of the solute (≈ stokes radius) and the mean pore size of the membrane (Pontalier *et al*, 1997). Then, it is not expected to depend on the solute concentration. Moreover, the values obtained in this work are similar to those previously obtained in comparable conditions by Bouchoux *et al*. (2005).

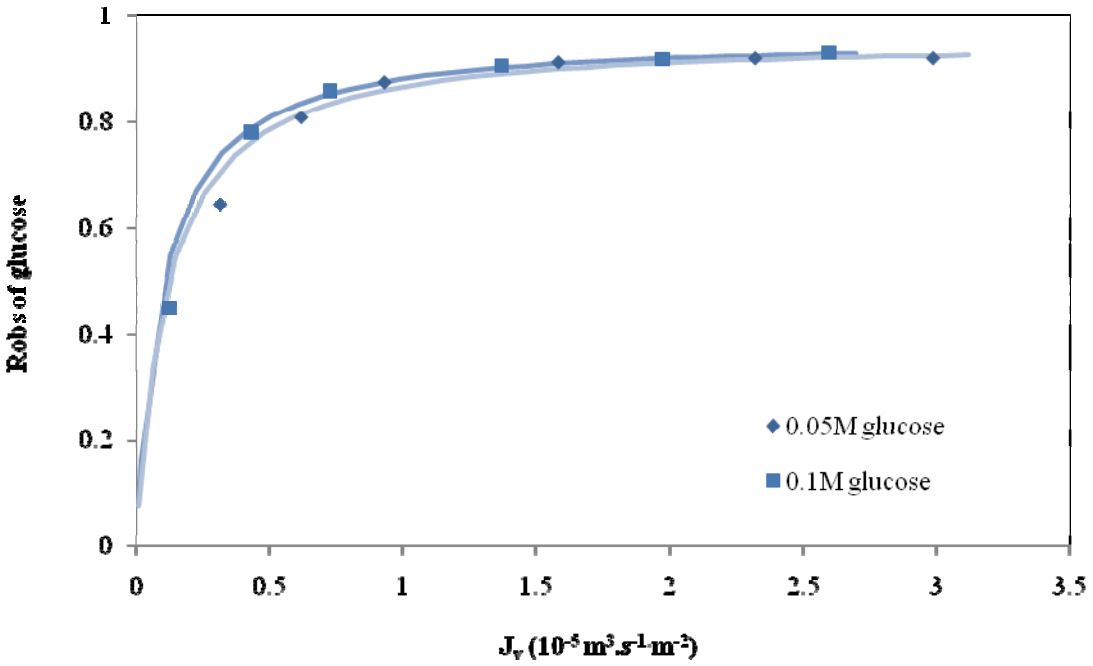


Figure 4.1 Retention of glucose as function of the permeate flux – influence of the glucose concentration.

4.1.2 Influence of the feed concentration on the salt retentions

The variations of the retention of lactate versus the permeate flux are plotted in Fig. 4.2 for different concentrations. A continuous decrease is observed with increasing concentrations. As previously explained, the transfer of a charged solute depends on the combination of steric hindrance effects and electrostatic interactions. It was also demonstrated in a former work that, for the conditions investigated here, the retention of lactate is mainly fixed by electrostatic repulsions (Bouchoux *et al.*, 2005). Then, increasing salt concentration results in a lower retention because of the screening effect (Kang *et al.*, 2004) that makes the electrostatic repulsion weaker. At low concentrations, the electrostatic repulsions are dominant. The lactate ions are repelled by the negative charges of the membrane, thus high salt retentions are obtained. At higher salt concentrations, the electrostatic repulsions become weaker and the salt retention decreases.

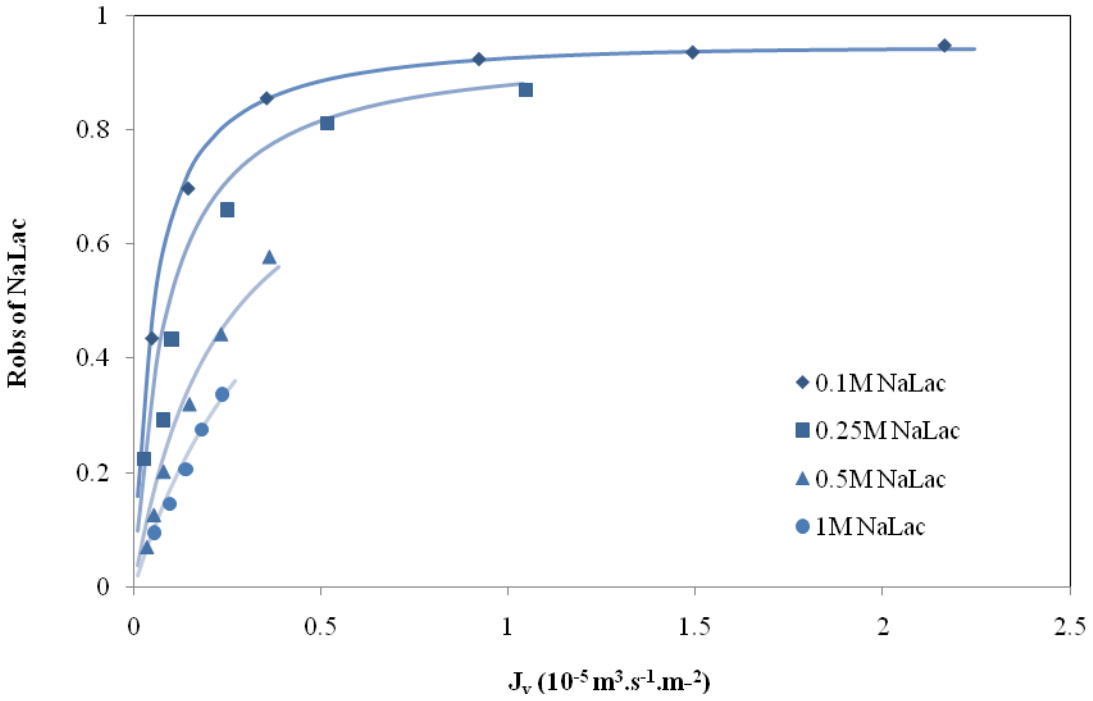


Figure 4.2 Retention of sodium lactate (NaLac) as function of the permeate flux – influence of NaLac concentration.

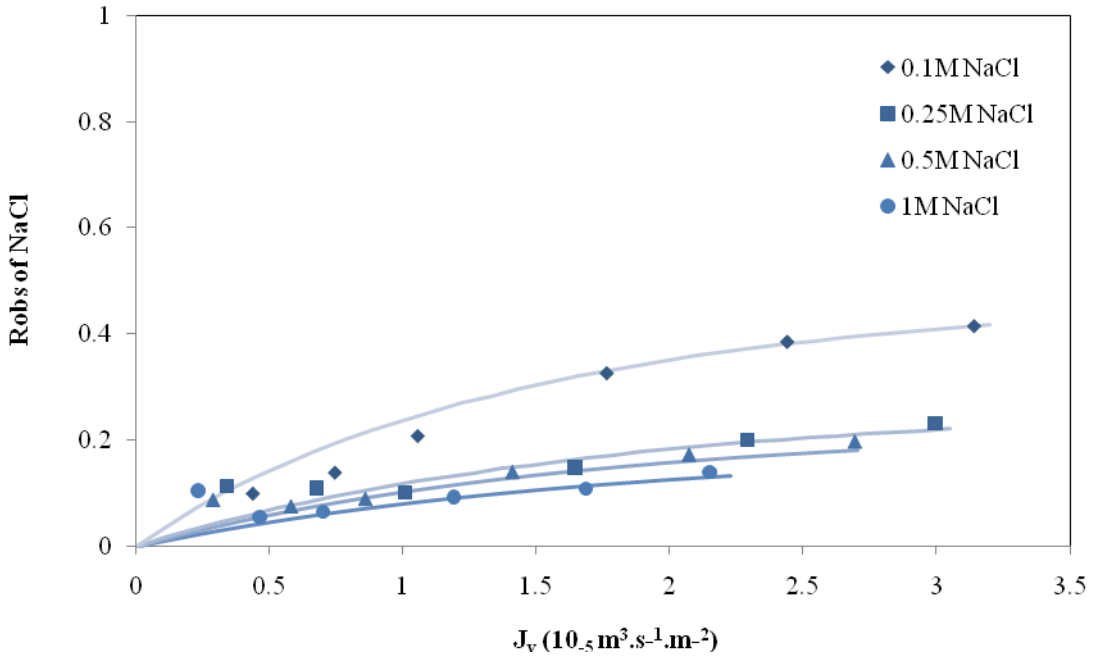


Figure 4.3 Retention of NaCl as function of the permeate flux – influence of NaCl concentration.

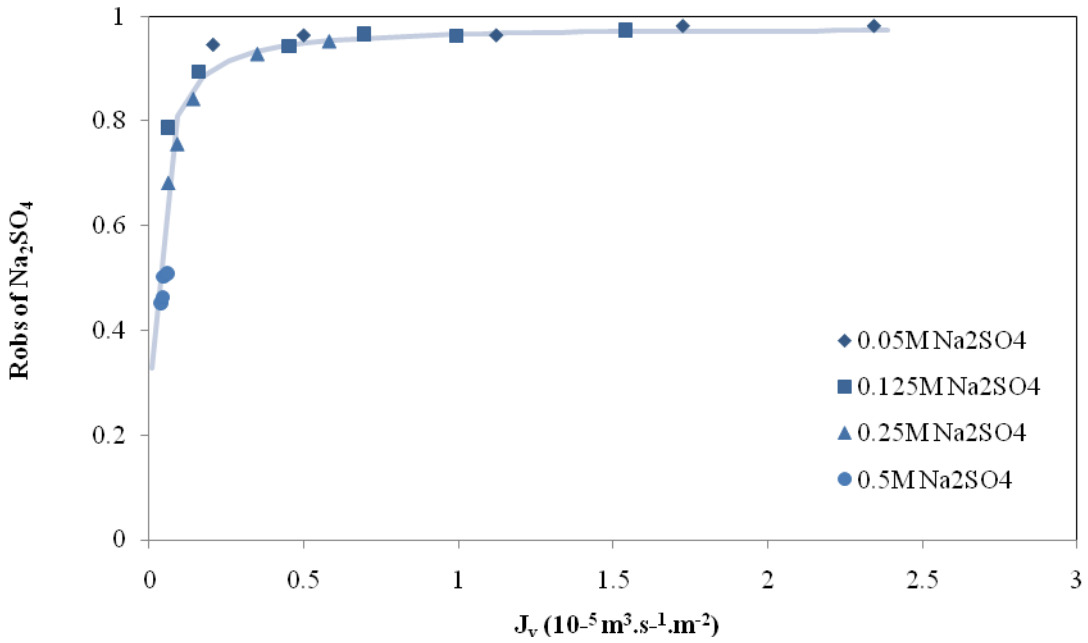


Figure 4.4 Retention of Na_2SO_4 as function of the permeate flux – influence of Na_2SO_4 concentration.

The relationship between observed retention of NaCl and the permeate flux at different NaCl concentrations is shown in Fig. 4.3. Similarly to that of sodium lactate, the observed retention of NaCl decreases with increasing NaCl concentration.

The plot of observed retention of Na_2SO_4 versus the permeate flux with different Na_2SO_4 concentrations is depicted in Fig. 4.4. On the contrary, the observed retention of Na_2SO_4 is quite independent of its concentration. It can be explained that the transfer of Na_2SO_4 depends mainly on the steric effects because Na_2SO_4 was almost totally retained and independent of Na_2SO_4 concentration. As explained earlier, the electrostatic repulsion is diminished at high salt concentration; however, Na_2SO_4 is still highly retained so that the size effect of Na_2SO_4 is predominant.

4.1.3 Discussions

The transfer of a neutral solute such as glucose is mainly due to steric effect, which is fixed by ratio between the solute and pore radius. The transfer of a charged solute results from the combination of steric effect and electrostatic interactions. The retention of NaLac in single-solute solution is high at low salt concentration since the electrostatic interactions are dominant; however, the retention decreases at high salt concentration because the electrostatic repulsion is less effective and the solute transfer is then mainly due to the steric effect. The retention of NaCl in single-solute solutions is low at all feed concentrations, because NaCl size might be small compared to the membrane pore size. However, the retention of NaCl decreases with increasing salt concentration. On the other hand, the retention of Na_2SO_4 in single-solute solutions is quite independent of its concentration because the size of SO_4^{2-} might be much larger than that of membrane pore.

The glucose retention is independent of its concentration while the NaLac retention decreases with increasing NaLac concentration. The glucose and NaLac retentions are identical at 0.1M glucose and 0.1M NaLac so that the separation is expected to be unachievable. On the other hand, the retention of NaLac at high concentration is much lower than that of glucose. At 0.25×10^{-5} $m^3.m^{-2}.s^{-1}$, the retention of NaLac is lower than that of glucose by 10%, 30%, and 40% for 0.25, 0.5, and 1M NaLac concentration, respectively. Therefore, the separation between glucose and NaLac is expected to be possible if both solutes exist together in the mixture containing high NaLac concentration.

4.1.4 Conclusions

This section studies the mass transfer mechanisms of charged and neutral solutes in nanofiltration by using Desal 5DK membrane. The experiments

were carried out with single-solute solutions. The influence of feed concentration and permeate flux on solute retention were investigated. The retention of glucose is independent of its concentration. The retentions of lactate ions and Cl^- decrease with increasing salt concentration due to the screening effect. However, the retention of SO_4^{2-} is independent of SO_4^{2-} concentration showing that it is fixed by size effects. The separation between glucose and NaLac at high NaLac concentration is expected to be feasible since NaLac retention is much lower than that of glucose.

4.2 Influence of the ionic composition on the transfer of glucose and lactate

This section studies the influence of the addition of salt on the transfer of glucose and lactate in binary-solute solutions. The experiments were carried out with the binary mixtures containing both charged/neutral solutes and charged/charged solutes at different salt concentrations. They were performed at constant feed concentration. The influence of the addition of a salt (NaLac, NaCl and Na_2SO_4) on glucose retention is first discussed. Then the results concerning the influence of the addition of a salt (NaCl and Na_2SO_4) on the lactate retention are focused. Finally, the separation glucose/ sodium lactate is discussed.

4.2.1 Influence of addition of a salt on glucose retention

The effect of presence of salt in feed solution on the glucose retention in binary-solute solutions is studied with different salts (NaCl and Na_2SO_4) at different concentrations. The salt retentions are measured as well. Then, the separation factor for the NaLac/glucose mixture is evaluated according to equation 2.5.

4.2.1.1 Influence of the addition of NaLac on glucose retention

The variations of the retention of glucose and lactate versus the permeate flux are plotted for different concentration of NaLac in Fig. 4.5. Fig.4.5 shows that both glucose and lactate retentions continuously decrease with increasing NaLac concentrations.

The glucose and lactate retentions obtained in binary solutions are also compared to those obtained in single solutions. It is observed that the lactate retentions obtained in single- and binary-solute solutions are only slightly different whereas the glucose retention decreases with increasing NaLac concentration.

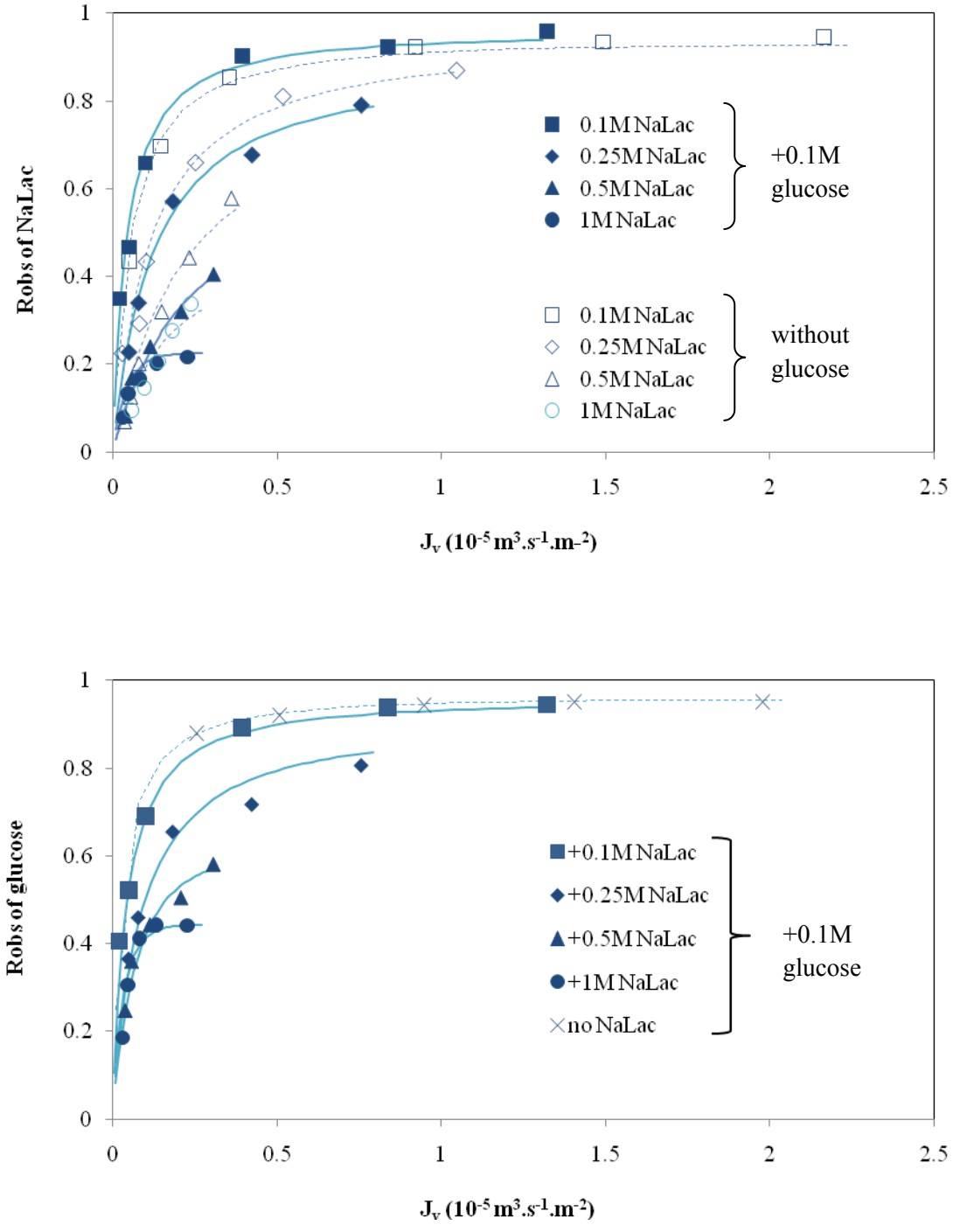


Figure 4.5 Retention of lactate (top) and glucose (bottom) as function of the permeate flux – influence of NaLac concentration (see legends) - feed solutions containing 0.1M glucose and 0.1to1M NaLac.

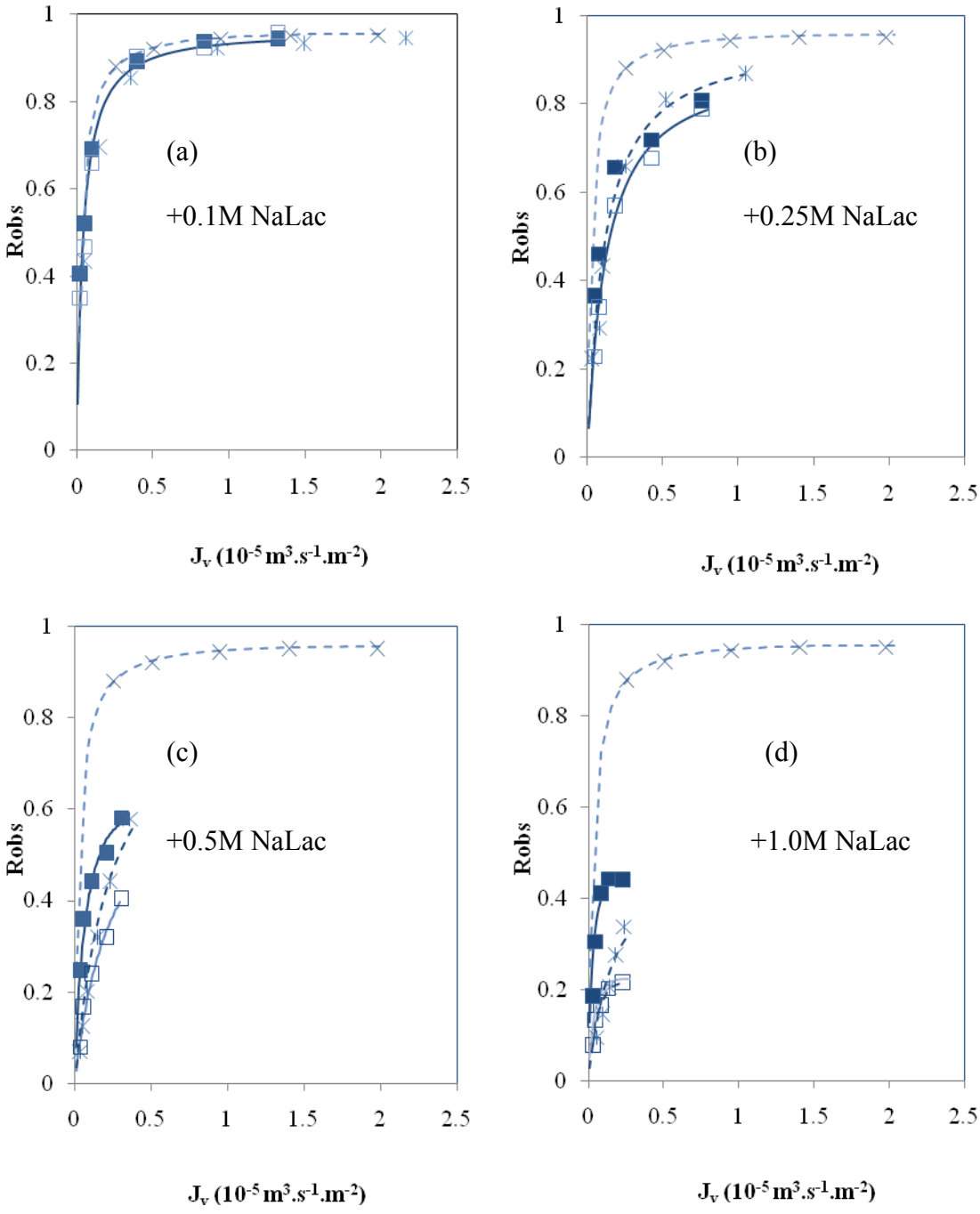


Figure 4.6 Retention of lactate and glucose as function of the permeate flux - feed solutions containing 0.1M glucose and 0.1to1M lactate. Single-solutions: (×) glucose and (*) lactate. Binary-solutions: (■) glucose and (□) lactate.

Fig. 4.6a shows the effect of the addition of 0.1M NaLac on the glucose retention. The retentions of lactate and glucose in the binary mixture that contains 0.1M NaLac and 0.1M glucose are not different from those observed in single-solute solutions. Both retentions are very close to each other. Fig. 4.6b shows the effect of presence 0.25M NaLac on glucose retention. The glucose retention is lower than that observed in single-solute solution; however, the retention of glucose and NaLac are similar. Fig. 4.6c shows the glucose retention in presence of 0.5M NaLac is much lower than that observed in single-solute solution and is little higher than that of lactate. Fig 4.6d shows the glucose retention in presence of 1M NaLac is very low; however, the glucose retention is higher than that of NaLac. This experiment was repeated in order to confirm the obtained results observed that the duplicate results were not different from those previous one higher than 5%.

4.2.1.2 Influence of the addition of NaCl

Fig. 4.7 shows the variation of glucose retention as function of permeate flux in presence of NaCl in binary-solute solutions. This experiment was carried out in order to check the extent of variation of glucose retention in the presence of other monovalent salt beside NaLac. The glucose retention slightly decreases with increasing NaCl concentration. The decrease is much less than that observed in the presence of NaLac (Fig. 4.5). Fig. 4.7 also shows the decrease of NaCl retention with increasing NaCl concentration. Fig. 4.8a-d shows that the glucose and NaCl retentions are slightly changed. The retentions of NaCl are not different from those observed in single-solute solutions indicating the presence of glucose does not affect NaCl retention.

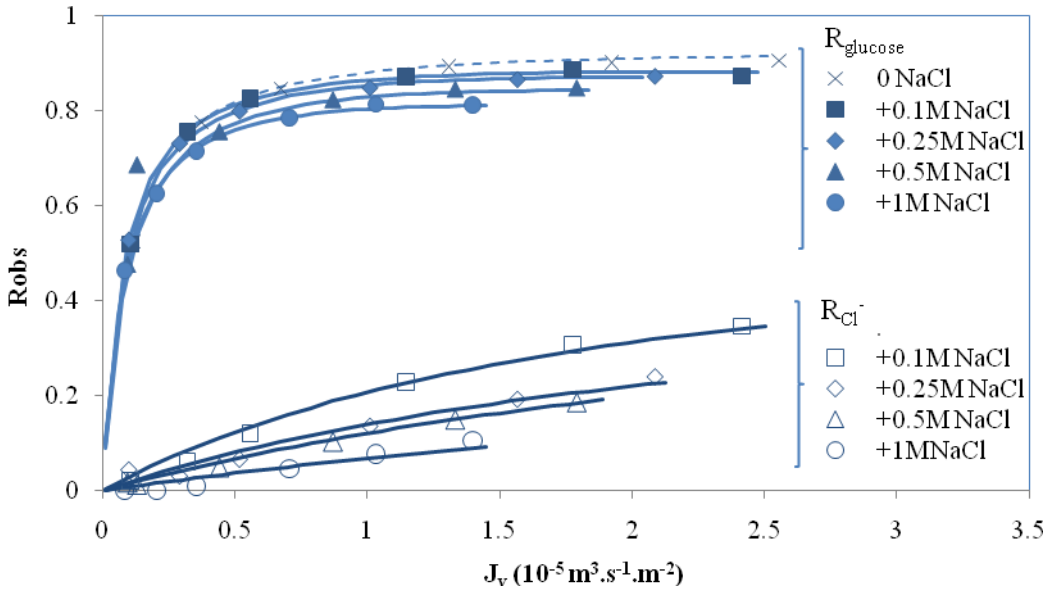


Figure 4.7 Retention of glucose and Cl^- as function of the permeate flux – influence of NaCl concentration (see legends) – feed solutions containing 0.1M glucose and 0.1M to 1M NaCl.

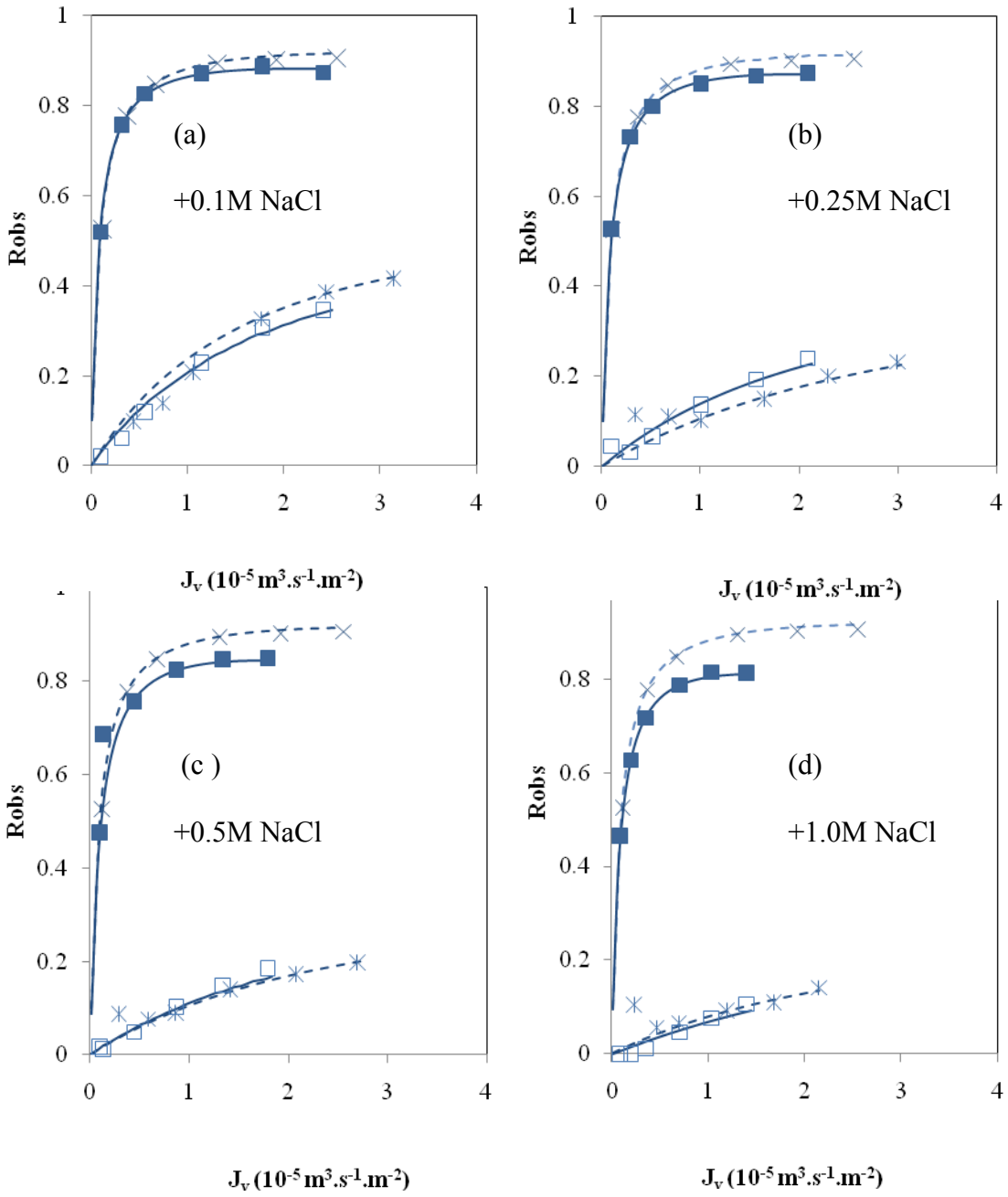


Figure 4.8 Retention of glucose and Cl^- as function of the permeate flux - feed solutions containing 0.1M glucose and 0.1to1M NaCl. Single-solutions: (×) glucose and (*) Cl^-. Binary-solutions: (■) glucose and (□) Cl^-.

4.2.1.3 Influence of the addition of Na_2SO_4

Fig. 4.9 shows the variation of glucose retention as function of permeate flux in presence of Na_2SO_4. Fig. 4.9 also shows the Na_2SO_4 retention is independent of Na_2SO_4 concentration. Figs. 4.10a-d show that both retentions of

glucose and Na_2SO_4 are quite independent of Na_2SO_4 concentrations and do not differ from those in single-solute solutions.

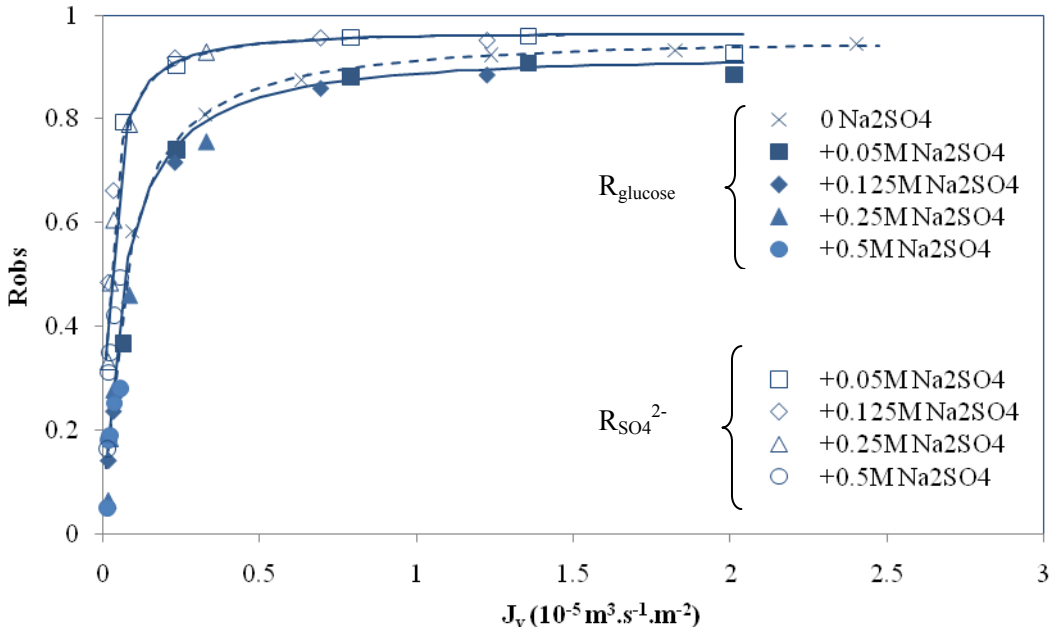


Figure 4.9 Retention of glucose and SO_4^{2-} as function of the permeate flux – influence of Na_2SO_4 concentration (see legends) - feed solutions containing 0.1M glucose and 0.05M to 0.5M Na_2SO_4.

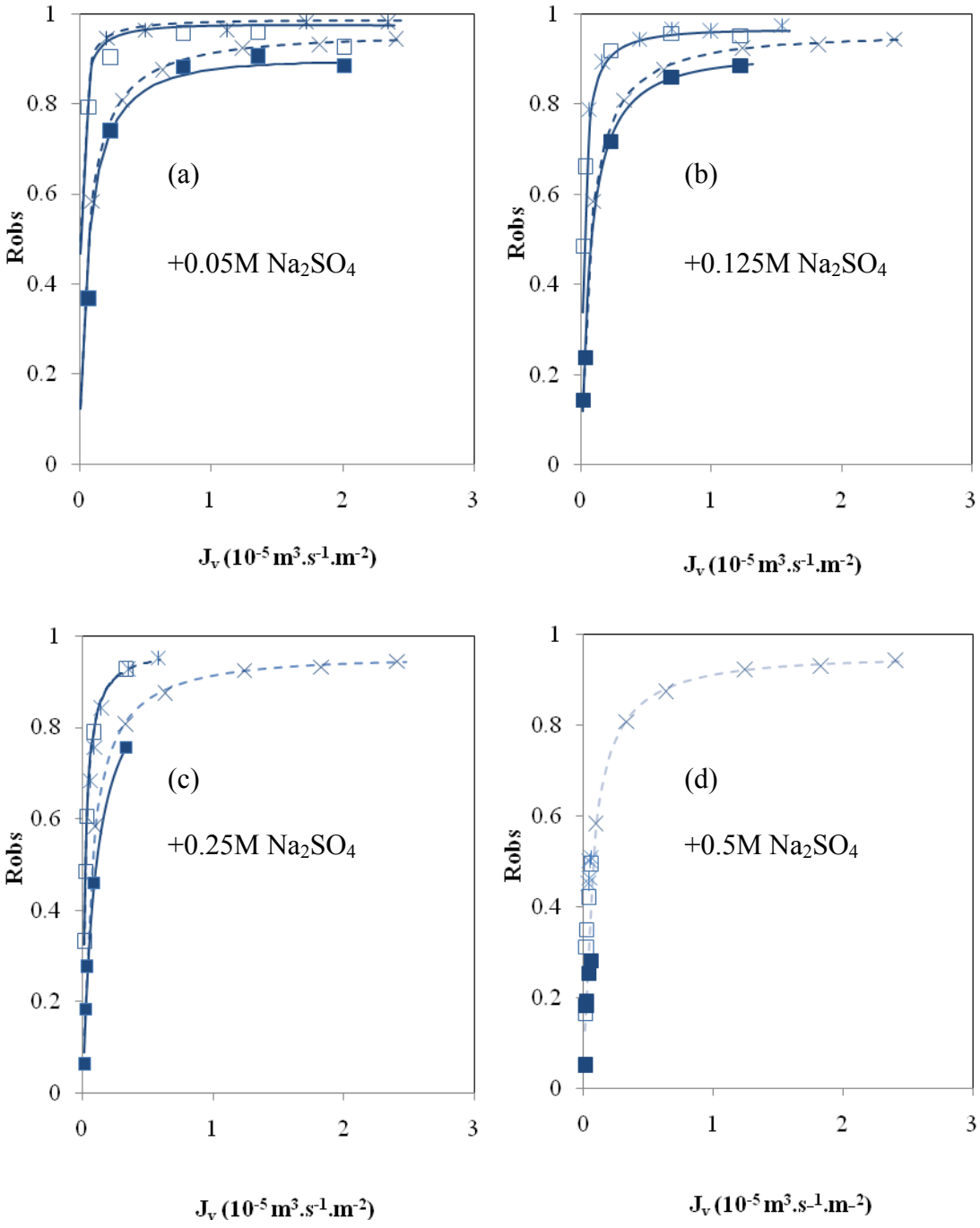


Figure 4.10 Retention of glucose and SO_4^{2-} as function of permeate flux - feed solutions containing 0.1M glucose and 0.05 to 0.5M Na_2SO_4. Single-soluitons: (×) glucose and (*) SO_4^{2-}. Binary-solutions: (■) glucose and (□) SO_4^{2-}.

4.2.1.4 Discussion

The experimental results show that the glucose retention decreases when adding a salt, like NaLac and NaCl. These results are in agreement with those already obtained in similar conditions (Bouchoux *et al*, 2005). Such an effect of the presence of charged species on the retention of neutral ones was reported in different situations, with organic as well as inorganic membranes and with different kind of solutes (Umpuch *et al*., 2010). In any case, it was pointed out that an increasing salt concentration results in lowering the neutral solute retention and that this decrease depends on the nature of the added salt. For instance, it was observed that the retention of glucose decreases more with the addition of sodium lactate than with that of sodium chloride at the same concentration (Bouchoux *et al*., 2005).

The retention of a neutral solute comes from steric effect, which is fixed by the solute and pore size. Then, a lower retention like that observed when adding a salt can be ascribed to an increase of pore radius, to a decrease of the solute hydrodynamic radius, ore more probably to a combination of both.

It was suggested that the addition of salt in solution can lead to an increase of the membrane charge density and to a higher concentration of counter-ions in the electrical double-layer at the pore surface (Hagmeyer *et al*., 1998; Straatsma *et al*., 2000). Then, because of the stronger repulsion foreces between the pore walls a pore swelling could appear on addition that the membrane material behaves like an electric polymer (Wang *et al*., 1997; Xu and Lebrun, 1999; Schaep *et al*., 2001; Wang *et al*., 2002; Bargeman *et al*., 2005; Nghiem *et al*, 2006). However, as already mentioned, the effect of salt on the transfer of a neutral solute has also been observed with ceramic membranes, which are not expected to swell (Bouranene *et al*., 2007).

On the other hand, the apperent size of neutral solutes, like carbohydrates, is also expected to be influenced by the ionic composition. Indeed, it was established for instance that the glucose solute becomes less hydrated when NaCl is added in the solution (Zhuo *et al*, 2000). Following the release of water contained in the hydration shell, the apparent size of the solute becomes smaller, so that a lower retention can be expected. According to that, more hydrated salts in solution mean a higher dehydration, and thus a lower size and a lower retention of the sugar. This tendency was found to be in good agreement with experimental results obtained with different satls (Bouchoux *et al*., 2005).

In the present work, it was shown that the glucose retention is not affected by the presence of Na_2SO_4. The corresponding retentions of sulphate were found to be very higher and almost independent of the concentration. These results can be put in parallel with those previously reported, showing a correlation between the decrease of the glucose retention resulting from the addition of a salt and the retention of the added salt (Bargeman *et al*., 2005). More precisely, the less the salt retention is, the more the decrease of the glucose retention is. In the same manner, the glucose retention was found to remain constant when adding a completely retained anion. However, the results obtained in the present work are different fromt those previously obtained in similar conditions (Bouchoux *et al*., 2005). It that former work indeed, the glucose retention was found to decrease with the addition of Na_2SO_4. The difference might be explained considering the glucose and sodium sulphate retentions, which were lower in the former study compared to the present one. Then, due to the lower sulphate retention, the addition of sodium sulphate is expected to have a more pronounced effect on the glucose retention.

Bouchoux *et al*. (2005) proposed to use a variable α, which depends on the flux, to characterize the influence of the salt addition on the glucose retention. This parameter is expressed in the following equation:

$$\alpha_{glucose} = 1 - \frac{R'_{glucose}(J_V)}{R_{glucose}(J_V)} \quad (4.1)$$

$$\alpha_{glucose} = \frac{R_{glucose} - R'_{glucose}}{R_{glucose}} \quad (4.2)$$

Where R and R' are the retention in single-solute solution (glucose) and binary-solutes solutions (glucose and salt), respectively. The α value equal to 0 means no effect of salt on the glucose retention whereas α value greater than 0 means that the glucose retention is affected by the presence of salt. Table 4.1 gives the values of α obtained at a given flux (0.2×10^{-5} $m^3.s^{-1}.m^{-2}$) for binary solutions containing glucose and different salt compositions. The values from previous work (Bouchoux *et al*., 2005) are also reported for comparison. The values in presence of NaLac are higher than those of NaCl and Na_2SO_4, respectively. The values of $\alpha_{glucose}$ were plotted versus the salt concentrations as shown in Fig. 4.11. The graph shows much stronger effect of NaLac on glucose retention reduction compared to NaCl and Na_2SO_4, respectively. The values (α) in presence of NaLac are similar to the ones reported by Bouchoux *et al*., (2005). The values (α) of NaCl are lower than that observed by Bouchoux *et al*., (2005) as shown in Table 4.1.

Table 4.1 Decrease of the glucose retention ($\alpha_{glucose}$ as defined by equation 4.1) in presence of sodium salts (NaLac, NaCl, and Na_2SO_4) at different concentrations compared with 0.1M glucose in single-solute solutions

Na^+ concentrations	**$\alpha_{glucose}$**		
(M)	**+NaLac**	**+NaCl**	**+Na_2SO_4**
0M	0	0	0
0.1M	0.06	0	0.01
0.25M	0.27	0.038	0.01
0.5M	0.37 (*0.30*)	0.066 (*0.13*)	0.01
1M	0.62 (*0.42*)	0.066 (*0.25*)	0.01

Remark: results obtained for $J_v = 0.2\times 10^{-5}$ $m^3.s^{-1}.m^{-2}$; the values in the bracket are those results obtained by Bouchoux *et al*. (2005) at the same flux.

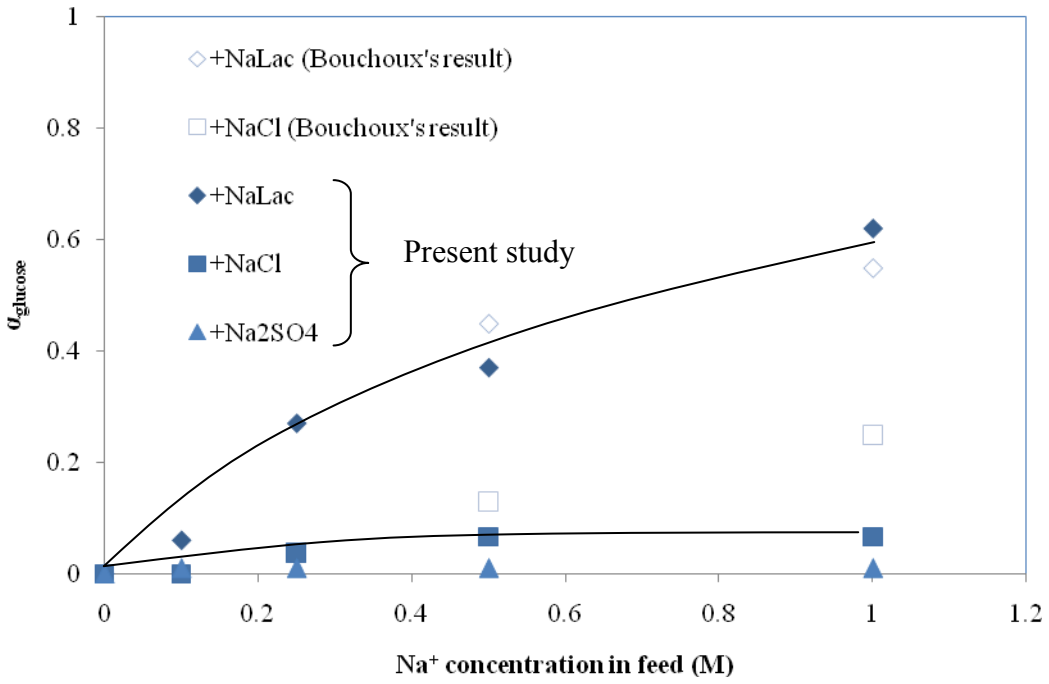


Figure 4.11 Variation of $\alpha_{glucose}$ as defined by equation 4.1 as function of the Na^+ concentration (NaLac, NaCl, and Na_2SO_4) - feed solutions containing 0.1M glucose - constant flux $J_v = 0.2\times10^{-5}$ $m^3.s^{-1}.m^{-2}$.

Since the effect of the addition of NaLac on glucose retention reduction is different from those of NaCl and Na_2SO_4 at similar Na^+ feed concentration. It appeares that the decrease of glucose retention depends on the salt concentration and the type of anion used. Bargeman *et al*., (2005) mentioned that the glucose retention reduction was correlated to the anion concentration in the permeate. Thus, the variations of glucose retention versus added anion concentration in the feed and in the permeate at different permeate fluxes are plotted in Fig. 4.12 to 4.15 for NaLac, NaCl and Na_2SO_4 respectively. As observed earlier, the glucose retention decreases with increasing anion concentration in the feed and permeate. In presence of NaLac, it shows a good tendency of glucose retention reduction in the range between 0.85 down to about 0.4. The correlation of observed glucose retention with lactate concentration in the permeate is slightly better than that with the concentration

in the feed. As already mentioned, the variations of the glucose retention with the addition of NaCl or Na_2SO_4 are much smaller compared to that of NaLac.

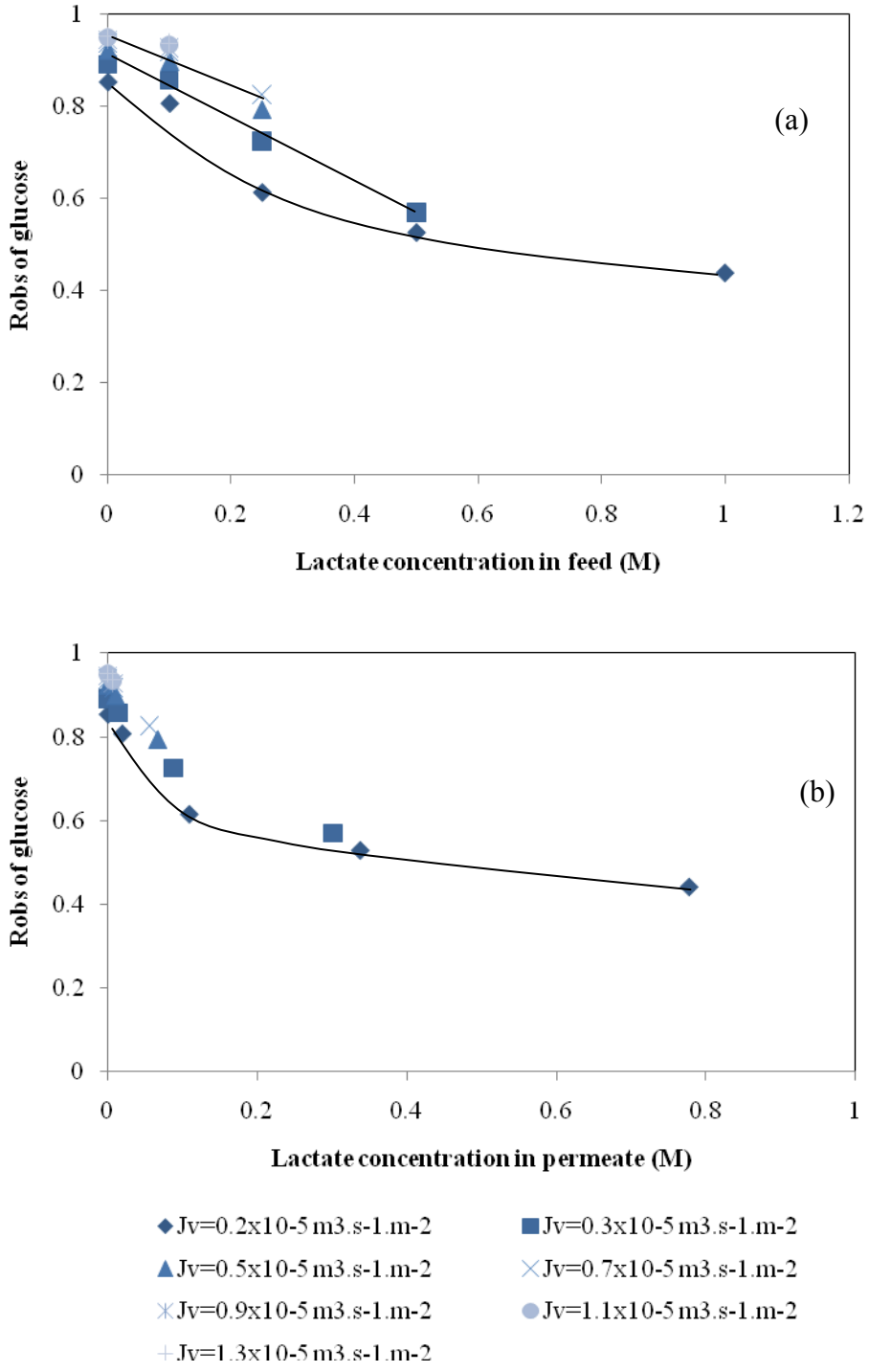


Figure 4.12 Retention of glucose as function of lactate concentration in the feed (a) and in the permeate (b) – influence of permeate flux (see legends) - feed solutions containing 0.1M glucose and 0.1to1M NaLac

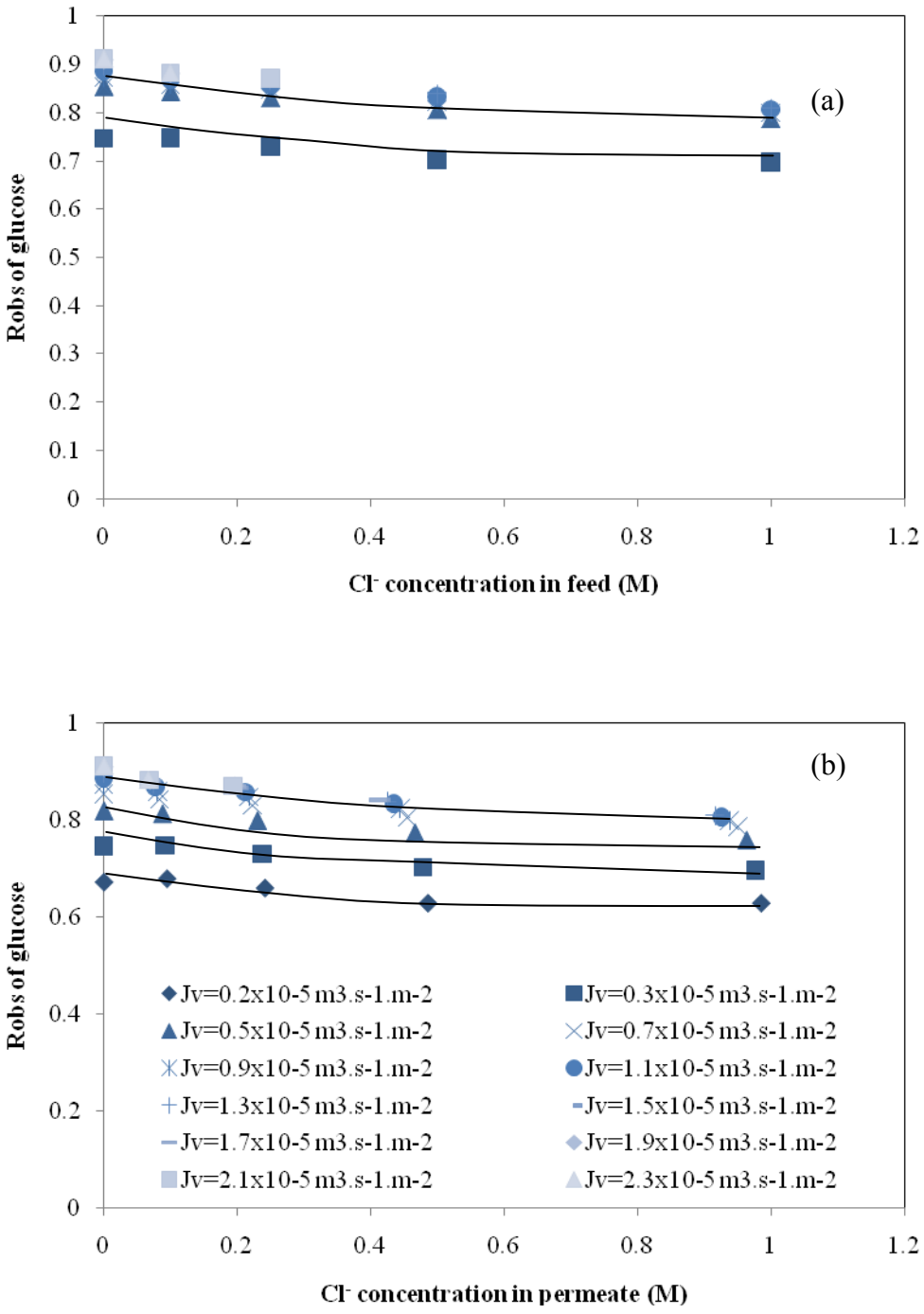


Figure 4.13 Retention of glucose as function of Cl^- concentration in the feed (a) and in the permeate (b) – influence of permeate flux (see legends) - feed solutions containing 0.1M glucose and 0.1to1M NaCl

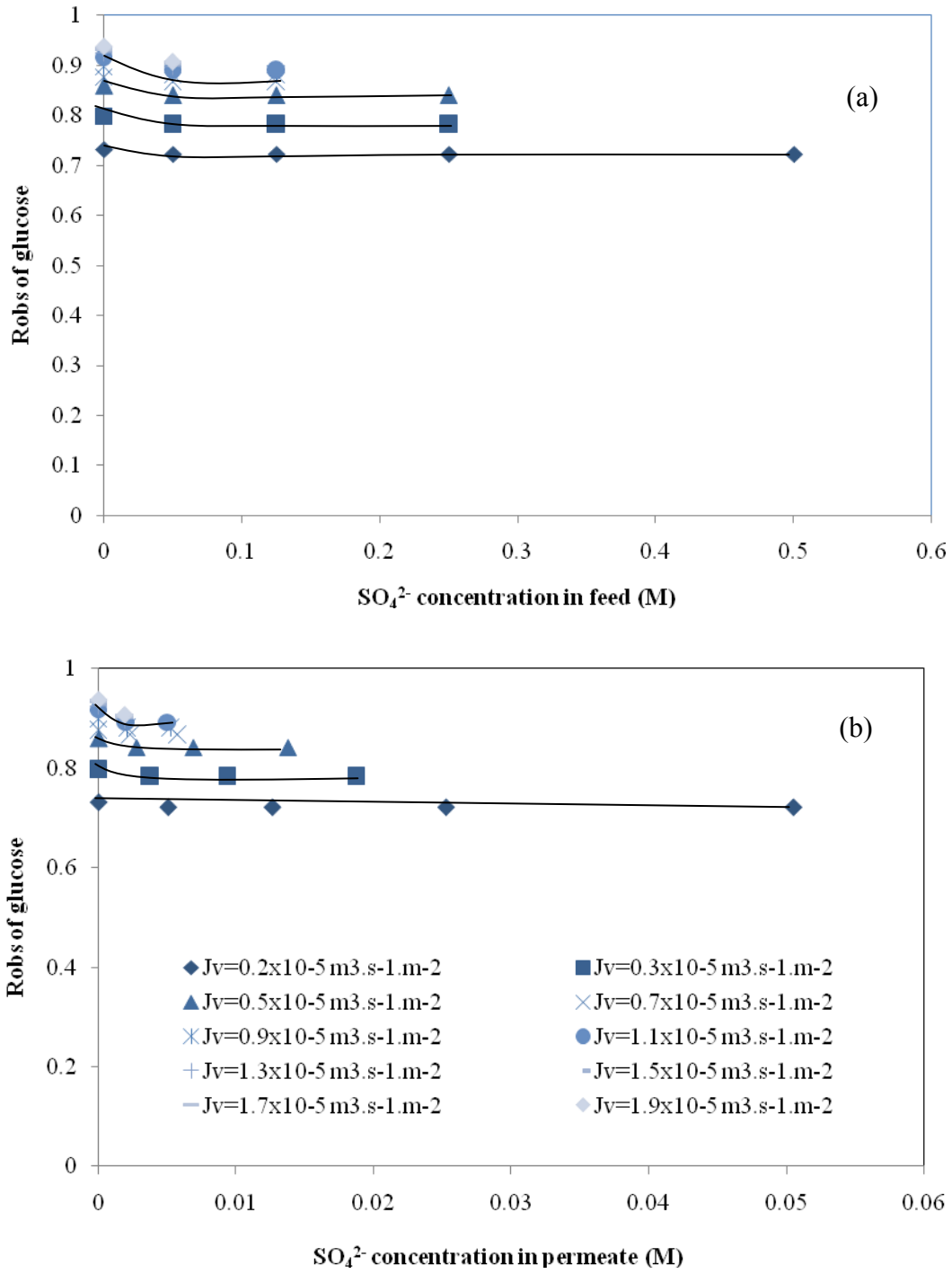


Figure 4.14 Retention of glucose as function of SO_4^{2-} concentration in the feed (a) and in the permeate (b) – influence of permeate flux (see legends) - feed solutions containing 0.1M glucose and 0.05 to 0.5M Na_2SO_4

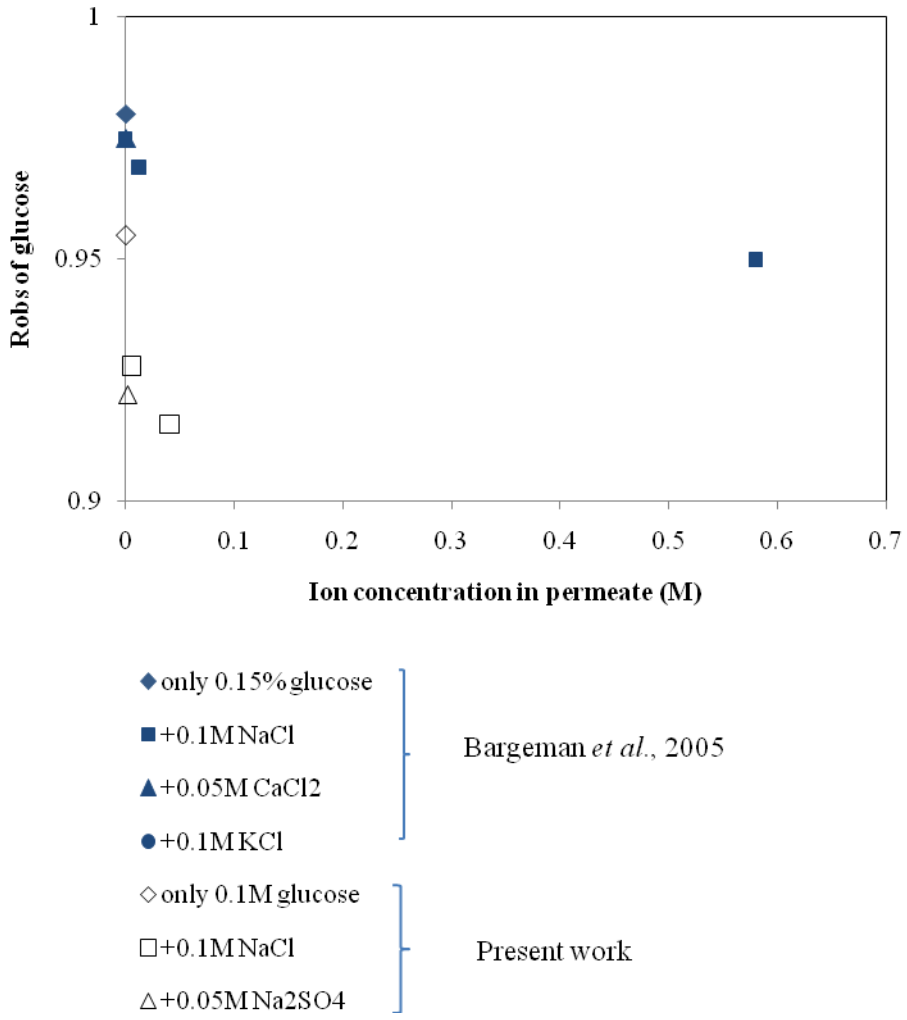


Figure 4.15 Retention of glucose as function of the salt concentration in the permeate at 2.0× 10^{-5} $m^3.s^{-1}.m^{-2}$ (see legends) - feed solutions containing either 0.1M glucose with 0.1M NaCl or 0.1M glucose with 0.05M Na_2SO_4 (see legends).

Fig. 4.15 shows the variation of glucose retention with the anion concentration in the permeate at different feed solutions. The added salt used in present work (empty symbols) were NaCl and Na_2SO_4 and those in Bargeman's work (Bargeman *et al*., 2005) (full symbols) were NaCl, $CaCl_2$ and KCl. The type of added salts between the two works are different that is Bargeman's work (2006) used the three salts that has different cations and seem anion (e.g. NaCl, $CaCl_2$, and KCl) whereas present work used the salts that has different anions and seem cation (e.g. NaCl and Na_2SO_4). As the cation and anion can not transport separately, they always

transport accoumpany due to the electroneutrality. Although three added salts with different cations were used in Bargeman's work whereas three different anions added salts were used in present work, the results from the both works are comparable. It was found that the retention of glucose in present work decreases with increasing anions concentration in permeate which is corresponded to those of Bargeman *et al*. (2005). The stronger effect of addition of salt on decrease of glucose retention was observed in our work compared to those in Bargeman's work. This might be explained that the experimental condition between Bargeman *et al*., (2005) and our work are different e.g. feed concentration of glucose and different type of salts used.

4.2.2 Influence of the addition of a salt on the lactate retention

The effect of the presence of another salt in feed solution on the lactate retention in binary-solute solutions is studied with different salts at different concentrations. The salt retentions were also measured to study the interaction between two electrolytes.

4.2.2.1 Influence of the addition of NaCl

Fig. 4.16 shows the variation of lactate retention versus the permeate flux in presence of NaCl. It is observed that the lactate retention decreases continuously with increasing NaCl concentration. Negative retention of Cl^- can be observed at low permeate flux. The retention of Cl^- first decreases to meet a minimum value and then increases with increasing permeate flux. The negative retention of Cl^- in presence of divalent ion such as SO_4^{2-} has been well documented (Krieg *et al*., 2004).

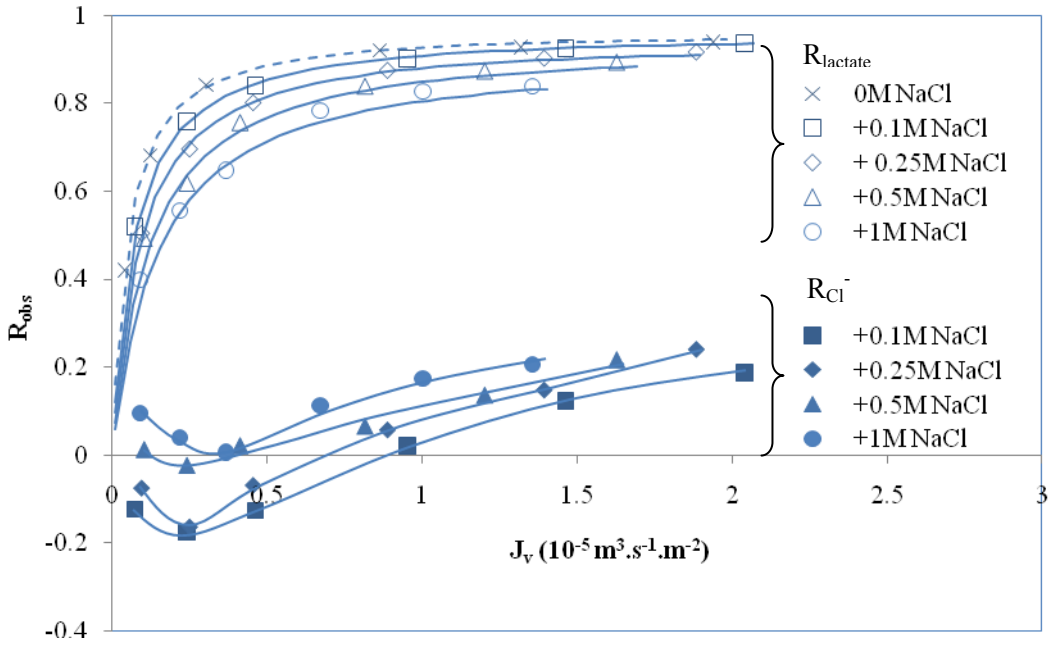


Figure 4.16 Retention of lactate and Cl^- as function of the permeate flux – influence of NaCl concentration (see legends) – feed solutions containing 0.1M NaLac and 0.1M to 1M NaCl.

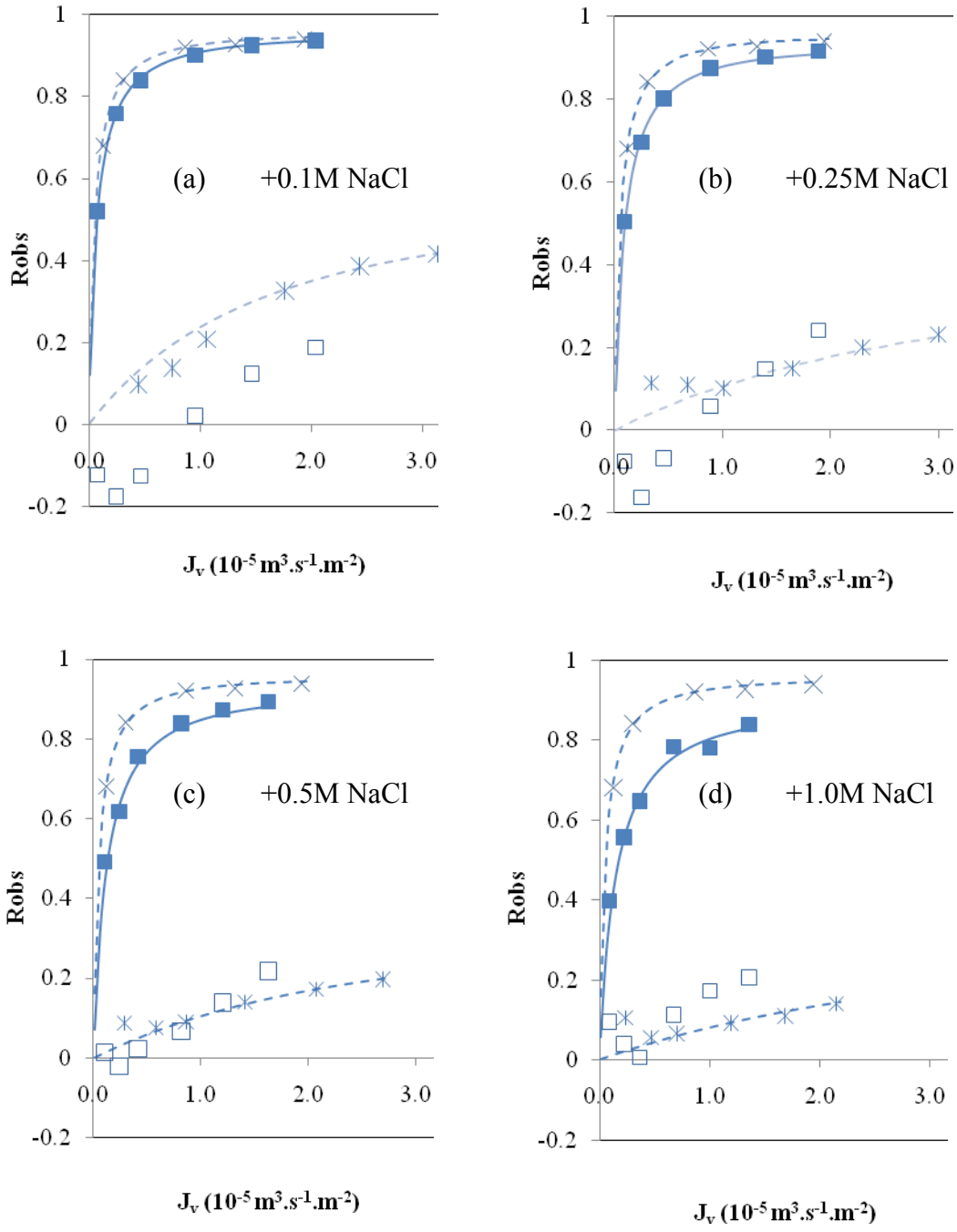


Figure 4.17 Retention of lactate and Cl^- as a function of the permeate flux - feed soluitons containing 0.1M NaLac and 0.1to1M NaCl. Single-solutions: (×) lactate and (*) Cl^-. Binary-solutions: (■) lactate and (□) Cl^-.

Fig. 4.17a shows the variation of lactate and Cl^- retentions in binary mixture that contains 0.1M NaLac and 0.1M NaCl. The retention of lactate is not different from that observed in single-solute solutions while the retention of Cl^- is lower than that of NaCl retention in single-solute solutions and the retention of Cl^- is negative at low flux. Fig. 4.17b shows the retention of lactate decreases in presence of 0.25M NaCl whereas the Cl^- retention increases. Fig 4.17c shows the Cl^- retention is less negative. Fig. 4.17d shows the lowest of lactate retention and Cl^- retention is positive at all permeate flux. It could be stated that the negative retention of Cl^- depends on concentration ratio between lactate and Cl^-.

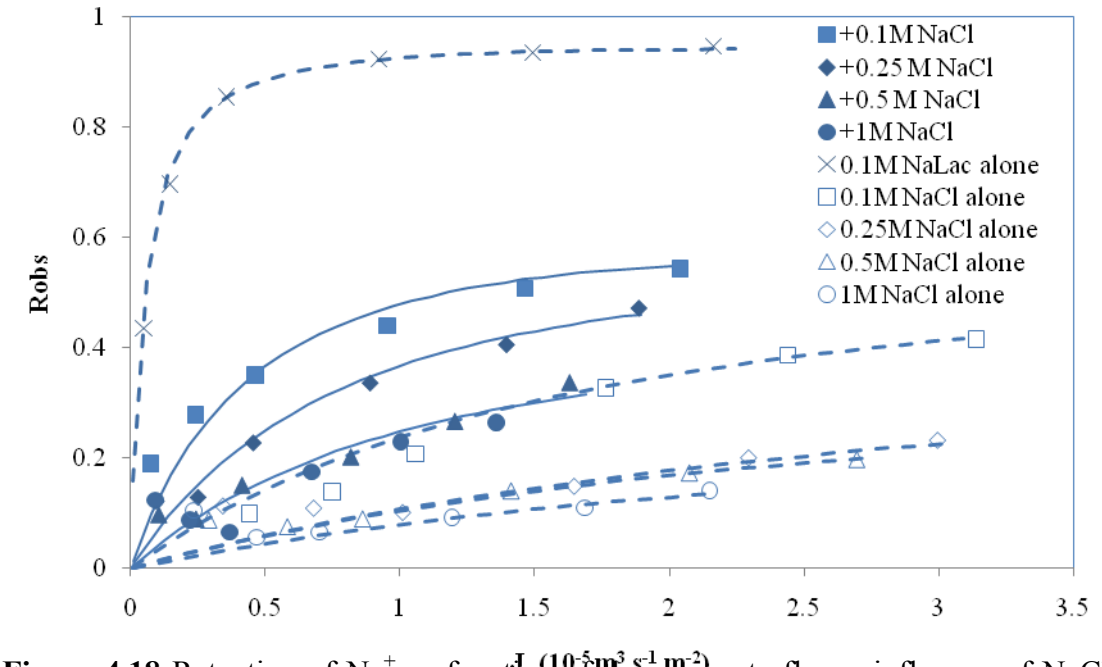


Figure 4.18 Retention of Na^+ as function of the permeate flux – influence of NaCl concentration (see legends) – feed solutions containing 0.1M NaLac and 0.1M to 1M NaCl.

Fig. 4.18 shows the variations of Na^+ retentions with increasing permeate flux in binary mixtures containing 0.1M NaLac and 0.1 to 1M NaCl. For the binary mixture containing 0.1M NaCl and 0.1M NaLac, the retentions of Na^+ which were obtained by both experimental measurement and mass balance calculation are

quite similar. The retention of Na^+ decreases with increasing NaCl concentration. The retention of Na^+ rapidly decreases when the low NaCl concentration contained in the feed solution. The decrease is more stabilized when high feed concentrations are filtrated. The decrease of Na^+ retention is in accordance with the decrease in the retention of lactate and Cl^- ions when more NaCl is added. Moreover, when the concentration of Na^+ is higher than 0.5M, the retention of Na^+ is not further decreased.

4.2.2.2 Influence of the addition of Na_2SO_4 on lactate retention

Fig. 4.19 shows the variation of lactate retention in addition of Na_2SO_4 at different Na_2SO_4 concentrations. The lactate retention decreases with increasing Na_2SO_4 concentration. The retention of SO_4^{2-} is independent of Na_2SO_4 concentration. Fig. 4.20a shows the retention of lactate in binary mixture that contains 0.1M NaLac and 0.05M Na_2SO_4 is lower than those observed in single-solute solutions whereas the retention of SO_4^{2-} is not different from those in single-solute solutions. Fig. 4.20b shows the retention of lactate in presence of 0.125M Na_2SO_4 decreases when more concentrated feed solution was carried out. Fig. 4.20c shows the negative retention of lactate in presence of 0.25M Na_2SO_4 in the feed solution at low flux. Finally, for increasing Na_2SO_4 concentration, the all retention of lactate is negative (Fig. 4.20d).

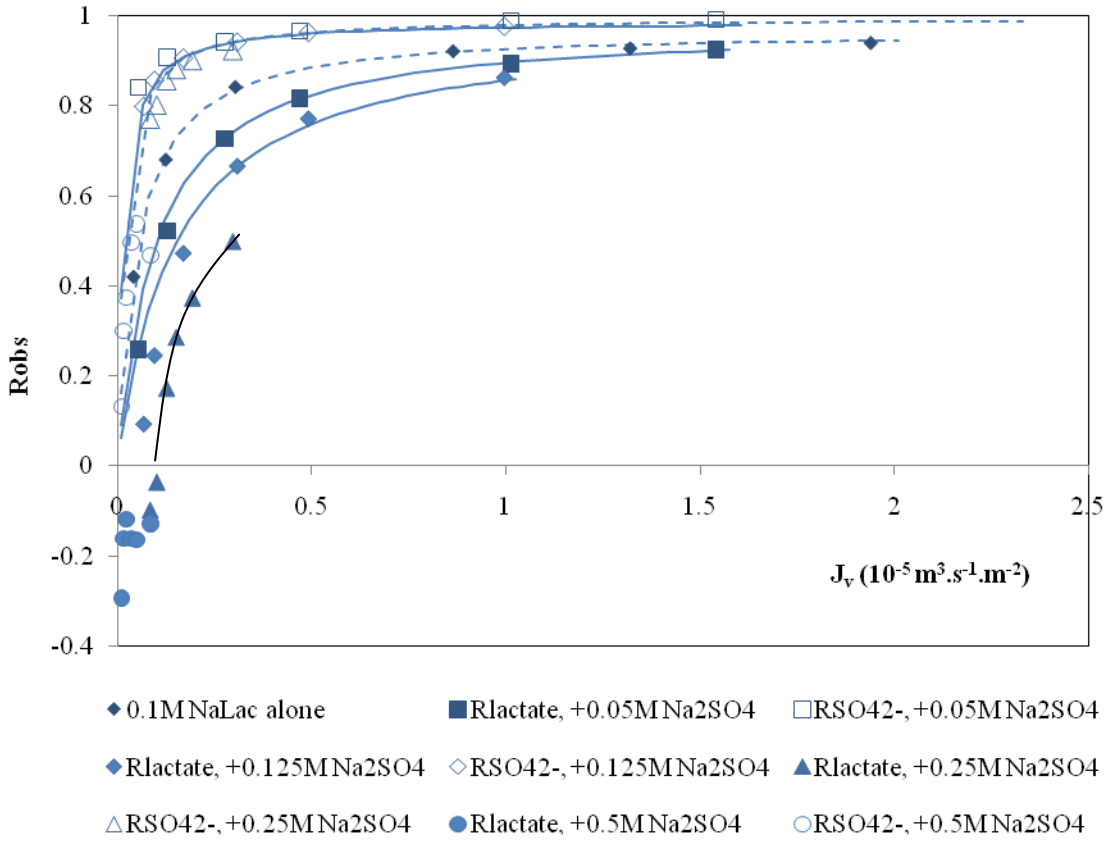


Figure 4.19 Retention of lactate (full symbols) and SO_4^{2-} (empty symbols) as function of the permeate flux – influence of Na_2SO_4 concentration (see legends) – fedd solutions containing 0.1M NaLac and 0.05M to 0.5M Na_2SO_4.

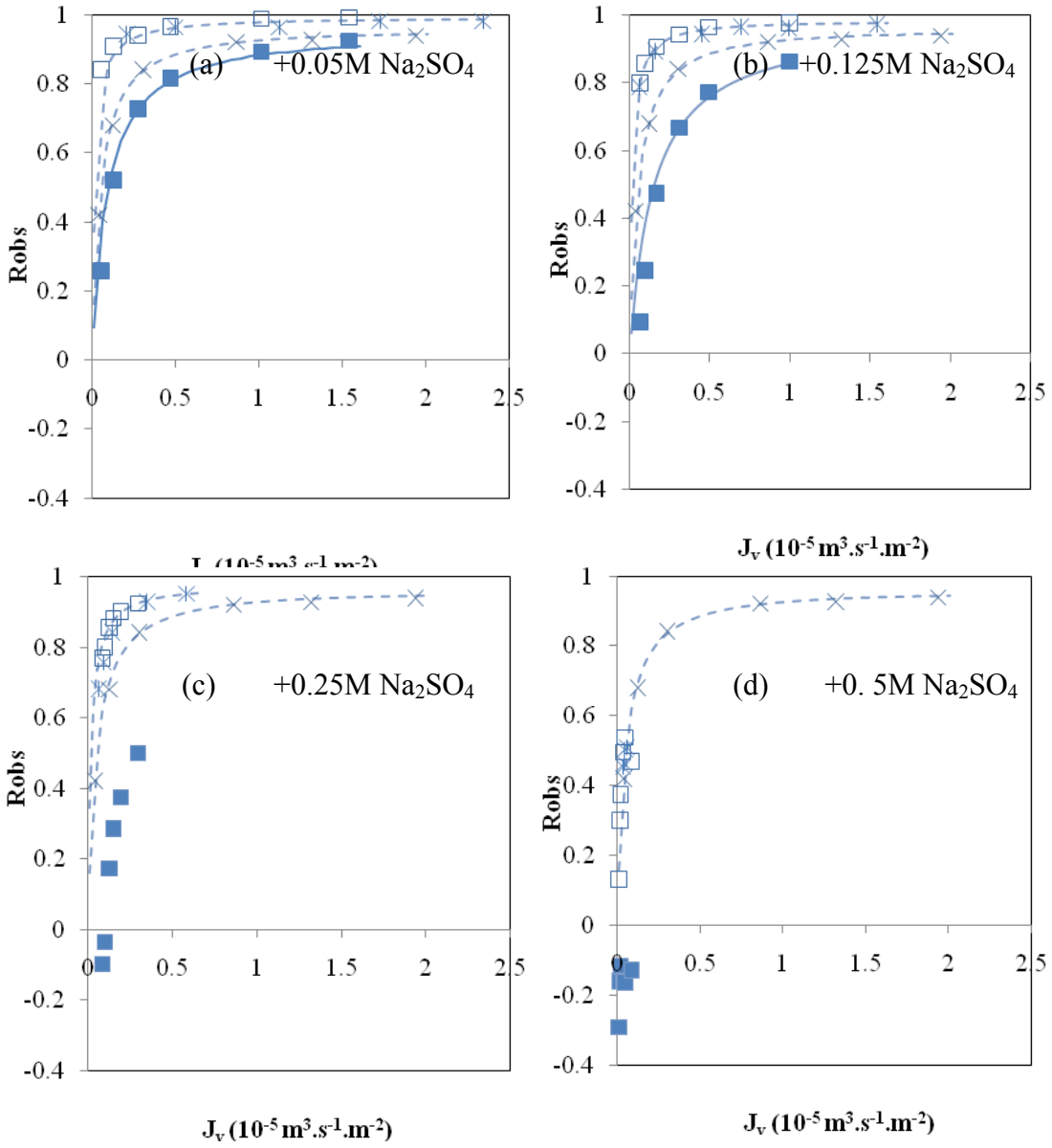


Figure 4.20 Retention of lactate and SO_4^{2-} as a function of the permeate flux – feed solutions containing 0.1M NaLac and 0.05to 0.5M Na_2SO_4: (×) lactate and (*) SO_4^{2-} retentions in single-solute solutions and (■) lactate and (□) SO_4^{2-} retention in feed binary mixtures.

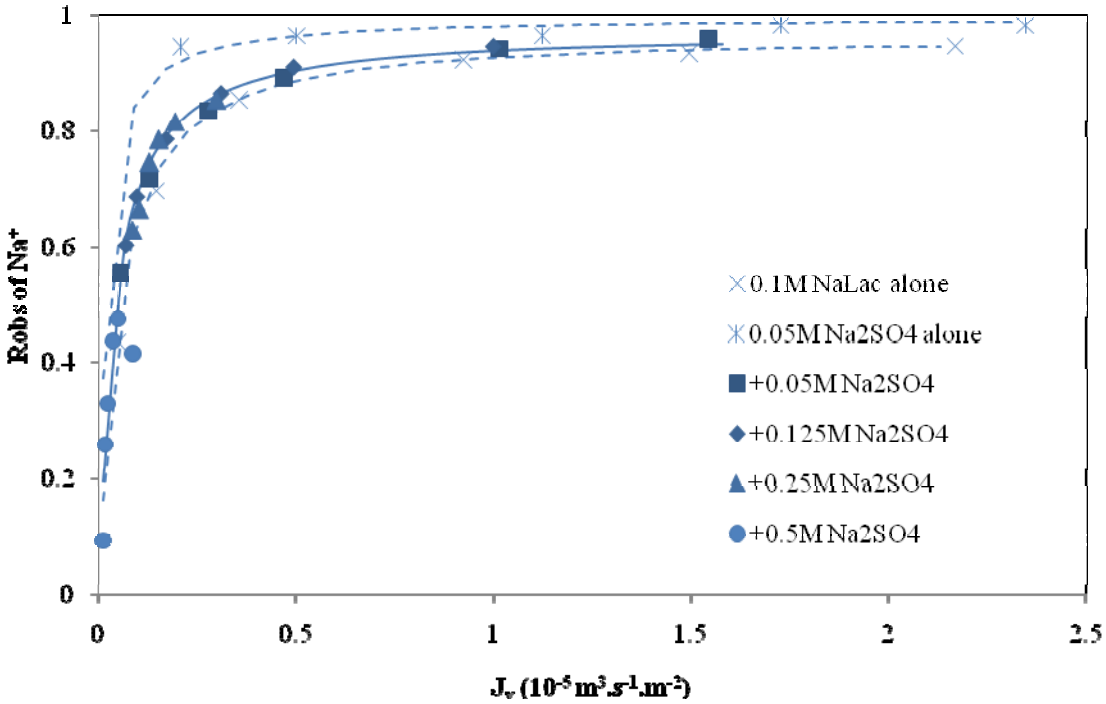


Figure 4.21 Retention of Na^+ (obtained by mass balance) as function of the permeate flux – influence of Na_2SO_4 concentration (see legends) – feed solutions containing 0.1M NaLac and 0.05M to 0.5M Na_2SO_4.

Fig. 4.21 shows the variation of Na^+ retention obtained by mass balance in binary mixtures containing NaLac and Na_2SO_4 with permeate flux. The Na^+ retention is rather independent of Na_2SO_4 concentration and is identical to 0.1M NaLac retention that observed in single-solute solutions.

4.2.2.3 Discussion

Fig. 4.16 shows that the lactate retention decreases with increasing NaCl concentration. This decrease is due to the screening effect. Indeed, it was demonstrated that at the corresponding NaLac concentration, i.e. 0.1M, the lactate retention is fixed by electrostatic repulsion due to the membrane charges. Then, the addition of a salt, like NaCl, which makes the electrostatic repulsion weaker, cuases lower lactate retention. On the other hand Fig. 4.16 shows that retentions of Cl^- are negative especially at low flux and having the lowest concentrations of NaCl.

Such negative retention means that the Cl^- concentration in the permeate is higher than that in the feed. Similar results were already reported in system such as solutions of two salts, sharing a common counter-ion e.g. NaCl and Na_2SO_4 (Bowen and Mukhtar, 1996; Mänttäri and Nyström, 2006; Tanninen *et al*., 2006). In such a case, negative retention of Cl^- are obtained because of the presence of SO_4^{2-} which is more, or even completely, retained by the membrane. In a solution of NaLac and NaCl, the lactate ions are repelled by negatively charged membrane (Desal 5DK) stronger than that of Cl^- because of the difference of their steric effects while the counter ions (Na^+) can pass through the membrane more easily. The excess concentration of Na^+ ions are obtained in the permeate. In order to maintain the electroneutrality on both sides of the membrane, Cl^- ions are continuously drown across the membrane to neutralize the charge imbalance and even the concentration of Cl^- ions in the permeate is higher than that in the feed. Thus, negative retention of Cl^- ions was obtained. All measured pH was in range of 6.5 - 7.0 so that the membrane charge was negative through out this work. This phenomenon can be described as follows.

In the situation investigated were, *i.e*. solution containing NaLac and NaCl, lactate is the more retained ion compared to Cl^- as observed in single salt solutions. In the other hand, Na^+ ions are only slightly retained. Then in order to maintain the electroneutrality, Cl^- ions are drawn to the permeate and the Cl^- concentration in the permeate can even be higher than that in the feed giving a negative retention of Cl^- ions. When the NaCl concentration increases, the lactate retention decreases as explained above. Then a higher concentration of lactate is present in the permeate and less amount of Cl^- ions is needed to neutralize the imbalance charge. As a result, the retention of Cl^- increases with the NaCl concentration.

. Fig. 4.19 gives the influence of the addition of Na_2SO_4 on the lactate retention. It shows that the lactate retention decreases in the presence of Na_2SO_4. On the other hand, the retention of SO_4^{2-} is very high and is independent of Na_2SO_4 concentration which is not different from that in the solution without NaLac. Moreover, negative values are obtained from the lactate retention at low flux for the highest concentrations. These results can be put in parallel with those obtained with the solution containing NaLac and NaCl. Indeed, negative retentions of the less retained anion i.e. lactate versus SO_4^{2-} in the situation considered in this paragraph, are pointed out. Kang *et al*. (2005) reported the negative retentoion of lactate in presence of succinate salt (divalent anion) using NF45 membrane. They purposed that the major factors providing the negative retention are concentration ratio between divalent anion and monovalent anion and the membrane surface charge. As the high concentration ratio and/or the low membrane surface charge, the negative retention is likely occurred. The negative retention in present work was obtained at the ratio between SO_4^{2-} and lactate was about 2.5 at low flux while Kang *et al*. (2006) reported the negative retention of lactate was at the ratio about 3.

The decrease of lactate retention in presence of NaCl and Na_2SO_4 can be also compared to that parameter previously used for glucose. For lactate, it is defined as:

$$\alpha_{lactate} = 1 - \frac{R'_{lactate}(J_V)}{R_{lactate}(J_V)} \quad (4.3)$$

$$\alpha_{lactate} = \frac{R_{lactate}(J_V) - R'_{lactate}(J_V)}{R_{lactate}(J_V)} \quad (4.4)$$

where R and R´ are the retention in single-solute solutions and binary-solute solutions, respectively. The values obtained for a given flux ($J_v = 0.2\times 10^{-5}$ $m^3.s^{-1}.m^{-2}$) are provided in Table 4.2. The lactate retention decreases in presence of NaCl. The maximum $\alpha_{lactate}$ in presence of NaCl is 0.32. The lactate retention strongly decreases in presence of Na_2SO_4. The maximum $\alpha_{lactate}$ in presence of Na_2SO_4 is 1.1.

Table 4.2 Decrease of lactate retention ($\alpha_{lactate}$ as defined by equation 4.3) in presence of other salts (NaCl and Na_2SO_4) at different concentrations compared with 0.1M lactate in single-solute solution

Na^+ concentrations	**$\alpha_{lactate}$**	
	+NaCl	**+Na_2SO_4**
0M	0	0
0.1M	0.08	0.28
0.25M	0.15	0.38
0.5M	0.24	0.61
1M	0.32	1.08

Remark: these results obtained at $J_v = 0.2 \times 10^{-5}$ $m^3.s^{-1}.m^{-2}$

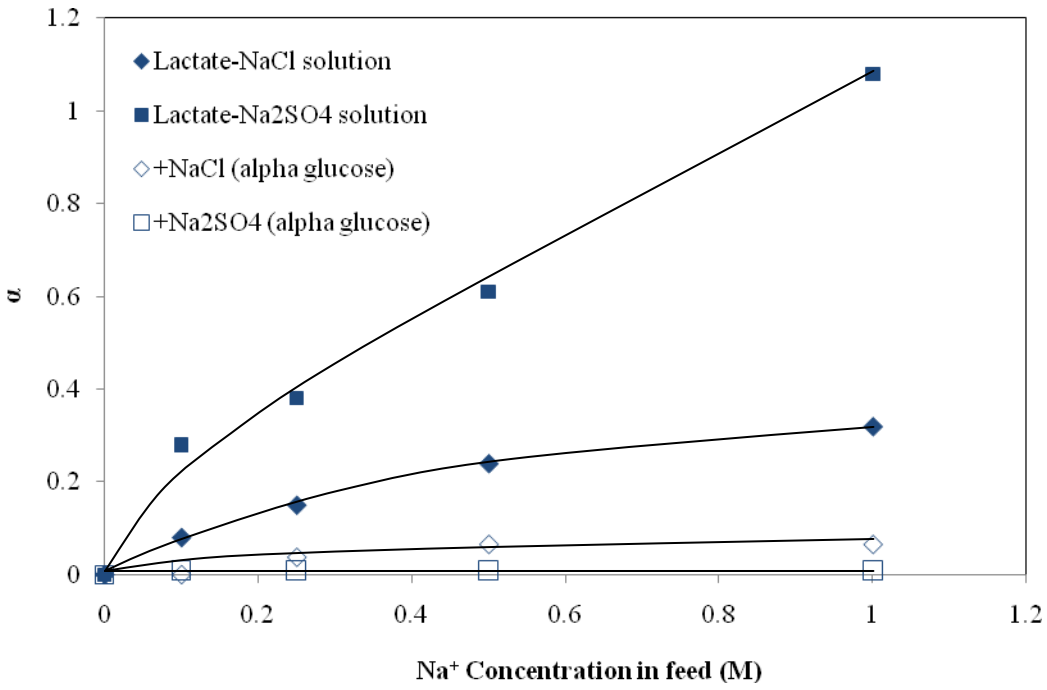


Figure 4.22 Variation of $\alpha_{lactate}$ as defined by equation 4.2 (full symbols) and $\alpha_{glucose}$ as defined by equation 4.1 (empty symbols) as function of the sodium salt concentration (NaCl and Na_2SO_4) – feed solutions containing either 0.1M lactate or 0.1M glucose with 0.1 to 1M NaCl and 0.05 to 0.5M Na_2SO_4 -constant flux $J_v = 0.2 \times 10^{-5}$ $m^3.s^{-1}.m^{-2}$.

Fig. 4.22 shows the variation of α value of both glucose and lactate versus Na^+ concentration. The graph indicates the difference effect of adding Cl^- or SO_4^{2-} salt on lactate and glucose retentions, which is expressed in α value. The α value represents the pronounce of adding salt effect on glucose and lactate retentions in which the higher α value means stronger effect of added salt on glucose or lactate retention. The $\alpha_{lactate}$ is higher than that of $\alpha_{glucose}$ can be observed. It can be mentioned here that the effect of adding NaCl and Na_2SO_4 is more effective on lactate retention than that of glucose. Especially, the difference between the both α values in adding of Na_2SO_4 is much higher than that of adding NaCl. The maximum differences

between α value of glucose and lactate are 1.07 and 0.31 in adding Na_2SO_4 and NaCl, respectively, obtained at 1M Na^+ concentration in feed as shown in Fig. 4.22. The difference in magnitude of decrease of glucose and lactate retentions in presence of NaCl might be able to improve their separation when the three solutes are present together in ternary-solute solution.

The decreasing of lactate retention is a function of total anion (lactate and Cl-) concentration in the feed and permeate (Fig. 4.23).

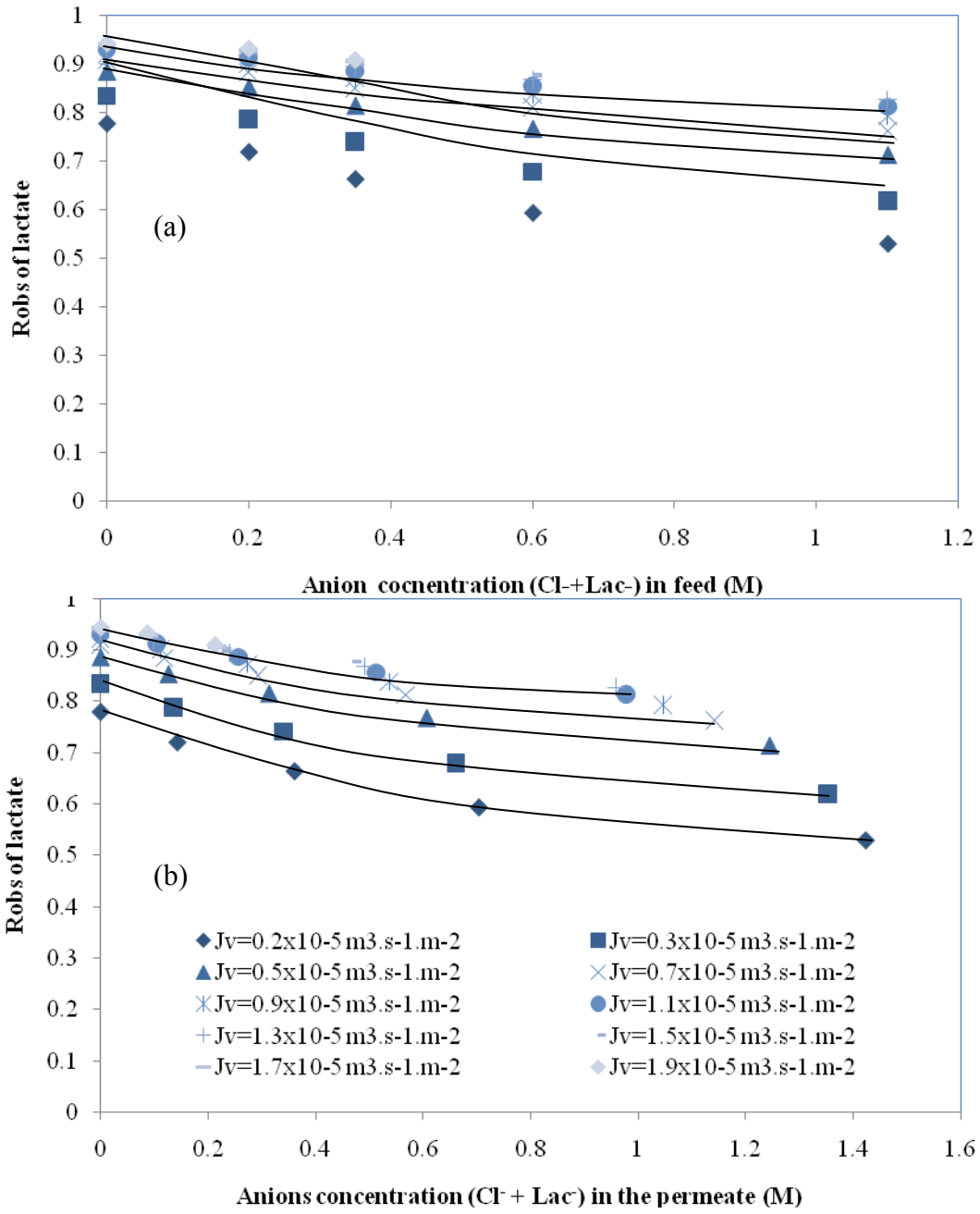


Figure 4.23 Retention of lactate as function of total anion concentration in the feed (a) and in the permeate (b) - influence of permeate flux (see legends) - feed solutions containing 0.1M NaLac and 0.1to1M NaCl

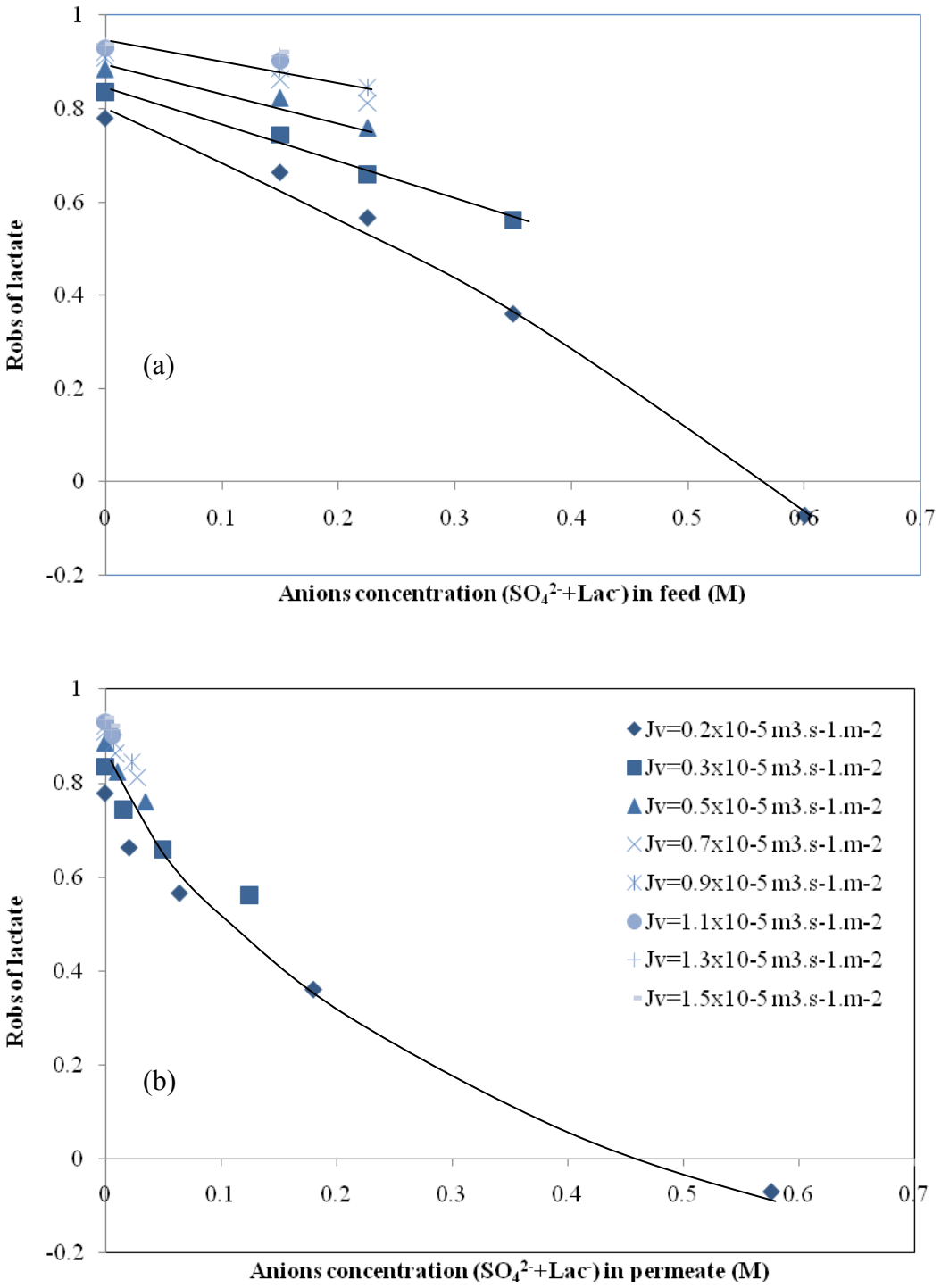


Figure 4.24 Retention of lactate as function of total anion concentration in the feed (a) and in the permeate (b) - influence of permeate flux (see legends) - feed solutions containing 0.1M NaLac and 0.05to0.5M Na_2SO_4

The addition of Na_2SO_4 causes much decrease of lactate retention. It can be observed that the decline of lactate retention in adding of Na_2SO_4

is stronger than those of NaCl (Fig. 4.24). The maximum decline of lactate retention in adding Na_2SO_4 is 85% with total anion concentration in the permeate and in the feed. The negative retention of lactate can be observed at high anion concentration in the permeate. The variation of lactate retention with anion concentration in permeate is more correlated than that in retentate.

From the results obtained from the retentions of lactate and glucose in binary solutions, one can calculate the separation factor, as expected in equation 2.5. The separation factor between lactate and glucose in binary-solute solutions was plotted versus permeate flux is shown in Fig. 4.25. The separation factor initial increases to meet a maximum and then decreases with increasing permeate flux. The separation factor of binary mixture containing high NaLac concentration is higher than those in presence of low NaLac concentration. Maximum separation factor is 1.43 in presence of the highest NaLac concentration in the feed. At addition 0.5M NaLac, separation factor almost reaches to maximum value (≈1.4) at permeate flux around 0.3×10^{-5} $m^3.s^{-1}.m^{-2}$ that can be achieved in the conditions investigated as shown in Fig. 4.26. It can be concluded that the separation between glucose and lactate is feasible in particular conditions i.e. maintaining low permeate flux (but not too low) and using high lactate concentration.

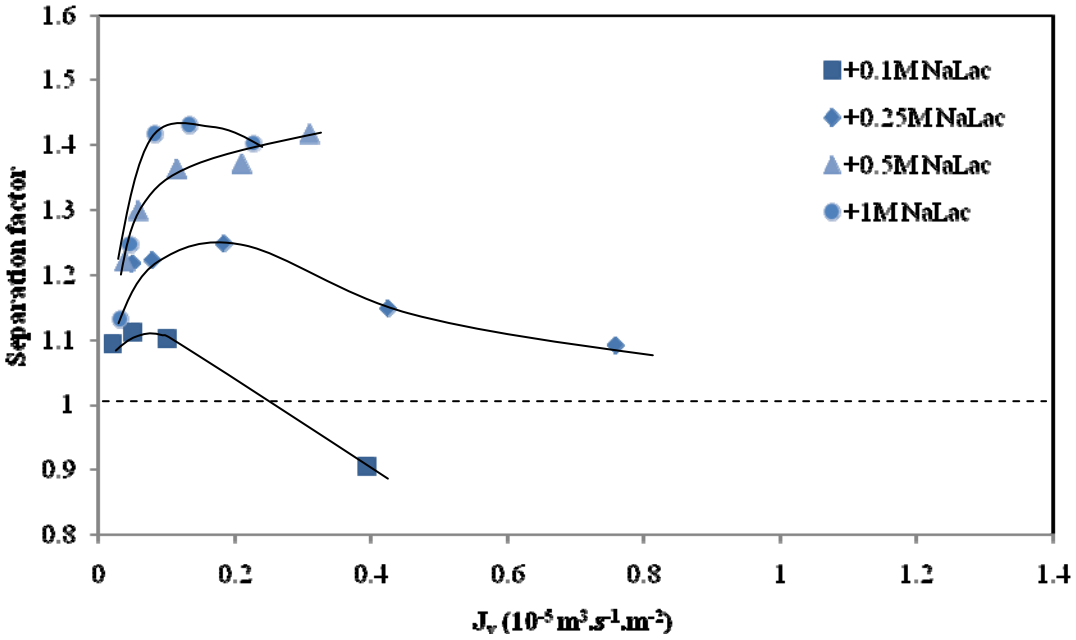


Figure 4.25 Separation factor of lactate as function of the permeate flux – influence of NaLac concentration (see legends) – feed solutions containing 0.1M glucose and 0.1 to 1M NaLac.

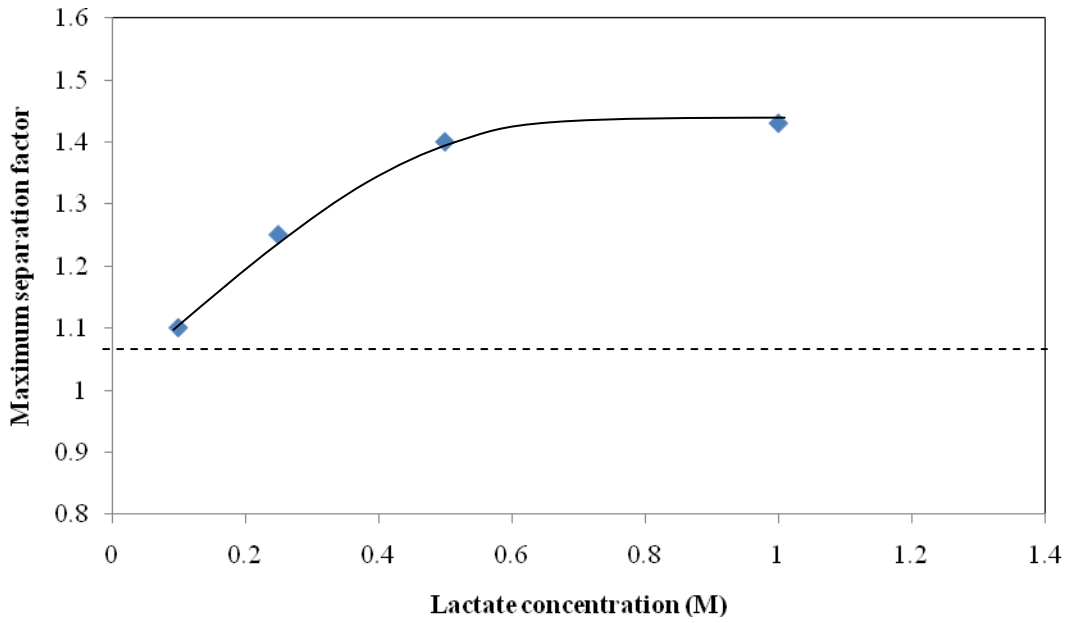


Figure 4.26 Maximum separation factor of lactate as function of lactate concentration in feed binary mixtures containing 0.1M glucose and 0.1to 1 M NaLac.

4.2.3 Conclusions

It was observed that the glucose retention is always lower when a salt is present in solution. The corresponding decrease depending on the salt is in the following order: NaLac > NaCl > Na_2SO_4 at the same Na^+ concentration. The decrease of glucose is more important with increasing the salt concentration. Different explanations were considered for the decrease of glucose retention *i.e*. pore swelling and/or decrease of glucose hydrodynamic radius in the presence of salt. The addition of Na_2SO_4 was found to slightly change the retention of glucose, while sulphate was almost totally retained by the membrane. This result is in accordance with those reported by Bargeman *et al*. (2005) showing that the glucose retention was slightly affected in presence of $CaCl_2$. The range of variation of glucose retention in this work was ranging between 0.98 (glucose alone) and 0.43 (presence of 1M NaLac) at the permeate flux of 2×10^{-5} $m^3.s^{-1}.m^2$.

The effect of the addition of salts on the lactate retention was also investigated. It was observed that the retention of lactate decreases when adding amounts of NaCl or Na_2SO_4. The effect of the addition salt on the lactate retention was found to be significant in the order: Na_2SO_4 > NaCl. A negative retention of Cl^- was observed at the high lactate concentration used and low flux. On the other hand, a negative retention of lactate was observed in presence of high Na_2SO_4 concentrations at low flux. Such results are in accordance with previous ones. Indeed, it was repeated that for solutions containing different ions can be obtained for the less retained one this was the case in the present for Cl^- in the NaLac, NaCl mixture and lactate in Nalac, Na_2SO_4 mixture.

Finally, the results have shown that the effect of addition NaCl or Na_2SO_4 on glucose and lactate retentions in binary mixtures are different. It was found to have a greater influence on the retention of lactate than the one of glucose.

4.3 Impact of the addition of an electrolyte on the separation between glucose and lactate

In this section, we study the effect of the addition of an electrolyte on the separation between glucose and lactate. Experiments are thus carried out with ternary-solute solutions containing glucose, lactate and other salts, at constant feed concentration.

It was shown in the former section that the separation was hardly achievable for a binary solution at 0.1M glucose and 0.1M lactate. Indeed, the maximum separation factor did not exceed 1.1. On the other hand, it was pointed out that the addition of a salt changes the glucose and lactate retentions but in a different manner. A better separation factor can thus be expected when adding a salt, as will be checked in this section.

Experiments are also performed with a higher lactate concentration, 0.5M for which a better separation factor (up to 1.4) was obtained in binary solutions. Again, we will investigate to what extend the addition of a salt can improve the separation.

The results concerning the retentions of glucose and lactate are first presented. Then the separation factor is calculated to evaluate the impact of the addition of salt on glucose/lactate separation.

4.3.1 Impact of the addition a salt on the glucose and lactate retentions

The influence of the addition of NaCl is first investigated. Fig. 4.27 shows the variations of glucose and lactate retentions versus permeate flux obtained with feed solutions containing 0.1M glucose, 0.1M NaLac and different NaCl concentrations (0.1 to 1M). It can be observed that the retentions of glucose and lactate decrease with increasing NaCl concentration. The decrease is more pronounced with lactate (up to 20%) than with glucose (up to 10%).

Fig. 4.28 shows the variation of Cl^- retention in the ternary-solute solutions containing 0.1M glucose, 0.1M lactate and 0.1 to 1M NaCl with the permeate flux. The retention of Cl^- first decreases to negative values at low flux to meet minimum value and then increases and becomes positive at flux greater than 0.6×10^{-5} $m^3.s^{-1}.m^{-2}$. The retention of Cl^- is less negative with increasing NaCl concentration. Moreover, the retention of Cl^- in the ternary solutions is similar to those obtained with the bianry solutions whereas the lower retention of ternary solution is observed at 1M NaCl.

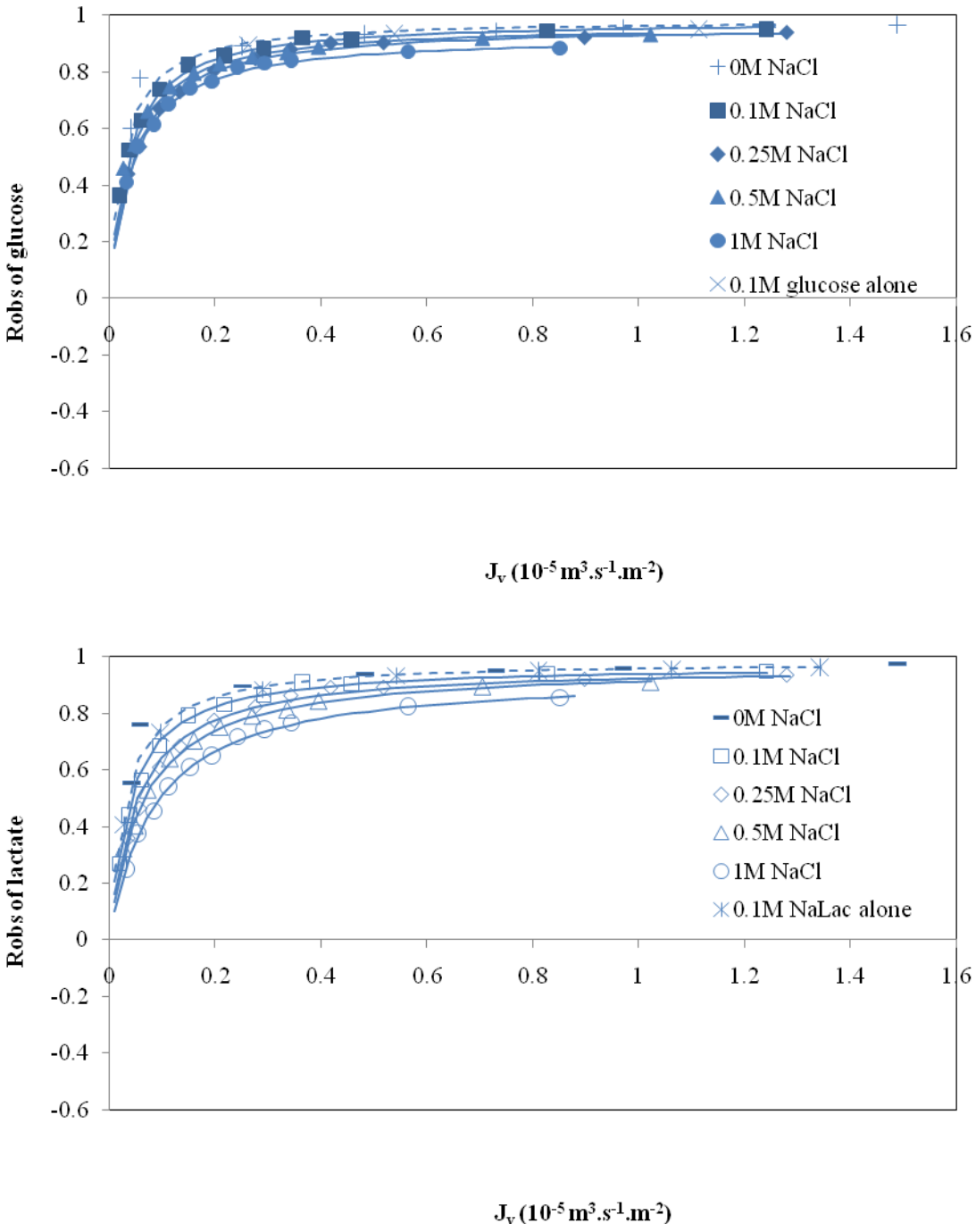


Figure 4.27 Retention of glucose (top) and lactate (bottom) as function of the permeate flux - influence of NaCl concentration - feed solutions containing 0.1M NaLac, 0.1M glucose and 0.1 to 1M NaCl.

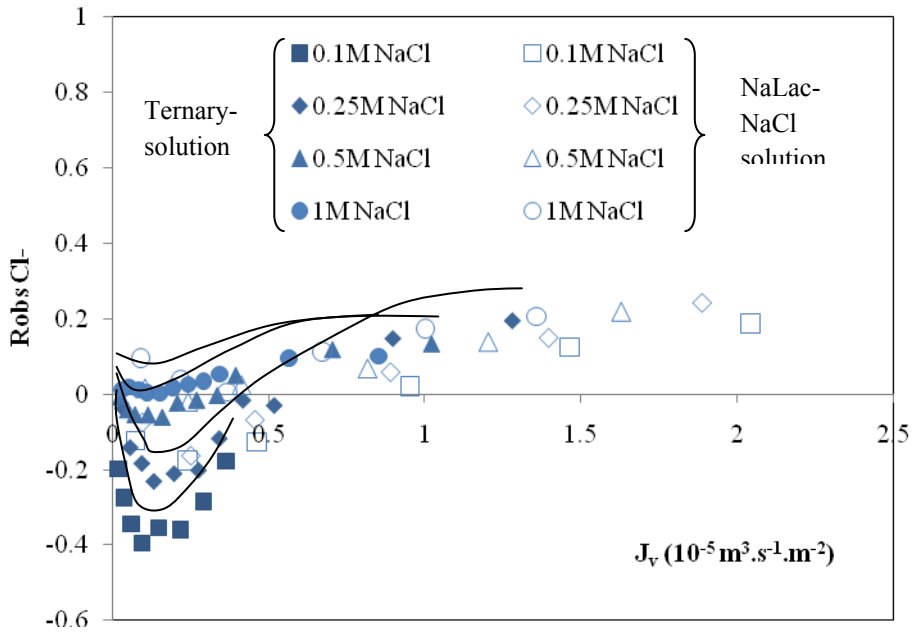


Figure 4.28 Retention of Cl^- as function of the permeate flux – influence of NaCl concentration - feed solutions containing 0.1M NaLac, 0.1M glucose and 0.1M to 1M NaCl.

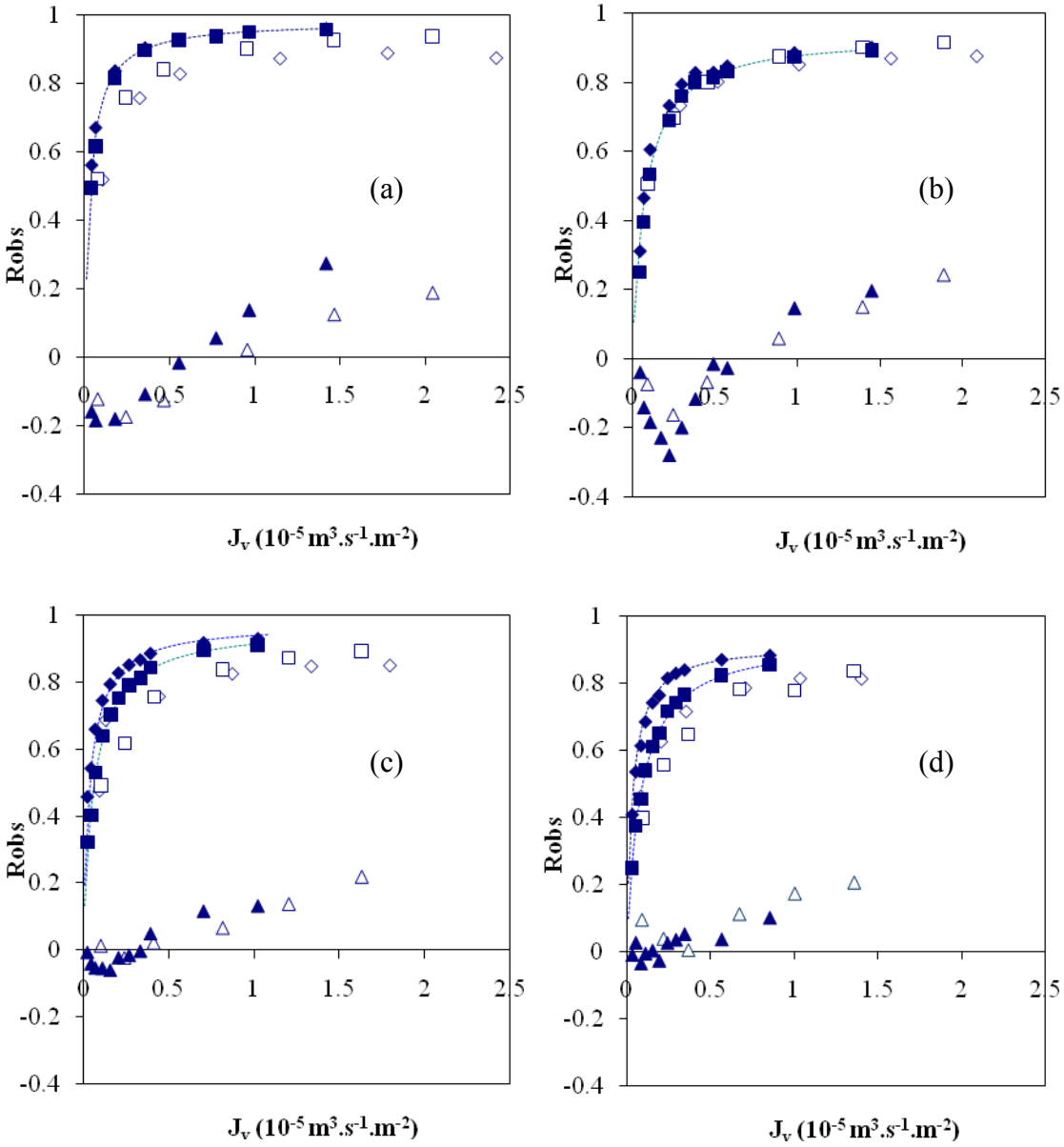


Figure 4.29 Retention of glucose, lactate and Cl^- as function of the permeate flux - influence of NaCl concentration - feed solutions containing 0.1M glucose, 0.1M NaLac and NaCl: 0.1M (a) 0.25M (b) 0.5M (c) 1M (d). Binary solutions: (◊) glucose for 0.1M glucose and 0.1-1M NaCl, (□) lactate for 0.1M lactate and 0.1 to 1M NaCl, and (Δ) Cl^- for 0.1M lactate and 0.1 to 1M NaCl. Ternary solutions: (♦) glucose, (■) lactate, and (▲) Cl^-.

Fig. 4.29 shows the glucose and lactate retentions obtained with ternary-solute solutions containing 0.1 to 1M NaCl. In order to focus the effect of adding NaCl on glucose and lactate retentions in ternary-solute solutions, the glucose retention is compared to those obtained with binary-solute solutions containing 0.1M glucose and 0.1 to 1M NaCl whereas the lactate retention is compared to those obtained with binary-solute solutions containing 0.1M lactate and 0.1 to 1M NaCl. The both results obtained from ternary-solute solutions and binary-solute solutions are comparable; however, the retentions of glucose and lactate obtained with ternary-solute solutions are slightly higher than those obtained with binary-solute solutions. It might be because the both results were not obtained with the same piece of membrane. Moreover, the difference between glucose and lactate retention is more obvious in presence of 0.5M NaCl (Fig. 4.29c) and 1M NaCl (Fig.4.29d) at flux below 0.1×10^{-5} $m^3.s^{-1}.m^{-2}$.

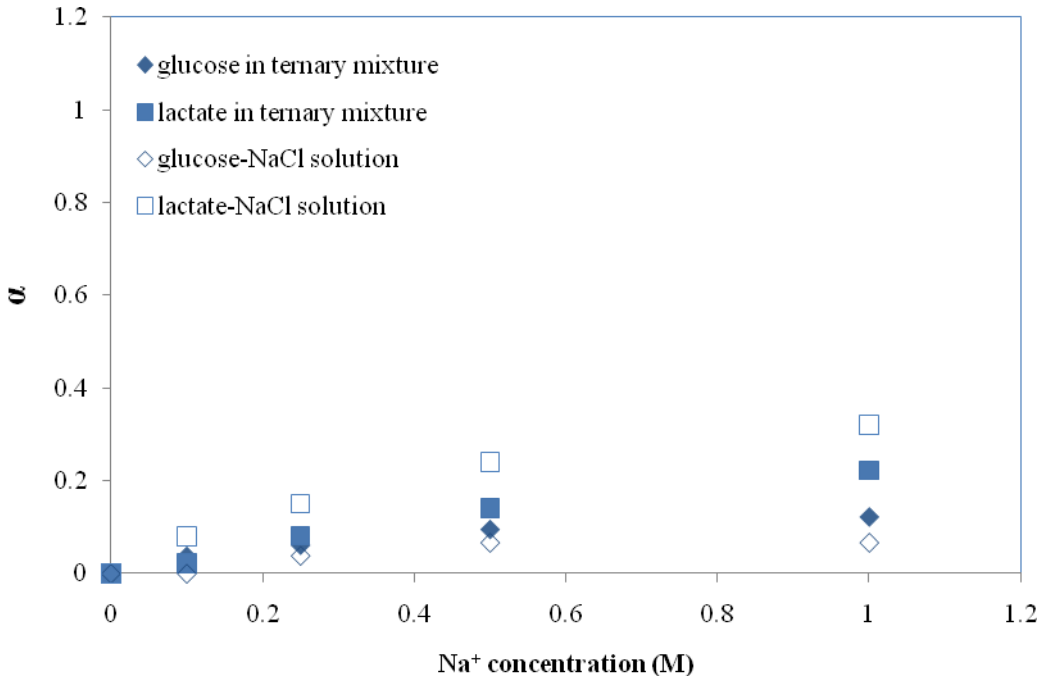


Figure 4.30 Variation of $\alpha_{glucose}$ and $\alpha_{lactate}$ as function of Na^+ concentration in feed solutions containing 0.1M NaLac, 0.1M glucose and 0.1M to 1M NaCl at 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$.

In order to have a quantitative evaluation of the influence of the addition of NaCl on the solute retentions, the values of the parameter α, as defined by equation 4.1 and 4.2, respectively (Note: R´ means retention obtained with ternary-solute solutions in this case). Fig. 4.30 shows the corresponding variations of $\alpha_{glucose}$ and $\alpha_{lactate}$ versus Na^+ concentration. As expected, higher values are obtained for $\alpha_{lactate}$ compared to $\alpha_{glucose}$; because of the higher sensibility of the lactate retention to the salt concentration. Moreover, the $\alpha_{glucose}$ and $\alpha_{lactate}$ values in ternary-solute solutions are accordance with those observed in binary-solute solutions. The difference does not exceed 10%. The maximum difference between $\alpha_{glucose}$ and $\alpha_{lactate}$ values of ternary-solute solutions is about 0.1 obtained at Na^+ concentration as 0.1M.

The influence of the ionic composition on the separation glucose/lactate is also studied for a higher lactate concentration. The experiments are carried out at 0.5M lactate concentration because the highest separation factor for glucose/lactate separation in binary solution is reached at this condition (SF_{max} = 1.4 see Fig. 4.26).

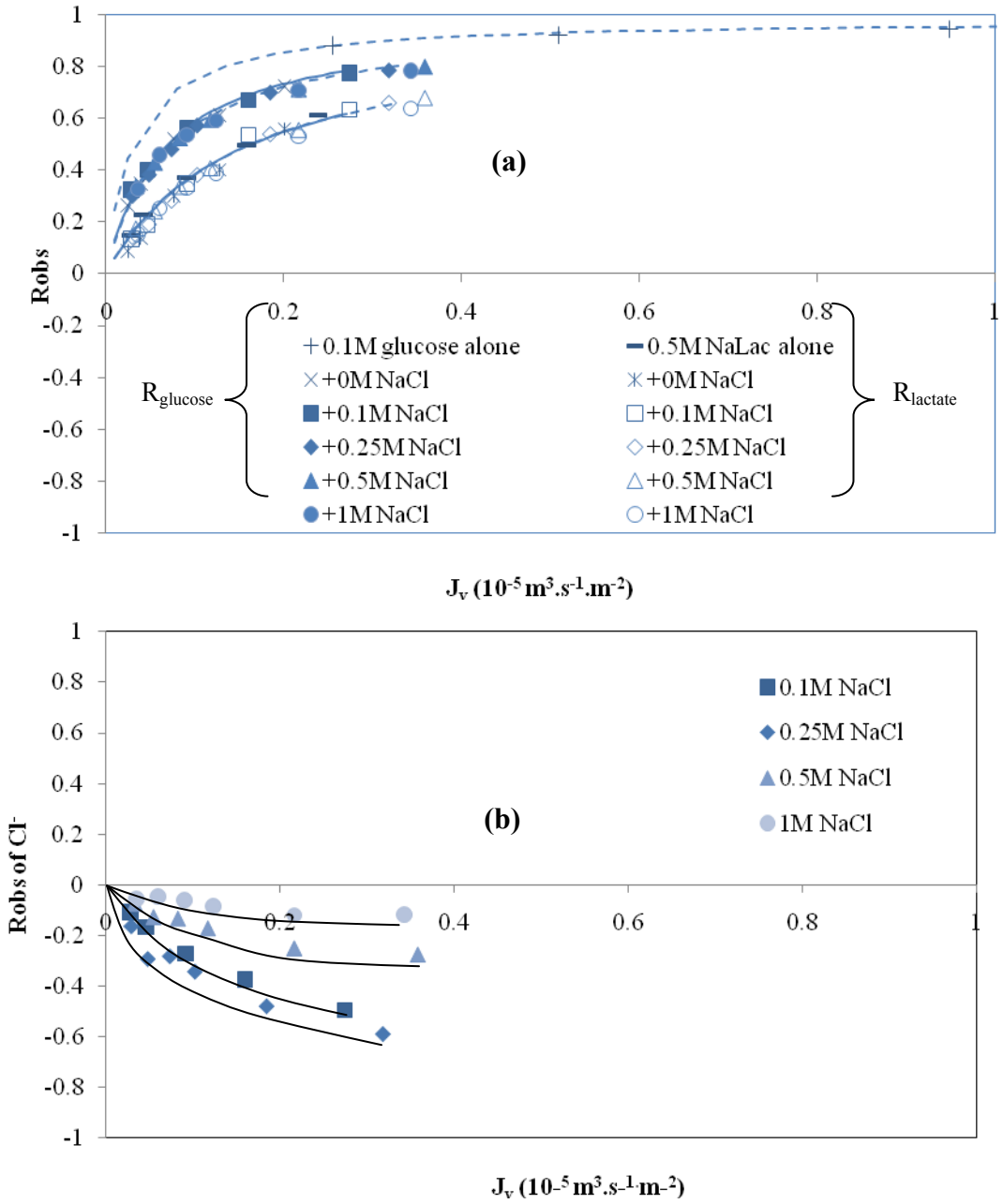


Figure 4.31 Retention of glucose and lactate (a) and Cl^- (b) as function of the permeate flux – influence of NaCl concentration - feed solutions containing 0.5M NaLac, 0.1M glucose and 0 to 1M NaCl.

Fig. 4.31a shows the variations of the glucose and lactate retentions versus the permeate flux for increasing concentrations of NaCl. One can observe that the glucose retention does not change when NaCl is added in the solution. It remains equal to the value obtained with the binary mixture NaLac/glucose, this value being

lesser than that obtained with a single solution of glucose. Then, once NaLac at 0.5M is present in the solution, further addition of NaCl has not effect on the glucose retention. This result can be explained by the presence of 0.5M NaLac in ternary-solute solutions which nearly attained the maximum effect of pore swelling and/or the decrease of glucose hydrodynamic radius. Thus, glucose retention is not affected by the addition of NaCl.

In the same manner, it is shown that the lactate retention remains constant. These results are different from those obtained with the lower lactate concentration, where retentions decrease with the addition of NaCl. They can be explained by weaker electrostatic interactions at 0.5M of lactate. Indeed, it has been previously observed that the lactate retentions at 0.5 and 1M are close because the electrostatic repulsions are negligible for lactate concentrations higher than 0.5M (see Fig. 4.5 section 4.2.1.1). Then, for higher salt concentration, the lactate retention is fixed by steric effect. Consequently, the separation of glucose (0.1M) and lactate (0.5M) cannot be improved since the addition of NaCl does not change the retentions of glucose and lactate.

Fig. 4.31b shows the corresponding variations of Cl^- retention with the permeate flux. It can be stated that the Cl^- retention is always negative and decreases with increasing permeate flux. Also, it becomes less negative with increasing NaCl concentration.

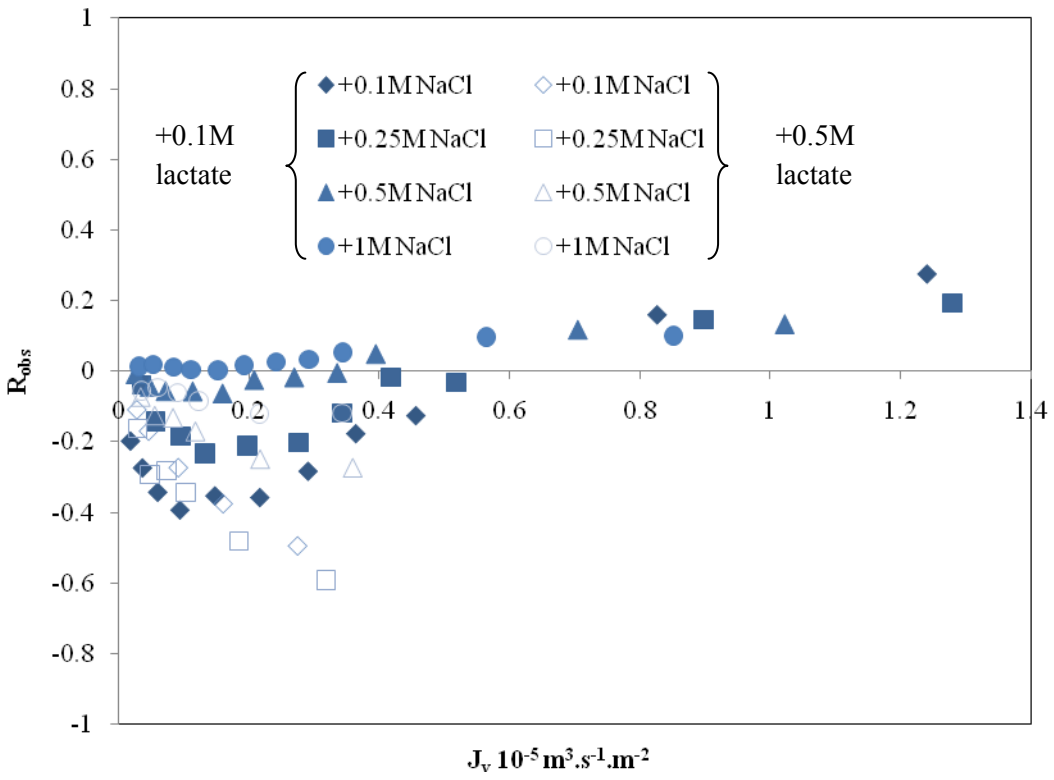


Figure 4.32 Retention of Cl^- as function of the permeate flux – influence of NaCl concentration - feed solutions containing 0.1M glucose, 0 to 1M NaCl and 0.1M lacate (Full symbol) and 0.5M lactate (Empty symbol).

In order to investigate the effect of lactate concentration on Cl^- retention in ternary-solute solutions, these results obtained with 0.5M lactate are compared to those previously obtained with 0.1M lactate (see Fig. 4.32). At 0.1M NaCl, the retentions of Cl^- of the both solutions containing 0.1M and 0.5M lactate are similar; however, the retention of Cl^- becomes more different when the concentration of NaCl higher than 0.25M. It could be explained that higher amount of lactate pushes more Cl^- toward the membrane causing decrease of Cl^- retention.

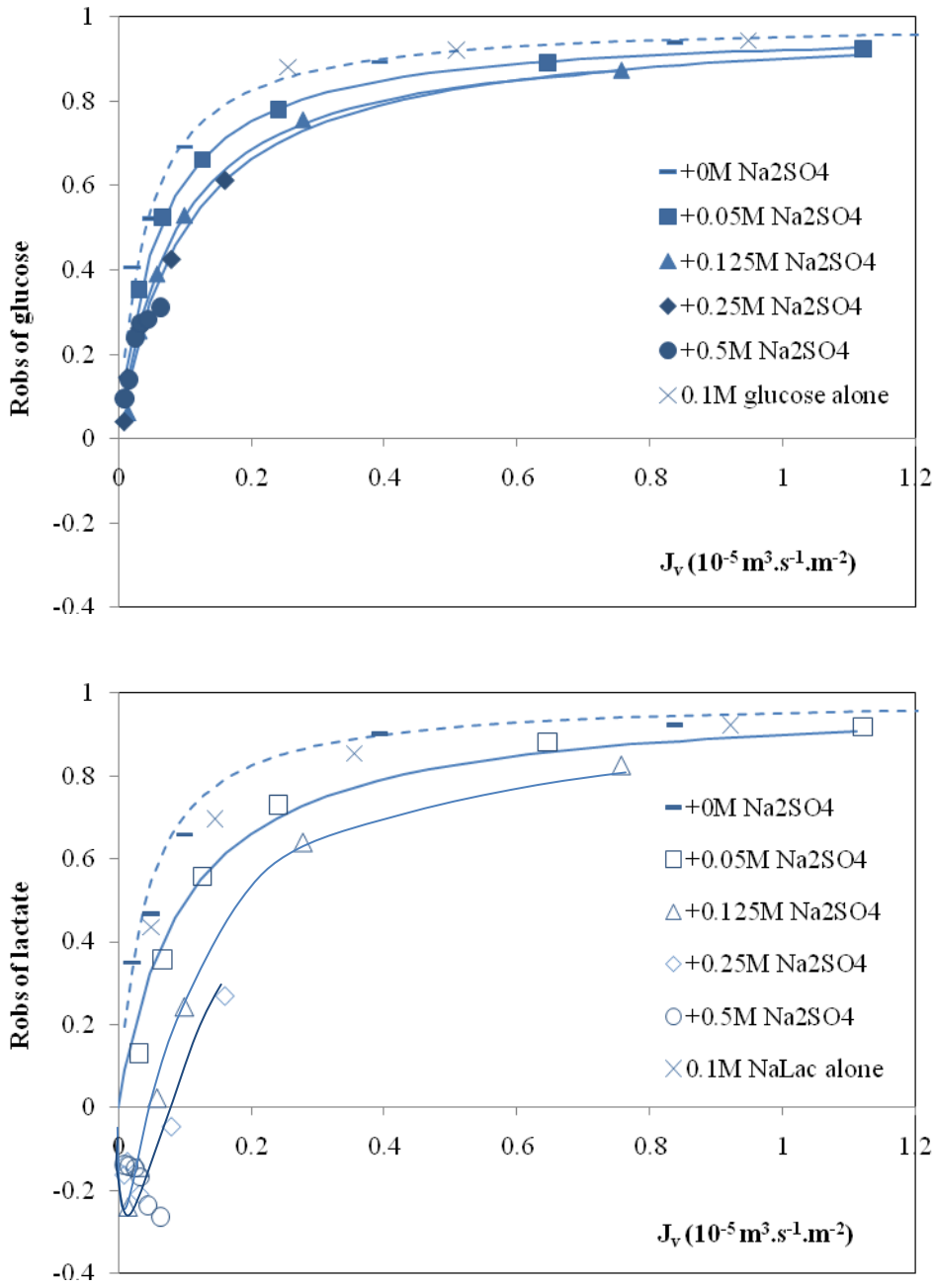


Figure 4.33 Retention of glucose (top) and lactate (bottom) as function of the permeate flux – influence of Na_2SO_4 concentration – feed solution containing 0.1M glucose, 0.1M NaLac and 0.05M to 0.5M Na_2SO_4.

Finally, the influence of the addition of Na_2SO_4 on glucose (0.1M) and lactate (0.1M) retention is studied. Fig. 4.33 shows the variation of glucose and lactate retentions with the permeate flux at different concentrations of Na_2SO_4. As expected, the retention of lactate strongly decreases with increasing Na_2SO_4 concentration,

while the retention of glucose slightly decreases. At high Na_2SO_4 concentration, the retention of lactate is negative at low flux. This indicates that the effect of addition Na_2SO_4 on lactate retention is stronger than that of glucose. The separation of glucose and lactate can thus be improved by maintaining low permeate fluxes and adding high Na_2SO_4 concentration.

Fig. 4.34 shows the comparison between the glucose and lactate retentions obtained with ternary-solute solutions containing 0.05 to 0.5M Na_2SO_4 and those obtained with binary-solute solution without adding Na_2SO_4. In order to focus the effect of adding Na_2SO_4 on glucose and lactate retention in ternary-solute solutions, the glucose and lactate retention are compared to those obtained from the binary-solute solutions containing 0.1M glucose and 0.05 to 0.5M Na_2SO_4 and binary-mixtures containing 0.1M lactate and 0.05 to 0.5M Na_2SO_4, respectively. The both results obtained from the ternary-solute solutions and the binary-solute solutions are comparable. However, the influence of the addition Na_2SO_4 on glucose retention in binary solutions Glucose/Na_2SO_4 (see Fig. 4.9) and in ternary solutions Glucose/Lactate/Na_2SO_4 (see Fig. 4.33) is slightly different. In binary-solution, the glucose retention is almost not affected by the presence of Na_2SO_4. This behavior has been previously explained by the fact that the retention of sulphate is high and independent of the Na_2SO_4 concentration.

On the contrary, in ternary solutions, Fig. 4.33 shows that the glucose retention slightly decreases for increasing salt concentration. Indeed, at $J_v = 0.2\times 10^{-5}$ $m^3.s^{-1}.m^{-2}$, the glucose retention decreases from 85% to 70% for increasing Na_2SO_4 concentration from 0 to 0.25M. Higher Na_2SO_4 concentrations do not decrease the glucose retention any more.

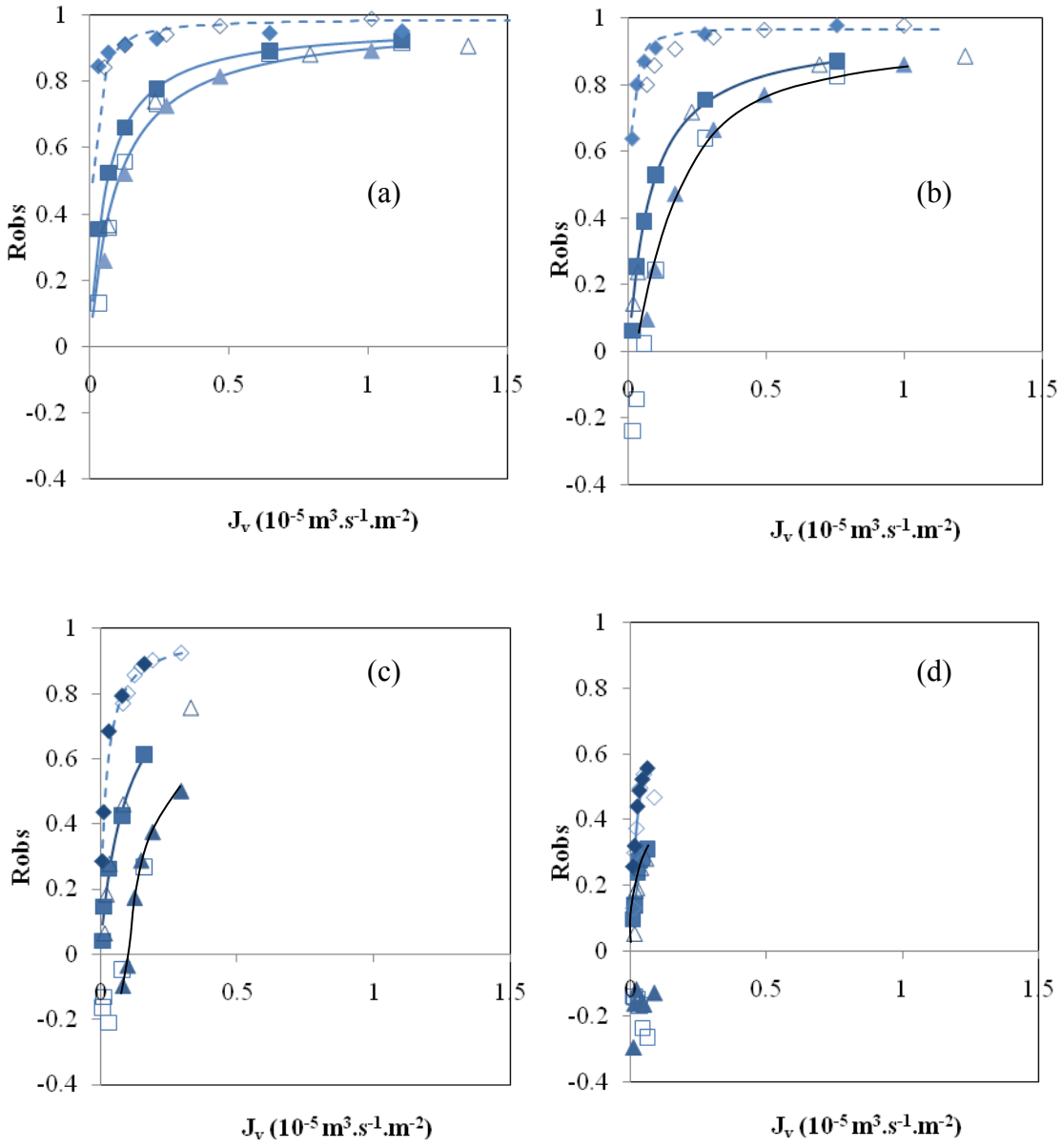


Figure 4.34 Retention of glucose, lactate and SO_4^{2-} as a function of the permeate flux - influence of Na_2SO_4 concentration - feed solutions containing 0.1M glucose, 0.1M NaLac and Na_2SO_4: 0.05M (a) 0.125M (b) 0.25M (c) 0.5M (d). Binary solutions: (◊) glucose for 0.1M glucose and 0.05 to 0.5M Na_2SO_4, (□) lactate and (Δ) SO_4^{2-} for 0.1M lactate and 0.05 to 0.5M Na_2SO_4. Ternary solutions: (♦) glucose, (■) lactate, and (▲) SO_4^{2-}.

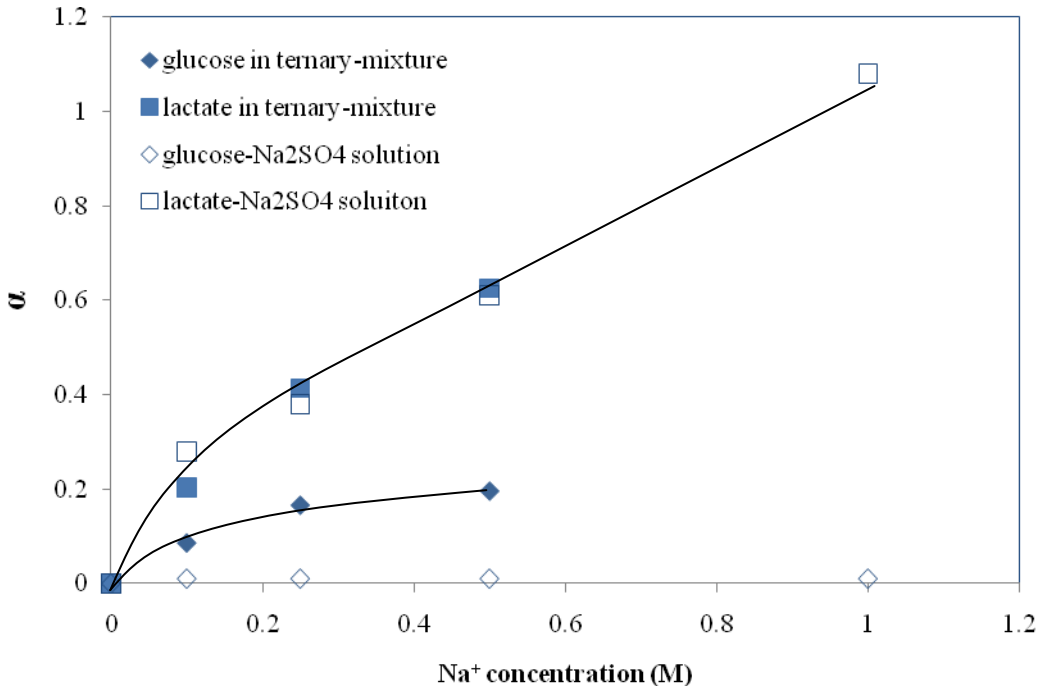


Figure 4.35 Variation of $\alpha_{glucose}$ and $\alpha_{lactate}$ as function of Na^+ concentration – feed solutions containing 0.1M glucose, 0.1M NaLac and 0.05M to 0.5M Na_2SO_4.

This difference is illustrated in Fig. 4.35 where the influence of the addition Na_2SO_4 on the variation of $\alpha_{glucose}$ and $\alpha_{lactate}$ values is plotted versus the Na^+ concentration. The $\alpha_{lactate}$ in ternary-solute solutions are not different from those in binary-solute solutions higher than 5% whereas the $\alpha_{glucose}$ is different from those in binary-solute solutions greather than 20%.

These results can be explained in regards to the lactate and sulphate retentions. According to Fig. 4.12, showing that the glucose retention decreases with increasing lactate concentration in the permeate or in another word glucose retention decreases with decrease of lactate retention. The enrichment of lactate concentration indicates extend effect of lactate on the membrane pore size e.g. pore swelling etc. The increase of membrane pore size causes decrease of glucose retention. Contrary to Fig. 4.9, showing that the sulphate retention remains high and independent of the Na_2SO_4 cocentration in binary-solutions, Fig. 4.36 points out that the sulphate

retention decreases for increasing salt concentration when concentration of Na_2SO_4 is over 0.25M. It could be explained that membrane pore size is affected adequately by enrichment of high enough lactate concentration in the permeate resulting in permeating of some sulphate into the permeate. Thus, the retention of sulphate slightly decreases. As explained previously, the higher salt concentration in the permeate result in a decrease of the glucose retention.

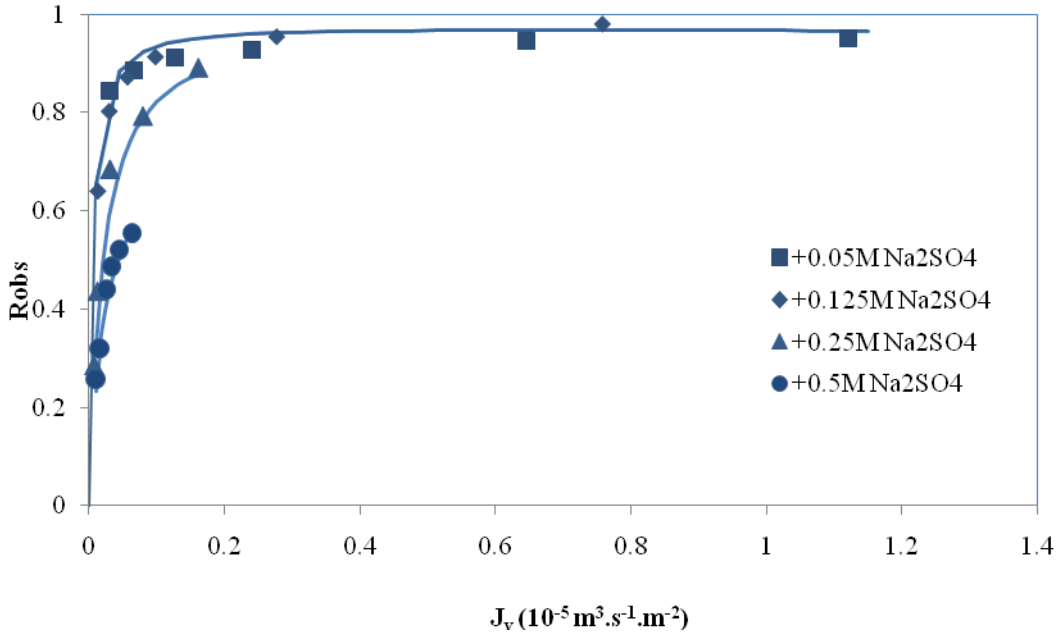


Figure 4.36 Retention of SO_4^{2-} as function of Na^+ concentration - influence of Na_2SO_4 concentration - feed solutions containing 0.1M glucose, 0.1M NaLac and 0.05 to 0.5M Na_2SO_4.

4.3.2 Influence of the addition of a salt on the glucose/lactate separation

The different impact of the salt addition on glucose and lactate retentions has been confirmed in ternary solutions. Indeed, experiments carried out with ternary solutions showed that the variations of glucose and lactate retentions in presence of NaCl or Na_2SO_4 at various concentrations are similar to that obtained in binary solutions. These results confirm that the addition of salt can improve the glucose/lactate separation. Then, in order to characterize the glucose/lactate separation, one can calculate the value of the separation factor for the different conditions investigated.

Lactate and glucose retentions decrease when NaCl is added. This decrease does not exceed 20% and is less for glucose than for lactate. The influence of the addition of NaCl on the retention has different origins, since the retention of any solute is not fixed by the same effects. Then, one can expect a better separation showing the separation factor obtained in ternary solutions with increasing NaCl concentration (Fig. 4.37). Indeed, the separation factor, which is close to 1 for the lowest NaCl concentrations, is improved for the higher concentrations. It passes through a maximum value; at permeate flux about 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$. This maximum value reaches 1.6, what means that for these conditions one can get in the permeate a solution which is enriched in lactate compared to that in the feed.

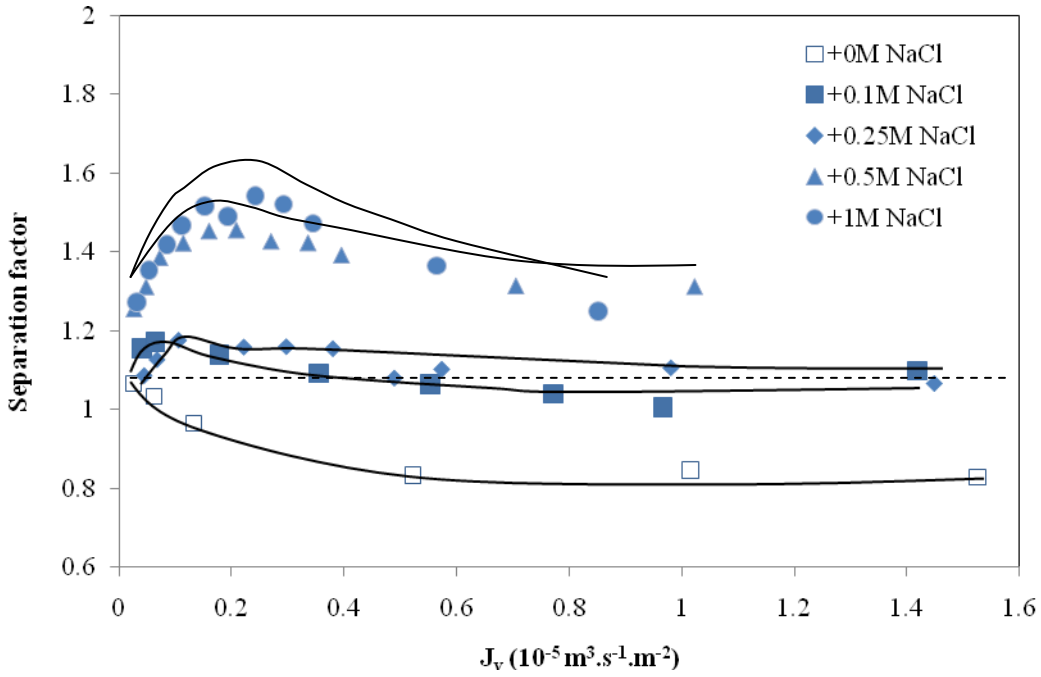


Figure 4.37 Variation of the separation factor as function of the permeate flux-influence of NaCl concentration - feed solutions containing 0.1M glucose, 0.1M NaLac and 0 to1M NaCl.

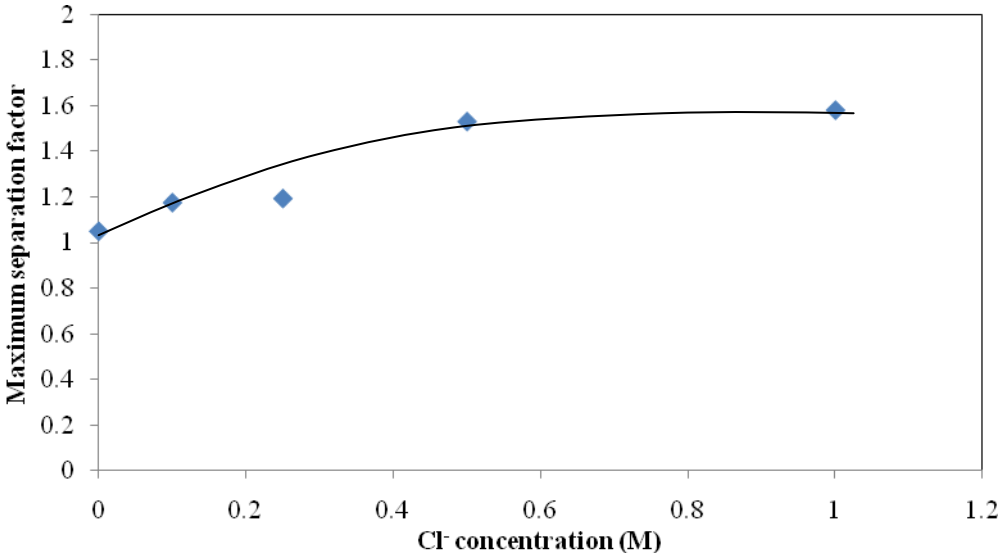


Figure 4.38 Variation of the maximum separation factor as function Cl^- concentration - feed solutions containing 0.1M glucose, 0.1M NaLac and 0 to 1M NaCl concentrations (Remarks: the values are not obtained at the same permeate flux).

The maximum values of the separation factor are plotted on Fig. 4.38 for the different NaCl concentrations. Since all the values are higher than 1, it means that for any composition, the NF permeate is enriched in lactate compared to the feed

solution. The maximum separation factor increases from 1.2, for the lowest NaCl concentration up to 1.6 for the highest one, which is 1M NaCl. For the conditions investigated here, the increase of the separation factor versus the chloride concentration seems to level off at this value close to 1.6.

In the same manner, Fig. 4.39 shows the results calculated for the higher lactate concentration from the retention previously plotted on Fig. 4.30. It is observed that the separation factor is almost independent of the NaCl concentration, as were the retention of glucose and lactate.

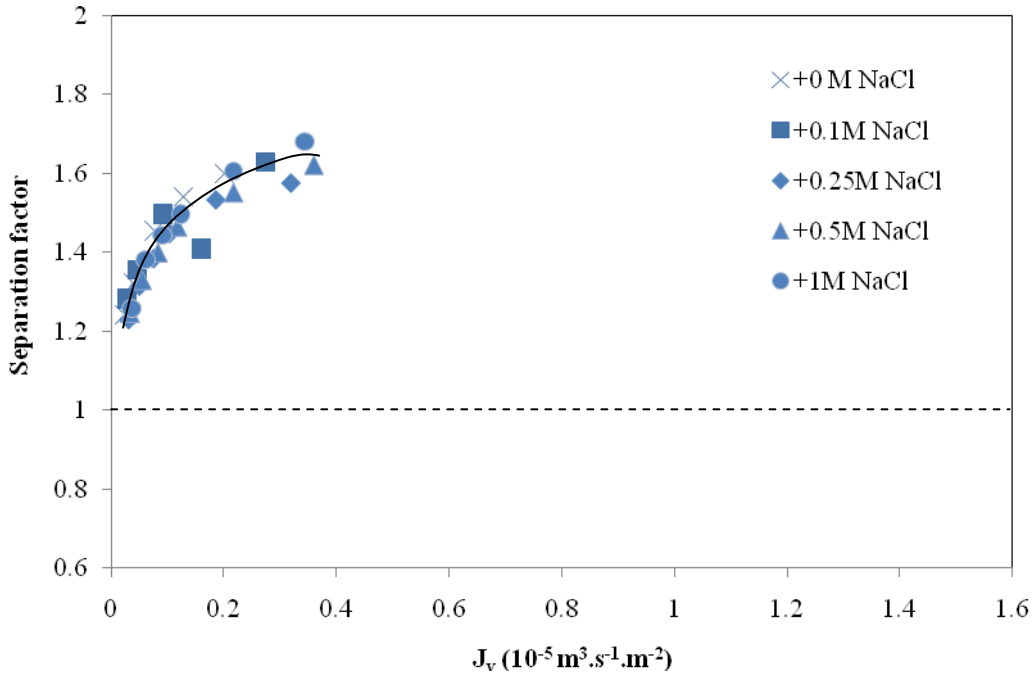


Figure 4.39 Variation of separation factor as function of the permeate flux-influence of NaCl concentration - feed solutions containing 0.1M glucose, 0.5M NaLac and 0.1to1M NaCl.

Indeed, it has been previously explained that the addition of NaCl does not change the retentions between glucose and lactate, the separation between glucose and lactate cannot be improved in this condition (see Fig. 4.31). However, one can observe that the values are comparable that obtained with lower lactate concentration

and the highest NaCl concentration (see Fig. 4.37). Again, the maximum separation factor to be achieved seems to be about 1.6.

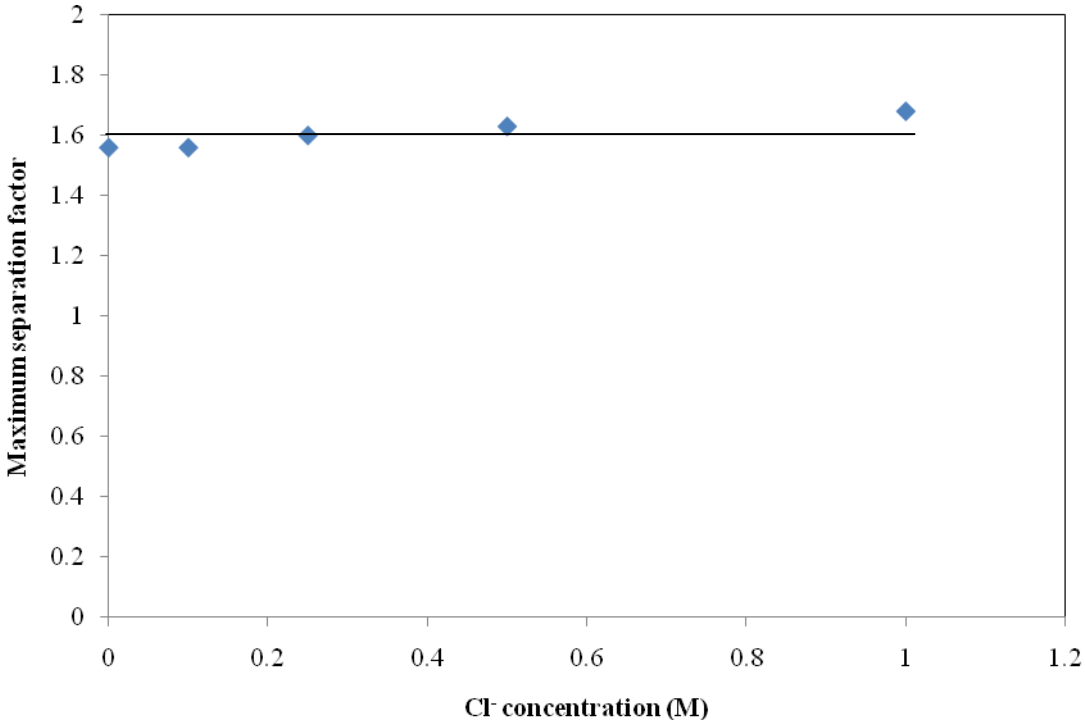


Figure 4.40 Variation of the maximum separation factor of lactate as function Cl^- concentration – feed solutions containing 0.1M glucose, 0.5M NaLac and 0.1 to 1M NaCl (Remarks: the values are not obtained at the same permeate flux).

Fig. 4.40 shows the maximum separation factor is almost constant at 1.6 with increasing Cl^- concentration in ternary mixtures containing 0.1M glucose, 0.1M NaLac and 0.1 to 1M NaCl.

The influence of the addition of Na_2SO_4 on the separation, represented by the separation factor, is also investigated. It was previously reported that in ternary-solute solution containing 0.1M glucose, 0.1M lactate and 0.05 to 0.5M Na_2SO_4 both glucose and lactate retentions are affected by the addition of Na_2SO_4. The retention of glucose slightly decreases with increasing Na_2SO_4 while that of lactate decreases. Moreover, negative values are obtained for the lactate retention at low permeate flux for the highest Na_2SO_4 concentrations. The separation between

glucose and lactate can be improved in this condition because the retention of lactate decreases much extent than that of glucose. Moreover, this improvement would be higher in the low permeate flux region and for the highest Na_2SO_4 concentration.

The effect of addition Na_2SO_4 on the separation factor between glucose and lactate in ternary-solute solutions is illustrated in Fig. 4.41 for the different Na_2SO_4 concentrations. Results obtained with the binary mixture, i.e. without Na_2SO_4 are also plotted for comparison. One can state that the general tendency is the same as that observed when adding NaCl (see Fig. 4.37). Indeed, the separation factor first increases with the permeate flux before passing through a maximum value. This maximum value increases with the Na_2SO_4 concentration, with a tendency to level off for Na_2SO_4 concentration higher than 0.25M. The maximum value of the separation is about 1.9. It is obtained at a flux about 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$ and with a Na_2SO_4 concentration about 0.25M. Therefore, in the conditions investigated here, the separation between glucose and lactate can be achieved with the addition of Na_2SO_4 at low permeate flux.

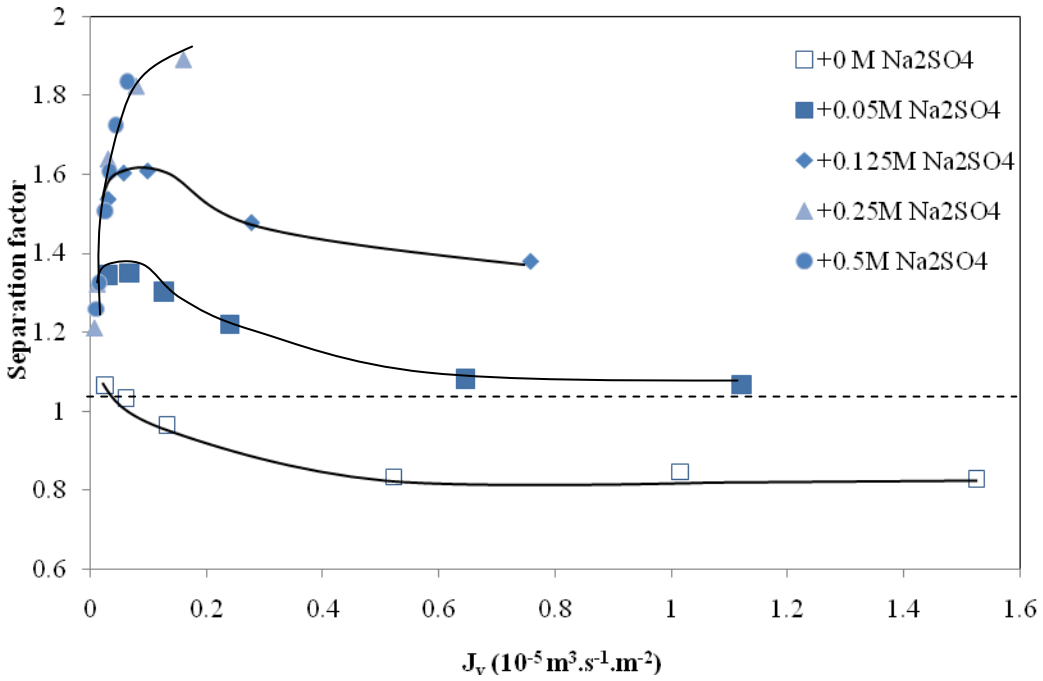


Figure 4.41 Variation of separation factor as function of the permeation flux – influnce of Na_2SO_4 concentration - feed solutions containing 0.1M glucose, 0.1M NaLac, and 0.05 to 0.5M Na_2SO_4.

The maximum value of the separation factor is further plotted on Fig. 4.42 versus the Na_2SO_4 concentration.

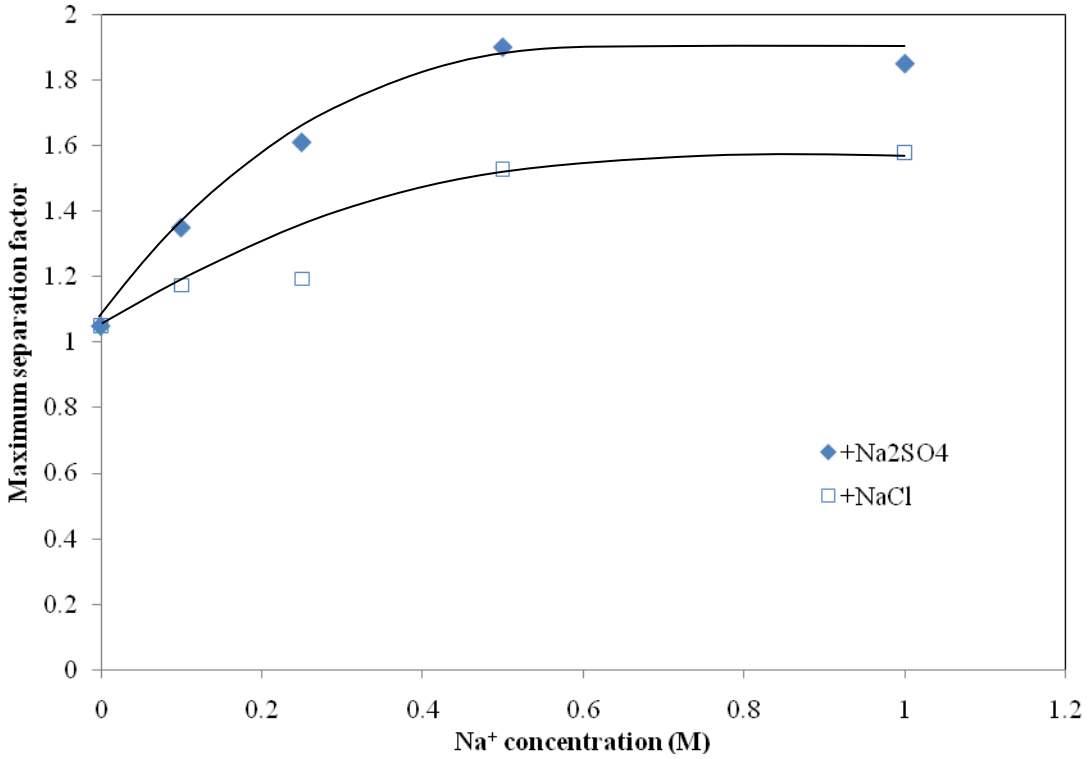


Figure 4.42 Variation of the maximum separation factor as function Na^+ concentration – feed solutions containing 0.1M glucose, 0.1M NaLac and 0.05 to 05M Na_2SO_4 (Remarks: the values are not obtained at the same permeate flux).

Fig. 4.42 shows the maximum separation factor increases with increasing Na_2SO_4 concentration in ternary mixtures containing 0.1M glucose, 0.1M NaLac and 0.05 to 0.5M Na_2SO_4 concentrations. All maximum separation factor is higher than 1. The maximum separation factor increases from 1.35 for the mixture containing 0.1M Na_2SO_4 to 1.9 for the mixture with adding 0.5M Na_2SO_4. Higher concentrations of Na_2SO_4 do not improve the separation any more. Moreover, the increase of the maximum separation factor with adding Na_2SO_4 is also compared to those with adding NaCl. The maximum separation factor with adding Na_2SO_4 is higher than that with adding NaCl. The maximum separation factor of both solutions begins level off at 0.5M Na^+ concentration.

The maximum values of the separation factor obtained for ternary solutions containing NaCl and Na_2SO_4 at two concentrations are reported in table 4.3. The one obtained without adding a salt is also reported for comparison. The corresponding permeate fluxes also given. As expected, the selectivity improvement at low NaCl concentration (0.1M) is poor. But it can reach up to about 35% for the highest NaCl concentration. The maximum value of the separation factor obtained in the conditions investigated in his work is about 1.9. It is obtained at a flux about 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$ with the addition of Na_2SO_4 at 0.25M. Higher concentrations of Na_2SO_4 do not improve the separation any more.

Then, besides improving the selectivity by adding Na_2SO_4, the permeate flux at the maximum separation factor increases by a factor of 4 compared to that one obtained in binary solution. Consequency, the addition of Na_2SO_4 also improves the productivity. It is also to be mentioned that such an addition of salt has a negligible influence on the salt composition in the permeate, which constitutes the enriched lactate solution, since sulphate is strongly retained by the nanofiltration membrane.

Table 4.3: Maximum separation factor in binary and ternary solutions and their corresponding permeate fluxes for glucose 0.1M and NaLac 0.1M.

Solutions	**Adding salt Concentration**	**SF_{max}**	**J_v (10^{-5} $m^3.s^{-1}.m^{-2}$)**
Binary solution			
Glucose/NaLac	No salt addition	1.1	0.05
Ternary solution			
Glucose/NaLac/**NaCl**	0.1M	1.2	0.1
	1M	1.6	0.2
Ternary solution			
Glucose/NaLac/**Na_2SO_4**	0.05M	1.3	0.05
	0.25M	1.9	0.2

4.3.3 Conclusions

This section studied the effect of the addition of a salt such as NaCl and Na_2SO_4 on the separation between glucose and lactate. As expected, the addition of NaCl into a solution containing 0.1Mglusoe/0.1M lactate slightly improves the separation. The retention of lactate decreases in higher extent than that of glucose, especially, at low permeate flux. The maximum separation factor increases from 1.1 (without adding NaCl) to 1.6 with adding 1M NaCl. On the other hand, the addition of NaCl in a solution containing 0.1M glusoe/0.5M lactate does not improve the separation. The addition of NaCl does not show any effect on glucose and lactate retention in this condition and the maximum separation factor is almost constant at 1.6 at 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$. It could be concluded that the separation depends on the concentration ratio between lactate and added NaCl. The separation is probably improved if the ratio is small.

As expected from binary solutions, the addition of Na_2SO_4 improves the separation between glucose and lactate. The retention of glucose slightly decreases with increasing Na_2SO_4 concentration whereas the retention of lactate strongly decreases. The lactate retention is negative at low permeation fluxes in presence of Na_2SO_4 at high concentrations (>0.125M). The maximum separation factor increases from 1.1 (without adding Na_2SO_4) to 1.9 with adding 0.5M Na_2SO_4. The achievable separation factor in this condition is close to 1.9. Again, the maximum separation factor was obtained at a flux about 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$. In order to obtain high separation, maintaining low permeate flux and high Na_2SO_4 concentration should be performed.

Then, the improvement that can be achieved by the addition of a salt is as much important as the retention of the added co-ions, sulphate in our case, is higher. For the conditions investigated in this study, a maximum separation factor of 1.9 was obtained after the addition of Na_2SO_4 at 0.25M into the glucose (0.1M)/ sodium lactate (0.1M) solution when no separation was achievable with the added salt free solution.

4.4 Impact of the addition of an electrolyte on the separation between glucose and lactate in a concentration mode

This section studies the performance of NF in lactate recovery in a concentration mode (only retentate is re-circulated to the feed tank and permeate is collected in the permeate tank). Section 4.4.1 deals with the design of concentration mode experiments from former results at constant feed concentration. The experiments are first conducted with the binary mixture containing 0.1M glucose and 0.1M lactate. The ternary mixtures containing 0.1M glucose, 0.1 or 0.2M lactate and 0.125M Na_2SO_4 are then investigated. The experimental results are presented in terms

of the variation of the feed concentration, the cumulative and instantaneous permeate concentration as well as the observed retention and the permeate flux versus the volume reduction factor V_0/V_R, where V_0 and V_R are respectively the initial solution and retentate volumes. The instantaneous values of retentions and flux will be compared to those obtained at constant feed concentration. The results obtained with ternary mixtures will be compared to those with binary mixture containing 0.1M glucose and 0.1M lactate.

An example of global mass balances of all solutes in concentration mode is shown in Table 3.5. The maximum mass balance error is lower than 5%.

4.4.1 Choice of the operating conditions

At constant feed concentration, it was observed that the separation factor obtained with the ternary mixtures containing glucose, NaLac, and added salts increases with the permeate flux to a maximum value and then decreases as shown in Figs. 4.38 and 4.42 for adding NaCl and Na_2SO_4, respectively. Furthermore, higher separation factors are also obtained with Na_2SO_4 compared to NaCl. Then, the objective of this part is to investigate the separation improvement that could be obtained by the addition of Na_2SO_4 when working in a concentration mode. Consequently, the experiments in concentration mode are designed to be in such conditions close to the maximum separation factor (ellipse region in Fig. 4.43). In the concentration mode, the permeate flux is expected to decrease with time because the increase of concentrations in the retentate increases osmotic pressure in the feed. The initial flux depends on two factors which are the concentration of Na_2SO_4 (both glucose and lactate concentrations are fixed to 0.1M) and applied pressure. Then, the initial Na_2SO_4 concentration and the applied pressure are selected to have an initial flux $J_{v,1}$ (see Fig. 4.43). The selected conditions are reported in Table 4.4. 0.125M

Na_2SO_4 is selected because it is the minimum Na_2SO_4 concentration providing a negative lactate retention (usually high negative lactate retention gives high separation factor), as observed in Fig. 4.33. Moreover, the value of the flux should decrease enough to reach the neagative retention which could improve the separation. On the other hand, it has been previously shown that the separation is achievable without addition of salt for increasing lactate concentrations at constant feed concentration. Then, experiments are also carried out for a higher lactate concentration (0.2M) to evaluate to what extent the separation could be improved by addition of Na_2SO_4.

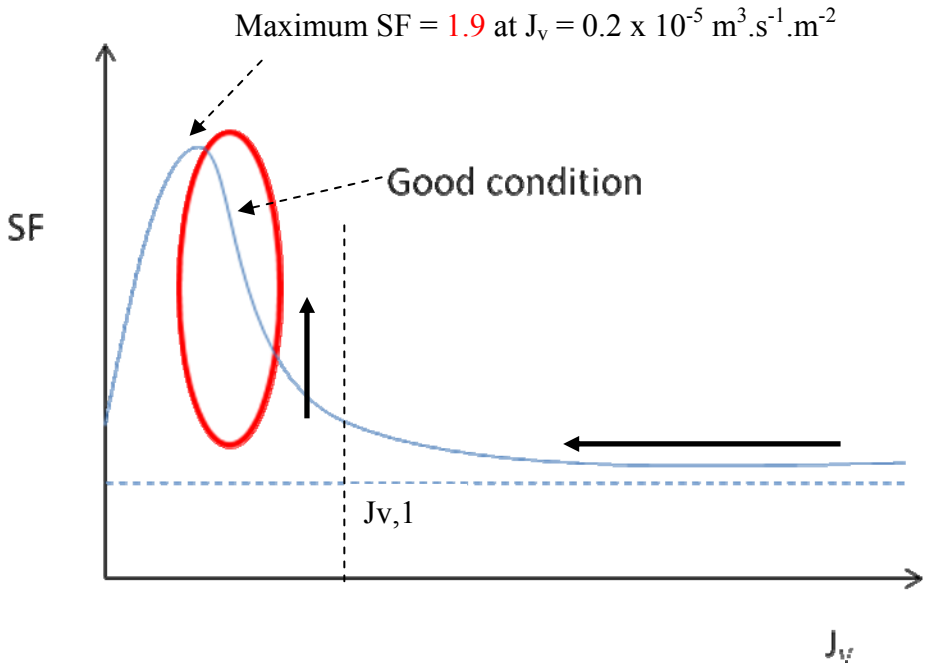


Figure 4.43 Typical variation of the separation factor of glucose and lactate in ternary-solute solutions containing 0.1M glucose, 0.1M NaLac and a high concentration of Na_2SO_4 (see Fig. 4.41).

Table 4.4 Designed conditions for experiments in a concentration mode

Batch no.	Initial volume (L)	Solutions	Pressure (bar)
1	3	0.1M glucose/ 0.1M NaLac	18
2	3	0.1M glucose/0.1M NaLac	7
3	5	0.1M glucose/0.1M NaLac/0.125M Na_2SO_4	18
4	5	0.1M glucose/0.2M NaLac/0.125M Na_2SO_4	18

4.4.2 Separation between glucose and lactate in binary-solute solutions

This section investigates the separation between glucose and lactate in binary mixture containing 0.1M glucose and 0.1M NaLac in the concentration mode. The results are compared with those obtained at constant feed concentration. Furthermore, they will be used as reference to investigate the influence of the addition salt working with ternary-solute solutions.

Two batches were carried out at the same feed solution and different applied pressures, which are 18 bar (batch no. 1) and 7 bar (batch no. 2). Figs. 4.44 and 4.45 show the corresponding variations of the glucose and lactate concentrations in the feed and permeate as well as the instantaneous ones in the permeate versus the volume reduction factor. The concentration in feed tank, permeate tank and instantaneous permeate concentration increase with increasing volume reduction factor.

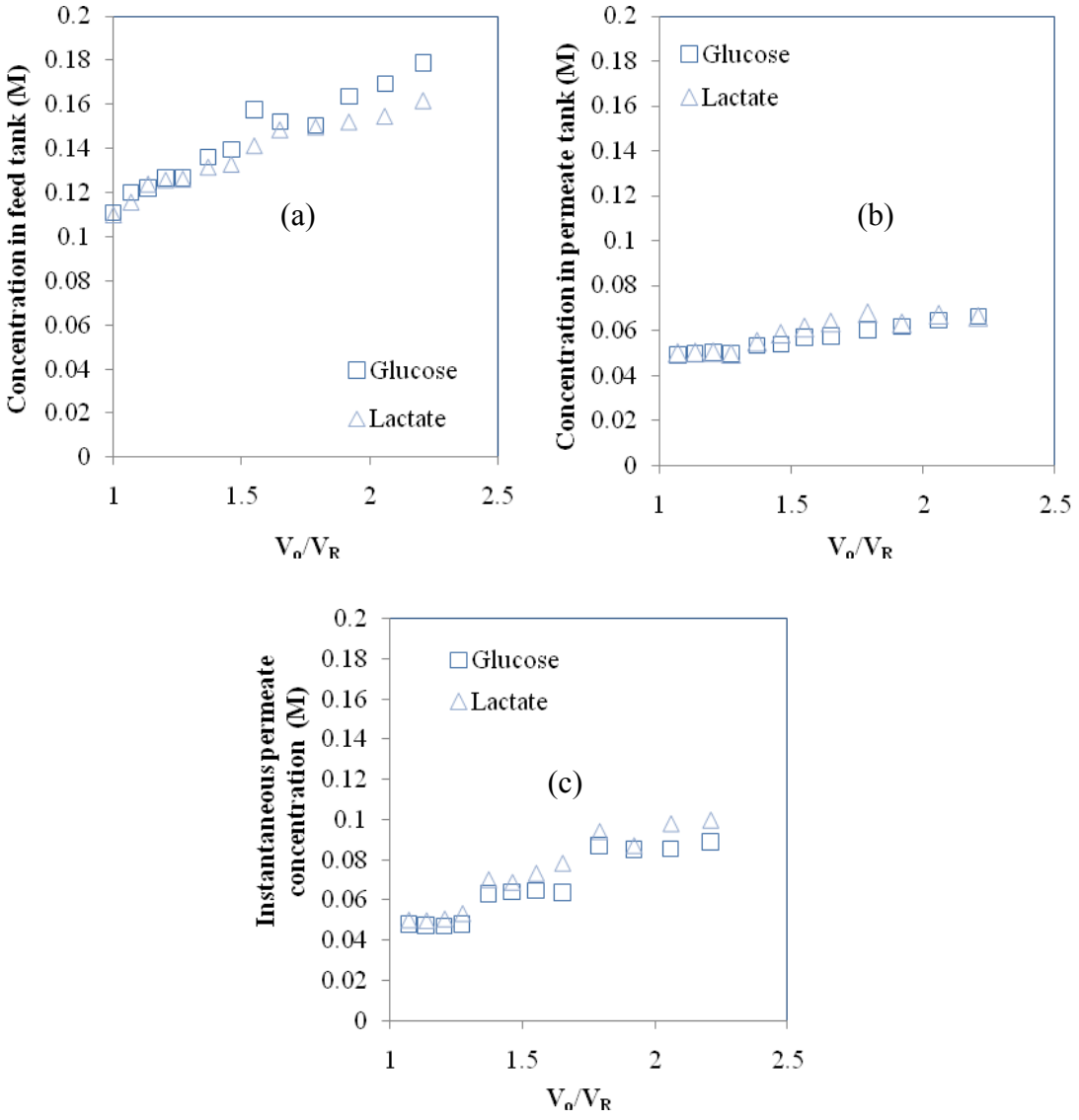


Figure 4.44 Concentrations of glucose and lactate as function of V_o/V_R in a concentration mode (Batch 1) a) concentration in retentate, b) concentration in permeate and c) instantaneous permeate concentration.

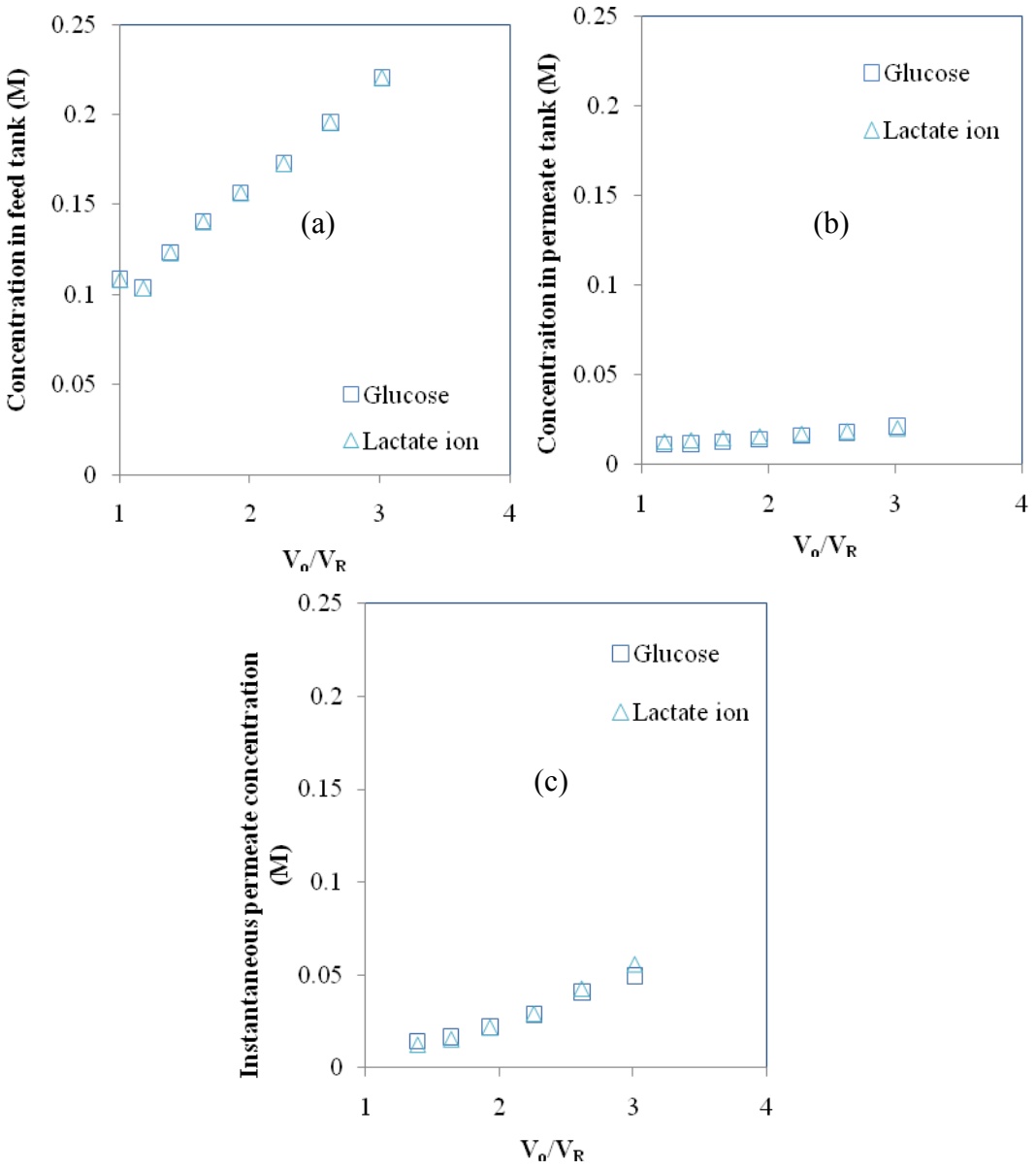


Figure 4.45 Concentrations of glucose and lactate as function of V_o/V_R in a concentration mode (Batch 2) a) concentration in retentate, b) concentration in permeate and c) instantaneous permeate concentration.

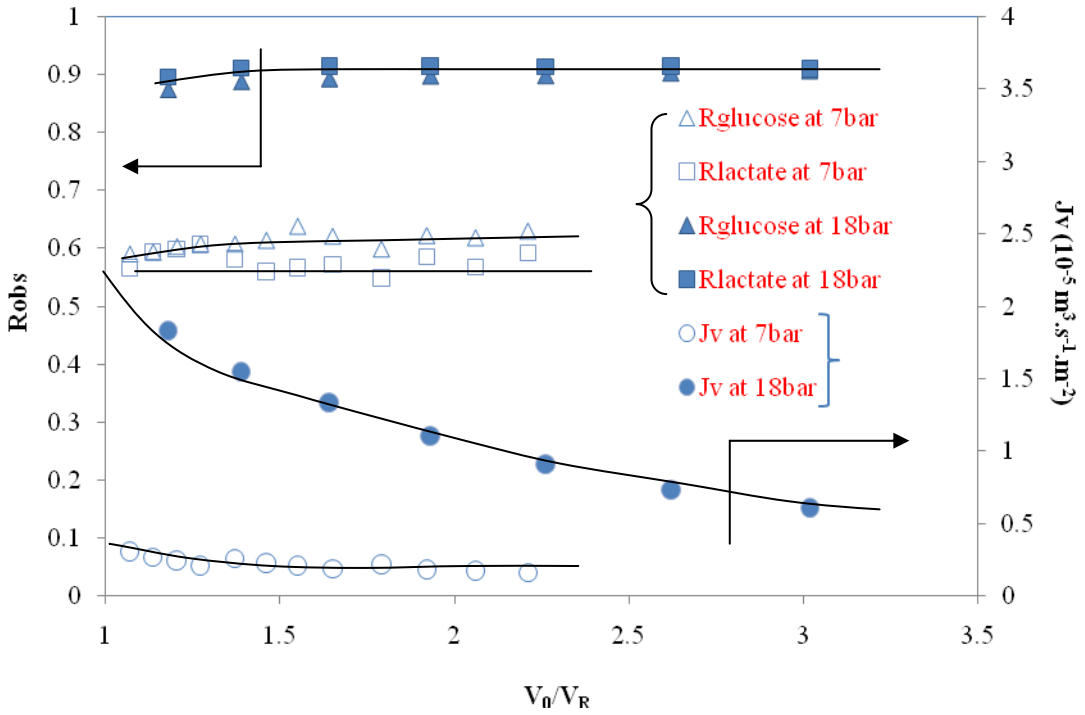


Figure 4.46 Retnetion of glucose and NaLac and instantaneous permeation flux as function of V_o/V_R in a concentration mode (Batch 1 and 2).

Glucose and lactate concentrations are close in both the feed and permeate. The instantaneous concentrations (e.g. example in Fig. 4.44c) allow calculating the instantaneous retention. This is confirmed in Fig. 4.46 showing that the variation of glucose and lactate retentions in binary mixture versus the volume reduction factor, V_0/V_R. At 18 bar (batch no.1) the result shows that both glucose and lactate retentions are high (approximately 0.9) and quite independent of the volume reduction factor (V_0/V_R). In these conditions, the permeate flux decreases from 2.5×10^{-5} to 0.5×10^{-5} $m^3.s^{-1}.m^{-2}$ for increasing V_0/V_R from 1 to 3.

These results are in agreement with those obtained with the binary solution containing glucose 0.1M and lactate 0.1M at constant feed concentration. Indeed, referring to section 4.2 and Fig. 4.6a, the glucose and lactate retentions are

similar and close to 90% within permeate flux about 0.5×10^{-5} $m^3.s^{-1}.m^{-2}$ and 1.5×10^{-5} $m^3.s^{-1}.m^{-2}$.

Decreasing the permeate flux, i.e. lowering the applied pressure, results in lower retentions with a slightly more important effect on the lactate one. In these conditions, glucose and lactate retentions are around 60% and 55% respectively. These values are also in accordance with that ones obtained at constant feed concentration. Indeed, it was observed that the difference between lactate and glucose retentions is less than 5% at permeate flux lower than 0.05×10^{-5} $m^3.s^{-1}.m^{-2}$ (see Fig. 4.6a).

Finally, as expected from the former experiments carried out at constant feed concentration, the separation between glucose and lactate is not achievable for such conditions, i.e. 0.1M glucose and 0.1M NaLac.

4.4.3 Impact of the addition of Na_2SO_4

4.4.3.1 Feed solution containing 0.1 M lactate

The accumulated concentrations of retentate and permeate in ternary solutions versus the volume reduction factor are represented in Figs. 4.47a and 4.47b, respectively. These figures show that the NF allows the lactate to pass through permeate stage more than glucose and SO_4^{2-}. As seen in the figure below, the lactate concentration increases in the permeate higher than that in the retentate whereas the amount concentration of glucose and SO_4^{2-} remains higher in the retentate stage. Fig. 4.47a shows the glucose and lactate concentrations in the feed increase and become more different with increasing volume reduction factor. The both concentrations become level off at V_0/V_R higher than 3. Fig. 4.47c shows the instantaneous glucose and lactate concentrations in the permeate increase and become more different until

V_0/V_R as 3.5 and then the both concentrations become closer until being identical at V_0/V_R as 4. It could be expected that the separation between glucose and lactate initial increases until V_0/V_R around 3 and then the separation will decrease and then finally the separation will be unachievable at V_0/V_R as 4.

According to the designed initial feed concentration, the accumulated SO_4^{2-} concentration in the retentate increases from 0.125M to meet 0.5M for increasing V_0/V_R from 1 to 4 whereas the lactate and glucose concentrations in the retentate increases from 0.1M to 0.15M for lactate and 0.25M for glucose, respectively. The concentration of SO_4^{2-} in the permeate remains negligible for volume reduction factor V_0/V_R lower than 2 and it slightly increases higher ones (0.02M at $V_0/V_R = 4$), while the lactate concentration is higher than that of glucose for the whole range of the volume reduction factor. As expected, the lactate is enriched in the permeate.

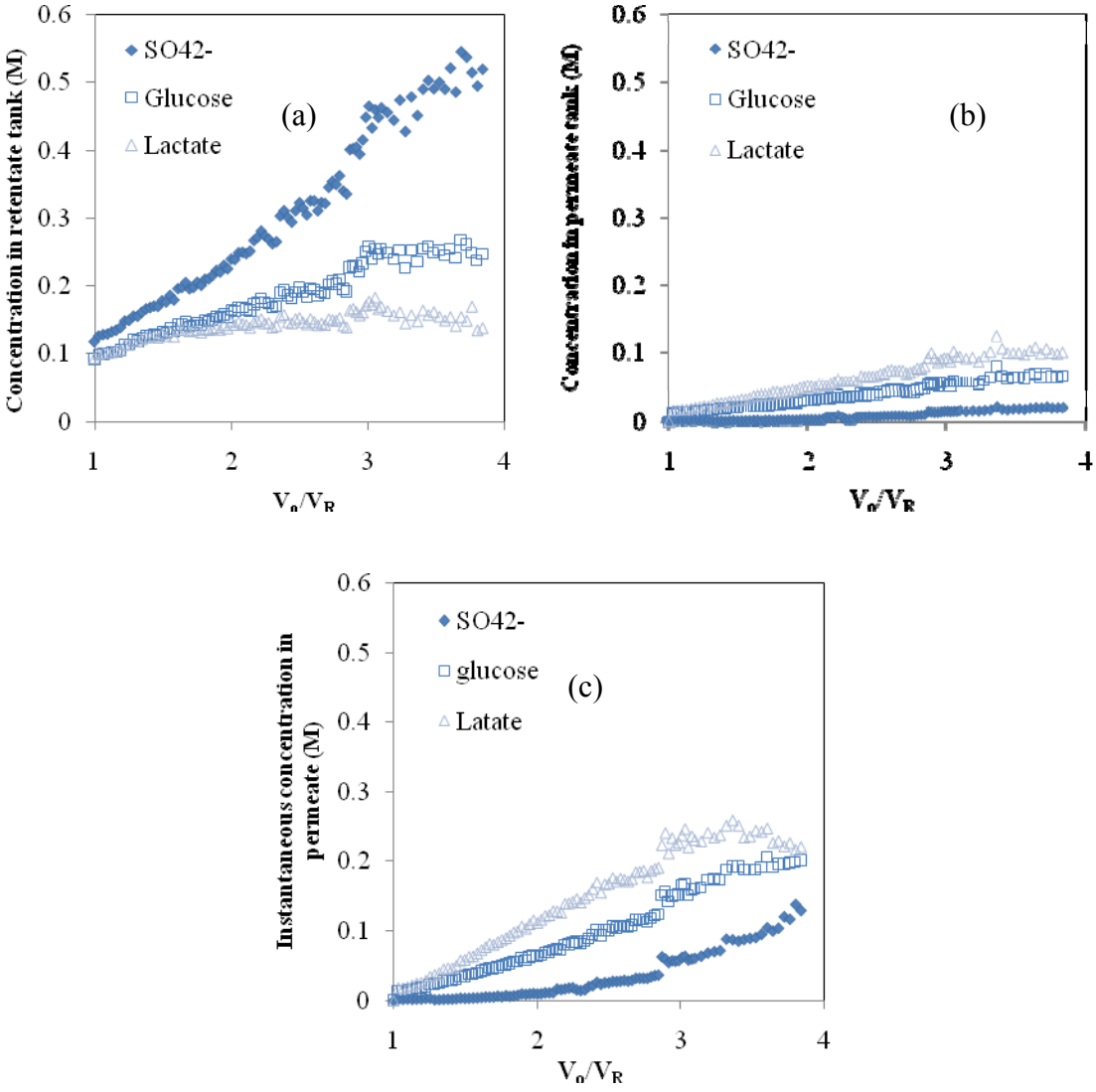


Figure 4.47 Concentration of glucose, lactate, and SO_4^{2-} as function of V_o/V_R in a concentration mode (Batch 3): a) concentration in retentate, b) concentration in permeate, and c) instantaneous permeate concentration.

Then, the observed retentions of glucose, lactate and SO_4^{2-} in ternary-solute solutions, calculated from the instantaneous concentrations, are reported in Fig. 4.48. The variation of the permeate flux versus the volume reduction factor V_0/V_R is also reported on this graph.

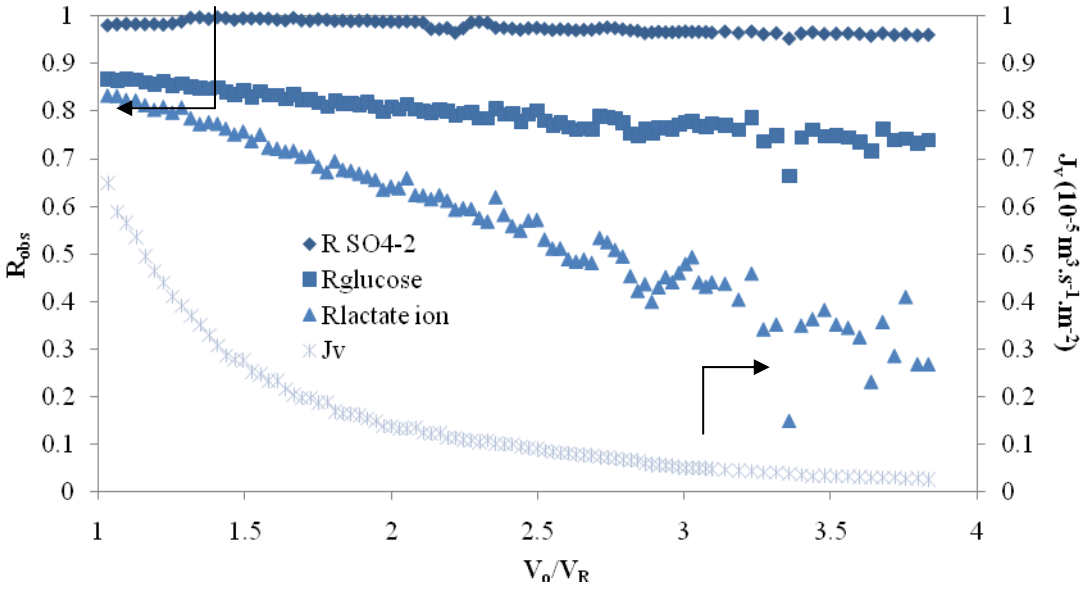


Figure 4.48 Instantaneous retention of glucose, lactate, and SO_4^{2-} and permeation flux as function of V_0/V_R in a concentration mode (Batch 3).

The retentions of the solutes decrease with increasing volume reduction factor, i.e., for decreasing permeate fluxes, in different manner. The retention of glucose slightly decreases with increasing volume reduction factor whereas the retention of lactate strongly decreases. These expected results are due to the increase of the sulphate concentration in the retentate for increasing volume reduction factor. Indeed, the sulphate retention remains constant and close to 100% for volume reduction factor lower than 2 and then slightly decreases for increasing V_0/V_R (96% at $V_0/V_R = 4$). The solute retention decreases could also be explained that the permeate flux will decrease when volume reduction factor increases. Indeed, it has been previously shown at the feed constant concentration that the solute retention increases for increasing permeate fluxes.

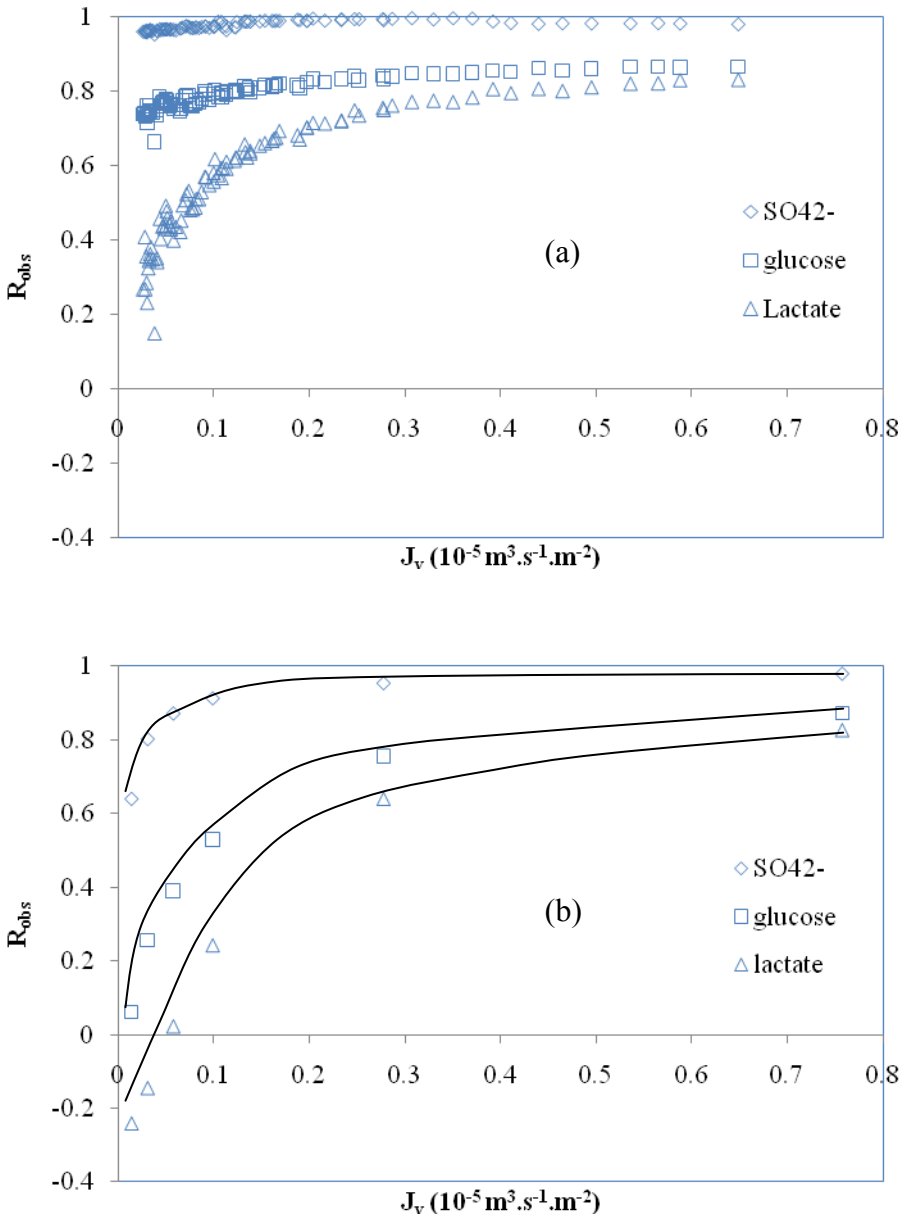


Figure 4.49 Instantaneous retention of glucose, lactate, and SO_4^{2-} as function of permeate flux: a) concentration mode (Batch 3) and b) constant feed concentration.

The instantaneous retentions of glucose, lactate and SO_4^{2-} (Batch 3) are compared to that obtained at constant feed concentration for the same initial solute concentration in Fig. 4.49. These results are in accordance for the initial conditions in the concentration mode (J_v = 0.65× 10^{-5} $m^3.s^{-1}.m^{-2}$) as well as for permeate fluxes higher than J_v = 0.25× 10^{-5} $m^3.s^{-1}.m^{-2}$. However, the solutes retentions in the concentration mode are higher than that obtained at constant feed concentration for

lower permeate fluxes. The result could be due to the higher solute concentration in the retentate.

From another point of view, the negative lactate retention at constant feed concentration was observed at the flux below 0.05× 10^{-5} $m^3.s^{-1}.m^{-2}$ (Fig. 4.49b) but the instantaneous lactate retention in a concentration mode does not meet negative value even the flux is below 0.05× 10^{-5} $m^3.s^{-1}.m^{-2}$ (Fig. 4.49a).

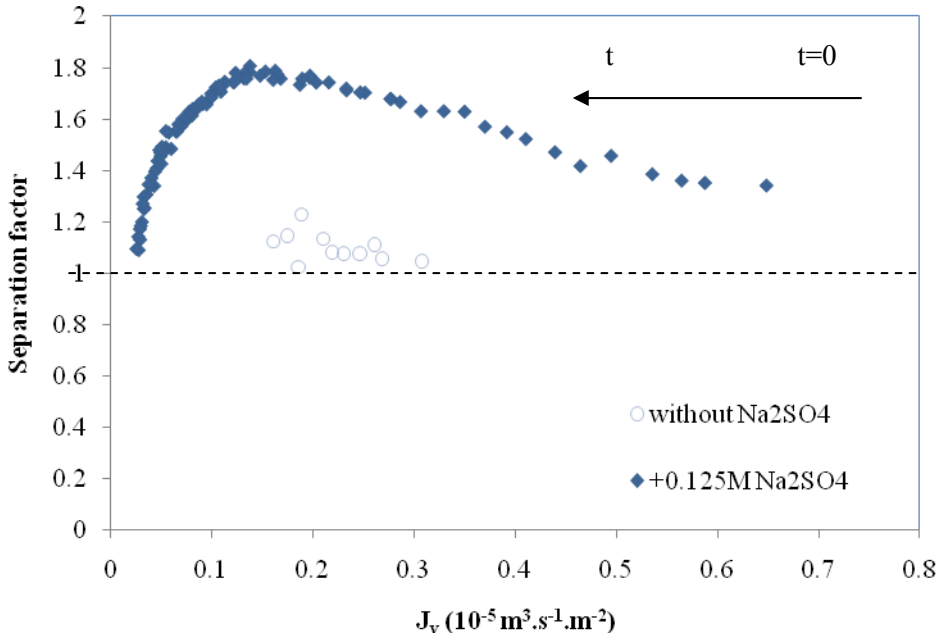


Figure 4.50 Separation factor (concentration ratio that calculated from instantaneous values in the permeate) as function of permeation flux – influence of addition of Na_2SO_4 (Batch 2 and 3).

For concentration mode result, the separation factor (the instantaneous concentration ratio of lactate divided by glucose in the permeate) is also evaluated to compare the separation efficiency of the NF operation. As expected from constant feed concentration results, the separation factor obtained in a concentration mode initially increases to maximum value and then decreases with decreasing permeate

flux (Fig. 4.50). At permeate flux around 0.15× 10^{-5} $m^3.s^{-1}.m^{-2}$, the separation factor reaches a maximum value at 1.8.

The separation factor values are compared to those obtained without adding Na_2SO_4. As expected, Fig. 4.50 shows that the separation factor of solution with Na_2SO_4 is higher than those of the solution without adding Na_2SO_4. Moreover, the maximum separation factor and the corresponding flux observed with the concentration mode and with the constant feed concentration mode are in the same range. The maximum separation factor and the corresponding permeate flux obtained at constant feed concentration are 1.9 and 0.2× 10^{-5} $m^3.s^{-1}.m^{-2}$, respectively, whereas those in concentration mode are 1.8 and 0.15× 10^{-5} $m^3.s^{-1}.m^{-2}$, respectively.

From the process point of view, it is also interesting to calculate the purity and the recovery of the lactate to evaluate the impact of the addition of salt on the separation efficiency.

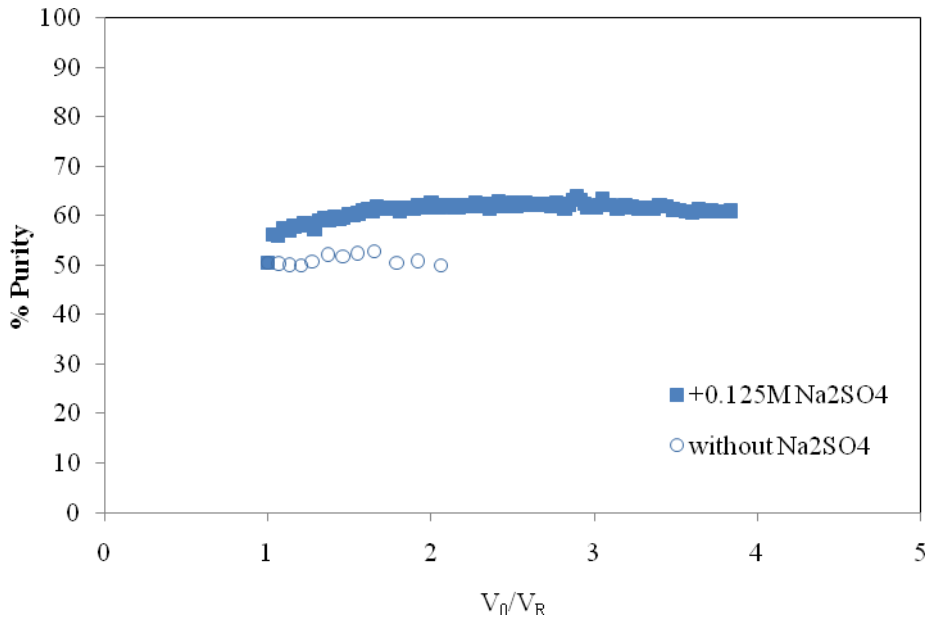


Figure 4.51 Purity of lactate as function of volume reduction factor (V_0/V_R) in a concentration mode – influence of the addition of Na_2SO_4 (Batch 2 and 3).

Fig. 4.51 shows lactate purity initial increases a maximum value and then decreases with increasing volume reduction factor. It was observed that the maximum purity is 65% (initial purity 50%) at permeate flux of 0.05× 10^{-5} $m^3.s^{-1}.m^{-2}$. Moreover, the lactate purity, obtained from the solution containing 0.125M Na_2SO_4 is 10% higher than that obtained without adding Na_2SO_4 in the same permeate flux region.

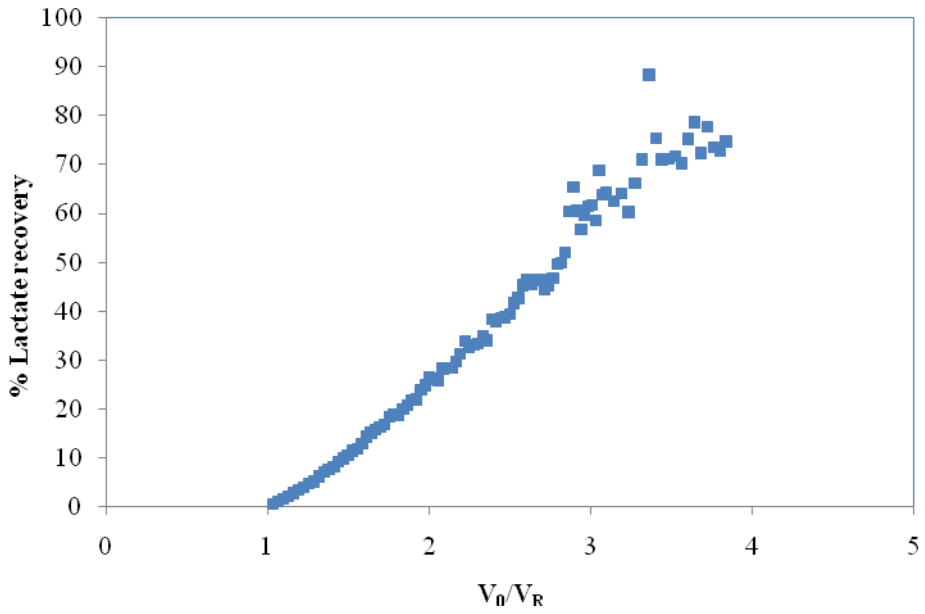


Figure 4.52 Recovery of lactate in the permeate as function of volume reduction factor (V_0/V_R) in a concentration mode (Batch 3).

Fig. 4.52 shows the variation of lactate recovery versus volume reduction factor in the ternary mixture with adding 0.125M Na_2SO_4 (batch no.3). As expected, it was observed that lactate recovery increases with time, i.e. for increasing volume reduction factor. The highest of recovery obtained in this study is 80% which correspond to volume reduction factor around 4. This value is not a limit and it by increasing the filtration time.

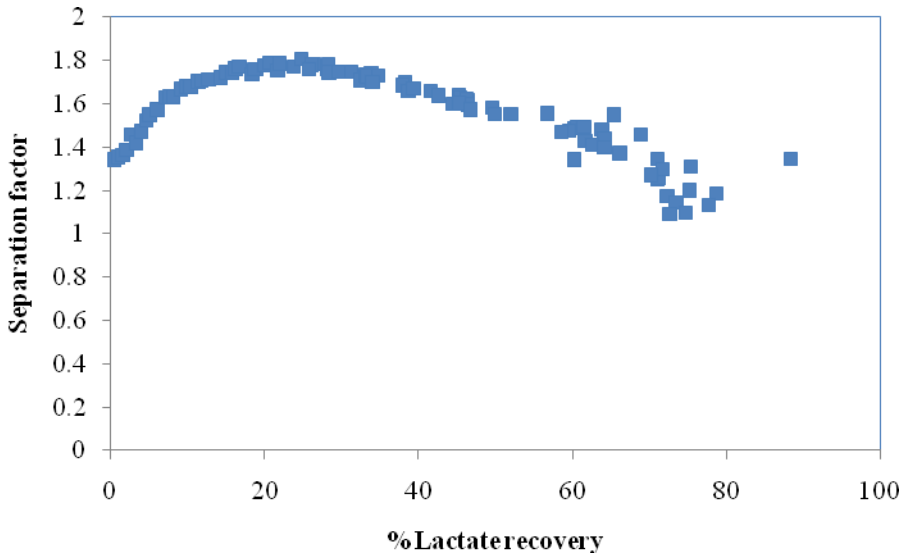


Figure 4.53 Separation factor as function of recovery of lactate in the permeate (Batch 3).

Fig. 4.53 shows the separation factor versus recovery of lactate in the ternary mixture with adding 0.125M Na_2SO_4 (batch no.3). It was observed that maximum separation factor is 1.8 at 25% recovery of lactate.

4.4.3.2 Feed solution containing 0.2M lactate

In order to evaluate the impact of lactate concentration on the separation improvement by adding Na_2SO_4, experiments are also carried out with higher initial lactate concentration, 0.2M. As observed in previous result from binary-solute solution containing glucose and lactate at constant feed concentration (Fig. 4.25) showing that the separation could be improved by increasing lactate concentration. Thus, the previous ternary solution (batch no.3) is modified by having higher initial lactate concentration (batch no. 4).

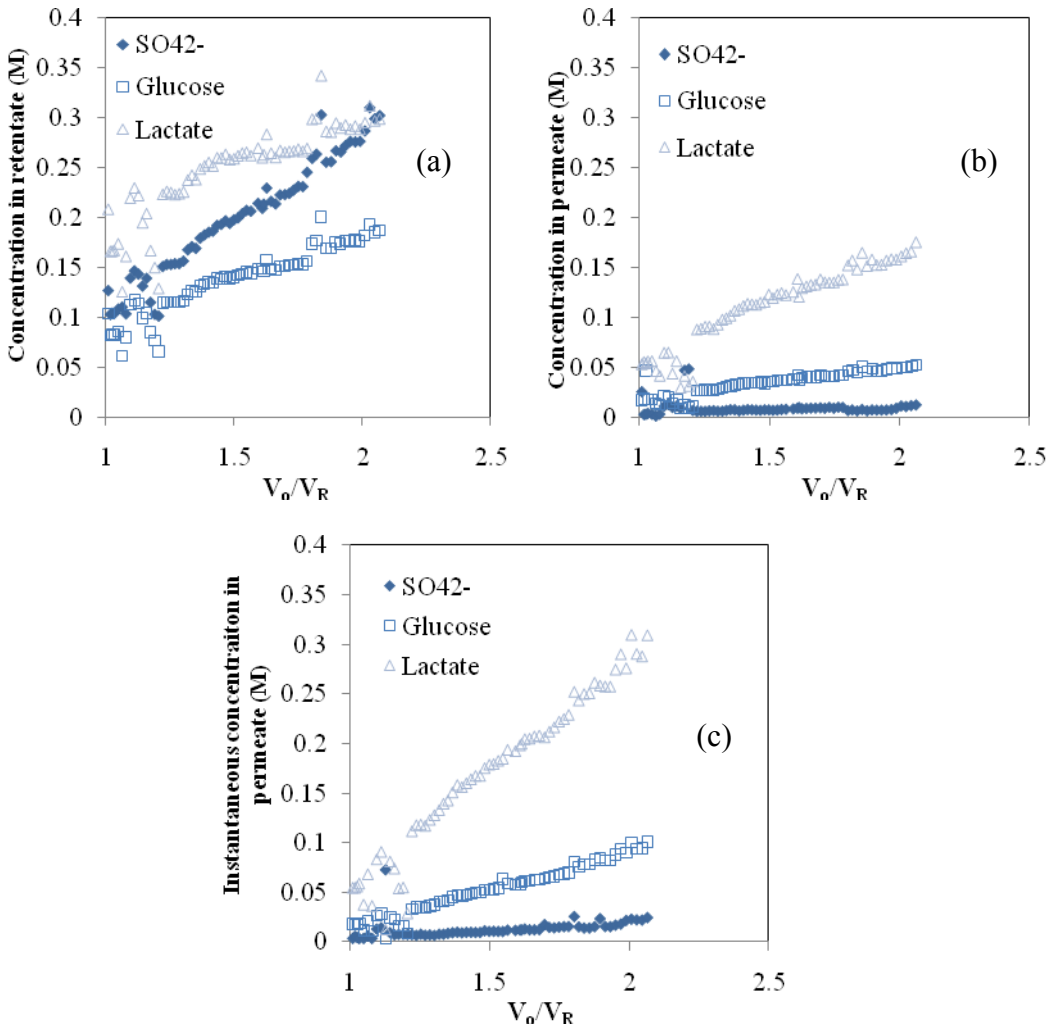


Figure 4.53 Concentration of glucose, lactate and SO_4^{2-} as function of V_0/V_R in a concentration mode (Batch 4) a) concentration in retentate, b) concentration in permeate, and c) instantaneous permeate concentration.

Fig. 4.53 shows that the concentration of the solutes (glucose, lactate and SO_4^{2-}) in retentate, permeate and instantaneous permeate increases with increasing volume reduction factor. Fig. 4.53a shows the both SO_4^{2-} and lactate concentrations in the retentate increase from 0.125M and 0.2M, respectively, to 0.3M for increasing V_0/V_R from 1 to 2. Fig. 4.53b shows the lactate concentration in the permeate increases higher than those of glucose and SO_4^{2-} remains negligible when volume reduction factor lower than 2. It should be mentioned here that the scattering

of the concentration values at the beginning of the experiment (V_0/V_R < 1.2) is abnormal low which might be due to analytical problem.

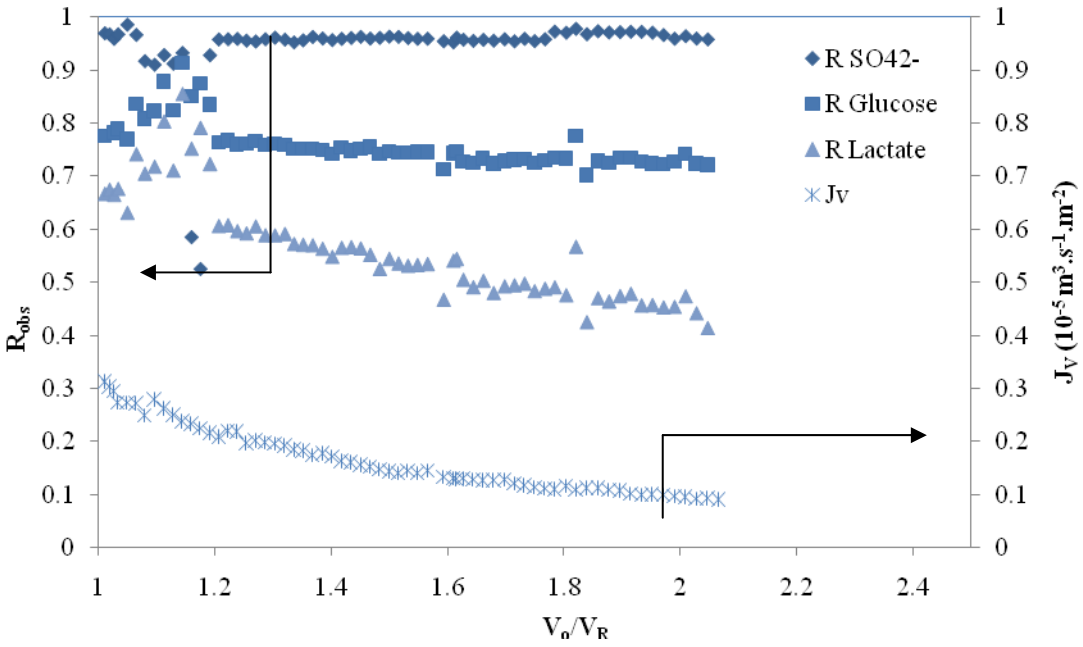


Figure 4.54 Instantaneous retention of glucose, lactate and SO_4^{2-} and permeation flux as function of V_o/V_R in a concentration mode (Batch 4).

Fig. 4.54 shows the variation of observed retention of glucose, lactate and SO_4^{2-} in ternary mixture containing 0.2M lactate with increasing volume reduction factor in concentration mode. The retention of glucose gradually decreases while lactate retention strongly decreases with increasing volume reduction factor. As expected, increase of lactate concentration results in lower retention of glucose and lactate compared to those obtained from initial feed solution containing 0.1M NaLac (see section 4.2 Impact of the adding of salt on glucose and lactate retention). The retention of SO_4^{2-} remains independent of volume reduction factor.

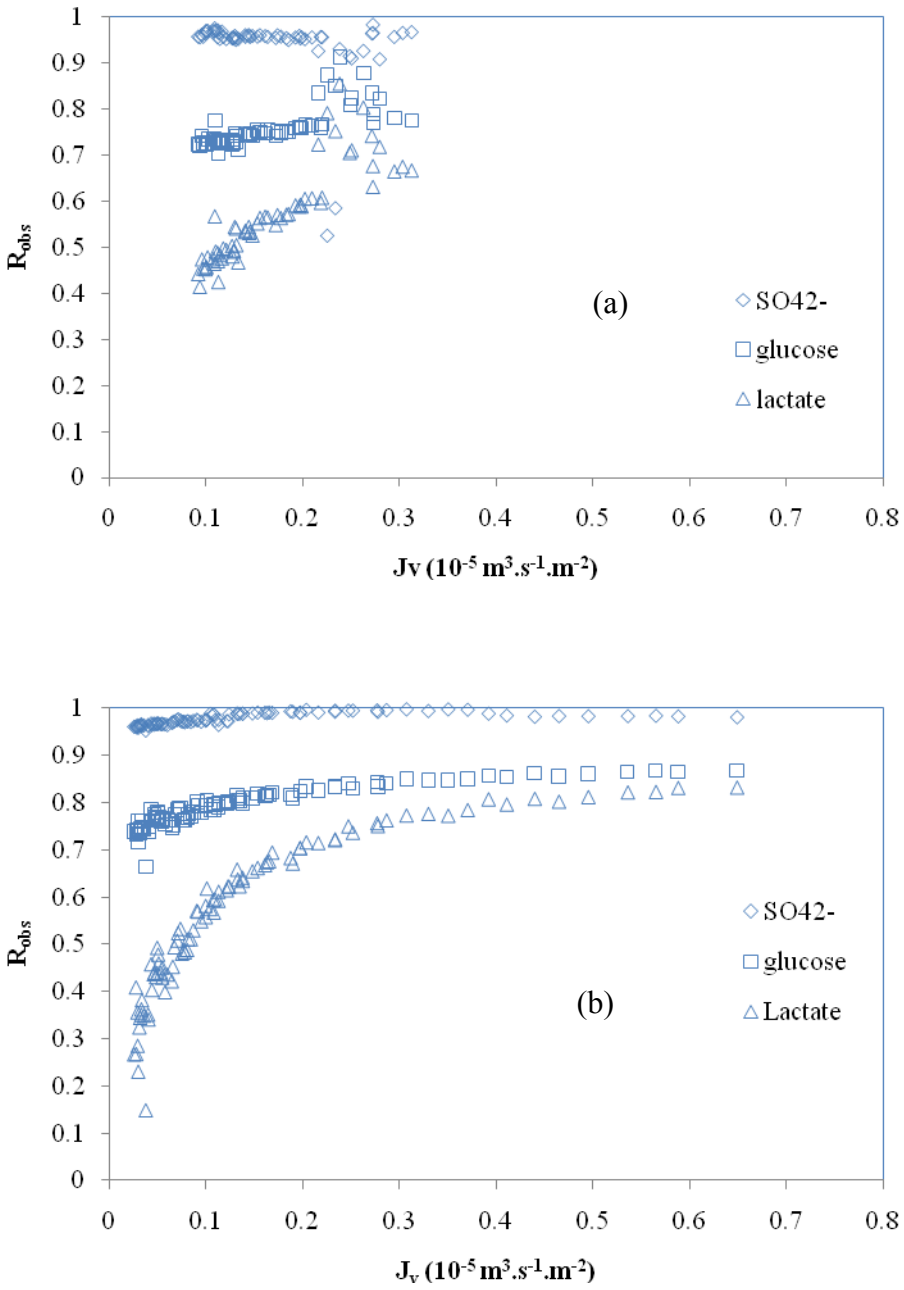


Figure 4.55 Comparison of instantaneous retention of glucose, lactate and SO_4^{2-} as function of permeate flux in a concentration mode – influence of the lactate concentration: (a) Batch 4 and (b) Batch 3.

Because the experiment at constant feed concentration with the ternary-solutions containing 0.1M glucose, 0.2M lactate and 0.125M Na_2SO_4 was not investigated and this experiment focus on the impact of increasing lactate

concentration on the separation of the ternary solution (batch no.3) , the retentions are only compared to those observed with previous experimental results from batch no.3.

Fig. 4.55 shows the retention of SO_4^{2-}, glucose and lactate in concentration mode of two different lactate ternary-solutions increases with increasing permeate flux. The retentions of SO_4^{2-} and glucose that obtained from both ternary-solutions are similar. On the other hand, the retention of lactate of ternary-solution containing 0.2M is 10% lower than that of ternary-solution containing 0.1M.

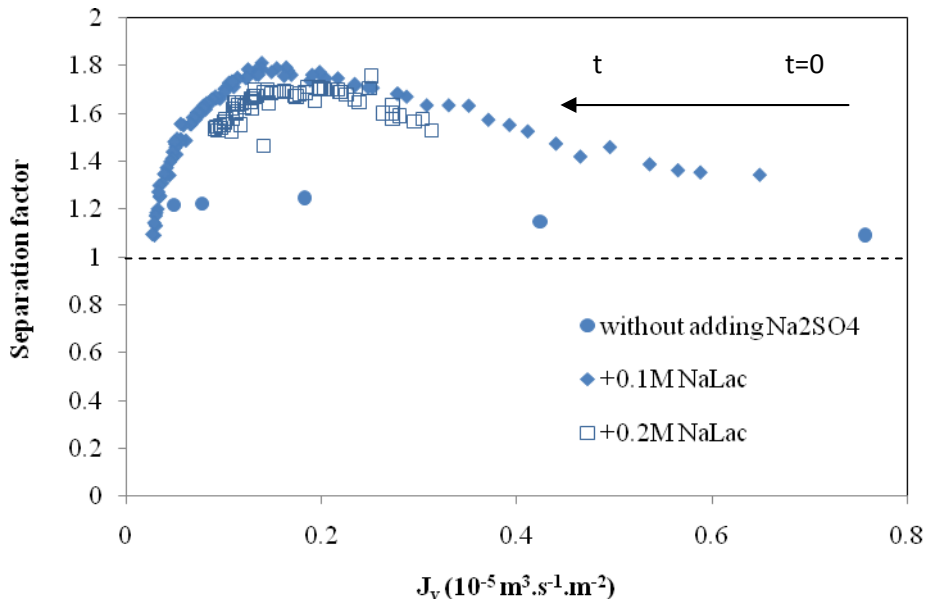


Figure 4.56 Separation factor as function of permeate flux in a concentration mode - influence of the lactate concentration (Batch 3 and 4). The solution without adding Na_2SO_4 contains 0.1M glucose and 0.25M lactate.

As expected, Fig. 4.56 shows the separation factor initially increases to maximum value and then tends to decrease with increasing permeate flux. At permeate flux around 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$, the separation factor of batch no.4 reaches to maximum value. As permeate flux below 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$, the separation factor becomes less. At permeate flux 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$, the maximum

separation factor of batch no.4 is 1.75 and the maximum separation factor obtained from binary mixture containing 0.25M lactate at constant feed concentration is 1.2. The maximum separation factor of the batch no.4 is approximately 50% higher than that of the binary mixture containing 0.25M lactate.

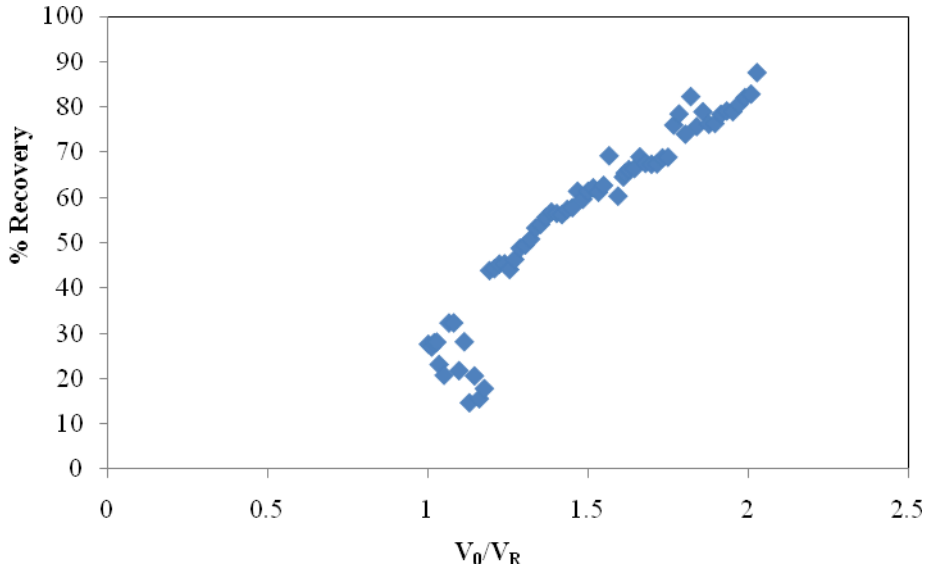


Figure 4.57 Recovery of lactate in the permeate as function of permeate flux in a concentration mode (Batch 4).

Fig. 4.57 shows that recovery of lactate increases with increasing volume reduction factor. The highest recovery of lactate is approximately 90%, which is observed at V_0/V_R as 2.

4.4.4 Discussion

The retentions of glucose and lactate in binary mixture containing 0.1M glucose and 0.1M NaLac do not differ more than 5% for both applied pressures, 7 and 18 bars (Fig. 4.46). The separation factor between glucose and lactate is approximately 1.1 (Fig. 4.50). These results confirm that the separation in binary mixture is not feasible which is in accordance with those obtained at constant feed concentration.

With the addition of 0.125M Na_2SO_4 (batch no.3) the retentions and separation factor are in accordance with those obtained at constant feed concentration. Firstly, the addition of Na_2SO_4 (batch no.3) causes glucose and lactate retentions to decrease in different magnitude. The reduction of lactate retention is higher than that of glucose (Fig. 4.49). However, the lactate retention did not reach the negative value even for fluxes are below 0.05×10^{-5} $m^3.s^{-1}.m^{-2}$, while (these fluxes gave negative lactate retention at constant feed concentration). Secondly, the initial feed concentration and permeate flux which are selected allow indeed the separation factor to meet the desired region, i.e. for permeate fluxes around 0.15×10^{-5} $m^3.s^{-1}.m^{-2}$ (ellipse in Fig. 4.43). The addition of Na_2SO_4 improves the separation (separation factor increases about 50%) and the maximum separation factor is closed to that obtained at constant feed concentration (Fig. 4.50).

The retention and separation factor for the ternary mixtures containing either 0.1M or 0.2M lactate are comparable. Firstly, the solution having 0.2M lactate (batch no. 4) reveals glucose retention is similar to those observed at 0.1M lactate (batch no.3); however, the lactate retention is 10% lower (Fig. 4.55). The influence of additional lactate from 0.1M to 0.2M will decrease the influence of Na_2SO_4 on lactate retention that can be observed from the smaller differential ratio between SO_4^{2-} and lactate concentration in the feed. Secondly, the separation factor obtained from both lactate solutions (0.1M or 0.2M) are similar (Fig.4.55). The result of experiment showing that 0.2M lactate concentration improves the separation. The separation factor obtained from 0.2M lactate is 30% higher than that obtined from binary-solution (0.1M glucose/0.25M lactate). The maximum separation factor obtained from 0.2M lactate is 10% less than that obtained from 0.1M lactate.

The results obtained in this work can be discussed in comparison with some ones previously reported. Kang *et al*. (2004) studied recovery of ammonium lactate from fermentation broth by using NF45 membrane in concentration mode. Kang *et al*. (2004) reported the lactate concentration in fermentation broth was concentrated from 0.84M to 0.92 M (increase 9.5%) in the retentate and 0.58 to 0.64M (increase 190%) in the permeate, respectively, at V0/VR as X(I am sorry. I will give this value to you next time.) as shown in Table 4.5. The maximum increase of lactate concentration in retentate and permeate in this work was 190% and 6300% in retentate and permeate, respectively, which were obtained with ternary mixture containing 0.1M glucose, 0.1M lactate and 0.125M Na_2SO_4. These results in this work and those reported by Kang *et al*. (2004) are in accordance even the experiments were carried out at different conditions.

Table 4.5 the increase of lactate concentration in a concentration mode of this work and other author

Batch no.	Increase concentration in retentate	Increase concentration in permeate	V_0/V_R
1	0.1M to 0.16M (60%)	0.045 to 0.06M (33.33%)	2.21
2	0.1M to 0.23M (130%)	0.01M to 0.02M (50%)	3.01
3	0.1M to 0.29M (190%)	0.01 to 0.64M (6300%)	3.83
4	0.2M to 0.3M (50%)	0.05 to 0.18M (260%)	2.05
Kang *et al*., 2004	0.84 M to 0.92M (9.5%)	0.58M to 0.64M (190%)	X

The purity of lactate reaches maximum value (65%) and then slightly decreases with increasing volume reduction factor. The increase of purity is because the separation slightly increases as observed from increase of separation factor. Then, the purity decreases very slightly because the separation factor passes the maximum and begins decrease. The recovery increases with increasing volume reduction factor which reaches to the maximum at 80%, which correspond to the lowest permeate flux around 0.03×10^{-5} $m^3.s^{-1}.m^{-2}$. This value could be increased by increasing the filtration time. This parameter indicates the extent of lactate enrichment in the permeate showing that the desired product contains in the permeate and the retentate contains the glucose and SO_4^{2-}. The separation between glucose and lactate in ternary mixture containing 0.1M glucose, 0.1M NaLac and 0.125M Na_2SO_4 with concentration mode can be improved.

4.4.5 Conclusions

The experiments in concentration mode of binary mixture containing 0.1M glucose and 0.1M lactate confirm separation is unachievable. The addition of Na_2SO_4 in ternary-solution affects the lactate retention to decrease in higher extent than that of glucose. The maximum separation factor increases 50% with the ternary-solutions adding 0.125M Na_2SO_4. The maximum purity is 65%. Moreover, at 0.2M lactate shows the separation factor is lower than that obtained at 0.1M lactate. The results (retention and separation factor) obtained in concentration mode are in accordance with those obtained at the constant feed concentration. It could be mentioned that the results that obtained at constant feed concentration could be used to predict the results in concentration mode.

4.4.6 Perspective

Furthermore, there is an assumption to reach higher percent recovery of lactate in this work that is addition of the diafiltration mode. The diafiltration mode is denoted that method in which the water is fed to feed tank to hold the total volume of feed solution unchangeable until the volume of pure water fed into feed tank being equal to the volume of feed solution at the beginning (Wang *et al*., 2002). The concentration mode is carried out first to reach the maximum instantaneous concentration ratio and then switch to the diafiltration mode to reach higher percent recovery. However, this assumption does not improve the percent purity.

4.5 Real fermentation broth

This section studies the performance of NF to recovery lactate with the real fermentation broth. The experimental results were compared with those of synthetic broth and also to investigate the effect of addition of a salt in the fermentation broth on the separation between glucose and lactate. First, a real fermentation broth was investigated and then it was modified to have the similar composition as those in synthetic solution regarding glucose and lactate (0.1M each) and then finally the modified borth was added with Na_2SO_4. The compositions of investigated broths were listed in Table 3.2. The experiments were investigated only at constant feed concentration.

4.5.1 Fermentation broth

Since there was some water existing in the piping of the experimental set-up, the real fermentation broth was diluted in the system and the final glucose and

lactate concentration in the broth was decreased from 9.87×10^{-4} to 6.9×10^{-4} M and from 0.989 to 0.7M, respectively. Fig. 4.58 shows the retention of glucose and lactate increases with increasing the permeate flux. As expected, the retention of glucose is higher than that of lactate when the high concentration of NaLac was applied. This means the separation of glucose and lactate can be achieved under this condition. The negative retention of Cl^- was observed, which was also observed with the model binary- and ternary-solute solutions containing two salts. However, since this section focuses on the separation between glucose and lactate so that the retention of Cl^- is only shown in the graph but it will not be discussed further in detail.

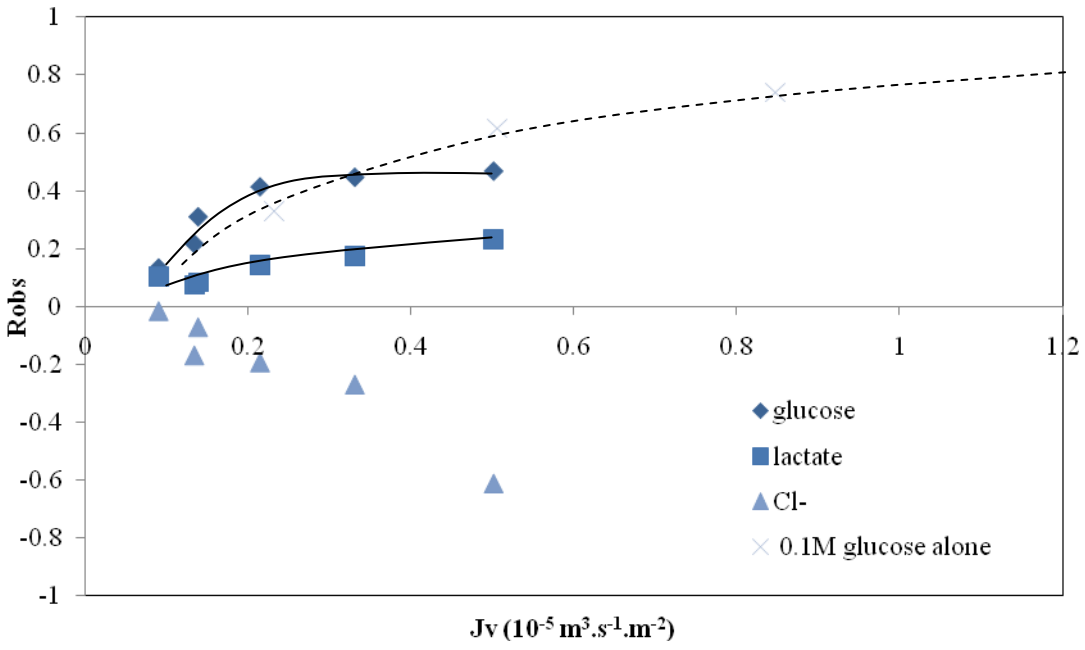


Figure 4.58 Retention of glucose, lactate and Cl^- as function of permeate flux in fermentation broth.

4.5.2 Modified fermentation broth

The experiment with modified fermentation broth was performed in order to compare the results obtained with the model solution with both glucose and lactate concentrations in the broth are diluted to 0.1M. Fig. 4.59 shows the retention

of glucose and lactate increases with increasing permeate flux. The retentions of glucose is higher than that of lactate. It could be mentioned here that the separation between glucose and lactate are achievable from the modified fermentation broth. The glucose and lactate retentions obtained with modified fermentation broth are different from those observed from model solutions, binary solution containing 0.1M glucose and 0.1M lactate. The retention of glucose is higher than those of model solution whereas the retention of lactate is lower.

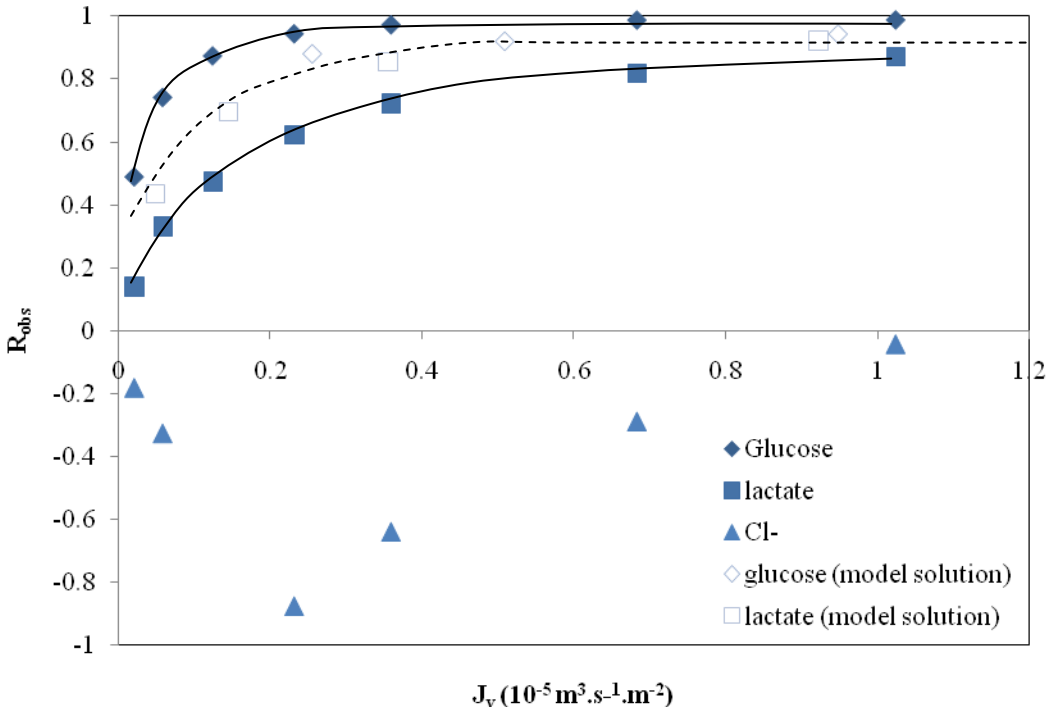


Figure 4.59 Retentions of glucose, lactate and Cl^- (see legends) as function of permeate flux in feed modified fermentation broth contianing 0.1M glucose and 0.1M lactate.

4.5.3 Modified fermentation broth with adding Na_2SO_4

The experiment of modified fermentation broth with adding Na_2SO_4 was investigated in order to investigate the influence of the addition of Na_2SO_4 on the separation between glucose and lactate in the real fermentation broth (Fig. 4.60). Both

glucose and lactate retentions increase with increasing the permeate flux. Again, the retention of glucose is higher than that of lactate. Negative retention of lactate can be observed at low flux. The retentions of glucose and lactate are lower than those of the modified broth without the addition of Na_2SO_4; however, the glucose and lactate retentions in presence of SO_4^{2-} are slightly different from those observed with the modified broth without Na_2SO_4 addition. This implies that the separation between glucose and lactate can be slightly improved further when there is SO_4^{2-} presents in the modified broth.

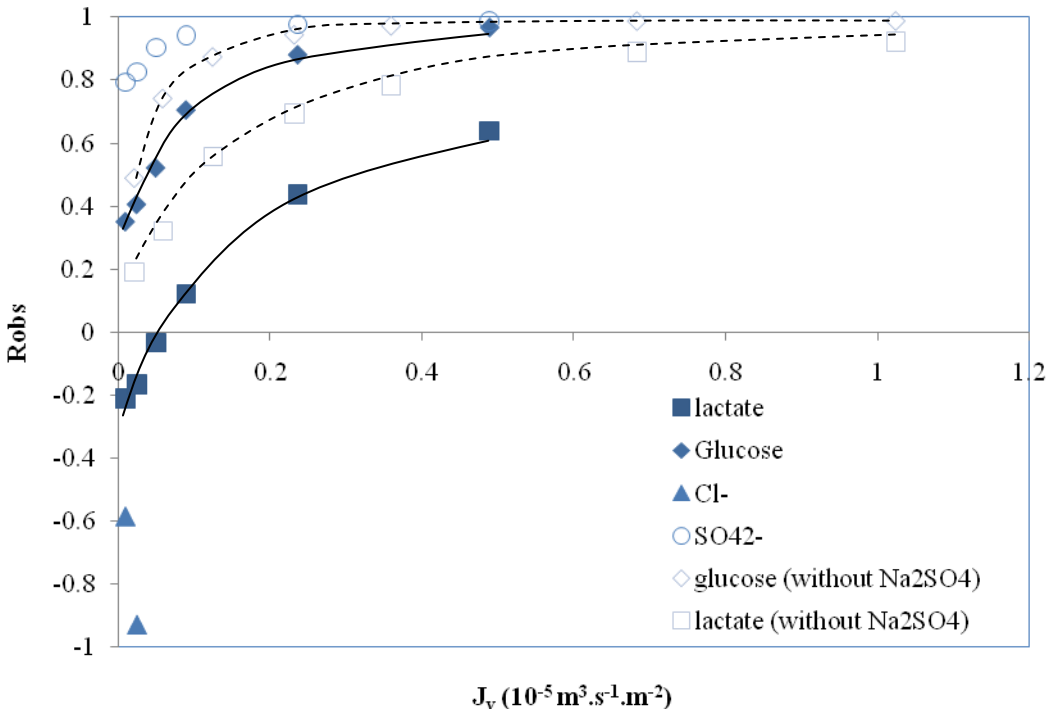


Figure 4.60 Variation of retentions of glucose, lactate and Cl^- (see legends) as function of permeate flux - modified fermentation broth containing 0.1M glucose, 0.1M lactate and with adding 0.125M Na_2SO_4.

4.5.4 Discussion

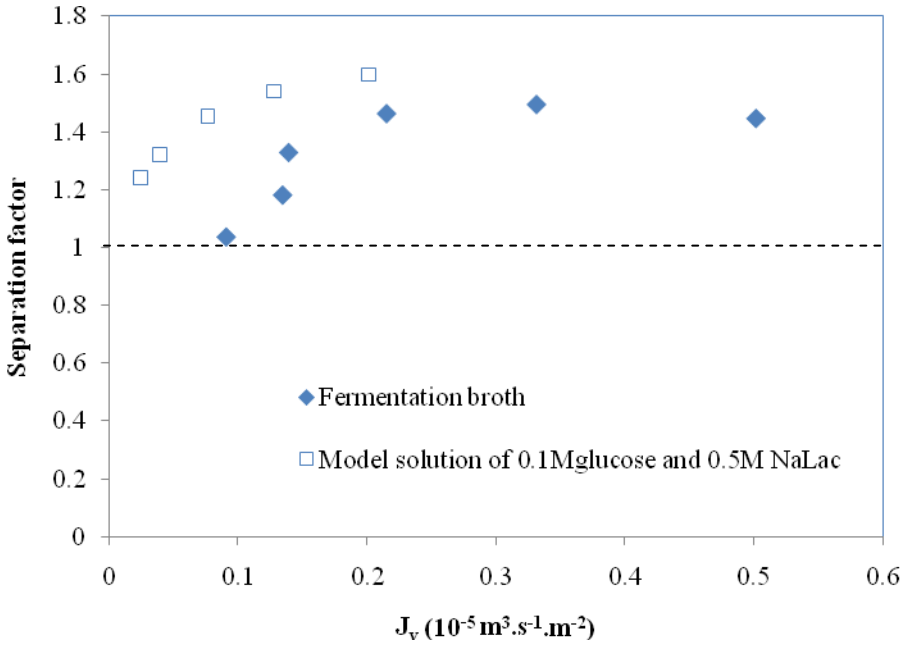


Figure 4.61 Separation factor of lactate as function of permeate flux in fermentation broth.

Fig. 4.61 shows the separation factor of the fermentation broth containing 0.7M lactate and 0.00069M glucose. It can be observed that all of separation factor is higher than 1 that means the separation can be achieved under this condition. The separation factor of model solutions containing 0.1M glucose and 0.5M NaLac, which is very close condition to the model solution, are compared with those of fermentation broth. The separation factor of fermentation broth is lower than those of the model solution at the permeate below 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$; however, the separation factor becomes more closer to that of model solution one at the permeate flux over 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$. The maximum separation factor in fermentation broth is 1.5 at permeate flux of 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$. It could be noted that the separation in the fermentation broth is feasible.

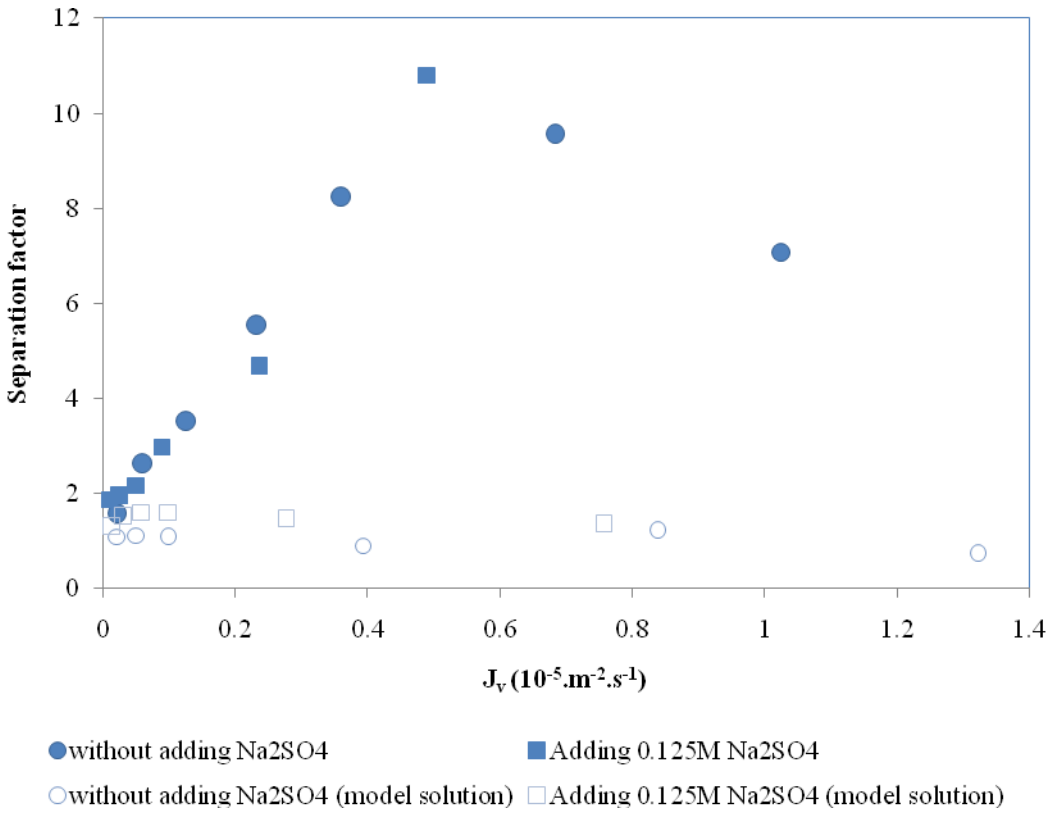


Figure 4.62 Separation factor of lactate as function of permeate flux in modified fermentation broth containing 0.1M glucose, 0.1M lactate and adding 0.125M Na_2SO_4.

Fig. 4.62 shows the separation factor of lactate obtained from the modified fermentation broth initially increases and then decreases with increasing permeate flux. The separation factor of modified fermentation broth is much higher than those obtained from synthetic binary-solute solution containing 0.1M glucose, 0.1M lactate. The maximum separation factor of lactate in modified fermentation broth is 10 at permeate flux of 0.7×10^{-5} $m^3.s^{-1}.m^{-2}$. The separation is achievable under this condition. The much higher of the separation factor is explained here. The fermentation broth does not only contain glucose and lactate but also other compounds, which were not be analyzed. The chromatogram of fermentation broth

sample contains many unknown peak as shown in the appendix C. These compounds could affect the retention of glucose and lactate in different magnitude.

Furthermore, Fig. 4.62 also shows the separation factor of lactate obtained from the modified fermentation broth with adding Na_2SO_4. The maximum of separation factor is 11 and is higher than those of the modified broth without adding Na_2SO_4 at permeate flux around 0.5×10^{-5} $m^3.s^{-1}.m^{-2}$. It shows that the addition of Na_2SO_4 further improves the separation. This can be explained by the modified fermentation broth is complex mixture which contains many other compounds as mentioned in the latter paragraph. The addition of SO_4^{2-} has much effect on the separation which is in accordance with those observed in the synthetic solutions. The results obtained from synthetic solution, simulated fermentation broth, are comparable to those with the real one. It is mentioned here that the separation between glucose and lactate in the modified fermentation broth can be predicted from those results of synthetic solutions.

4.5.5 Conclusions

The separation between glucose and lactate in the fermentation broth containing high lactate concentration (0.7M) is achievable by using NF. As the fermentation broth concentration was modified to 0.1M glucose and 0.1M lactate, the separation between glucose and lactate is surprisingly achieved. It might be due to the unknown compounds containing in the broth could affect the retentions of glucose and lactate. The addition of Na_2SO_4 into the modified fermentation broth helps further improve the separation. The results that obtained from the experiments with fermentation broth and with synthetic solutions are comparable.

CHAPTER V

CONCLUSIONS

This work studies the separation and purification of lactic acid from fermentation broth by nanofiltration. The mechanisms of charged and neutral solutes transfer through nanofiltration membrane, interaction between neutral/electrolyte and electrolyte/electrolyte, the efficiency of nanofiltration on lactic acid recovery, and performance NF on the separation in real fermentation broth were investigated. The experiments were classified into 4 parts depending on the type of solution used such as single-, binary- and ternary-solute solutions and fermentation broth. The experiments are concluded as following:

1) Single-solute solutions, the mechanisms of charged and neutral solutes transfer through nanofiltration membrane were investigated. The retention of glucose is independent of its concentration because of the size effects. The retentions of NaLac and NaCl decrease with increasing salt concentration due to the screening effect. However, the retention of Na_2SO_4 is independent of Na_2SO_4 concentration showing that it is fixed by size effects. The separation between glucose and NaLac is expected to be feasible since NaLac retention is much lower than that of glucose at high NaLac concentration.

2) Binary-solute solutions, the influence of adding salt on the glucose and lactate retentions was investigated. The effect on glucose retention is mentioned first,

the retention of salts does not only decreases with increasing salt concentration but also that of glucose. The glucose retention decreases in presence of the salt which is in sequence: NaLac > NaCl > Na_2SO_4 at the same Na^+ concentration. Different explanations concerning reduction of glucose retention were taken to describe the decrease of glucose retention *i.e.* pore swelling and/or decrease of glucose hydrodynamic radius in the presence of salt.

Another, the retention of lactate decreases in presence of other salts e.g. NaCl and Na_2SO_4. The lactate retention decreases with adding other salt in the order: Na_2SO_4 > NaCl. At low flux and high concentration of higher retained solute, the less retained solute decreases even meets the negative retention which is due to influence of electroneutrality. The binary mixtures containing Cl^- and lactate, the less retained ion Cl^- meet the negative and the binary mixture containing lactate and SO_4^{2-}, the less retained lactate meet negative retention. The decrease depends on the concentration ratio between higher retained solute and less retained solute and permeate flux.

Other, for binary mixtures containing glucose and lactate, the separation between glucose and lactate is achievable in particular condition such as low permeate flux and high NaLac concentration. The effect of addition NaCl or Na_2SO_4 on glucose and lactate retentions in binary mixtures are different in magnitude. The retention of lactate decreases in higher extent than that of glucose at similar added salt concentration. It could be expected that the addition of NaCl or Na_2SO_4 can improve the separations.

3) Ternary-solute solutions, the influence of addition salt on the separation between glucose and lactate and the performance of NF on the separation between glucose and lactate were investigated by performing at feed constant concentration and under concentration mode, respectively. The effect of addition of salt on the separation is mentioned first. The addition of NaCl can slightly improve the

separation which is the separator factor increases from 1.05 (without adding NaCl) to 1.6 (in presence of 1.0M Cl^-). The addition of NaCl is less effective when high lactate concentration (0.5M) contained in the solution. The addition of Na_2SO_4 improves the separation which is the separation factor increases from 1.05 (without adding Na_2SO_4) to 1.9. The maximum separation factor was always obtained at permeate flux about 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$. The separation is achievable in the condition such as maintaining low permeate flux and high SO_4^{2-} concentration.

Secondly, under concentration mode, the separation is unachievable in binary mixture containing 0.1M glucose and 0.1M lactate. The addition of Na_2SO_4 into a glucose/lactate solution improves the separation and the highest of percent purity as 65% and the maximum of percent yield as 80% were obtained. Again, the maximum of separation factor was obtained at 0.2×10^{-5} $m^3.s^{-1}.m^{-2}$. However, the addition of Na_2SO_4 is less effective when the higher lactate concentration (0.2M) is in the solution. The results obtained in concentration mode are in accordance with those obtained at the constant feed concentration. It could be mentioned that the results that obtained in constant feed concentration mode could predict the results under concentration mode.

4) The experiments with fermentation broth were carried out at feed constant concentration. The separation efficiency was carried out with origin fermentation broth first and then the broth was modified to meet the composition of those at feed concentration and then finally the modified broth was added by Na_2SO_4 to see the effect on the separation. Firstly, the separation in the origin fermentation broth containing high lactate concentration (0.7M) is achievable as expected. Secondly, the fermentation broth composition was modified to meet 0.1M glucose and 0.1M lactate showing the separation is surprisingly achievable, which is different from those obtained from model solution. The maximum separation factor is 10 for the modified

broth and 1.05 for the model solution containing 0.1Mglucose and 0.1M lactate. It is likely happened which comes from the fact that there are many unknown substances contained in the modified broth and they can affect the glucose and lactate retention. Finally, the modified fermentation broth with adding Na_2SO_4 shows the improvement of the separation which is the separation factor increase to 11. The both results obtained from experiments with fermentation broth and model solutions are comparable.

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APPENDICES

APPENDIX A: PURE WATER PERMEABILITIES

The pure water permeabilities, which usually are determined before performing an experiment, are ploted as function of number of experiments. List of experiments before determining pure water permeability is also presented in table as following.

Table 1A List of experiments before determining pure water permeability of 1st membrane

No.	Type of solutions	Pure water permeabilities (10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)
1	0.1M glucose	0.136
2	0.1M glucose	0.141
3	0.1M glucose + 0.125M NaCl	0.145
4	0.1M glucose + 0.25M NaCl	0.121
5	0.1M glucose + 0.5M NaCl	0.104
6	0.1M glucose + 1M NaCl	0.2
7	0.1M glucose + 0.5M NaCl	0.157
8	0.1M glucose	0.160
9	0.1M NaLac	0.233
10	0.5M NaLac	0.159
11	0.5M NaLac	0.160
12	0.1M NaLac	0.273
13	0.1M NaLac	0.260
14	0.1M NaLac	0.150
15	0.1M NaLac	0.173
16	0.25M NaLac	0.179
17	1M NaLac	0.157
18	0.1M glucose	0.168
19	0.1M glucose	0.188
20	0.1M NaLac	0.139
21	0.1M glucose	0.139

Table 2A List of experiments before determining pure water permeability of 2nd membrane

No.	Type of solutions	Pure water permeabilities (10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)
1	0.1M glucose	0.138
2	0.1M glucose	0.154
3	0.1M NaLac	0.144
4	0.25M NaLac	0.154
5	0.1glucose + 0.1M NaLac	0.141
6	0.1M glucose + 0.5M NaLac	0.141
7	0.1M glucose + 0.1M NaLac	0.168
8	0.1M glucose + 0.1M NaCl + 0.1M NaLac	0.153
9	0.1M glucose + 0.1M NaCl + 0.1M NaLac	0.147
10	0.1M glucose + 0.2M NaCl + 0.1M NaLac	0.155
11	0.1M glucose	0.170

Table 3A List of experiments before determining pure water permeability of 3rd membrane

No.	Type of solutions	Pure water permeabilities (10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)
1	0.1M glucose	0.136
2	0.1M glucose + 0.1M NaCl + 0.1M NaLac	0.147
3	0.1M glucose + 0.05M Na_2SO_4 + 0.1M NaLac	0.157
4	0.1M glucose + 0.1M NaCl + 0.1M NaLac	0.167
5	0.1M glucose + 0.25M NaCl + 0.1M NaLac	0.175
6	0.1M NaLac	0.177
7	0.05M glucose, 0.1M glucose	0.185
8	0.1M glucose + 0.1M NaLac	0.223
9	0.1M glucose + 0.25M NaLac	0.207
10	0.1M glucose + 0.5M NaLac	0.202
11	0.1M glucose + 1M NaLac	0.206
12	0.1M glucose	0.204
13	0.1M glucose + 0.25M NaLac	0.230
14	0.1M glucose + 0.5M NaLac	0.277
15	0.1M glucose	0.234

Table 4A List of experiments before determining pure water permeability of 4th membrane

No.	Type of solutions	Pure water permeabilities (10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)
1	0.1M glucose	0.137
2	0.1M NaLac	0.148
3	0.1M glucose + 0.1M NaLac	0.175
4	0.1M glucose + 0.1M NaCl + 0.1M NaLac	0.178
5	0.1M glucose + 0.125M NaCl + 0.1M NaLac	0.193
6	0.1M glucose + 0.25M NaCl + 0.1M NaLac	0.179
7	0.1M glucose + 0.5M NaCl + 0.1M NaLac	0.187
8	0.1M glucose + 1.0M NaCl + 0.1M NaLac	0.202
9	0.1M glucose + 0.5M NaLac	0.139
10	0.1M glucose	0.141

Table 5A List of experiments before determining pure water permeability of 5th membrane

No.	Type of solutions	Pure water permeabilities (10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)
1	0.1M glucose	0.139
2	0.5M NaLac	0.141
3	0.1M glucose + 0.5M NaLac + 0.1M NaCl	0.177
4	0.1M glucose + 0.5M NaLac + 0.25M NaCl	0.175
5	0.1M glucose + 0.5M NaLac + 0.5M NaCl	0.176
6	0.1M glucose + 0.5M NaLac + 1M NaCl	0.174
7	0.1M glucose + 0.05M Na_2SO_4 + 0.1M NaLac	0.176
8	0.1M glucose + 0.125M Na_2SO_4 + 0.1M NaLac	0.180
9	0.1M glucose + 0.25M Na_2SO_4 + 0.1M NaLac	0.178
10	0.1M glucose + 0.5M Na_2SO_4 + 0.1M NaLac	0.196
11	0.1M glucose	0.179
12	0.05M NH_4Lac	0.182
13	0.1M NH_4Lac	0.187
14	0.1M glucose + 0.1M NH_4Lac	0.186
15	0.25M NH_4Lac	0.192
16	0.1M glucose + 0.25M NH_4Lac	0.213

Table 6A List of experiments before determining pure water permeability of 6th membrane

No.	Type of solutions	Pure water permeabilities (10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)
1	0.1M glucose	0.144
2	0.1M glucose	0.118
3	0.1M glucose	0.150
4	0.1M NaLac	0.148
5	0.1M glucose + 0.1M NaLac	0.163
6	0.05M Na_2SO_4	0.170
7	0.125M Na_2SO_4	0.169
8	0.05M Na_2SO_4 + 0.1M NaLac	0.170
9	0.125M Na_2SO_4 + 0.1M NaLac	0.174
10	0.25M Na_2SO_4	0.177
11	0.25M Na_2SO_4 + 0.1M NaLac	0.176
12	0.1M NaCl + 0.1M NaLac	0.176
13	0.25M NaCl + 0.1M NaLac	0.176
14	0.5M NaCl + 0.1M NaLac	0.183
15	1M NaCl + 0.1M NaLac	0.187
16	0.5M Na_2SO_4	0.190

Table 6A (Continued) List of experiments before determining pure water permeability of 6th membrane

No.	Type of solutions	Pure water permeabilities (10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)
17	0.1M glucose	0.189
18	0.1M NaCl + 0.1M glucose	0.182
19	1.25M NaCl + 0.1M glucose	0.185
20	0.05M Na_2SO_4 + 0.1M glucose	0.189
21	0.125M Na_2SO_4 + 0.1M glucose	0.194
22	0.25M Na_2SO_4 + 0.1M glucose	0.194
23	0.25M NaCl + 0.1M glucose	0.185
24	0.5M NaCl + 0.1M glucose	0.191
25	1M NaCl + 0.1M glucose	0.191
26	0.1M NaCl	0.195
27	0.5M Na_2SO_4 + 0.1M glucose	0.196
28	0.1M glucose	0.193
29	0.5M Na_2SO_4 + 0.1M NaLac + 0.1M glucose	0.187
30	0.25M Na_2SO_4 + 0.1M NaLac	0.191

Table 7A List of experiments before determining pure water permeability of 7th membrane

No.	Type of solutions	Pure water permeabilities (10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)
1	0.1M glucose	0.140
2	0.1M NaLac	0.188
3	0.1M glucose + 0.2M NaLac + 0.125M Na_2SO_4*	0.188
4	0.1M glucose + 0.2M NaLac + 0.125M Na_2SO_4*	0.219
5	0.1M glucose + 0.1M NaLac + 0.125M Na_2SO_4*	0.224
6	0.1M glucose + 0.25M NaLac*	0.225
7	0.1M glucose + 0.1M NaLac*	0.212
8	0.1M glucose + 0.1M NaLac*	0.204
9	0.1M glucose + 0.1M NaLac*	0.206

Remarks: * carried out under concentration mode

Table 8A List of experiments before determining pure water permeability of 8th membrane

No.	Type of solutions	Pure water permeabilities (10^{-5} $m^3.s^{-1}.m^{-2}.bar^{-1}$)
1	0.1M glucose	0.096
2	fermentation broth	0.092
3	Modified fermentation broth	0.103
4	Modified fermentation broth with adding Na_2SO_4	0.111

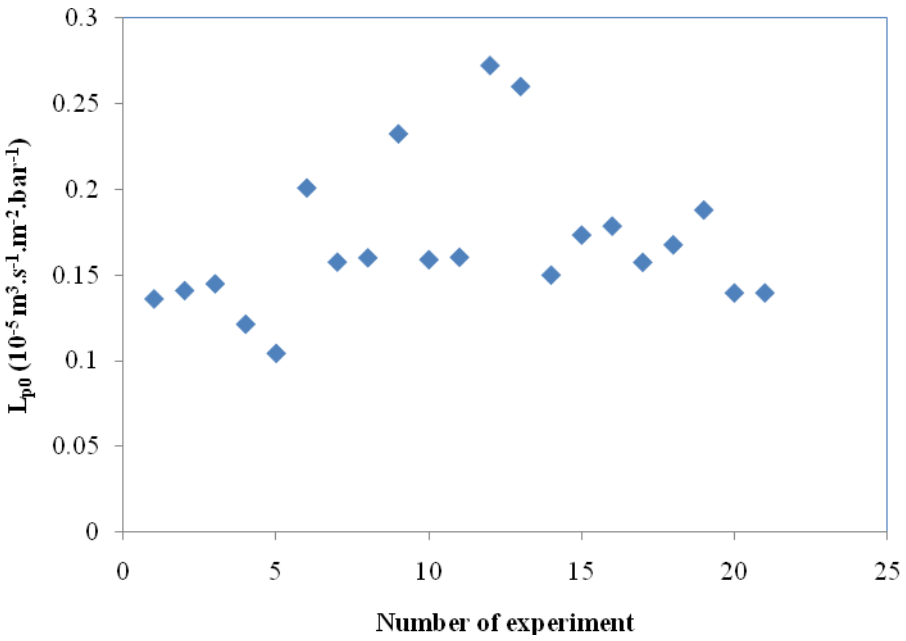


Figure 1A Variation of the hydraulic permeability Lp_0 measured before each experiment of 1st NF membrane.

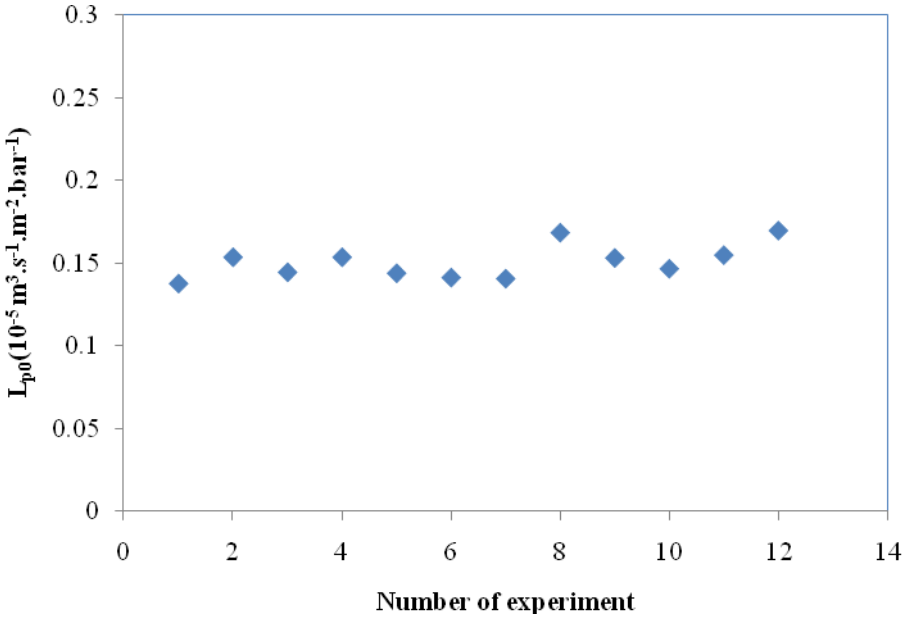


Figure 2A Variation of the hydraulic permeability Lp_0 measured before each experiment of 2nd NF membrane.

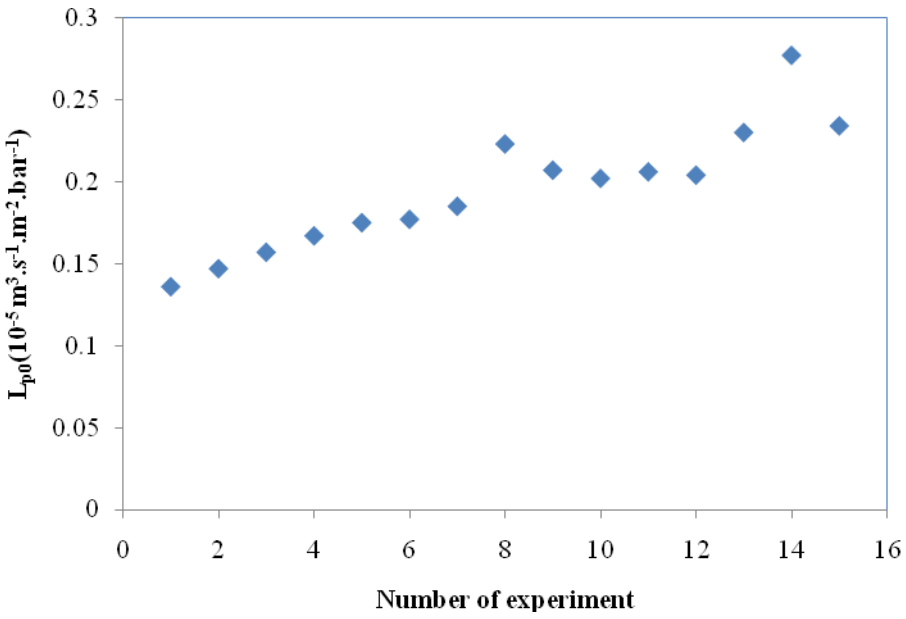


Figure 3A Variation of the hydraulic permeability Lp_0 measured before each experiment of 3rd NF membrane.

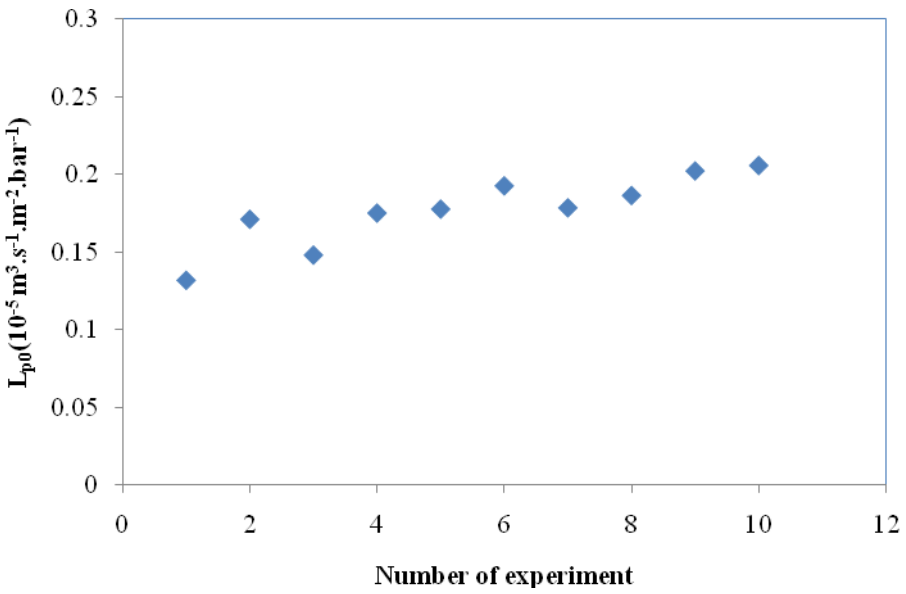


Figure 4A Variation of the hydraulic permeability Lp_0 measured before each experiment of 4th NF membrane.

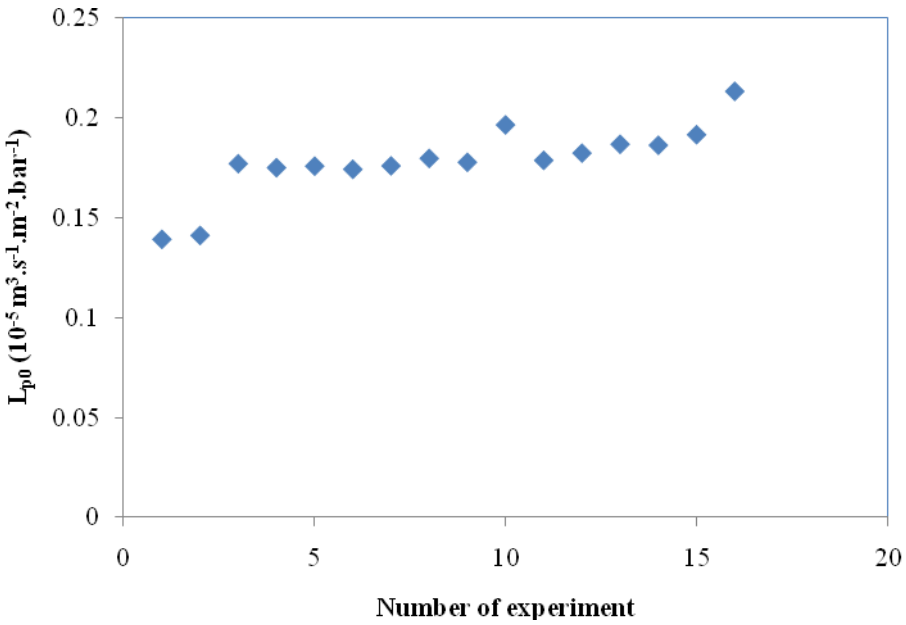


Figure 5A Variation of the hydraulic permeability Lp_0 measured before each experiment of 5th NF membrane.

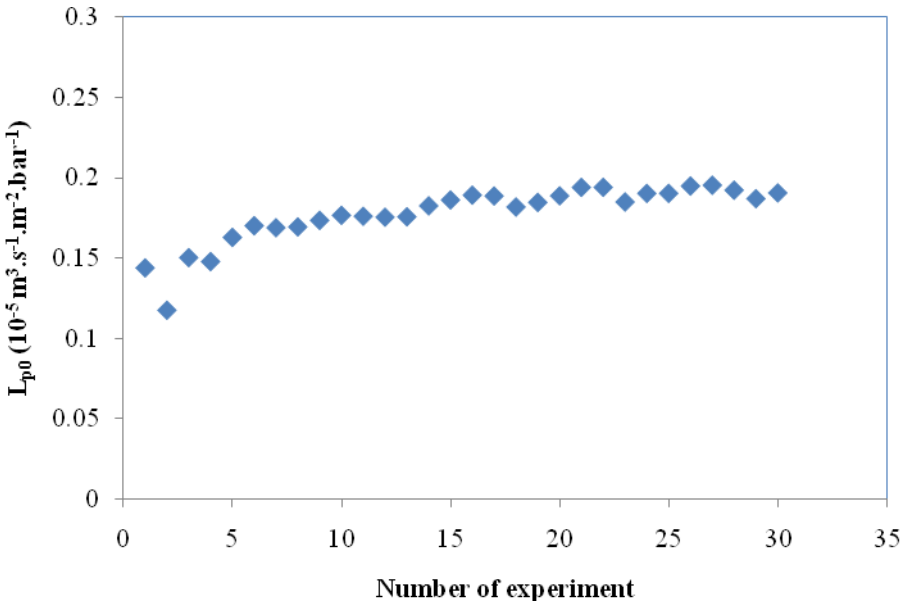


Figure 6A Variation of the hydraulic permeability Lp_0 measured before each experiment of 5th NF membrane.

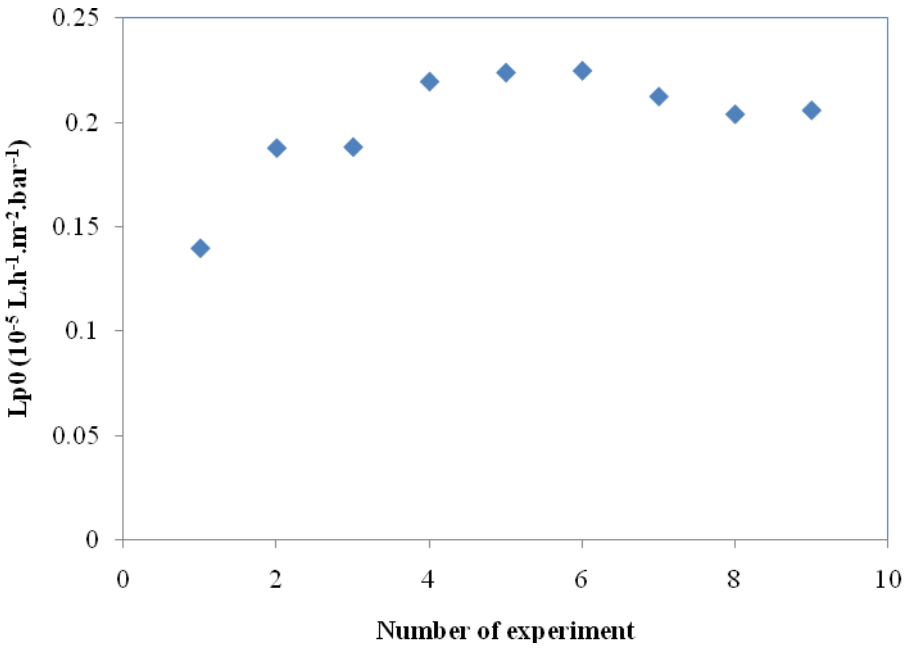


Figure 7A Variation of the hydraulic permeability Lp_0 measured before each experiment of 7th NF membrane.

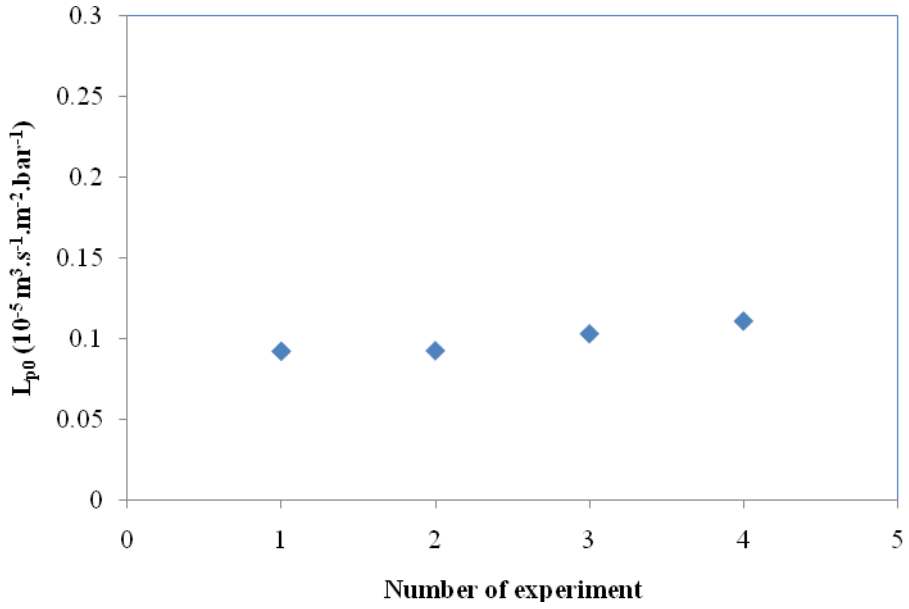


Figure 8A Variation of the hydraulic permeability Lp_0 measured before each experiment of 8th NF membrane.

APPENDIX B: CROMATOGRAMS

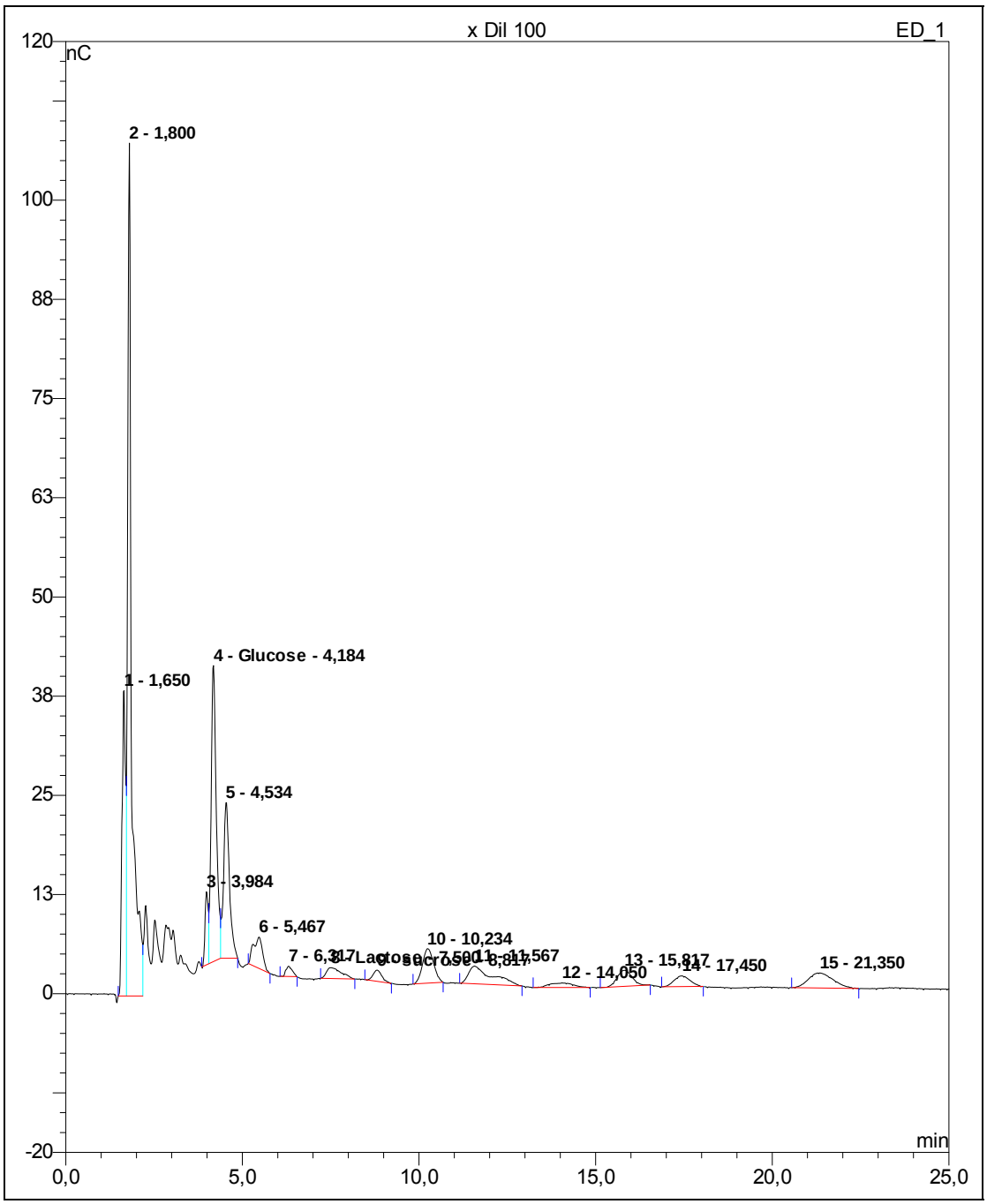


Fig. 1B Chromatogram of the fermentation broth with HPLC (Dionex)

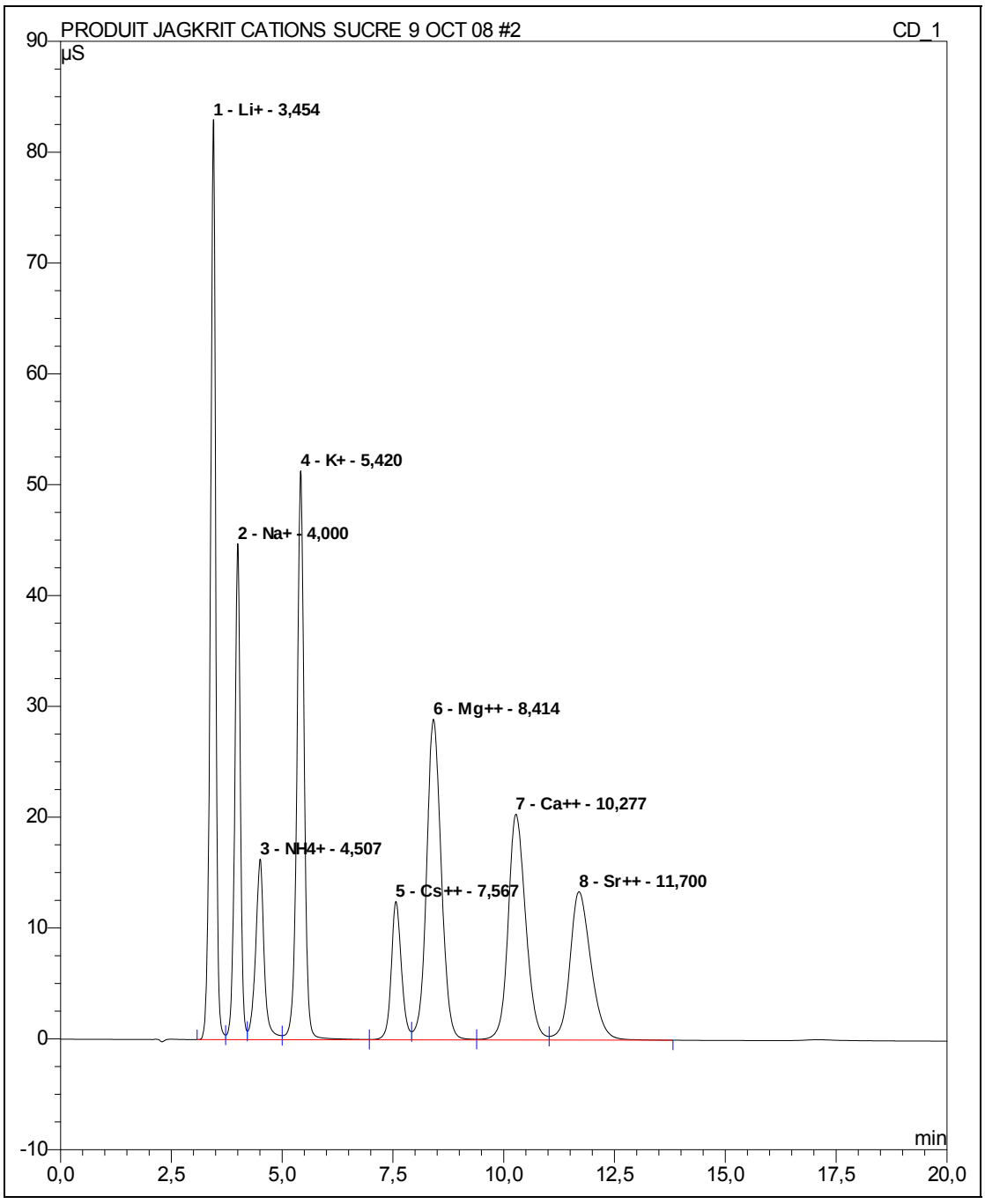


Fig. 2B Chromatogram of the cation calibration curve with HPLC (Dionex)

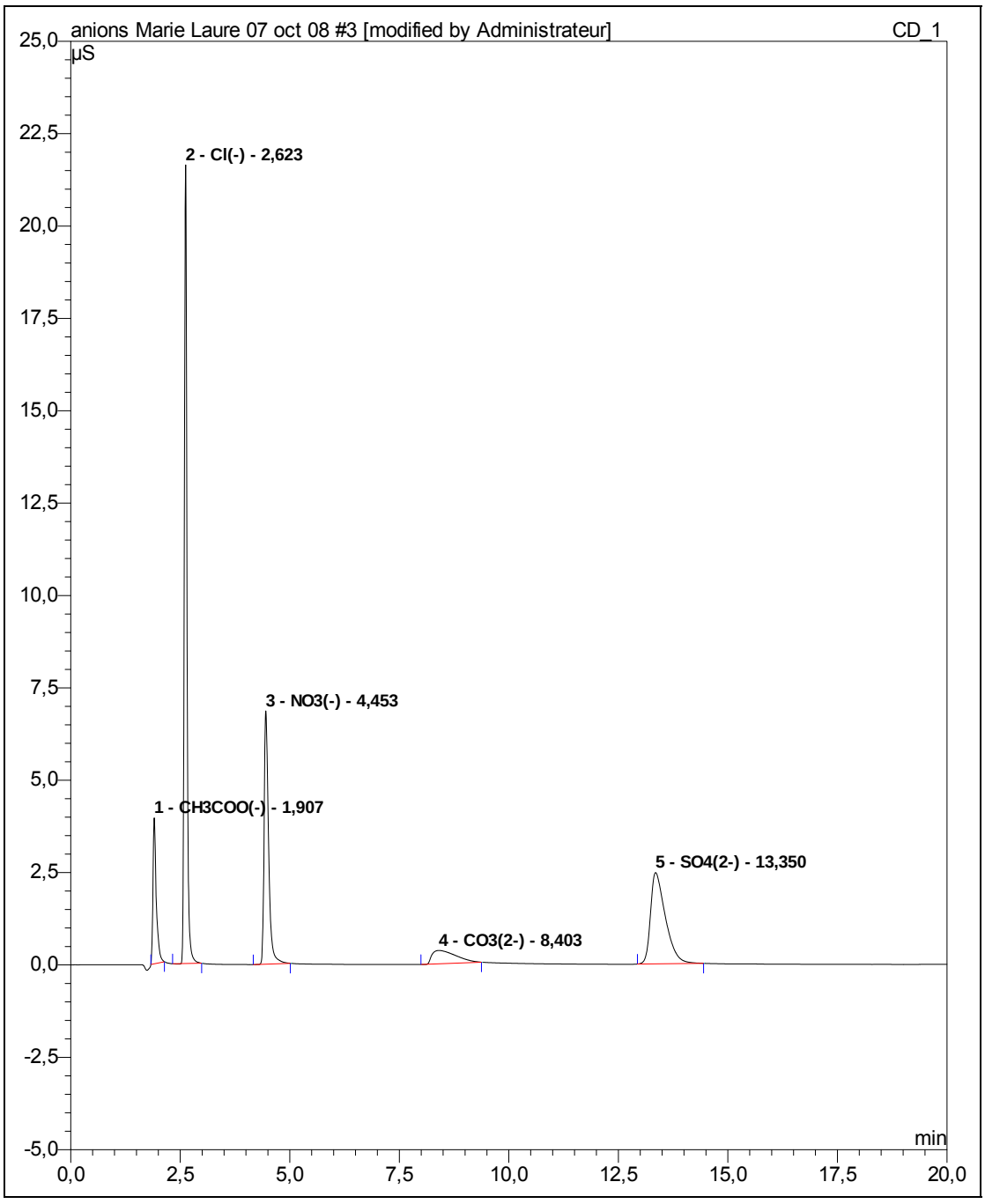


Fig. 3B Chromatogram of the anion sample with HPLC (Dionex)

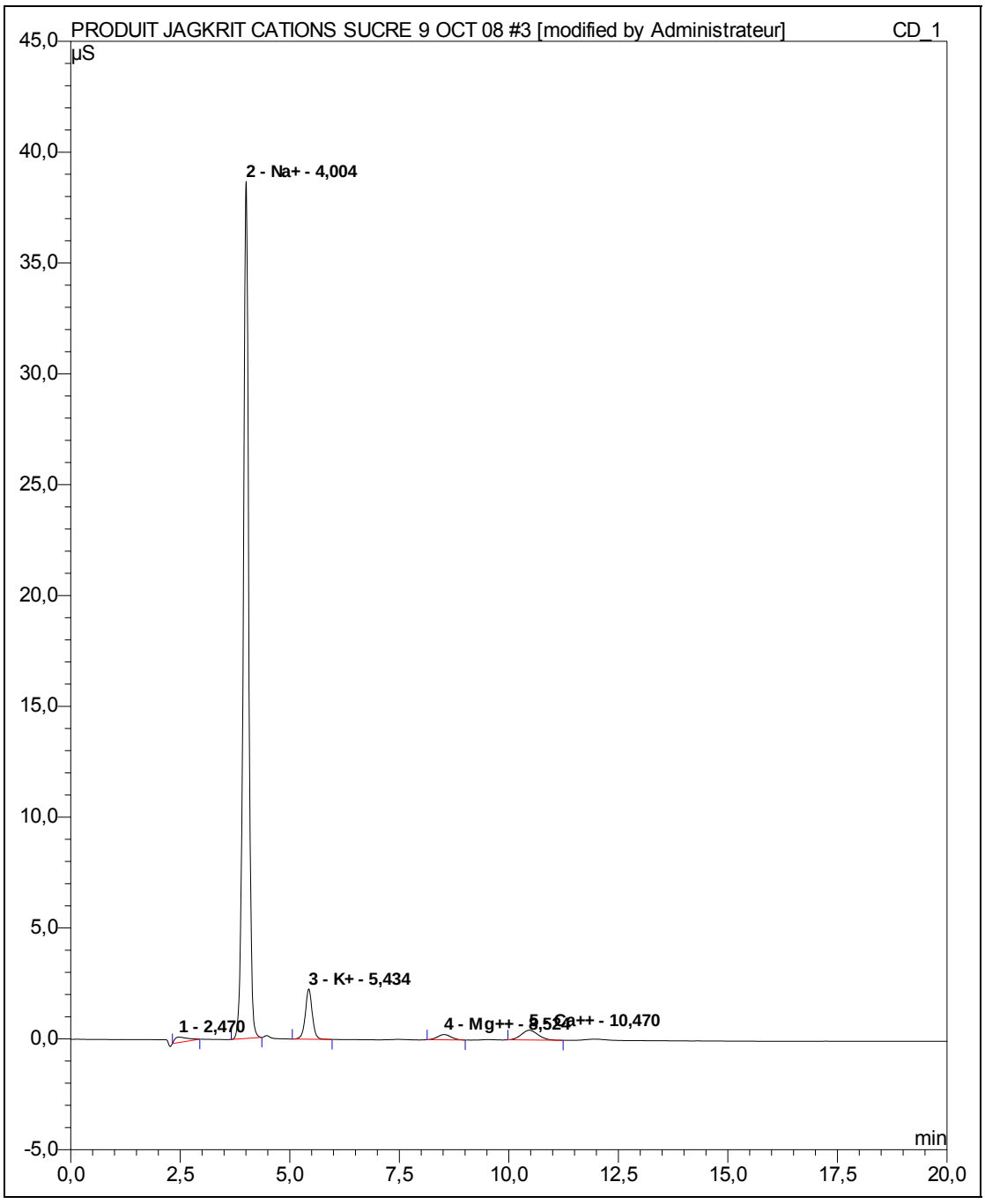


Fig. 4B Chromatogram of the cation sample with HPLC (Dionex)

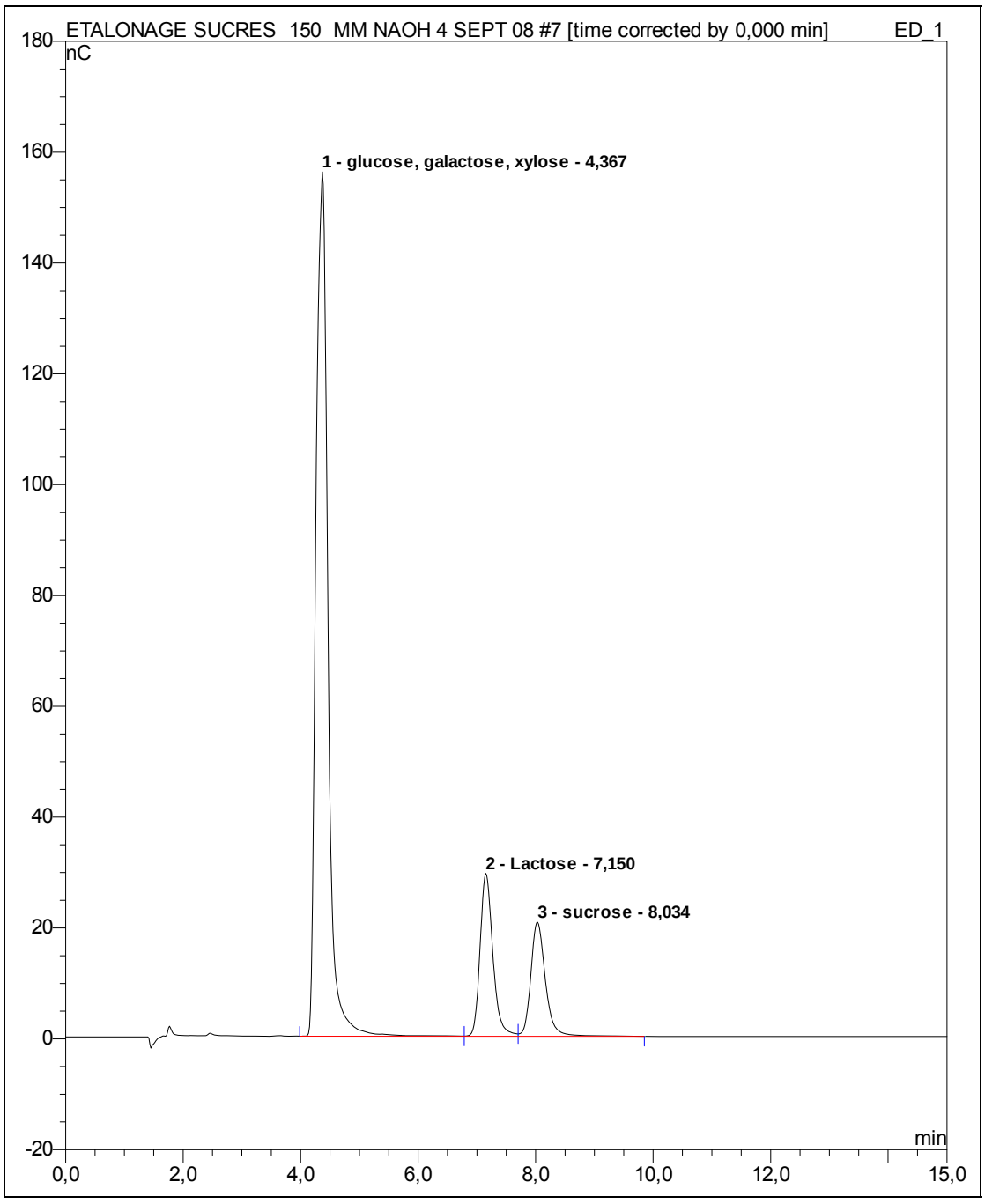
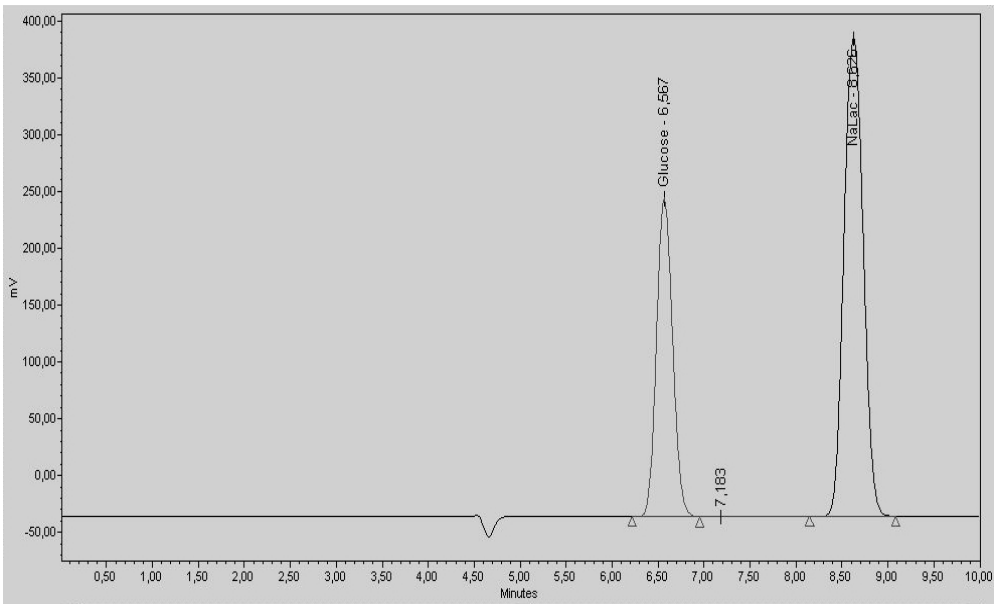


Fig. 5B Chromatogram of the sugar sample with HPLC (Dionex)



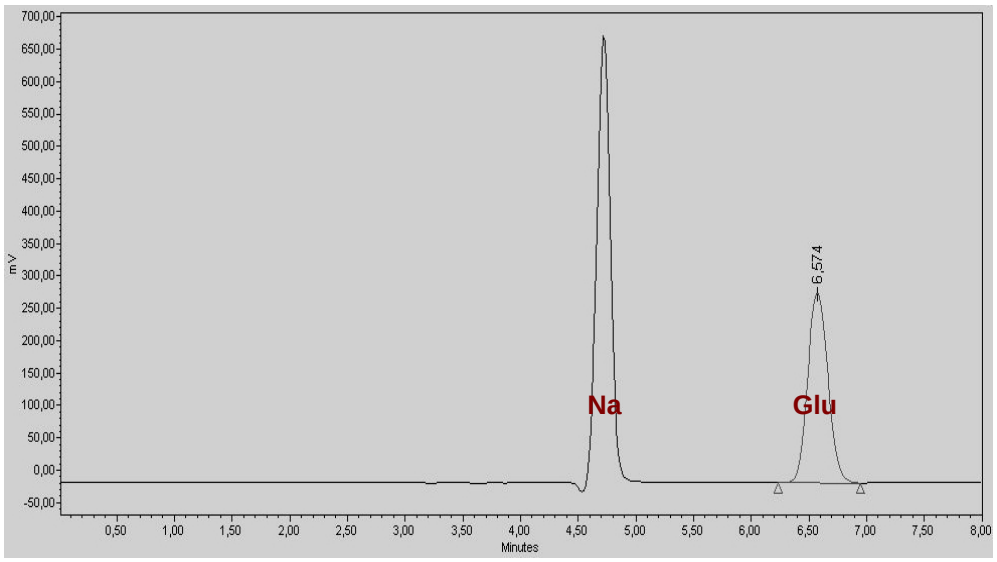


Fig. 6B Chromatogram of the cation and sugar sample with HPLC (Shodex)

APPENDIX C: LIST OF PRESENTATION

Oral Prsentation

C. Umpuch, S. Galier, S. Kanchanatawee, H. Roux-de Balmann, **Intégation de la nanofiltration de la sélectivité par ajout de sels**, 12ième Congrès SFGP, Marseille, 14-16 Octobre 2009.

C. Umpuch, S. Galier, S. Kanchanatawee, H. Roux-de Balmann, **Influence of additive mineral salt on the purification performances of glucose/sodium lactate solution by nanofiltration**, Euromembrane 2009, Montpellier, 6-10 Septembre 2009.

C. Umpuch, S. Galier, S. Kanchanatawee, H. Roux-de Balmann, **Purification of glucose/sodium lactate solutions by nanofiltration: selectivity improvement by the addition of a mineral salt.** ICOM 2008, Honolulu, Hawaii, USA, 12-18 Juillet 2008.

C. Umpuch, S. Galier, S. Kanchanatawee, H. Roux-de Balmann, **The separation of glucose/lactate in single- and mixed-solute solution by nanofiltration.** TSB2008, Maha Sarakham, Thailand, 14th -17th October 2008.

Poster Presentation

S. Galier C. Umpuch,, S. Kanchanatawee, H. Roux-de Balmann, **Séparation Glucose/Lactate par Nanofiltration : Comment améliorer la sélectivité en ajustant la composition ionique,** Marseille, 6-8 octobre 2010**.**

APPENDIX D: LIST OF PUBLICATIONS

Pubilication in an international journal

C. Umpuch, S. Galier, S. Kanchanatawee, H. Roux-de Balmann (2010), Nanofiltration as a purification step in production process of organic acids: Selectivity improvement by addition of an inorganic salt, **Process Biochemistry** (article in press).

Pubilication in a national journal

S. Galier, C. Umpuch, S. Kanchanatawee, H. Roux-de Balmann (2010), Séparation Glucose/Lactate par Nanofiltration : Comment améliorer la sélectivité en ajustant la composition ionique, **Récents Progrès en Génie des Procédés**: 100.

BIBLIOGRAPHY

Mr. Chakkrit Umpuch was born on April 6, 1981 in Lampang, Thailand. He graduated with the Bachelor's degree and Master's degree in Chemical Engineering, Suranaree University of Technology, Nakorn Ratchasima, Thailand in 2003 and 2005, respectively. Then, he started Ph.D. in school of biotechnology, Institute of Agricultural Technology in the previous university. He received a financial support from Duo-Thailand, Duo-France and French government scholarship during 2006-2008 to do his thesis research in Laboratore de Génie Chimique, University Paul Sabatier, Toulouse, France. He participated two international conferences such as ICOM2008, Hawaii, USA and TSB2008, Mahasarakarm, Thailand.