L(+)- Lactic Acid Purification From Fermentation Broth Using Ion Exchange Resins

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Zelal POLAT

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Department: Biotechnology and Bioengineering
Major: Biotechnology

121003

İzmir Institute of Technology
İzmir, Turkey

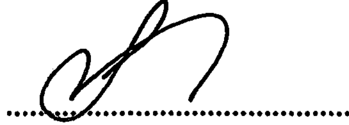
October, 2002

We approve the thesis of **Zelal POLAT**

Date of Signature

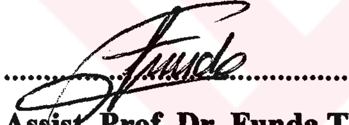
.... **04.10.2002**

Prof. Dr. Şebnem HARSA
Supervisor
Department of Food Engineering



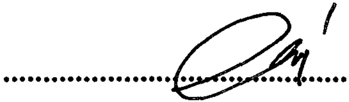
.... **04.10.2002**

Prof. Dr. Semra ÜLKÜ
Co-Supervisor
Department of Chemical Engineering



.... **04.10.2002**

Assist. Prof. Dr. Funda TIHMINLIOĞLU
Department of Chemical Engineering



.... **04.10.2002**

Assist. Prof. Dr. Oğuz BAYRAKTAR
Department of Chemical Engineering

.... **04.10.2002**

Prof. Dr. Şebnem HARSA
Head of Interdisciplinary
Biotechnology and Bioengineering Program

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T.C. YÜKSEKÖĞRETİM KURULU
DOKÜMANTASYON MERKEZİ

ABSTRACT

Lactic acid exists in two optically active form, D(-) and L(+)-lactic acid. It has been used in food, leather, textile, pharmaceutical and cosmetic industries. Moreover, L(+)-lactic acid constitutes the raw material for the production of poly-L-lactic acid which is used in biomedical applications.

The aim of this study was to recover and purify the microbially produced L(+)-lactic acid from the fermentation media efficiently and economically. Among the various downstream operations, ion exchange chromatography was used since it is highly selective and yields a low cost product recovery within a short period of time. The additional goals were to investigate the end product purity, to obtain new data on the adsorption/desorption behaviours of lactic acid and to investigate the applicability of the system for industrial usage.

In this project, *Lactobacillus casei* NRRL B-441 was used for the production of L(+)-lactic acid from whey by a 12 hours fermentation process at pH 5.5 and 37 °C. The product concentration was 50 g/l with 100% L(+)-lactic acid content. Then, a suitable resin with high sorption capacity and rapid equilibrium behavior was selected. The selected resin was Dowex marathon WBA, a weakly basic anion exchanger in OH form. It reached the equilibrium state in 15 minutes. The batch sorption experiments were done at pH 7.0 and 30 °C and sampling was continued for 20 hours. Furthermore, the effect of temperature and pH was investigated and their influence was found to be unimportant. All the adsorption/desorption experiments were applied both to model lactic acid and to biomass free fermentation broth. The ion exchange equilibria of lactic acid and L(+)-lactic acid in fermentation broth on Dowex marathon WBA were explained by the Langmuir isotherm. The maximum exchange capacity (q_m) for model lactic acid was 0.25 g La/g wet resin, while L(+)-lactic acid in fermentation broth has a q_m value of 0.04 g La/g wet resin. The equilibrium loading and exchange efficiency of L(+)-lactic acid in fermentation broth were reduced as a result of competition by other ionic species. The competing ions inhibit the binding of L(+)-lactic acid to the free sites of ion exchanger. Moreover, column operations were applied to recover sorbed lactic acid from the ion exchanger. 2.0 M HCl was found to be a suitable eluting agent to recover the bound L(+)-lactic acid with a flowrate of 1 ml/min at ambient temperature. About 95 % of bound L(+)-lactic acid was recovered from Dowex marathon WBA.

ÖZ

Laktik asit doğada D(-) ve L(+) olmak üzere iki formda bulunur ve gıda, deri, tekstil, ilaç ve kozmetik endüstrilerinde kullanılmaktadır. Bununla birlikte, L(+)-laktik asit, biyomedikal uygulamalarda kullanılan poli-L-laktik asitin üretimi için de hammadde teşkil etmektedir.

Bu çalışmada mikrobiyal yolla üretilen L(+)-laktik asitin fermantasyon ortamından verimli ve ekonomik olarak ayrılması amaçlanmıştır. Çeşitli alt akım işlemlerinden iyon değiştirme kromatografisi seçiciliğinin yüksek olması ve düşük maliyetle kısa sürede ürün eldesi sebebiyle kullanılmıştır. Ayrıca, son ürün saflığının araştırılması, laktik asitin adsorpsiyon/desorpsiyon davranışları üzerine yeni verilerin eldesi ve sistemin endüstriyel kullanımda uygulanabilirliğinin araştırılması ilave amaçlar arasındadır.

Bu projede, pH 5.5 ve 37 ^{o}C`de 12 saat süren fermantasyon yoluyla peynir suyundan L(+)-laktik asit üretimi için *Lactobacillus casei* NRRL B-441 kullanılmıştır. %100 L(+)-laktik asit içerikli ürün konsantrasyonu 50 g/l`dir. Ardından yüksek sorpsiyon kapasitesi ve hızlı denge davranışına bağlı olarak uygun reçine seçilmiştir. Dowex marathon WBA, OH formunda bulunan zayıf bazlı anyon değiştirici, dengeye 15 dakika içerisinde ulaşmıştır. Kesikli sorpsiyon deneyleri yaklaşık olarak pH 7.0 ve 30°C`de yapılmış, 20 saat boyunca örnek alınmıştır. Ayrıca sıcaklık ve pH`nın etkisi incelenmiş ve etkisi önemsiz bulunmuştur. Bütün adsorpsiyon/desorpsiyon deneyleri model laktik asit ve fermantasyon sıvısına uygulanmıştır. Dowex marathon WBA, üzerindeki laktik asit ve fermantasyon sıvısındaki L(+)-laktik asitin iyon değiştirme dengesi Langmuir izotermi ile açıklanmıştır. Model laktik asit için maksimum değişim kapasitesi (q_m) 0.25 g La/g yaş reçine ve fermantasyon sıvısında 0.04 g La/g yaş reçinedir. Fermantasyon sıvısındaki L(+)-laktik asitin dengede yüklenmesi ve değiştirme etkinliği diğer iyonik türlerin rekabetinin sonucu olarak azalmıştır. Mevcut iyonlar L(+)-laktik asitin iyon degiştiricinin serbest bölgelerine bağlanmasını engellemiştir. Bununla birlikte, adsorplanan laktik asitin iyon değiştiriciden geri kazanılması için kolon işlemleri uygulanmıştır. Bağlı L(+)-laktik asitin geri kazanılması için 1 ml/dk ve ortam sıcaklığında 2 M HCl uygun elüsyon ajanı olarak seçilmiştir. Bağlı L(+)-laktik asitin % 95`i Dowex marathon WBA`dan geri kazanılmıştır.

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CHAPTER 1

INTRODUCTION

Lactic acid is a beneficial chemical, which is used in many application areas. In food industry, it is used as acidulant, preservative and antimicrobial agent. It has been also utilized in leather, textile, pharmaceutical and cosmetic industries for many years. There are two isomers of lactic acid that are present in nature, L(+) and D(-) forms. L(+)-lactic acid is biodegradable and can be metabolized by the body and this property leads the application of lactic acid in biomaterial applications. L(+)-lactic acid is the raw material for the production of poly-L-lactic acid. To obtain high quality poly-L-lactic acid using L(+)-Lactic acid with high purity is very important.

Lactic acid is produced by chemical synthesis and by microbial fermentation. By the chemical synthesis method, racemic (DL) mixture of lactic acid is produced. By microbial production method L(+) and D(-)-lactic acid can be produced according to the type of microorganism which may be homofermentative or heterofermentative. This is an important advantage of the microbial production method compared to the chemical synthesis method. At the end of the fermentation process, lactic acid exists in the complex medium of fermentation broth. In order to obtain high yield from the fermentation process operating conditions are very important. The fundamental parameters are the type of microorganism, the best working temperature, pH, agitation speed, the type and the amount of initial carbon source, nitrogen source and some additional salts. The carbon sources can be supplied in pure form or as a constituent of crude feedstock. Whey is a crude feedstock used as a carbon source, which is the by-product of dairy industry. Thus lactose in whey is consumed to produce lactic acid and the latter will be converted to a valuable marketable product. However, at the end of the fermentation process, besides lactic acid whey proteins, biomass, salts and other impurities are present and lactic acid should be recovered from that complex media. As high cost of lactic acid purification process limits the utilization of this chemical, in large scale applications a system with less raw material and fewer unit operations are needed.

Ion exchange is one of the downstream processing techniques. Furthermore, this technique provides low cost, proceeds in shorter time period, has high yield and has

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good selectivity property. Because of those forthcoming properties of ion exchange technique, it is subjected to various purification studies. In order to purify lactic acid by ion exchange, the selection of the resin, the determination of operation conditions, the effect of competition ions present in the media, the efficiency of the elution step and the performance of ion exchanger after serial applications are of great importance. Besides the exchange capacity of the ion exchangers, the equilibrium properties are also necessary in designing a large scale process. In this study, the ion exchange characteristics were studied in terms of ion exchange isotherms and kinetics. Besides the sorption isotherm, the breakthrough curve, washing and elution conditions and column separation process for lactic acid were also described.

Sorption of lactic acid by ion-exchange resins is a process, which achieves good selectivity and specifity. This functions well in dilute processing streams and complex aqueous solutions such as fermentation broth (Van`t Hul and Gibbons, 1997).

The objective of this study was to investigate the use of ion exchangers containing basic functional groups in the primary recovery of lactic acid from fermentation broth which is formed by utilizing whey as carbon source. Moreover, additional objectives were to determine the amount of L(+)-and D(-)-lactic acid inside the produced lactic acid, to determine the ion exchange behavior of the selected resin towards the lactic acid isomers and to investigate the competition effect of ions that are present in fermentation media on ion exchange reaction.

CHAPTER 2

LACTIC ACID

2.1. Historical Perspective

Lactic acid (LA) was first produced by Charles E.Avery in USA in 1881. The first successful uses in the leather and textile industries began about 1894 and the production levels were about 5000 kg y^{-1} on a 100 % basis. In 1942, about half of the 2,7x10^6 kg y^{-1} produced in the US was used by the leather industry, and an emerging use in food products consumed about 20%. United States production peaked at 4.1x10^6 kg y^{-1} during World War 2 and leveled off to about 2.3x10^6 kg y^{-1}. A 90x10^6 kg y^{-1} market for lactic acid in the plastics industry was predicted in the late 1940s and early 1950s which encouraged a large but unsuccessful research effort to reduce costs and increase purity. A decade later, the need for heat stable lactic acid to produce stearoyl-2-lactlylates for the baking industry opened the way for a synthetic route to lactic acid. The 1982 world-wide production of lactic acid is 24-28x10^6 kg y^{-1}. More than 50% of the lactic acid production is used in food industry as an acidulent and a preservative. The production of stearoyl-2-lactylates consumes another 20%. The rest of the lactic acid is used by the pharmaceutical industry or is used in numerous industrial applications (Vickroy, 1985).

2.2. Properties of Lactic Acid

Lactic acid is an organic acid (∝-hydroxy-propionic-acid) with two isomeric forms. L(+) and D(-) lactic acid are two optical isomers. Fermentation is the most adequate means to obtain the pure isomers L(+) or D(-) lactic acid, and actually it is possible to choose a lactic acid bacterium capable of producing one of the stereoisomers because of its taxonomic characteristics (Raya-Tonetti *et al*, 1999).

Both isomeric forms of LA can be polymerized and polymers with different properties can be produced depending on the composition. Of the 80,000 tonnes of lactic acid produced worldwide every year about 90% are made by lactic acid bacterial fermentation and the rest is produced synthetically by the hydrolysis of lactonitrile.

Fermentative production has the advantage that by choosing a strain of lactic acid bacteria (LAB) producing only one of the isomers, an optically pure product can be obtained, whereas synthetic production always results in a racemic mixture of lactic acid (Hofvendahl and Hahn-Hägerdal, 2000).

Lactic acid was first isolated from sour milk by the Scheele in 1780. The two optically active forms of lactic acid are shown in Figure 2.1.

Figure 2.1. Isomeric forms of lactic acid.

Table 2.1. Physical properties of lactic acid (Vickroy, 1985)

Molecular weight	90.08 g/mole
Melting Point D(-) or L(+) DL(varies with composition)	52.8-54°C 16.8-33°C
Boiling point DL	82°C at 0.5 mmHg
Dissociation constant(K_a at 25°C)	$1.37x10^4$
Heat of combustion(ΔH_c)	1361 kj mol^{-1}
Specific heat(C_p at 20°C)	190 J mol^{-1} °C

2.3. Applications of Lactic Acid

Lactic acid is a useful product in the food industry as a biologically produced acidulent and preservative. Furthermore lactic acid is widely used as a starting material for chemical synthesis, because of its optical activity and its hydroxyl and carboxyl moieties. Lactic acid has the potential of becoming a very large volume chemical intermediate, produced from renewable resources for use as a feedstock for biodegradable plastics and other environmentally friendly green compounds. But until now, the extensive use of lactic acid in chemical industry is hampered by the high pro-

duction costs of optical pure (biologically produced) lactic acid (Börgardts *et al.*, 1998).

Nowadays, the industrial application of this acid as a precursor for poly(lactic acid) polymers requires one of the isomers to produce high quality products for biomedical applications and drug delivery (Raya-Tonetti *et al.*, 1999). Poly-L(+)-lactic acid is a polymer used in medical applications such as sutures, scaffold materials for artificial organs and implantable drug delivery systems.

There is an increasing interest in the biotechnological production of lactic acid for its use in the food, pharmaceutical and cosmetic industries. Recently this compound has been considered a potential feedstock for biodegradable lactide polymers (polylactic acids). In particular both the polymers and the co-polymers of L-lactide are especially attractive for biomedical applications, because of their biocompatibility, body absorbability and their reasonable blood compatibility (Vaccari *et al.*, 1993).

Polymer is an indispensable part of our life-style in the industrialized world. However, these petrochemical-based synthetic polymers have contributed to an increasing difficulty in controlling environmental problems due to its durability and non-biodegradability. One solution for these problems is the use of biodegradable polymers as substitutes for petrochemical-based polymers. Polylactide, which is known to be biocompatible and biodegradable, can be used as biodegradable polymer for bulk product and is now widely used for many biomedical applications. For example in the surgical suture producton, in drug delivery systems, and in the internal bone fixation applications (Choi and Hong, 1999).

2.4. Production Technology

2.4.1. Chemical Production

The synthetic manufacture of lactic acid on a commercial scale began around 1963 in Japan and in the United States. Synthetic lactic acid production is based on the hydrolysis of lactonitrile by a strong acid such as HCl as is shown in Figure 2.2 and Figure 2.3.

Figure 2.2. Hydrolysis of lactonitrile

An ammonium salt is formed as a by-product of this reaction. Lactonitrile was obtained along with acetaldehyde:

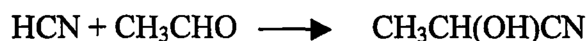

Figure 2.3. Lactic acid synthesis

The synthetic lactic acid contains no residual sugars and does not discolor significantly upon heating. (Helfferich, 1962)

2.4.2. Microbial Production

Lactic acid bacteria (LAB) consist of the Gram-positive genera: *Carnobacterium, Enterococcus (Ent), Lactobacillus(Lb); Lactococcus (Lc), Leuconostoc (Leu), Oenococcus, Pediococcus (Ped), Streptococcus (Srr), Tetragenococcus, Vagococcus,* and *Weissella.* LAB are cocci, with the exception of lactobacilli and carnobacteria which are rods, unable to synthesize ATP by respiration, and that have lactic acid as the major end product from energy-conserving fermentation of sugars. Most LAB are facultatively anaerobic, catalase negative, nonmotile and nonspore forming bacteria. They have high acid tolerance and survive at pH 5.0 and lower. Their acid tolerance gives them a competitive advantage over other bacteria. The optimal temperature for growth varies among the members of genera from 20 ^{0}C to 45 ^{0}C. Most of them are considered GRAS (Generally Regarded As Safe), but some strains of LAB e.g. *Streptococci* are pathogenic. All LAB genera belong to the *Clostridium* which in addition to give LA is also metabolized into formate and acetyl-CoA by pyruvate formate lyase (PFL). In the presence of oxygen PFL is inactivated, and an alternative pathway of pyruvate metabolism becomes active via pyruvate dehydrogenase (PDH), resulting in the production of carbon dioxide, acetyl-CoA and NADH. LAB are also capable of forming other products, e.g. flavors such as diacetyl and acetoin or bacteriocins (Hofvendahl and Hahn-Hägerdal, 2000).

2.4.2.1. Raw Materials

The lactic acid production has received much attention due to its numerous uses in the food and biochemical industries. The substrates such as, soybean milk, corn, sulfite waste liquor and potatoes have been used for lactic acid production (Srivastava *et al*, 1992). In lactic acid fermentation the selection of the substrate depends on the following criterias; fast fermentation rate, high lactic acid yields, low cost, low levels of contamination, little or no by product formation, ability to be fermented with little or no pre-treatment and year round availability (Vick Roy, 1985). Generally lactic acid is produced from carbohydrates. The most widely used sources are glucose, lactose, maltose, sucrose that can be supplied either in pure form or as a constituent of a crude feedstock. The most widely used crude feedstocks are molasses and whey. Molasses is the by-product of sugar industry and contains high levels of sucrose.

Cheese whey is the yellowish green liquid waste generated during the manufacture of cheese. It contains high levels of organic matters and its direct discharge into the drain, without further treatment, constitutes an environmental hazard. To convert this waste into an asset many researchers have analyzed a large number of potential utilizations of either whole cheese whey or its major components (lactose and proteins). Among them, the biological conversion of its lactose content into lactic acid using appropriate species of *Lactobacillus* has the double advantage of alleviating a pollution problem and at the same time, producing a marketable product (Rincon *et al*, 1997).

In order to make protein concentrates it may be exposed to ultrafiltration. The filtrate is whey permeate, which still contains almost the total lactose and salts derived from milk, resulting in a high COD of 50-60 kg 0_2/t. The disposal of the whey permeate is still a problem. Because of the high chemical oxygen demand(COD) it is not economic and mostly not possible to treat it in sewage treatment plants. A promising way to use whey permeate in high quantities is the fermentation of the lactose to lactic acid by lactic acid bacteria (Börgardts *et al.*, 1998).

Table 2.2. Gross composition of liquid and dried whey

Component	Fluid whey	Dried whey
Total solids, %	6.35-7.0	96.3-96.5
Protein, %	0.8-0.9	13.0-75.0
Lactose, %	4.85-5.1	68.0-75.0
Fat, %	0.3-0.5	0.8-1.0
Lactic acid, %	0.05	0.2
Ash, %	0.5-0.6	7.3-9.6

It is also possible to use renewable resources as substrates, such as starch and cellulose in fermentative production. Renewable resources do not give any net contribution of carbon dioxide to the atmosphere as the limited oil and fossil-fuel-based sources. Cellulose, hemicellulose and starch are the most abundant compounds in the world and when hydrolyzed to mainly glucose they are fermentable by a number of microorganisms. Hemicellulose, in contrast to starch and cellulose, contains pentoses, which give rise to by-products such as acetate and ethanol, decreasing the lactic acid yield. Fermentative lactic acid production from renewable resources comprises the following steps: First pretreatment of substrate including hydrolysis to sugars, next fermentation of sugars to lactic acid, then separation of bacteria and solid particles from the broth, and finally purification of lactic acid (Hofvendahl and Hahn-Hägerdal, 2000).

2.4.2.2. Fermentation Process

More than half of the total consumption of lactic acid is produced in industrial scale traditionally in simple batch fermentations with low productivities. In order to get higher productivities and therefore lower production costs, techniques have been developed for raising the concentration of biomass in the reactor by separating the fluid and cell residence time. This leads to higher volumetric productivities. For this purpose, the most promising bioreactor system seems to be the stirred tank reactor with cell recycle. The biomass is separated by filtration from the fermentation broth and recycled to the reactor. The product stream is free of solids and can directly be processed for product recovery. In the last years many researchers have worked with the investigation of this type of reactor and high lactic acid productivities have been achieved. But there

are still problems with fouling of the filtration membrane and the stability of the culture over long fermentation times with a high recycling ratio remains to be proved (Börgardts *et al.*, 1998).

As with most polymerizations, monomer purity is highly critical in the synthesis of polylactides and a purity of 99.9% or higher is usually required with the starting lactide material. At present, batch fermentation is generally employed in industrial sca1e lactic acid production. About 70% of total lactate produced although continuous processes with cell recycling or cell immobilization systems have been proposed. The restriction imposed by lactic acid on its fermentation has been avoided by an extractive fermentation technique employing ion-exchange resin (Vaccari *et al.*, 1993).

The commercial production of lactic acid through fermentation process has always been in competition with its chemical synthesis process. Lactic acid produced through the fermentation process has to cope with the problems of purification to meet the required quality standards. An attempt to improve the fermentative production is possible by proper design of an industrial process involving low capital cost for the plant. Also, the low energy costs both in its fermentation and purification are required. In the commercial interest, the investment cost should be minimised, which is possible only when the cell density in fermenter is high. It means that the inhibitory effect of the product on process kinetics must be minimised. Based on these requirements, the extractive bioconversion technique is one of the approaches to achieve the commercially viable lactic acid production (Srivastava *et al*, 1999).

To increase volumetric production in lactic acid fermentations, high cell density has been achieved through growth of biofilm on activated carbon or cell immobilization in gelatin beads. Removal of inhibitory product has been achieved using both liquid extractants and solid adsorbents either in a product stripping side stream or added directly to the continuous stirred tank reactor. *In situ* product removal during the fermentation has the potential to minimize process waste streams by obviating the need for reactor pH control and lactic acid precipitation (Kaufman *et al*, 1994).

Lactic acid fermentation is well known for its sensitivity. Several integrated fermentation-separation systems have been used to reduce end-product inhibition and thus to improve overall process efficiency. These systems attempted to increase the productivity of lactic acid and biomass. However, these two targets are interrelated: an increase in biomass concentration will improve the productivity of lactic acid since lactic acid is a partial growth-associate product, while high lactic acid concentration will

cause the inhibition of microbial cell growth which in turn results in a low production rate of lactic acid (Ye *et al*, 1996).

2.5. Recovery Techniques

Highly purified lactic acid as monomer is required for the chemical synthesis of polylactide. However, the purification of lactic acid obtained from bacterial fermentation is difficult due to its low vapor pressure (boiling temperature of 122 °C at 0.0164 atm), its tendency to undergo self-esterification and the presence of troublesome impurities. The conventional methods for the recovery of pure lactic acid were crystallization, extraction with a solvent and filtration-carbon treatment-evaporation (Choi and Hong., 1999).

The technological interest of lactic acid is geared to an adequate system for recovery and purification which can achieve high yield and purity considering that the concentration of lactic acid produced by bioconversion results in a high cost process. Conventional lactic acid purification from fermentation broth consisted in recovering lactate as a calcium salt, but this technique is expensive and produces wastes with an important contaminant load. Actually alternative processes have been developed such as electrodialysis, solvent extraction and ion exchange. The extractive fermentation of lactic acid was recently proposed as an alternative technology. A fluidized bed column for integrative protein purification from unclarified culture broth was developed to diminish the overall time of conventional downstream processing and increase the product yield (Raya-Tonetti *et al*., 1999).

Cross-flow filtration has been demonstrated to be an efficient process for high cell density culture with high productivity of lactic acid by continuous removal of fermentation broth from fermenter and recycling biomass. A productive process may not be achieved in practice since nutrients, especially the carbon source, are not efficiently utilized due to removal of fermentation broth for control of lactic acid concentration. Also a low concentration of lactic acid in the effluent from the fermenter is a burden on the recovery of lactic acid in downstream processes. To improve the performance of conventional biomass recycling fermentation, the spent broth should be re-used through recovery of lactic acid *in situ* with an appropriate downstream process. The spent broth from which lactic acid was extracted is then re-used for subsequent cell recycle fermentations after being supplemented with some necessary nutrients and a carbon

source. A novel integrated fermentation system was developed in this study to carry out biomass recycle and efficient reutilization of broth media by coupling a biomass recycling fermentation to an ion-exchange resin column (Ye *et al.*, 1996).

Since it is the product recovery cost rather than the feedstock or fermentation cost that dictates the production economics of the process, considerable recent work has been carried out to study methods of purification. One method that has received widespread attention is the extraction with organic solvents. However, since this method presents a number of problems related to the toxicity of the solvent, its selectivity, distribution ratio of the solute and solvent recovery, other separation processes have been investigated. For example, a number of authors have analyzed the application of liquid membrane and reverse osmosis and Prigent (1983) has proposed and studied lactic acid recovery in a two-stage process consisting of ultrafiltration and electrodialysis. However, in spite of all these developments at the scientific level, to date, few have found their way into commercial practice. The reason for the apparent lack of success in industrial developments is that they are at present fairly costly. An alternative to all these methods that would eliminate much of downstream processing costs associated with the concentration of the acid, would be a technology focused on the purification of the fermentation broth to obtain a lactic acid solution suitable for use as preservative in the food industry. Taking into account that the fermentation broths do not contain lactic acid but lactate (because during the fermentation process to produce lactic acid a neutralizing agent is used to keep the medium at an optimum pH value), ion exchange using cationic resins could be a possible method for obtaining a lactic acid solution from the broths. Although NaOH is a common neutralizing agent in lactic fermentations, the Na^+ ion becomes a common cation to be removed from the fermentation broths (Rincon *et al*, 1997).

The traditional recovery process of lactic acid from fermentation broth is quite complicated, in the conventional processes, lactic acid has been recovered from the fermentation beer by precipitation of calcium lactate with calcium hydroxide. In this recovery scheme, calcium lactate is precipitated, recovered by filtration, and converted to lactic acid by addition of sulfuric acid. The dilute lactic acid product is then sequentially purified using activated carbon, evaporation and crystallization. These stages account for up to 50% of the production costs. On the other hand, the accumulation of lactic acid product in the fermentation broth often inhibits further product formation. Reactor productivities are low and the products are obtained in dilute

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form. The effects of end product inhibition can be reduced by removing toxic metabolic products from the broth *in situ* by several methods. Fermentation products, such as lactic acid, can be removed from the broth by conventional liquid-liquid extraction. This process requires high volumes of solvent. Like many such processes, the product recovery and purification contribute significantly to the cost of production. As an alternative, Liquid Surfactant Membrane(LSM) extraction has been proposed. Interest in liquid surfactant surfactant membranes over other separation techniques is the large surface area available for mass transfer which results in a fast rate of separation. In spite of these apparent advantages, very few industrial applications have been reported so far. Several drawbacks were shown to hinder implementation, mainly complexity of operation and swelling in LSMs. The use of supported liquid membranes for the recovery of lactic acid offers unique advantages. Some of the advantages are lower energy consumption, higher separation factors in a single stage and the ability to concentrate lactic acid during the separation. However, supported liquid membrane often suffers from membrane instability (Zihao and Kefeng, 1995).

In recent years fermentation processes have become more industrially successful because of the increasing demand for naturally produced lactic acid. The major drawback of processes is the high cost of recovery, purification and concentration of the acid, which represents a considerable portion of the lactic acid production costs.

The growth of microorganisms may be limited either by the availability of nutrients or by the accumulation of inhibitory metabolic products. Thus, lactic acid inhibition of fermentation can be avoided by extractive fermentation techniques. Studies were performed by attaching an ion exchange resin-packed column to a fermenter for the separation of lactic acid. The recirculation of the fermentation broth through the resin to the fermenter minimized the inhibition of the batch fermentation by lactic acid. An innovative process for the recovery of lactic acid from fermentation broths is described. Fermentation was carried out in a Continuous Stirred Tank Fermenter (CSTF) with an ion exchange resins system for the removal of lactic acid from the fermentation broth. Lactate was adsorbed as it was formed on to a strongly acidic anion exchange resin in the carbonate form placed inside a column and eluted with ammonium carbonate during the course of fermentation The resulting ammonium lactate was passed through a strong cation exchange resin in the H^+ form to obtain lactic acid. By this means, it was possible to maintain an actively growing culture in a medium of low lactate concentration. The utilization of the fermenter-ion exchange

resins system offers the advantages of eliminating the inhibitory effects of lactate at the same time, reducing the costs of recovery and purification of the lactic acid from the fermentation broth (Monteagudo and Aldavero, 1999).

CHAPTER 3

PURIFICATION BY ION EXCHANGE

Lactic acid inside fermentation media exists in lactate form due to the consumption of bases for the adjustment of fermentation pH. Therefore downstream operations are required so as to obtain lactic acid. Ion exchange chromatography has the advantages as less unit operations requirement, feasibility of the technique and attractive from economical perspective among the other recovery techniques. In spite of the complex fermentation media lactic acid with high purity is obtainable.

The use of polylactic acid (PLA) for biodegradable plastics and controlled-release drugs and pesticides is a potential multimillion dollar market. The commercial success of PLA hinges on the purity (heat-stable grade) and the cost of the fermentation-produced lactic acid. Therefore, one of the major challenges in lactic acid production is to reduce the cost of the acid recovery and purification, which could amount to almost 50% of the final product cost (Evangelista *et al.,* 1994).

Adsorption is a process suitable for the recovery of substances produced in dilute concentrations and complex aqueous solutions, such as fermentation broth. A substantial number of publications in the last 12 year have addressed the use of ion exchangers and other adsorbents in the recovery of organic acids. The manufacture of lactic acid currenly uses ion exchange adsorbents mainly for demineralization of crude lactic acid (Davison and Scott, 1992).

The adsorption of lactic acid, and carboxylic acids in general, on basic adsorbents is strongly affected by the pH of the process stream. This is because of the pH effect on the equilibrium of the undissociated and dissociated acid forms. Therefore, the adsorbent that will maximize the recovery of lactic acid is expected to depend on the processing pH as well as on the adsorbent`s basicity.

Evangelista and his collegues (1994) aimed to investigate the use of polymeric adsorbents containing basic functional groups in the primary recovery and/ or purification of lactic acid from fermentation broth. The uptake mechanism of the selected resins occurs by both adsorption and absorption; thus, the lactic acid uptake was referred to as sorption, and the ion exchange resins as sorbents (Evangelista *et al.,* 1994).

3.1. Fundamental Concepts in Ion Exchange Process

Ion Exchange Chromatography (IEC) is the most utilized chromatographic technique for the separation and purification of substances. The enhanced interest on ion exchange applications is due to its versatility, high resolving power, high capacity and its straightforward basic principle. Charge density and the distribution of charges on the sample improves the resolving power. Ion exchange is a reversible reaction wherein an ion from solution is exchanged for a similarly charged ion attached to an immobile solid particle, the ion exchanger.

Ion exchange resembles sorption in that, in both cases, a dissolved species is taken up by a solid. The characteristic difference between the two phenomena is that ion exchange, in contrast to sorption, is a *stoichiometric* process. Every ion removed from the solution is replaced by an equivalent amount of another ionic species of the same sign. In sorption, on the other hand, a solute (an electrolyte or non-electrolyte) is taken up without being replaced by another species (Helfferich, 1962).

The competitive binding of ions of one kind, for oppositely charged chromatographic medium is the basis of ion exchange chromatography. Several factors affect the interaction between the substance and the ion exchanger :

- the net charge and surface charge distribution of the substance
- the ionic strength and the nature of the particular ions in the solvent
- pH, (proton activity)
- temperature
- other additives to the solvent (such as organic solvents)

A kind of energy is gained by the formation of ionic bond between the substance and the ion exchanger which is expressed by the Coulombic law:

$$\Delta E = \frac{Z_A . Z_B . e^2}{r_{AB}} \qquad (3.1)$$

Z_A, Z_B → number of units

ΔE → change in energy

r_{AB} → distance between two charges

If the two charges are of the same sign there is an increase in the enegy formed by the interaction of ions. On the other hand this energy decreases when the charges differentiate in sign. If the sample is highly charged then it will bind to the oppositely charged ion exchanger more strongly. In the similar way if the ion exchanger is highly charged those with a higher degree of substitution with charged groups, then the exchanger will bind to the analyte more effectively than weakly charged ones. Conditions for the process are very important. For instance the pH which alter the effective charge on either the substance or the ion exchanger will affect their interaction. The terms weak and strong ion exchangers derive from the pK_a of their charged groups. At pH`s far from the pK_a, binding will be equally strong to either a weak or a strong ion exchanger (Janson and Ryden, 1989).

The principle of ion exchange process is so simple. It is a reversible reaction wherein an ion from solution is exchanged for a similarly charged ion attached to an immobile solid particle which is called ion exchange resin.

The pH is one of the most important parameters for binding as it determines the effective charge on both the solute and the ion exchanger. During the process pH is adjusted by the addition of buffer salt because binding to an ion exchanger occurs, if there is a net charge of opposite sign to that, on the ion exchanger. The ion exchanger should normally only be used in conditions where its charges will not be significantly titrated by small shifts in pH (Janson and Ryden, 1989).

The influence of the concentration of ions in the solution is an other important parameter. The analyte competes with the other ions in the solvent in order to bind on the stationary phase. At low concentrations of competing ions analyte binding occurs through multiple charge interactions on the ion exchanger. At higher concentrations of competing salt ions, the analyte will start to be displaced from the ion exchanger in the order of their binding strength, the least strongly binding being displaced and eluted from the column first. In the process, the type of ion is important. In elution the ions which interact specifically with charged groups on the analyte are effective (Janson and Ryden, 1989).

3.2. The Stationary phase-The Ion Exchangers

The ion exchangers, are insoluble solid materials which carry exchangeable cations or anions. These ions can be exchanged for a stoichiometrically equivalent

amount of other ions of the same sign when the ion exchanger is in contact with an electrolite solution. Carriers of exchangeable cations are called *cation exchangers* and carriers of exchangeable anions *anion exchangers.* Certain materials are capable of both cation and anion exchange. These are called *amphoteric ion exchangers* (Helfferich, 1962).

The first ion exchangers were synthetic resins designed for applications such as demineralization, water treatment, and recovery of ions from wastes. Such ion exchangers consist of hydrophobic polymer matrices highly substituted with ionic groups, and have very high capacities for small ions. Due to their low permeability these matrices have low capacities for macromolecules such as proteins. In addition, the extremely high charge density gives very strong binding and the hydrophobic matrix tends to denature labile biological materials. Thus despite their excellent flow properties and capacities for small ions, these types of ion exchangers are unsuitable for use with biological samples (Pharmacia, 1980).

The exchange happens without any chemical alteration to the insoluble matrix and electroneutrality is maintained both within the matrix and in the solution surrounding the matrix. The exchange is stoichiometric, it is essentially a redistribution of counter-ions between the external solution and he matrix interior and, as a rule, it is reversible. Stoichiometric and reversible, ion exchange exhibit all the characteristics of the chemical equilibria but here diffusional processes, rather than chemical reaction mechanisms govern this approach to equilibrium (Addo,1988).

The matrix may be based on inorganic compounds, synthetic organic polymers (called resins), polysaccharides (such as celluose), water insoluble electrolytes (liquid ion exchangers) etc. (Addo.,1988). The matrix determine its chromatographic properties such as resolution, capacity and recovery as well as physical properties such as its mechanical strength and flow properties. The nature of the matrix will also effect its behavior towards biological substances and the maintenance of biological activity (Pharmacia, 1980).

They consist of a framework which is held together by chemical bonds or lattice energy. This framework carries a positive or negative charge which is compensated by ions of opposite sign, the so-called *counter ions.* The counter ions are free to move within the framework and can be replaced by other ions of the same sign. The framework of a cation exchanger may be regarded as a macromolecular or crystalline polyanion, that of an anion exchanger as a polycation (Helfferich,1962).

The ion exchanger consists of an insoluble porous matrix to which charged groups have been covalently bound. The charged groups are associated with mobile counter-ions. These counter-ions can be reversibly exchanged with other ions of the same charge without altering the matrix. It is possible to have both positively and negatively charged exchangers (Figure 3.1, Figure 3.2). Positively charged exchangers have negatively charged counter-ions(anions) available for exchange and so are termed anion exchangers. Negatively charged exchangers have positively charged counter-ions (cations) and are termed cation exchangers (Pharmacia, 1980).

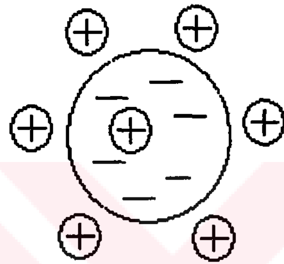


Figure 3. 1. Cation exchanger

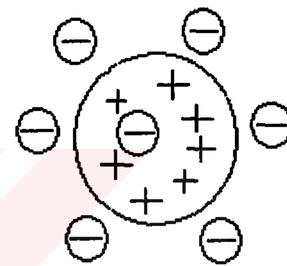


Figure 3. 2 Anion exchanger

In ion exchange chromatography, the structure of the matrix is very important. It determines to a large extent the separation performance of the technique and is often the most costly element of the process (Janson and Ryden, 1989). The properties of an ideal matrix for ion exchange may be summarised as:

a) The matrix should have no intrinsic chemical groups which may cause interactions which are difficult to control.
b) It shoud have high porosity with pores large enough to allow macromolecules access to the adsorption surface; and to give a large adsorption surface.
c) It should have good mechanical strength, preferably be rigid and incompressible; an essential requirement for good hydraulic properties.
d) It should be easy to derivatise with chemical functional groups whilst retaining good mechanical properties after the chemical treatment.
e) It should be chemically and mechanically stable to varying operating conditions with the minimum of the swelling and contraction over a wide range of solution pH and ionic strength.

f) It should have small sized spherical particles in a narrow size distribution to minimise irregularity in packing characteristics, flow disturbances and to improve exchange kinetics.
g) Low cost of the material coupled with long life to enable large scale application to be economic.

3.2.1. Functional Groups and Acid-Base Properties

In the case of producing ion exchangers, several functional groups are used. Most common ones are the amines in anion exchangers and carboxylic acids in the cation exchangers. Strong anion exchangers contain quarternary amines and strong cation exchangers sulfonates. Ion exchanger is a matrix with acidic or basic group substitutions. The basic ion exchangers contain positive groups and are called as anion exchangers. Similarly acidic ion exchangers that contain negative groups and called cation exchangers. The pH is the critical parameter that determines the charge of the ion exchanger.

The matrices are (Janson and Ryden, 1989):

1-Hydrophobic polystyrene-based or partly hydrophobic polymethacrylate-based polymers which are called resins that are crosslinked with divinylbenzene.

2-Hydrophilic synthetic and naturally occurring polymers such as cellulose, dextran, agarose etc.

3-Various synthetic hydrophilic polymers which make hard or moderately hard beads for high pressure applications.

4-Silica gels.

Matrix consists of irregular, macromolecular and three-dimensional network of hydrocarbon chains that are high molecular weight polymers. The presence of charged groups is a fundamental property of an ion exchanger. The type of group determines the type and strength of the ion exchanger; their total number and avalability determines the capacity. There is a variety of groups which have been chosen for use in ion exchangers; some of these are shown in Table 3.1.

Table 3.1 Functional groups used on ion exchangers (Pharmacia, 1980)

Anion exchangers	**Functional group**
Diethylaminoethyl (DEAE)	$-OCH_2CH_2N^+H(CH_2CH_3)_2$
Quaternary aminoethyl (QAE)	$-OCH_2CH_2N^+(C_2H_5)_2CH_2CH(OH)CH_3$
Quaternary ammonium 8q)	$-CH_2N^+(CH_3)_3$

Cation exchangers	**Functional group**
Carboxymethyl (CM)	$-OCH_2COO^-$
Sulphopropyl (SP)	$-CH_2CH_2CH_2SO_3^-$
Methyl sulphonate (S)	$-CH_2SO_3^-$

Sulphonic and quarternary amino groups are used to form strong ion exchangers; the other groups form weak ion exchangers. The term strong and weak refer to the extent of the variation of ionization with pH and not the strength of binding. Strong ion exchangers are completely ionized over a wide pH range whereas with weak ion exchangers, the degree of dissociation and thus exchange capacity varies much more markedly with pH (Pharmacia, 1980).

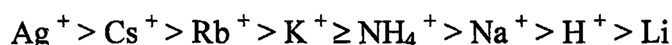
Functional groups ionize into fixed ions and counter ions. Fixed ion is attached to the polymer chain and it is immobile. Fixed ions in cation exchangers;

Fixed ions in anion exchangers (Helfferich, 1962);

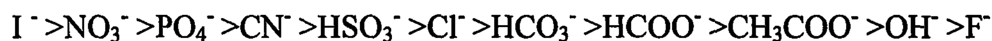
Counter ion is mobile and can diffuse through the swollen resin and exchange with an ion of like charge surrounding solution.

The activity series of some anionic and cationic counterions

Cations:



Anions



$I^- > NO_3^- > PO_4^- > CN^- > HSO_3^- > Cl^- > HCO_3^- > HCOO^- > CH_3COO^- > OH^- > F^-$

All cation exchangers have a limiting pH below which they cannot be used. Ion exchangers have significant part of groups on which ion exchangers have lost their charge at some pH values. The pK is suggested as a lowering limit for their practical use (Harris, 1989).

3.2.2. General Properties of Synthetic Ion Exchangers

3.2.2.1. Capacity

Ion exchangers are characterized in a quantitative manner by their capacity in common usage, is defined as the number of counter-ion equivalents in a specified amount of the material. Capacity and related data are primarily used for two purposes: for characterizing ion-exchange materials and for use in numerical calculation of ion exchange operations. There are various definitions about capacity.

Weight Capacity: The specific amount expression in the capacity definition is given as the amount which weighs 1 gram without sorbed solutes and solvent, i.e., in dry form, the value obtained is called the (scientific) weight capacity and is expressed in miliequivalents per g dry resin (Helfferich, 1962).

Volume capacity: For technical applications and design purposes, number of ionogenic groups per *volume of packed bed.* This so-called technical volume capacity is given in equivalents per liter of (fully swollen) bed. The volume occupied by a given amount of the ion exchanger depends the experimental conditions (ionic form of the resin, composition of solution with which the resin is in contact, etc.). The volume capacity usually refers to a settled bed which is in H^+ or Cl^- form, contains no solutes and is fully water-swollen (Helfferich, 1962).

Concentration of fixed ionic groups: The weight or volume capacity is an excellent means of characterizing an ion exchanger but there are some circumstances in which quantities that reflect the condition (swelling state, etc.) of the resin in the particular process are needed to be known. Due to this requirement, a quantity called concentration of fixed ionic groups is defined which is usually given in milliequivalents per gram of solvent in the resin (weight normality), or per milliliters of swollen resin (volume normality, molarity). The concentration of fixed ionic groups refers to the

particular state of the resin under the given experimental conditions and thus is inherently a variable rather than a characteristic constant (Helfferich, 1962).

There are two more capacity definitions to consider. The first, the total capacity or ion exchange capacity, refers to the amount of charged groups per unit mass of the ion exchange matrix and is generally expressed in units of milliequivalents of charged groups per dry gram of exchanger (meq/g). The second capacity is the available capacity which is the actual capacity of the adsorbent obtained under actual operating conditions. In the context of lactic acid adsorption, the available capacity is defined as the amount of lactic acid adsorbed by a given quantity of the exchanger. The usual units are mg lactic acid/ g dry ion exchanger. Two definitions of available capacity are necessary, the distinction being between the total available capacity of the adsorbent for lactic acid measured in batch-wise equilibrium conditions, and the dynamic capacity measured in a column system. The adsorption capacity can also be expressed as a rate by introducing a time factor (Addo, 1988). Since the degree of ionisation of the ion exchange functional groups and lactic acid is influenced by solution conditions, the lactic acid adsorption capacity will also depend on pH, ionic concentration and, to an extent, temperature. The factors influencing the capacity of an ion exchanger for lactic acid is summarised in Table 3.2.

Table 3.2. The influencing factors on capacity

Parameters	Main influence on
Matrix pore size distribution Lactic acid molecular weight	Accessibility to functional groupswithin the matrix; lactic acid adsorption rate
Chemical and physical structure of matrix. Degree of substitution of functional groups	Selectivity of the ion exchanger
Solution pH; Temperature	Charge of functional groups; lactic acid adsorption rate, selectivity
Solution ionic concentration, concentration of other charged species	Competition for charged sites; selectivity
Column flow rate; agitation in batch reactors	Lactic acid adsorption rate

3.2.2.2. Porosity

Porosity influences ion exchanger properties, mainly the capacity and selectivity. The capacity would be much lower than it really is if the exchange resins had no pores and if only the functional groups at the surface were active for ion exchange. More recently developed ion exchangers are based on matrices with large pores. The analyte, which has high molecular weight, does not penetrate the matrix and only binds to sites near the surface of the particles (Janson and Ryden, 1989). The porosity of the matrix influences the choice of ion exchanger. Media with larger pore sizes are required when larger analyte molecules are present.

3.2.2.3. Structure

The most important class of ion exchangers are the organic ion exchange resins. They are typical gels. Their framework, the so-called matrix, consists of an irregular, macromolecular, three-dimensional network of hydrocarbon chains. The matrix carries ionic species. Cation exchangers have negatively charged fixed ions and can bind positively charged mobile ions. Anion exchangers have positively charged fixed ions.

As a rule, the pores are occupied not only by counter ions but also by solvent and solutes which can enter the pores when the ion exchanger is in contact with a solution. Uptake of solvent may result in swelling of the ion exchanger. Uptake of solutes is usually called sorption, though it is essentially a distribution of the solute between two liquid phases, namely, the pore liquid and the solution outside. Sorption of an electrolyte increases the counter-ion content of the ion exchanger. The sorbed counter ions, which are present in addition to those compensating the framework charge, are accompanied by an equivalent amount of so-called *coions*. These are mobile ions with charges of the same sign as the framework charge. The counter-ion content of an ion exchanger thus depends not only on the magnitude of the framework charge but also on its co-ion content. The common definitions of ion exchange capacities refer, for this reason, to ion exchangers which are free of co-ions (Helfferich, 1962).

3.2.2.4. Swelling

Ion exchangers, both inorganic and organic, are able to sorb solvents in which they are placed. While taking up solvent, the ion exchanger usually expands or swells.

Ion exchangers swell to only a limited degree; an equilibrium is attained beyond which swelling does not proceed. Swelling equilibrium is a balance of opposing forces. The tendency of the polar and ionic constituents of the resin to surround themselves with solvent and thus to stretch the matrix meets with an incresing resistance by the latter. Equilibrium is attained when the elastic forces of the matrix balance the dissolution tendency (Helfferich,1962).

Swelling and shrinking of ion exchanger adsorbents are complex phenomena which have so far received little attention. They occur more with the non-rigid polysaccharide-based media. There is evidence that the capacity for lactic acid of the more compressible media is reduced under flow conditions in packed columns which compress the packed bed. Excessive shrinking during desorption conditions can also reduce recoverable lactic acid capacity (Addo, 1988)

3.2.2.5. Stability

The chemical, thermal and mechanical stability and the ion exchange behavior of the resins depend chiefly on the structure and the degree of crosslinking of the matrix and on the nature and number of fixed ionic groups. The chemical and thermal stability of the resins are not unlimited. The most frequent causes for resin deterioration are chemical and thermal degradation of the matrix, for example, by thermal hydrolysis. Most of the present commercial resins are stable in all common solvents, except in the presence of strong oxidizing or reducing agents, and withstand temperatures up to slightly more than 100 ^{0}C. Strong base anion exchange resins begin to deteriorate above 60^0 C (Helfferich, 1962).

3.2.2.6. Selectivity

In ion exchange equilibrium, the concentration ratios of the competing counter ion species in the ion exchanger and in the solution are not the same. As a rule the ion exchanger prefers one species to other. This shows that the redistribution of the counter ions is not purely statistical. The preference for one species may have several species

1. The electrostatic interactions between the charged framework and the counter ions depend on the size and, in particular, on the valence of the counter ion.

2. In addition to the electrostatic forces, other interactions between the ions and their environment are effective.

3. Large counter ions may be sterically excluded from the narrow pores of the ion exchanger.

All these effects depend on the nature of the counter ion and thus may lead to preferential uptake of a species by the ion exchanger. The ability of the ion exchanger to distinguish between various counter ion species is called selectivity (Helfferich, 1962).

3.2.3. Equilibrium Properties

When an ion exchanger in A form (where A is an arbitrary counter ion) is placed in a solution of an electrolyte BY, counter ion A will migrate from the exchanger into the solution and counter ions B* from the solution into the ion exchanger, i.e., an exchange of the counter ions takes place. After a certain time, *ion-exchange equilibrium* is attained. Both the ion exchanger and the solution contain both counter-ion species, A and B. The concentration ratio of the two counter ions, however, is not necessarily the same in both phases (Helfferich,1962).

Equilibria between ion exchangers and solutions are one of the most important factors in ion exchange chromatography. The equilibrium relationship determines the distribution of solutes between stationary phase and mobile phase. It is influenced by various factors, such as ionic strength of the solution, analyte concentration, pH and types of ion exchangers. The dependency of the distribution coefficient of sample on ionic strength and pH is closely related to the elution pattern, that is peak position, peak width, etc. (Yamamoto *et al.,* 1988).

The ion exchanger shows a preference for certain ions and exhibits selectivity. The most simple example of selectivity in ion exchange is the almost complete exclusion of all co-ions(mobile ions in solution with the same polarity as the bound ionic groups) from the internal matrix. With counter-ions, the basis of selectivity are multiple and complex, but the most influential factors include: 1-the type, size and valency of the ions ; 2-the type and concentration of functional groups in the matrix as well as the matrix properties; and 3-the total concentration and the concentration ratio of ions both capable and incapable of exchange; as well as the type and quantity of other substances in the solution.(Helfferich, 1962). Selectivity is a very important property of an ion exchanger. It forms the basis of all the applications of materials since the mechanism by which the separation is obtained on an ion exchanger is one of selective reversible adsorption. Several theories have been proposed in an attemt to obtain greater insight into the

conditions which determine selectivity (Addo, 1988). None of these theories furnishes quantitative information, but three important general rules for practical use do emerge. They are :1)at low concentrations of aqueous solution and at room temperature most ion exchangers give preference to polyvalent ions, particularly larger organic ions, in the absence of any matrix exclusion effects; 2)the selectivity of hydrogen ions (H^+) and hydroxide(OH^-) depends on the acid or base strength of the functional groups of the exchanger; 3)the greater the degree of cross-linking, the more selective is the ion exchanger. Though simply stated, these three points have a complex major influence on the application of ion exchangers (Addo,1988).

If we symbolise an anion exchange matrix carrying functional groups with any number n of positive charges as M^{n+}, and use the symbols A^- and B^- to represent monovalent counter-ions in solution, ion exchange can be described as follows:

$$M^{n+}.n\,A^- + n\,B^- + n\,C^- \rightleftharpoons M^{n+}.n\,B^- + n\,A^- + n\,C^- \qquad (3.2)$$

$M^{n+}.n\,A^-$, : Anion exchange group
$n\,B^-$, : exchangeable counter-ions
$n\,C^-$, : co-ions

The concept of selectivity is modelled using an equilibrium or selectivity coefficient K derived empirically from the equilibrium states of the ion exchange system. For example for the ion exchange reaction represented in Equation (3.2) a practical selective coefficient is obtained by applying the law of mass action,

$$K= [M^{n+}]\,[A^-]^n / [B^-]^n\,[M^{n+}] \qquad (3.3)$$

and the equilibrium distribution of the counter-ions in the ion exchanger over a range of ion concentrations is characterised by a mass action isotherm. The magnitude of K determines whether the equilibrium is favorable or unfavorable in terms of the partitioning of ions between the liquid phase and the matrix phase (Addo, 1988).

Ion exchange equilibrium of organic species may be described by vigorous mathematical expressions. The analysis of ion exchange is based on equilibria and on mass balances. The equilibria are presented as ion exchange isotherms.

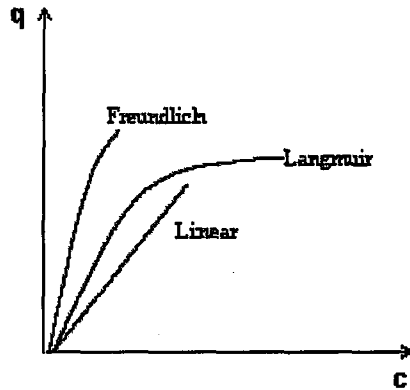
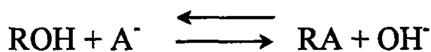


Figure 3.3. Equilibrium isotherms

As is shown in Figure 3.3 it is a graphical representation relating the concentration of the solute in the solid and liquid phases at equilibrium when a fluid phase containing two phases is reached for various solute concentrations at a constant temperature. Any specific set of experimental conditions (solution concentration, relative amounts of counter ions, etc.) corresponds to one point on the isotherm surface.

Separation performance is highly dependent upon the curvature of a particular isotherm. In this respect, if the curve is convex upward, equilibrium is referred to as favorable, and if it is concave upward then it is unfavorable meaning that at equilibrium the exchange is predominantly in the forward direction of the reaction.



$$ROH + A^- \rightleftharpoons RA + OH^-$$

For the former case and in the backward direction in the latter case. The case in which the tendency for the forward direction exactly equals that for the backward reaction is called a linear equilibrium.

Ion exchange equilibrium can be characterized by the adsorption isotherm. Simplest types of isotherms are the Langmuir and Freundlich isotherms. The simplest and the most widely used theoretical model was first developed by Langmuir. It describes the adsorption of one component as a monolayer.

The Langmuir model was originally developed to represent chemisorption on a set of distinct localized adsorption sites. A simple kinetic derivation is given below.
The basic assumptions on which the model is based are:

1) molecules are adsorbed at a fixed number of well-defined localized sites.

T.C. YÜKSEKÖĞRETİM KURULU DOKÜMANTASYON MERKEZİ

2) each site can hold one adsorbate molecule

3) all sites are energetically equivalent

4) there is no interaction between molecules adsorbed on neighboring sites.

Considering the exchange of molecules:

Rate of adsorption $k_a c(1-q)$

Rate of desorption $k_d q$

Where q_m is the total number of sites per unit weight or volume of adsorbent and q/q_m is the fractional coverage. At equilibrium the rates of adsorption and desorption are equal:

$$[q / 1-q] = k_a c / k_d = bc \qquad (3.4)$$

where $b= k_a / k_d$ is the adsorption equilibrium constant.Commonly ;

$$q/q_m = bc/1+bc \qquad (3.5)$$

this expression shows the correct asymtotic behavior for monolayer adsorption since at saturation $c \longrightarrow \propto, q \longrightarrow q_m$

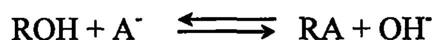
Commonly suggested procedures for testing the fit of the Langmuir model to experimental data involve plotting either c/q against cor 1/q against 1/c.

$$(c /q)=(1/ bq_m) + (c / q_m) \qquad (3.6)$$

or

$$(1 /q)=(1/ q_m) + (1/ bq_m)*(1/ c) \qquad (3.7)$$

so it is evident that the model parameters b and q_m may be easily obtained from the slopes and intercepts of such plots (Ruthwen, 1984)



$$ROH + A^- \rightleftharpoons RA + OH^-$$

The Langmuir equation may be written for the reaction given above as:

$$K= [OH^-] [RA] / [A^-] [ROH] \qquad (3.8)$$

$$[ROH] + [RA] = q_{max}$$

$[A^-] + [OH^-] = C_o$

$[RA] = q$

$[A^-] = C$

K is expressed as

$$K= [(C_o - C)q] / [C (q_{max}-q)] \quad (3.9)$$

$$q = [q_{max}KC] / [KC + (C_o-C)] \quad (3.10)$$

The Freundlich isotherm is the other most common emprical isotherm. The expression of the Freundlich equation is given below:

$$q = K_F C^n \quad (3.11)$$

K_F: Freundlich constant

n: Constant

C: Equilibrium solute concentration in solution

q: equilibrium solute concentration in resin phase

K_F and n are parameters which should be determined experimentally. Linear transformation of the Freundlich equation to estimate the constants is achieved by taking logarithms of both sides (either with base 10 or e) and obtaining the corresponding ln q vs ln C graph. If the exchange is favorable, then $n<1$; if unfavorable, then $n>1$ (Zihao and Kefeng, 1995).

Conventional models for ion-exchange processes are based on a number of simplifying and therefore limiting assumptions. A common assumption is that the maximum uptake of ions by an ion-exchange resin is constant and determined by the number of functional groups on the resin matrix. The usual second assumption is a strict stoichiometric coupling among different components involved in the ion-exchange process. This implies that each ion released from the resin phase has to be replaced by another component from the liquid phase with an equivalent charge. Furthermore, it is assumed that only counterions, with a charge opposite to that of the functional groups,

can enter the resin phase. Co-ions are assumed to be excluded from the resin phase due to the strong repulsive forces between like charges. Thus, a cation is supposed not to enter an anion-exchange resin, and a cation-exchange resin is supposed to repel anions effectively. The electroneutrality condition determines the quantity of counterions that will enter the resin; in any case, their total number of charge equivalents must equal the resin capacity (Jansen *et al.*, 1996).

3.2.4. Kinetics

After the nature of ion exchange equilibria, the next important question is how fast equilibrium is established in a given system (or kinetics). In the study of kinetics the objective is to establish the mechanisms of ion exchange and the rate determining step in the system.

Fundamental to the design and operation of any adsorption process is an understanding of the position of the system at infinite time or equilibrium and how fast equilibrium conditions can be approached. The study of how fast an adsorption process proceeds is the basis of kinetics.

1-Theoretical analysis of kinetics:

The rate of ion exchange is determined by diffusional processes. This is conveniently modeled using the concept of a diffusing liquid boundary layer surrounding the ion exchange particle which is assumed to be a quasi-homogeneous phase. For simple isotopic exchange of small ionic species, diffusion occurring through these two media is described in terms of Fick`s law where flux is proportional to the concentration gradient; and the respective diffusion coefficients are constant, though not of the same magnitude. Mathematical solutions are obtainable under ideal limiting cases of film diffusion and particle diffusion controlling, and for the intermediate cases. When true ion exchange is involved, diffusion of a species is coupled with other processes, say with diffusion of a different species, because electroneutrality must be conserved. Such processes can not be described in terms of constant diffusion coefficients. Further complications arise from the selectivity of the ion exchanger, specific interactions, sorption and desorption and changes in swelling and swelling pressure. Solutions have been obtained for a few of the ideal limiting cases applying the Nerst-Plank flux equation (Addo, 1988).

With macromolecular polyelectrolytes, it becomes impossible to derive rigorous theoretical kinetic models to describe stoichiometric exchange due to their size, complex structure, charge distribution and multivalent interaction with the solid matrix. Therefore, a simple approach is to treat the phenomena purely as a sorption process governed by the rates of diffusion in the external solution and in the solid phase during transport from the adsorption sites, or to use a simple reaction kinetics model (Addo, 1988).

2- Rate equations for interface mass transport:

The transfer of solutes from dilute aqueous solution to an adsorption site on an ion exchanger takes place in a number of stages:

(i) transport of solutes from the bulk solution to the solution-adsorbent interface-(bulk diffusion);

(ii) mass transport across the boundary layer-(film diffusion);

(iii) diffusion through the pores of the adsorbent-(pore diffusion);

(iv) adsorption onto the internal surface (surface adsorption);

(v) movement of mobile adsorbed solutes along the pore surface without detaching (surface diffusion)

One or more of the stages may offer the greater resistance to mass transport, the greater the resistance offered by a stage the greater the concentration gradient across it (Addo, 1988).

3.3. Procedures in Ion Exchange Separations

Adsorption chromatography depends upon on interactions of different types between solute molecules and ligands immobilized on a chromatography matrix. The first type of interaction to be successfully employed for the separation of macromolecules was that between charged solute molecules and oppositely charged moieties covalently linked to a chromatography matrix. The technique of ion exchange chromatography is based on this interaction (Pharmacia, 1980).

Before starting the purification besides the establishment of suitable adsorption and elution conditions other factors should be considered. For example, the need for matrix pre-treatment, the adsorption mode and the elution mode (Harris, 1989).

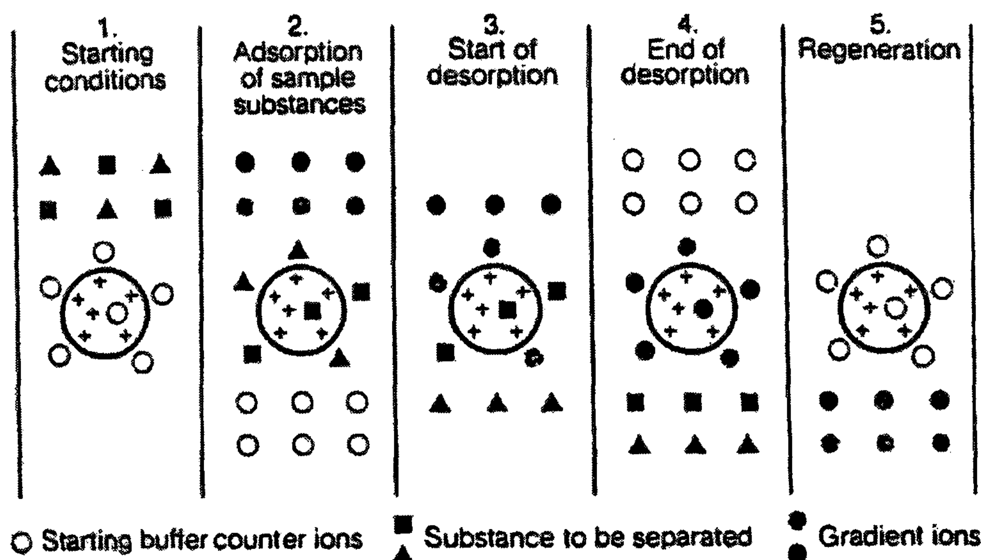


Figure 3. 4. Procedures in ion exchange

The theory of separation on ion exchange chromatography depends on the reversible adsorption of charged solute molecules to an immobilised ion exchange group of opposite charge. Most ion exchange experiments are performed in five main stages. These steps are schematically illustrated in Figure 3.4.

The first stage is equilibration in which the ion exchanger is brought to a starting state, in terms of pH and ionic strength, which allows the binding of the desired solute molecules. The exchanger groups are associated at this time with exchangeable counter-ions (usually simple anions or cations).

The second stage is sample application and adsorption in which solute molecules carrying the appropriate charge displace counter ions and bind reversibly to the gel. Unbound substances can be washed out from the exchanger bed using starting buffer.

In the third stage, substances are removed from the column by changing to elution conditions unfavorable for ionic bonding of the solute molecules. This normally involves increasing the ionic strength of the eluting buffer or changing its pH. In Figure 3.4 desorption is achieved by the introduction of an increasing salt concentration gradient and solute molecules are released from the column in order of their strengths of binding, the most weakly bound substances being eluted eluted first.

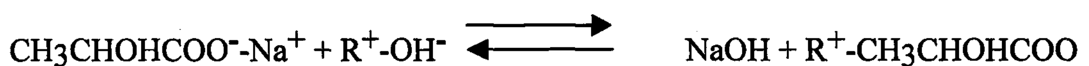
The fourth and fifth stages are the the removal from the column of substances not eluted under the previous experimental conditions and re-equilibration at the starting conditions for the next purification.

Separation is obtained since different substances have different degrees of interaction with the ion exchanger due to differences in their charges and charge densities. These interactions can be controlled by varying conditions such as ionic strength and pH. The differences in charge properties of biological compounds are often considerable, and since ion exchange chromatography is capable of separating species with very minor differences in properties, e.g. two proteins differing by only one charged amino acid, it is a very powerful separation technique.

In ion exchange chromatography one can chose whether to bind the substances of interest and allow the contaminants to pass through the column, or to bind contaminants and allow the substance of interest to pass through. Generally the first method is more useful since it allows a greater degree of fractionation and concentrates the substances of interest.

Ion exchange separations may be carried out in a column or by a batch procedure. Both methodologies are performed using the definite stages of equilibration, sample adsorption etc.

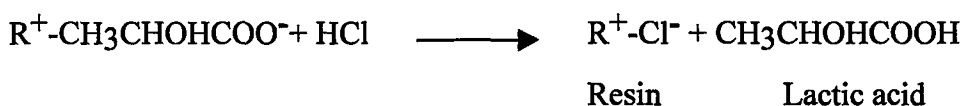
Ion-exchange :



$$CH3CHOHCOO^{-}\text{-}Na^{+} + R^{+}\text{-}OH^{-} \rightleftharpoons NaOH + R^{+}\text{-}CH3CHOHCOO$$

Sodium lactate Resin

Elution:



$$R^{+}\text{-}CH3CHOHCOO^{-} + HCl \longrightarrow R^{+}\text{-}Cl^{-} + CH3CHOHCOOH$$

Resin Lactic acid

Equilibration:

$$R^{+}\text{-}Cl^{-} + NaOH \longrightarrow R^{+}\text{-}OH^{-} + NaCl$$

3.3.1. Matrix Pre-treatment

Preparation of the ion exchanger for use may involve removal of fines, swelling, washing and conversion to the correct counter-ion.

- Fines removal
- Swelling (necessary for supports supplied in dry form)
- Washing (remove contaminants)

Counter-ion conversion (the exchanger should be pre-treated if the eluent to be used contains different counter-ions (Harris, 1989).

3.3.2. Adsorption Method

Having determined the capacity and selected the optimum pH and ionic strength for both adsorption and elution steps, a choice should be made between using ion exchange in a batch or column mode (Harris, 1989). Adsorption may be carried out in a batch (stirred tank) mode or in a packed bed (column) mode.

3.3.2.1. Batch Adsorption

Batch fractionation is carried out in free solution and although inferior to column seperations in efficiency, it is ideally suited to the initial treatment of large volumes of sample. Furthermore, it does not suffer from the problems of bed swelling and shrinkage which are sometimes encountered in column seperations. Elution followed by batch adsorption may be carried out in batch mode or the ion exchanger slurry may be packed into a column and than eluted. The entire capacity of the ion exchanger should be used during the adsorption step (Harris, 1989).

In a batch adsorption system, a fixed volume of lactic acid solution at a specific concentration is contacted with the ion exchanger and agitated for a fixed time, typically 30-60 minutes or until equilibrium is achieved. After this the solution is drained off and the ion exchanger is washed before eluted by adding a suitable eluent.

The amount of ion exchange medium required to reduce the feed concentration to an acceptable specified level is determined by the equilibrium adsorption characteristics of the lactic acid/ion exchanger system i.e. the adsorption isotherm. A batch adsorption step can be depicted on the equilibrium diagram as illustrated in Figure 3.5. Two shapes of the adsorption isotherm are considered, both are presented as

favorable isotherms which are the most common in ion exchange systems. Isotherm A is more favorable than isotherm B because the solute exhibits a higher affinity for the solid phase in the adsorption system exhibited by isotherm A. Thus the partition coefficient α defined as the concentration ratio between the adsorbed lactic acid and the total lactic acid present in the system from Scopes (1982) theory, will be closer to unity and the dissociation constant K_d for the lactic acid –matrix interaction will be close to zero. For an adsorption step initiating at a lactic acid concentration C_0 and terminating at a final equilibrium concentration C_1 in a batch volume V, the amount of ion exchange medium w required is obtained by a mass balance;

$$(q - q) = - V (c - c_o) / W \qquad (3.12)$$

It can be seen that less adsorbent is required in system A than in system B to meet the equilibrium effluent requirement. If the same amount of adsorbent is used in both systems, ie adsorption along operating line 1, then more lactic acid is left in solution at equilibrium, with system B and recovery efficiency here is poor.

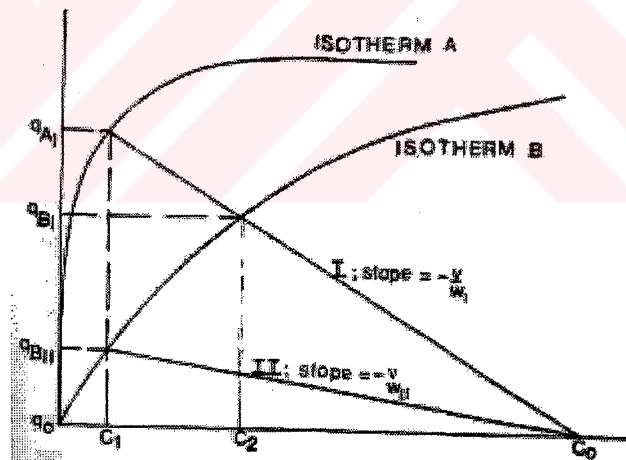


Figure 3.5. Effect of isotherm shape on batch processing efficiency

Two points arise from these considerations. The first is that to achieve high adsorption recovery, the value for α the partition coefficient must be high and as close to 1 as possible. The second is that the amount of ion exchange adsorbent is very important, especially when the partition coefficient has a low value. Conditions which

might transform Isotherm A into type B may be due to changes in pH, ionic strength of solution, or temperature.

3.3.2.2. Column Adsorption

As with batch adsorption packed bed ion exchangers can be used to purify the analyte by adsorption of contaminants. This allows the analyte to pass through the column without binding. While this is an acceptable means of purification, no concentration of the analyte result. Usually, the required analyte is adsorbed onto the support in preference to contaminants and then eluted with concentration and / or fractionation (Harris, 1989).

A column adsorption process involves the continuous application of lactic acid feed solution to the inlet of the bed until a breakthrough occurs and lactic acid is detected at the column exit. The plot of the lactic acid concentration in the column effluent as a function of time, or volume passed is called a breakthrough curve. As in any adsorption system, the shape and position of the breakthrough curve characterises the adsorption efficiency of the system. Factors affecting the shape and position of the breakthrough curve are adsorption effects (feed concentration, adsorption isotherm) and non-equilibrium factors such as the medium particle size, packed bed height and fluid velocity (linear flow rate) which affect the mass transfer process. The equilibrium or dynamic capacity of packed bed is obtained from the breakthrough curve (Helfferich, 1962). This is illustrated in Figure 3.6. and Figure 3.7. where two breakthrough curves are considered.

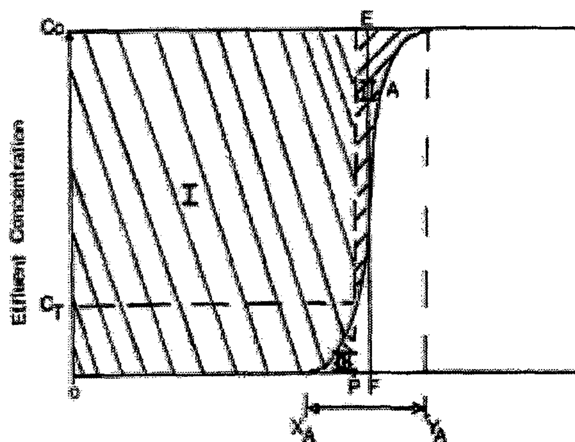


Figure 3.6. Breakthrough curve for ideal adsorption

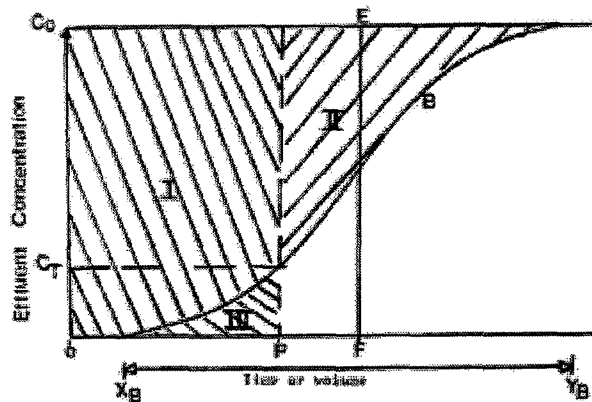


Figure 3.7. Breakthrough curve for less efficient adsorption

Curve A represents a near ideal adsorption process which results in a sharp breakthrough curve, whilst curve B shows a less efficient breakthrough curve. The total area behind the breakthrough curve represents the maximum equilibrium or dynamic capacity of the column. This is the binding capacity of the adsorbent under real working conditions as opposed to the available capacity measured under equilibrium batch conditions. The dynamic capacity neasured under column conditions is generally lower than the available capacity.

In a normal adsorption process, the feed flow to the column is usually terminated at some specified outlet concentration rather than loading the column to full capacity. Representing this concentration as C_T, then the breakthrough curve provides information on (1) how much of the column capacity has been used (shaded area 1), the unused capacity (shaded area 2), how much lactic acid is lost in the effluent (area3 under curve) and the processing time. It can be seen that the sharper breakthrough curve A results in a greater utilisation of the total dynamic capacity of the column with less wastage of lactic acid in the effluent (Addo, 1988).

Factors which contribute to the formation of less efficient breakthrough curves (ie curve B) are non-ideal liquid flow (channeling) through the bed, mixing (axial dispersion and dead spaces) and finite mass transfer and adsorption rates especially those involved with intraparticle mass transfer (Helfferich, 1962).

3.3.3. Elution Method

The type of elutions are mainly classified in two groups. Static and dynamic ion exchange differentiate according to the volume of the sample and eluent.

Static ion exchange: The analyte is initially fully adsorbed to the bed and then completely eluted by displacement into the mobile phase using a small volume of a strong eluent. This is useful method for the concentration of analyte from large volume of sample.

Dynamic ion exchange: All the sample components migrate but separate depending on their relative equilibrium distributions between stationary and mobile phases. Three choices of elution conditions exists (Harris, 1989).

Isocratic elution: The starting buffer is used throughout the separation which may give good resolution of similar analytes, but results in large elution volumes. The sample volume should be 1% to 5% of the bed volume. This is because the sample is only loosely bound and not concentrated during the adsorption step.

Step-wise elution: This elution type is achieved by using a sequential, discontinuous change in pH and / or salt concentration. The column volume used is determined by the exchanger capacity and the sample volume. The column length is smaller.

Gradient elution: The composition of eluent is changed continuously. Total volume of eluent should be about five times the bed volume. Elution through pH change usually uses a stepwise method. Elution by a change in pH may also be combined with an increase in ionic strength.

3.3.4. Regeneration and Storage

Regeneration of samples involves the removal of tightly bound contaminants and the conversion of the support to the required counter-ion form ready for equilibration and protein adsorption.

Ion exchangers stored in a wet state are susceptible to microbial degradation. This is particularly true for polysaccharide based matrices. Recommended antimicrobial agents (for anion exchangers include 0.002% chlorohexidine) are used. If these preservatives can not be used, high concentration of organic solvents such as 70% are recommended (Harris, 1989).

Lactic acid containing feedstoks are capable of causing fouling of the ion exchange media. This fouling may reduce capacity and permeability. The problem can

be minimised through periodic cleaning. However if cleaning is too frequent, it results in loss in adsorption capacity.

CHAPTER 4

MATERIALS AND METHODS

4.1. Materials

Lactobacillus casei NRRL B-441 was used in lactic acid fermentation. This organism was kindly provided by United States Department of Agriculture, National Center for Agricultural Utilization Research. Whey powder with the lactose content of 62% was used as carbon source for the fermentation process. It was obtained from Pınar Dairy Products, Inc (İzmir, Turkey).

Litmus milk was used as culture propagation medium, lyophilized form of *Lactobacillus casei* NRRL B-441 was activated in this medium. 100 g litmus milk powder was dissolved in 1 litre distilled water. 20 ml of suspension was poured into 25 ml heat stable glass bottles and were sterilized at 121^{0}C for 15 minutes in an autoclave (Hirayama, Japan). The chemicals used during this study and their properties are given in Appendix A. Moreover, a representative picture of *Lactobacillus casei* NRRL B-441 in litmus milk was taken by using Scanning Electron Microscopy (SEM) and is shown in Appendix, Figure D.2.

Six different type of ion exchangers were used in this study. Basic properties of these commercial anion exchangers are given in Table 4.1. Other chemicals for the fermentation and purification processes are given in Appendix A. All the solutions were prepared by using distilled water and deionized water.

Table 4.1. Properties of commercial anion exchangers used in this study

Amberlite I-6766	Strongly basic anion exchanger, cross linkage 6 %, wet mesh.100-200
Amberlite IRA-400	Strongly basic anion exchanger, Gel form, cross linkage.8 %, wet mesh:16-50
Dowex-1	Strongly basic anion exchanger, Gel form, cross linkage.4 %, wet mesh:20-50
Amberlite IRA-67	Weakly basic anion exchanger, Gel form, wet mesh: 16-50
Amberlite IRA-402	Strongly basic anion exchanger, Gel form, cross linkage.6 %, wet mesh:16-50
Dowex marathon WBA	Weakly basic anion exchanger, wet mesh: 25-50

4.2. Methods

4.2.1. Fermentation studies

4.2.1.1 Culture Propagation

10 % (v/v) *Lactobacillus casei* NRRL B-441 was transferred into 20 ml sterile litmus milk suspensions. The microorganism was incubated at 37 ^{0}C for 24 hours by 15 days intervals at least in the incubator (Sanyo) and stored in the refrigerator at 4 ^{o}C. The maintenance of the culture was done by transferring to fresh litmus milk every 15 days at least. 24-hour-old fresh cultures were used as the inocula for the fermentation experiments.

4.2.1.2 Lactic Acid Fermentation

Fermentations were carried out under the conditions that were determined by Büyükkileci, 2000. The components of fermentation media are given in Table 4.2. Sartorious and AND HM-200 balances were used to weigh the components.

Table 4.2. Ingredients used for the preparation of the fermentation media

Component	Concentration (g/l)
Whey powder	80
Yeast extract	5
KH_2PO_4	0.5
K_2HPO_4	0.5
$MgSO_4$	0.2
$MnSO_4.H_2O$	0.05
NaOH	10 N

The 5 litre jacketed fermenter having a 3 litre working volume was used for the fermentation studies (Bioengineering, type ALF). The fermenter was equipped with pH, temperature and agitation speed controllers. The automation was provided by the

computer loaded with software FERM which controlled the latter parameters. The medium components given in Table 4.2 were sterilized separately and added to the fermenter. 250 ml erlen meyer flasks were used for shake flask experiments. Three flasks, each with a working volume of 125 ml were used as the inocula for the fermenter. Totally 375 ml seed culture was prepared in shake flasks containing identical components of fermentation medium given in Table 4.2. Seed culture was prepared in order to provide adaptation of microorganisms for the fermentation and for initializing the exponential growth phase. Fermentations were carried out at 37 ^{0}C, with a stirring rate of 200 rpm, at pH 5.5. Appropriate amount of samples of were removed at various time intervals. The samples were centrifuged and diluted with the mobile phase used in HPLC (High Pressure Liquid Chromatography) measurements.

4.2.2. Purification studies

4.2.2.1 Preparation of Ion Exchanger

Preparation: Several commercially available resins were used for the purification of lactic acid in this study. Their basic properties are presented in Table 4.1. For the achievement of desired capacity and ionic form the ion exchangers were treated with 1.25 N NaOH at a volume of 500 ml. The ion exchangers were maintained in NaOH solution over night. Pretreatment was carried out in a chromatography column with the dimensions 3 cm diameter and 23 cm in height (Sigma). Samples were throughly washed with deionized water until the conductivity and the pH values decreased to 1.0 µS and 7.0 respectively. The conductivity measurements for the effluents were done after the washing of resin was throughly completed. The pH was decreased around 7.0.

Resin Density Determination: The density of wet resin was obtained from the slope of the cumulative mass against the volume when aliquots of hydrated resin were repeatedly added to a graduated cylinder with water while the volume and mass increase were noted (Jansen *et al.,* 1996).

4.2.2.2 Preparation of Lactic Acid Solutions

Model Lactic Acid Solutions: Lactic acid solutions were prepared from a racemic mixture of lactic acid about 32%. The stock solution was obtained from METU (Middle East Technical University).

Fermentation Broth: The fermentation broths were centrifuged (Hettich) at 6000 rpm at 10 ^{0}C for 20 minutes in order to get biomass free broths. Supernatants were collected and used for purification studies.

4.2.2.3 Batch Sorption

4.2.2.3.1 Ion exchange kinetics

Ion exchange kinetic experiments for six different resins were performed in batch mode in well stirred vessels (20ml). Model lactic acid solutions and fermentation broth of different concentrations (in the range of 1-5%) were added to 3g of hydrated resins. The solutions at pH 7.0 were agitated continuously in an orbital shaker at 30°C and 180 rpm to ensure homogenous dispersion. The decrease in lactic acid concentration in solution due to sorption was analysed by pipetting small amounts of solution (100µl) at short time intervals starting from the initial time until the equilibrium was reached. Then, samples were centrifuged and analysed for final lactic acid concentration. The sorbed amount of lactic acid was calculated from the differences between initial and equilibrium concentrations.

The effect of pH and temperature on lactic acid/ion exchanger system kinetics were also determined, similarly. Amberlite I-6766 and Dowex marathon WBA resins were used to observe the effects of pH on the ion-exchange kinetics at the pH values of pH: 11.5, 10.3, 9.9, 9.3, 8.3, 7.0 and 2.2. The pH adjustments were done by 1M H_2SO_4 and 1.25 N NaOH solutions dropwise.

Temperature dependence experiments with Amberlite IRA-400, Amberlite IRA-402 and Dowex marathon WBA to generate lactic acid/ion exchanger system dynamics were performed at 20 ^{o}C, 30 ^{o}C and 40 ^{o}C. Since the resins were not stable at higher temperatures, the kinetics experiments were not carried out above 40 ^{o}C.

T.C. YÜKSEKÖĞRETİM KURULU DOKÜMANTASYON MERKEZİ

4.2.2.3.2. Ion Exchange Isotherms

The equilibrium ion exchange experiments were conducted in batch mode for Amberlite I-6766 and Dowex marathon WBA resins at pH 7.0 and 30 °C. When Amberlite I-6766 resin was used in experiments, the concentration ranges of lactic acid in model solutions and in fermentation broth varied from 20 to 50 g/l and 10 to 40 g/l in sorption medium, respectively. All reactions were carried out in stirred solutions (total volume about 20 ml) with 3 g hydrated resin at 180 rpm, in an orbital shaker (Gerhardt).

In the experiments carried out with Dowex marathon WBA resin, the concentration values of model lactic acid solutions were 30; 40; 50; 85; 115; 145 and fermentation broth was diluted to correspond with 20, 30, 40, 50 g/l lactic acid concentrations.

Lactic acid solutions contacted with the resins were maintained over 20 hours until the equilibrium was approached. Samples(100 µl) were taken, centrifuged in a Hettich, model EBA 12R centrifuge and diluted with 5mM H_2SO_4 for the further analyses. The initial and equilibrium concentration of lactic acid were determined in each run. The initial and residual lactic acid concentrations (C_{eq}) in solution were determined by using HPLC and the sorbed lactic acid amount per gram of hydrated resin (q_{eq}) was calculated from the mass balance. Equilibrium ion exchange isotherms were prepared by plotting the amount of lactic acid sorbed per mass of hydrated resins as a function of residual concentration of lactic acid at equilibrium. The mean values of duplicates of each concentration were plotted and fitted to both Langmuir and Freundlich isotherm equations.

4.2.2.4. Column Sorption

4.2.2.4.1. Breakthrough Curve

10 gram of dry Dowex marathon WBA was equilibrated according to the resin preparation procedure given in section 4.2.3. Next, it was adjusted to pH 7.0 with deionized water. 100 ml model lactic acid solution and biomass free fermentation broths of having 50 g/l concentration value were fed to the column at room temperature at a flow rate of 1 ml/min. The flow rate was regulated with adjustable clamps. The

peristaltic pump was connected with the inlet part of the column for feeding the hydrated Dowex marathon WBA with lactic acid solutions. The programmable biocollector was connected to the outlet section of the column to obtain samples at desired time intervals. 1 ml of effluents were fractionated for further analyses.

4.2.2.4.2 Elution Conditions

Batch elution studies were carried out to determine the suitable eluting agent. 50 ml model lactic acid solution and fermentation broth at 50 g/l were loaded inside the column. 1 gram of lactic acid loaded resin were contacted with 10 ml eluting agent selected among the literature. Mainly, methanol, HCl and H_2SO_4 were studied at 0.5 M, 1.0 M, 1.5 M ionic strengths. These tubes were shaken at 200 rpm and lactic acid concentrations inside the tubes were analysed at different time periods. Then, the eluting agents which gave maximum recovery results were studied in column operations.

4.3. Analyses

4.3.1. HPLC Studies

Lactic acid and lactose analyses were done by using HPLC. The mobile phase was 5 mM H_2SO_4 for Aminex HPX-87H column and 3 mM $CuSO_4$ for Nucleosil chiral column. Mobile phase was filtered through 45 μm cellulose acetate filter papers after solution preparation. The system was equipped with Perkin Elmer Series 200 Pump, Series 200 refractive index detector, Series 900 interface, Series 200 Diode array detector and a computer. The degassing unit was connected to the Helium gas.

Lactic acid analyses were verified with an enzymatic method by using UV-VIS Spectrophotometer (Shimadzu). The data obtained with UV-VIS Spectrophotometer were confirmed with the results of HPLC.

The lactic acid and lactose analyses were done by heating the column at 45 ^{0}C whereas for the isomeric determination of lactic acid this value was 60 ^{0}C. All the standard solutions were prepared with the mobile phase of the column. The samples were diluted with mobile phase where dilution was required.

Calibration was held by chemicals with high purity. Calibration curves for lactic acid, L(+)-lactic acid, D(-)-lactic acid and lactose are shown in Appendix B. The R squared value of lactic acid calibration curve at HPLC is greater than 0.999.

The parts of the HPLC system used in this study and the properties of the column and the analysis conditions are given in Table 4.3 and Table 4.4.

Table 4.3. The parts of HPLC system

Pump	Perkin Elmer Series 200
Detector	Series 200 Refractive index and Diode Array
Interface	Series 900
Computer	Controlling unit of HPLC system
Software	Turbochrom
Column oven	Metatherm
Degassing unit	Helium
Mobile phase Reservoirs	4 different containers suitable both for isocratic and gradient elution

Table 4.4. The properties of the column and analysis conditions for HPLCmeasurements

Property	Specifications	Specifications
Type of Analysis	Lactose Lactic acid	D(-) Lactic acid L(+) Lactic acid
Retention Time	Lactose : 7.6 min Lactic acid : 12.4 min	D(-) Lactic acid : 5.6 min L(+) Lactic acid : 5.8 min
Column	Aminex HPX-87H ion exclusion Column (Biorad Laboratories)	Nucleosil chiral (Macherey-Nagel)
Column Length	300 mm	250 mm
Column Diameter	7.8 mm	4 mm
Particle Size	9 µm	5 µm
Guard cartridge	Micro-Guard cation –H cartridge (30x4.6)	CC 8/4 nucleosil 100-5 chiral –2/3G
Mobile Phase	5 mM H_2SO_4	3 mM $CuSO_4.5H_2O$
Flow rate	0.6 ml/ min	0.6 ml/min
Temperature	45 ^{0}C	60 ^{0}C
Detector	Refractive index	Diode array detector
Elution Type	Isocratic Elution	Isocratic Elution

4.3.2. ICP Analyses

The analysis of elements inside fermentation media were carried out by Inductively Coupled Plasma(Varian,ICP-AES, Axial Liberty) (Miller, 1996). 1 gram of homogenized fermentation samples were placed into test tubes. These samples were taken from the inlet and outlet of the column. Next 1 ml HNO_3 about 65% in concentrated form and 1 ml deionized water were added into test tubes. Test tubes were placed in a water bath and heated at 80 ^{0}C overnight. The following day digests were treated with 30% H_2O_2, added dropwise and heated at 100 ^{0}C for several hours, repeating the H_2O_2 treatment until sample digests were clear. The digests were heated again overnight at 80 ^{0}C. Then 1 ml of 37 % HCl was added and the digests were heated for 3-4 hours. The digests were filtered trough ashless 7 cm(D) Whatman No:41 filter paper and they were diluted to a final volume of 50 ml. The wavelengths used for ICP measurements are tabulated in Table 4.5. and the instrument operating conditions of ICP-AES are described in Table 4.6.

Table 4.5. The wavelengths used for element analyses by ICP

Element	Wavelength (nm)
Ca	317.933
Cu	324.754
Fe	259.940
K	766.490
Mg	279.079
Mn	257.610
Na	588.995
Ni	231.604
Zn	213.856

Table 4.6. ICP-AES (Axial Liberty) operating conditions

Argon gas flow	15 l/min
Argon auxiliary flow	1.50 l/min
PMT Voltage	650 V
Sample uptake	30 sec
Rinse time	10 sec
Spray chamber	Glass cyclonic
Nebulizer	Glass nebulizer

CHAPTER 5

RESULTS AND DISCUSSION

5.1. Fermentation

Fermentation experiments were carried out in a 5 litre fermenter under batch conditions at optimum pH and temperature (as given by Büyükkileci (2000)) in order to obtain high concentration of lactic acid for later use in purification experiments. The homofermentative lactic acid bacteria *Lactobacillus casei* NRRL B441 was used. In this study the effects of pH, temperature and carbon source were not investigated, since there is sufficient information on these aspects for *Lactobacillus casei* fermentations from whey powder in Büyükkileci (2000). Basic process information such as lactose consumption and lactic acid production were obtained experimentally. Lactic acid and lactose concentrations were analyzed by using HPLC equipped with an enantiomeric separation column. The results showed that the fermentation broth of *L. casei* secreted about 100% L(+)-lactic acid. The main factor on the fermentative production of L(+) and D(-)-lactic acid content was the type of microorganism rather than substrate, and operating conditions.

Figure 5.1 and Figure 5.2 give average experimental results obtained from different fermentation batches conducted at pH 5.5 and 5.0, respectively. In both cases, the temperature of 37 °C and stirring rate of 200 rpm were kept constant. In Figure 5.1, fermentation was completed in 12 hours and approximately 50 g/l lactic acid was produced. During the first hours of fermentation, the lactic acid is produced exponentially and reached to the concentration value of 40 g/l at 8th hour. In another fermentation run shown in Figure 5.2, the fermentation pH was 5.0; fermentation time was prolonged and the full conversion of lactose to obtain the same amount of lactic acid was observed within 24 hours. At the 18th hour of fermentation, the microorganism reduced the speed of lactic acid production, however, in the subsequent 2 hours an increase on the production of lactic acid was succeeded. The lactic acid production reached its maximum level of 50 g/l after 22nd hour of fermentation.

During the fermentation process, lactose was converted into lactic acid. Furthermore, accumulation of lactic acid in fermentation broth reduced the pH. High

acidic medium prevents the microorganism to grow and decreases the production of lactic acid, therefore, it is concluded that the control of pH is important, which is 5.5 for this study. The pH adjustment was done by the addition of 10N NaOH to the fermentation medium at pH 5.0 and pH 5.5. As it is easily seen from Figure 5.2 at pH value 5.0, fermentation takes 24 hours, whereas it takes 12 hours at pH 5.5. It is important to complete the process in shorter time from the point of consuming less energy, time, and for labor savings. The adjustment of pH can also be done by extractive fermentation instead of base additions, where an ion exchanger column recovers the produced lactic acid. Lactic acid recovered broth is supported with nutrients and is pumped back to the fermenter. Thus, a continuous production of lactic acid can be achieved.

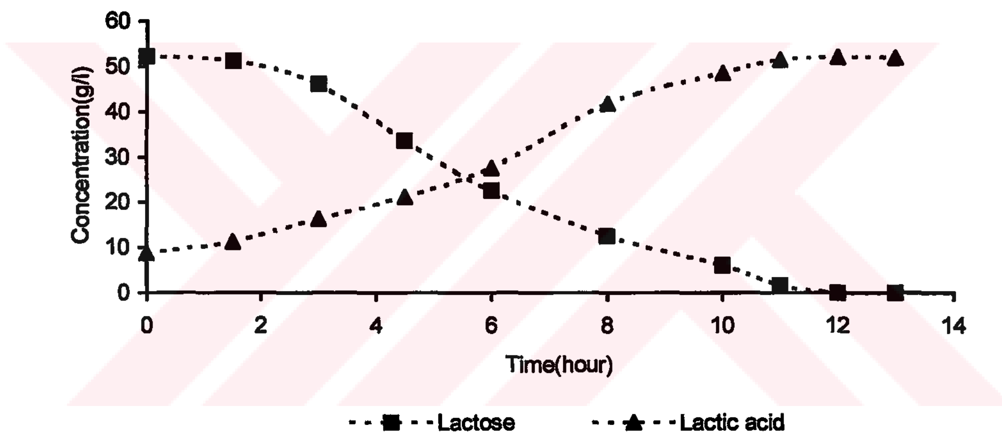


Figure 5.1. Fermentation of whey powder at pH 5.5, T= 37^{0}C

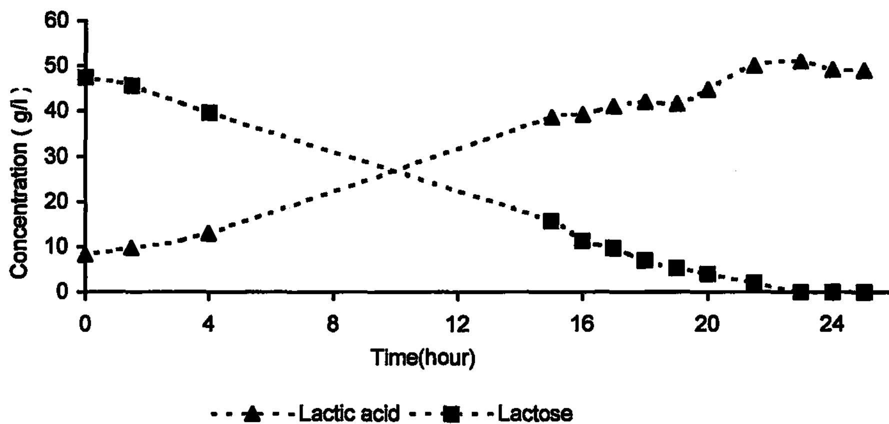


Figure 5.2. Fermentation of whey powder at pH 5.0, T=37^{0}C

In the literature, generally, studies were carried out with synthetic sugars such as lactose and glucose rather than whey fermentation, due to the formation of less complex medium. Fewer researches were based on whey fermentation. Senthuran et al, used cheese whey as the culture medium for the lactic acid fermentation. The pH of the whey was adjusted to 6.5, followed by the addition of Neutrase (10 ml/l), an enzyme from *Bacillus subtilis* with endoprotease activity. After proteolysis at 45 °C for 5.5 h, the whey was boiled for about 10 minutes and centrifuged to remove the precipitated, unhydrolyzed proteins. The supernatant, containing 30 g/l lactose and 5.3 g/l hydrolyzed protein, was used for lactate fermentations after supplementation with nutrients (Senthuran *et al.*, 1996).

A homofermentative strain maximizes the proportion of lactic acid produced. In contrast to the synthetic racemate, an optically pure product can be produced by fermentation by choosing the proper organism and growth conditions (Hofvendahl *et al.*, 1999). Inoculations of *Lactobacillus casei, Lactobacillus rhamnosus and Lactobacillus plantarum* improved the fermentation quality, especially *Lactobacillus casei* and *Lactobacillus rhamnosus.* Inoculations of *Lactobacillus casei* and *Lactobacillus rhamnosus* increased L(+)-lactic acid and reduced D(-)-lactic acid of silage, greatly enhancing the proportion of L(+)-lactic acid in total lactic acid. This result should probably be attributed to the fact that they produce L(+)-lactic acid only and their activities inhibited or weakened the development of other Lactic Acid Bacteria (LAB) (Zhang *et al.,* 2000).

When comparing batch and continuous fermentation modes, the former gave higher lactic acid concentrations and yields in most of the studies. This is mainly due to that all substrate is used in the batch mode, whereas a residual concentration remains in the continuous one. On the other hand, the continuous one resulted in higher productivities (Hofvendahl *et al.*, 1999). The fermentation was carried out in the batch mode. Thus, all the lactose inside whey powder was consumed at the end of 12 hours. Therefore, competition of lactose molecules with lactic acid was eliminated.

5.2. Resin Selection By Batchwise Sorption

The choice of an appropriate resin for recovery and purification would be a compromise of its exchange rate, exchange capacity, and selectivity (Zihao and Kefeng,

1995). Therefore, six anion exchangers were selected from the literature that were used used in numerous studies for the recovery and purification of lactic acid; and they were screened for their sorption performances. The selection of the best resin was carried out in two consecutive steps. The rates of approach to equilibrium and the equilibrium loadings of model lactic acid solutions for six resins were measured in batchwise operations. The series of experiments were run at different lactic acid concentrations (initial concentrations: 2-5 %). Effects of pH, temperature and lactose concentration were also investigated and results are presented in this section.

5.2.1. Ion Exchange Kinetics

It is important to understand the dynamic characteristics of ion-exchange chromatography for design and scale-up purposes. Batch kinetics data has been regarded as accurate and extensive enough to allow a more detailed analysis to explain ion exchange behaviors (Rincon *et al.,* 1997). From the standpoint of an industrial application of this process; the kinetic curves obtained are important to provide essential information required for process design. Batch kinetic experiments were carried out with six different commercially available ion exchangers in this study. To make comparisons between the kinetic behaviors of selected resins, the uptake values at equilibrium were measured as a function of time, under identical conditions. The experimental kinetics results showed the best behaved resin.

To evaluate resin behavior towards the L(+) and D(-)-lactic acid isomers distribution, purification assays were carried out using a racemic mixture of lactic acid.

5.2.1.1. Effect of Different Lactic Acid Concentrations

The effect of different lactic acid concentrations on uptake of lactic acid by different ion exchangers were investigated. Figures 5.3 and 5.4 show the results of Amberlite I-6766 for model lactic acid and fermentation broth, respectively. Other Figures 5.7-5.12 illustrate sorption kinetics of model lactic acid solutions on Amberlite IRA-400, Dowex-1, Amberlite IRA-67, Amberlite IRA-402, and Dowex marathon WBA resins respectively.

Five different concentrations of lactic acid solutions in 20 ml were prepared by using 32 %(w/v) model lactic acid solution. The initial concentration values were 10,

20, 30, 40, 50 g/l. These solutions were treated with 3 g. hydrated ion exchanger (Amberlite I-6766). The resin and lactic acid solutions were exposed to equilibration at 30 °C by shaking at 180 rpm. Samples of 100 μl were collected at various time intervals and diluted with 5 mM H_2SO_4, the mobile phase of HPLC system, under operating conditions. This procedure was applied throughout all uptake experiments. The equilibrium uptake experiments were conducted upto 20 hours. However, to observe the initial period better, a graphical representation of lactic acid uptake on Amberlite I-6766 for 250 minutes is given in Figure 5.3. As seen from the Figure 5.3, the vast majority of lactic acid uptake occurred during the first minute of contact, with lower accumulation occurring during the following time periods. The equilibrium lactic acid concentrations from model solution on resin phase (q) in these uptake experiments were: 0.06; 0.08; 0.1; 0.1 and 0.12 g La/g wet resin for the inital lactic acid concentrations of 10; 20; 30; 40; 50 g/l, respectively. As the lactic acid concentration increased, the amount of sorbed lactic acid in time increased.

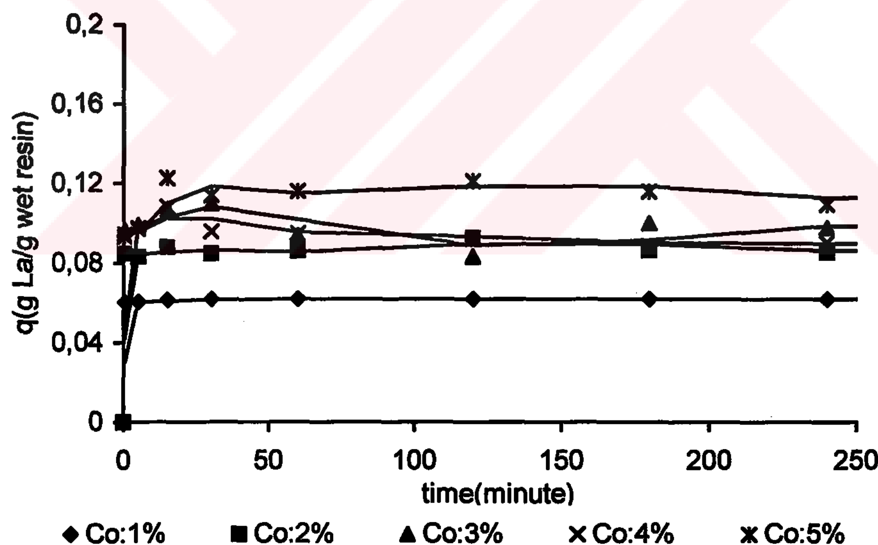


Figure 5.3. Uptake of Lactic Acid from model solution by Amberlite I-6766 at pH=6.5 by shaking at 180 rpm at 30 °C

The biomass in fermentation broth was separated by centrifugation at 6000 rpm for 20 minutes. The supernatant was used for preparing lactic acid solutions. Four different lactic acid solutions having 10, 20, 30, 40 g/l initial lactic acid concentrations

were prepared. However, to observe the initial period better, a graphical representation of lactic acid uptake on Amberlite I-6766 for 250 minutes is given in Figure 5.4. The figure shows the rapid sorption outcomes within one minute where small amounts were sorbed among the rest of the experiment time. The equilibrium lactic acid concentrations in the resin phase were around 0.036 g La/g wet resin for the initial concentration of 10 g/l and 0.046; 0.056; 0.076 g La/g wet resin for the initial lactic acid concentrations of 20; 30; 40 g/l, respectively.

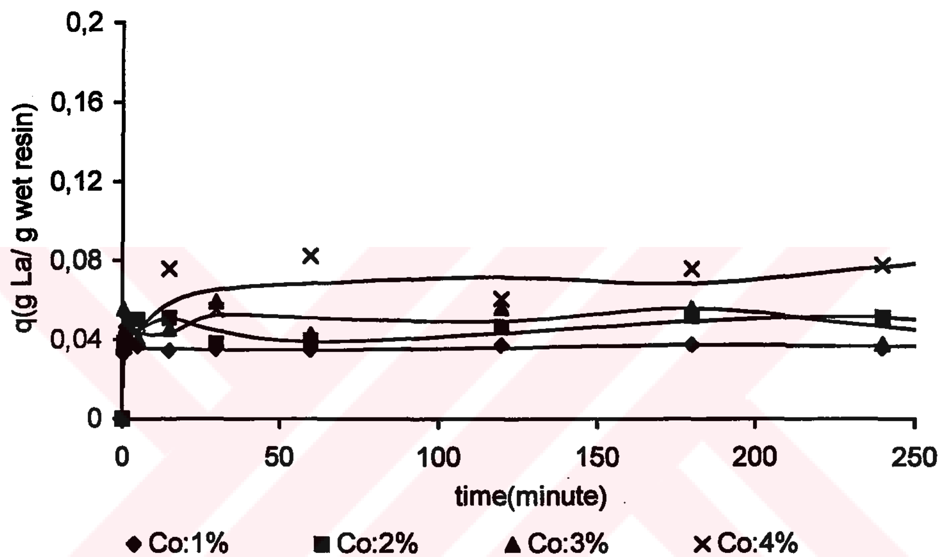


Figure 5.4. Uptake of Lactic Acid from biomass free fermentation broth by Amberlite I-6766 at pH≅5.5 by shaking at 180 rpm at 30 °C .

The uptake values of Amberlite I-6766 for both model solution and fermentation broth are summarized in Figure 5.5. The compared initial lactic acid concentration ranges were about 10 g/l lactic acid for Co 1, 20 g/l lactic acid for Co 2, 30 g/l lactic acid for Co 3, 40 g/l lactic acid for Co 4. Total final uptake value of lactic acid in fermentation broth was about 50 % of that obtained for model solution at the end of 20 hours. Besides the rich composition of whey powder, fermentation medium was fortified with additional nutrients and salts to get high microbial growth and to increase the productivity. Hence, the equilibrium lactic acid uptake by Amberlite I-6766 for fermentation broth decreased and the decrease in uptake amount was due to the competition of ions present inside the fermentation medium.

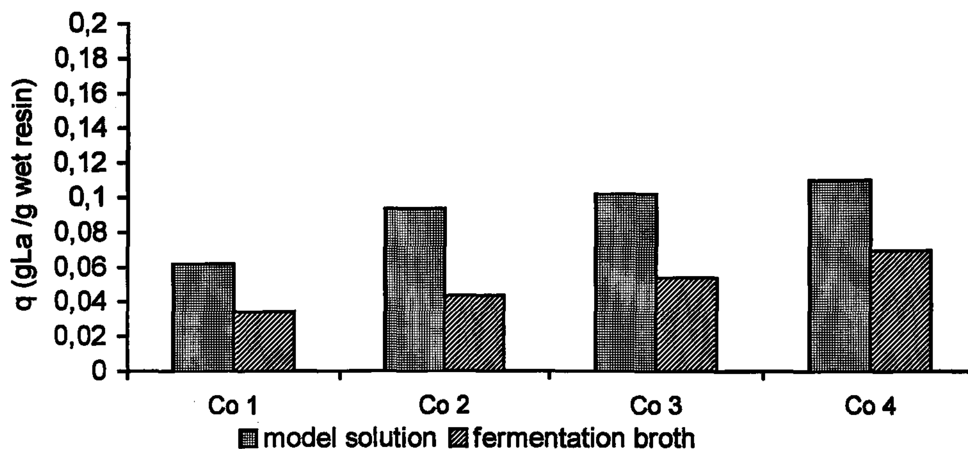


Figure 5.5. Comparison of lactic acid uptake between model solution and fermentation broth by Amberlite I-6766 at pH≅6.5 by shaking at 180 rpm at 30 °C.

The time course for sorption of lactic acid by Amberlite I-6766 was examined to observe a clear equilibration period. As the equilibration was occurring within 60 seconds, samples were collected between narrower time intervals. The samples were obtained at every 10 seconds within one minute and every minutes within five minutes in order to see how fast it is to approach sorption equilibrium. The experiment was held for lactic acid solution having 45 g/l initial concentration. The rapid sorption of lactic acid on Amberlite I-6766 was observed. The equilibrium lactic acid concentration on anion exchanger at the end of five minutes was measured as 0.05 g La/ g wet resin (shown in Figure 5.6) where it was already measured as about 0.05 g La/ g wet resin at the first 10 seconds of contact. This shows that the final concentration value was very close to the value obtained at the first 10 seconds of the experiment. In this case, the equilibrium of lactic acid with the anion exchanger Amberlite I-6766 occurred in a very rapid manner.

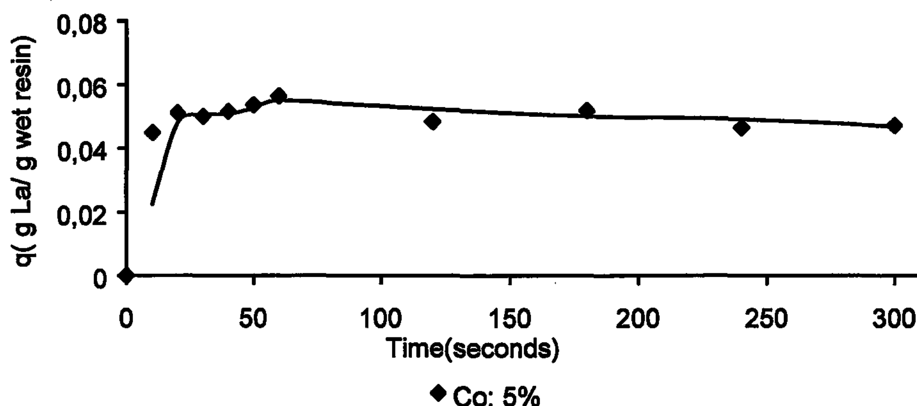


Figure 5.6. Uptake of Lactic Acid from model solution on Amberlite I-6766 at pH≅7 by shaking at 180 rpm at 30 °C

Lactic acid solutions at two different initial concentrations were prepared. Generally, lactic acid concentration in fermentation medium under the selected operating conditions was about 50 g/l. In order to make better comparison, model solution having an initial concentration of about 50 g/l was selected for uptake experiments. The other initial concentration was selected as 20 g/l. As seen from the Figure 5.7, lactic acid solutions at two different initial concentrations were prepared. The equilibrium was reached within 15 minutes on Amberlite IRA-400 and there was not any important fluctuations. The equilibrium uptake of lactic acid by Amberlite IRA-400 was approximately 0.12 g La/g wet resin. Senthuran et al, have used Amberlite IRA-400 for the adsorption of lactic acid in their study, about 186 g of lactic acid could be sorbed per kg of resin, at pH 6.0 (Senthuran *et al.,* 1997).

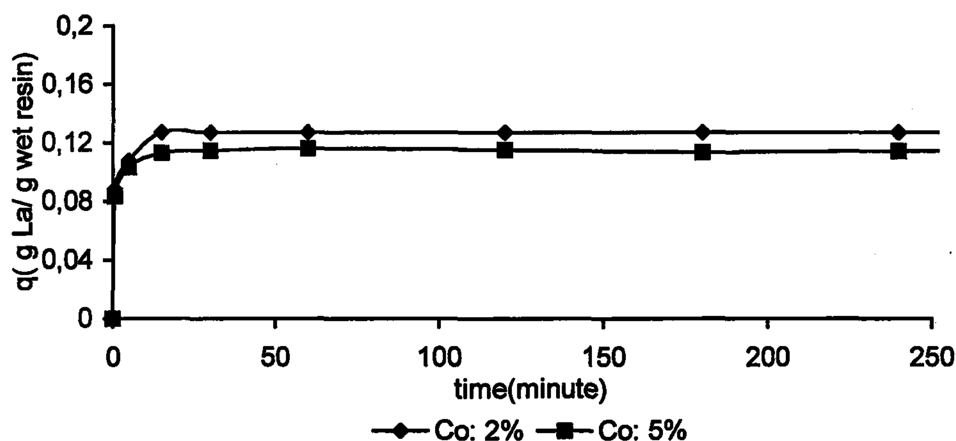


Figure 5.7. Uptake of Lactic Acid from model solution by Amberlite IRA-400 at pH≅7 by shaking at 180 rpm at 30 °C

The effect of initial model lactic acid concentration on the uptake behavior for strongly basic anion exchanger Dowex-1 was investigated. Co 1 and Co 2 were prepared and batch kinetic experiments were carried out for 20 hours. The experimental results are plotted for 250 minutes in Figure 5.8. High amount of lactic acid uptake by Dowex-1 occurred within 15 minutes. It was seen that there was not significant fluctuations on equilibrium lactic acid concentrations at the end of 20 hours. The equilibrium uptake of lactic acid in the first 15 minutes were measured as 0.13 g La/ g wet resin for the initial concentration represented as Co ; 20 g/l La and 0.165 g La/ g wet resin for Co ; 50 g/l. The equilibrium concentration values in the liquid phase for the initial concentration values of 20 g/l and 50 g/l were ; 2.51 g/l and 28.16 g/l, respectively.

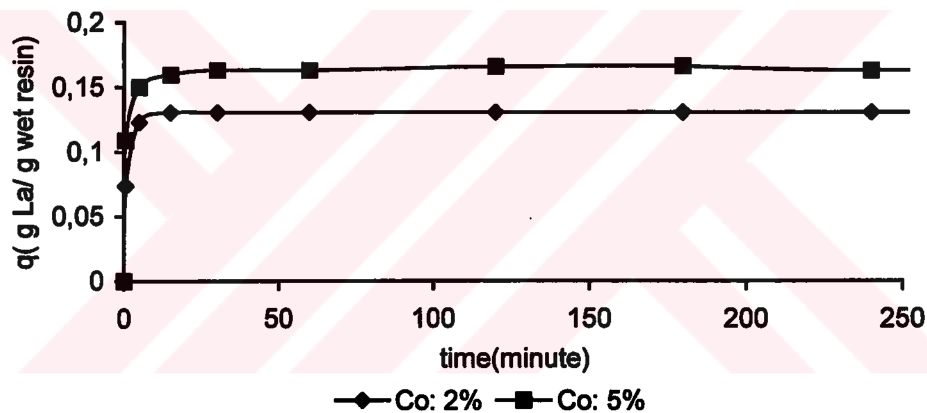


Figure 5.8. Uptake of Lactic Acid from model solution by Dowex-1 at pH≅7 by shaking at 180 rpm at 30 °C

Weakly basic anion exchanger, Amberlite IRA-67, at pH 7.0 was treated with model lactic acid solutions at 30°C by shaking at 180 rpm. The aim was to determine the change of uptake due to the difference in initial lactic acid concentrations (Figure 5.9). The sampling procedure was identical with the other batch kinetic studies. The equilibration was observed for 20 hours. The time period to reach the equilibrium for Amberlite IRA-67 was approximately 1 hour. This equilibration time value was the highest value among the other ion exchangers studied. The capacity values for batch kinetics experiments are given as 0.18 g La/g wet resin for the initial concentration (Co) of 50 g/l lactic acid solution, whereas it is 0.14 g La/g wet resin for Co 20 g/l.

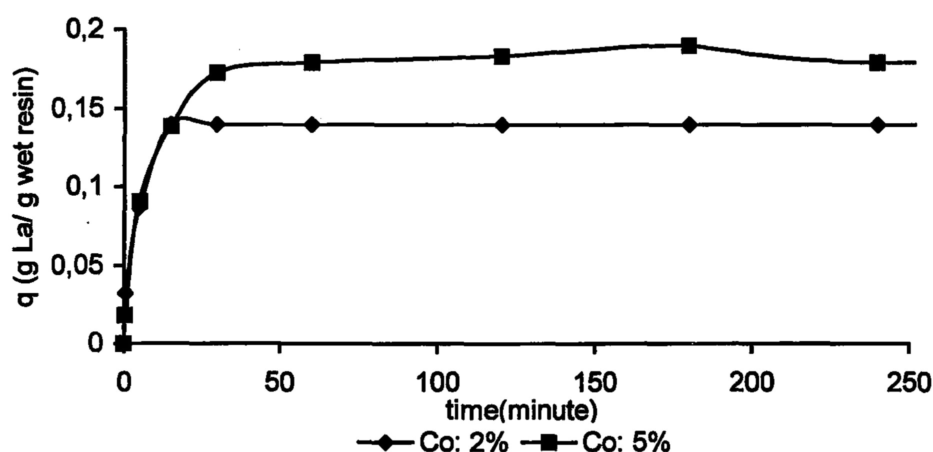


Figure 5.9. Uptake of Lactic Acid from model solution by Amberlite IRA-67 at pH≅7 by shaking at 180 rpm at 30 °C

Amberlite IRA-402 is a strongly basic anion exchanger. The effect of initial concentrations were investigated. The aim was to learn how fast the equilibrium was reached in case of Amberlite IRA-402 resin under operating conditions. Initial concentrations were Co 1; 20 g/l and Co 2; 50 g/l . Figure 5.10 represents that most of the lactic acid uptake occurred in the first 15 minutes of contact. About 90 % of total final sorption occurred during this initial period. The equilibrium lactic acid uptake of Amberlite-402 was determined as 0.12 g La/g wet resin for the initial concentration (Co) of 20 g/l and 0.14 g La/g wet resin for Co 50 g/l.

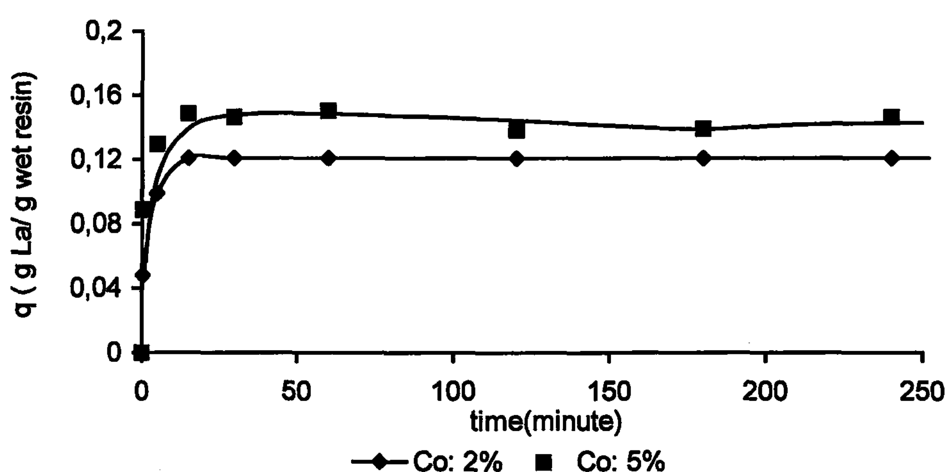


Figure 5.10. Uptake of Lactic Acid from model solution by Amberlite IRA-402 at pH≅7 by shaking at 180 rpm at 30 °C

Dowex marathon WBA, weakly basic anion exchanger in OH form was contacted with various concentrations of 20 ml lactic acid solution for 20 hours. The equilibrium kinetics are illustrated in Figure 5.11. More than 95% of total sorption occurred within 15 minutes. The equilibrium uptakes of lactic acid were 0.07g La/g wet resin for the initial lactic acid concentration of (Co) 10 g/l, 0.13g La/g wet resin for Co 20 g/l, 0.15 g La/g wet resin for Co 30 g/l, 0.16 g La/g wet resin for Co 40 g/l, 0.18 g La/g wet resin for Co 50 g/l.

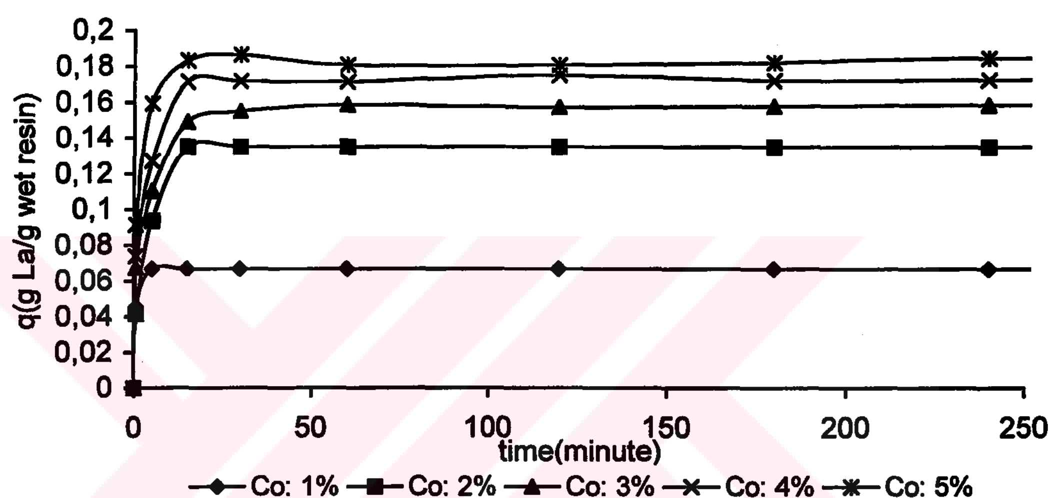


Figure 5.11. Uptake of Lactic Acid from model solution by Dowex marathon WBA at pH≅7 by shaking at 180 rpm at 30 °C

The results of the kinetics experiments are summarized in Table 5.1. As it is seen, the initial concentration of lactic acid solution did not make great differences on the uptake of lactic acid by the ion exchangers of Amberlite IRA-400, Amberlite IRA-402, and Dowex marathon WBA. The equilibration time for those anion exchangers were almost same. The sorption capacities of lactic acid by Amberlite I-6766, Dowex-1, and Amberlite IRA-67 were higher when compared with the former anion exchangers. Having the shortest equilibration time is an important criteria as well as having the highest capacity in ion exchange chromatography. In this case, as it is shown in Table 5.1 Amberlite I-6766 reached to equilibrium more rapidly when compared with the studied anion exchangers. However, this resin was eliminated due to the insufficient amount required for the following experiments. The company (Sigma) unfortunately announced that they have stopped the production of anion exchanger. The results of

kinetic experiments conducted with Dowex marathon WBA were better. Hence the Dowex marathon WBA resin was chosen as a promising initial candidate based on its highest uptake of lactic acid and fast dynamic property. Because of this reason, Dowex marathon WBA was investigated in detail during the following studies.

Table 5.1. Comparison of kinetic results by initial concentration and equilibration time

Anion exchanger	*q (g La/g wet resin)* Co: 20 g/l	*q (g La/g wet resin)* Co: 50 g/l	Equilibration time
Amberlite I-6766	0.08	0.12	1 minute
Amberlite IRA-400	0.115	0.125	15 minutes
Dowex-1	0.13	0.165	15 minutes
Amberlite IRA-67	0.14	0.18	60 minutes
Amberlite IRA-402	0.12	0.14	15 minutes
Dowex marathon WBA	0.16	0.18	15 minutes

The effect of medium components present in fermentation broth on lactic acid uptake by Dowex marathon WBA was examined. About 5 % (w/v) of lactic acid solution was prepared from biomass free fermentation broth. The broth was centrifuged at 6000 rpm for 20 minutes and the supernatant was used for the experiment. The equilibration time was 15 minutes. The equilibrium lactic acid uptake by Dowex marathon WBA was determined as 0.04 g La/g wet resin (Figure 5.12.).

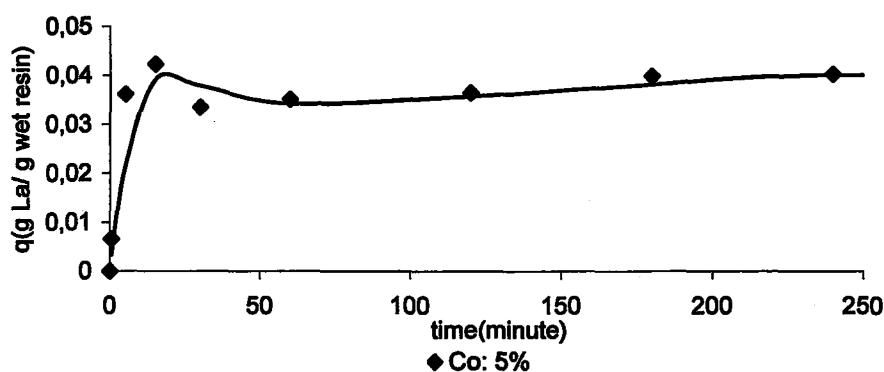


Figure 5.12. Uptake of Lactic Acid from fermentation broth by Dowex marathon WBA at pH≅7 by shaking at 180 rpm at 30 °C

In order to investigate the influence of other metabolites and ions (present in the *L. casei* fermentation broths) on the ion exchange process, experiments conducted with model lactic acid solutions and L(+)-lactic acid in fermentation broth were compared. The equilibrium uptake amount of lactic acid by Dowex marathon WBA changed according to the type of solution. The amount of sorbed lactic acid decreased in the presence of fermentation medium components. To make a comparison, a graphical representation was given in Figure 5.13. The amount of sorbed lactic acid from fermentation broth was about five fold less than the model solution. The reason for that behavior could be the interfering effects of the fermentation broth constituents such as proteins and salts.

The results showed that the equilibrium uptake of lactic acid from the fermentation broth by the Dowex marathon WBA resin was found to be reduced during an ion exchange process. Higher equilibrium uptakes were obtained for the model lactic acid solutions. It is likely that in this case the anions of strong mineral acids, e.g.Br^-, Cl^-, HSO_4^-, and SO_4^{-2} have strong affinities for Dowex marathon WBA. As given above, the other anions in the fermentation broth may change the equilibrium uptake of lactate ions on Dowex marathon WBA and introduce competition with the lactic acid for ion exchange sites. In the literature similar studies were done for comparing the affinity of the ion exchangers towards lactic acid when exposed to fermentation broth. Zihao and Kefeng, (1995) obtained that the competing ions inside the fermentation broth decreased the equilibrium uptake amount.

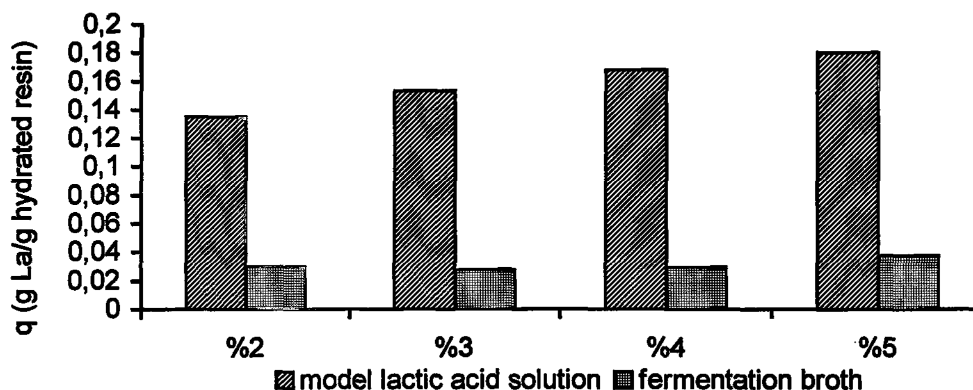


Figure 5.13. Comparison of lactic acid uptake between model solution and fermentation broth by Dowex marathon WBA at pH≅7 by shaking at 180 rpm at 30°C

5.2.1.2. Effect of Temperature

The effect of temperature on the uptake of lactic acid was investigated for three different arbitrary selected ion exchangers. The results are given in Figure 5.14, Figure 5.15, and Figure 5.16 for Amberlite IRA-400, Amberlite IRA-402 and Dowex marathon WBA resins, respectively. The temperature effect was investigated at two different initial lactic acid concentrations, 20 g/l and 50 g/l.

Figure 5.14 shows the uptake of lactic acid on Amberlite IRA-400 at 20 °C, 30°C, 40 °C. Lactic acid molecules showed less tendency to interact with Amberlite IRA-400 at 20 °C. At other temperature values, the uptake amount of lactic acid did not change significantly.

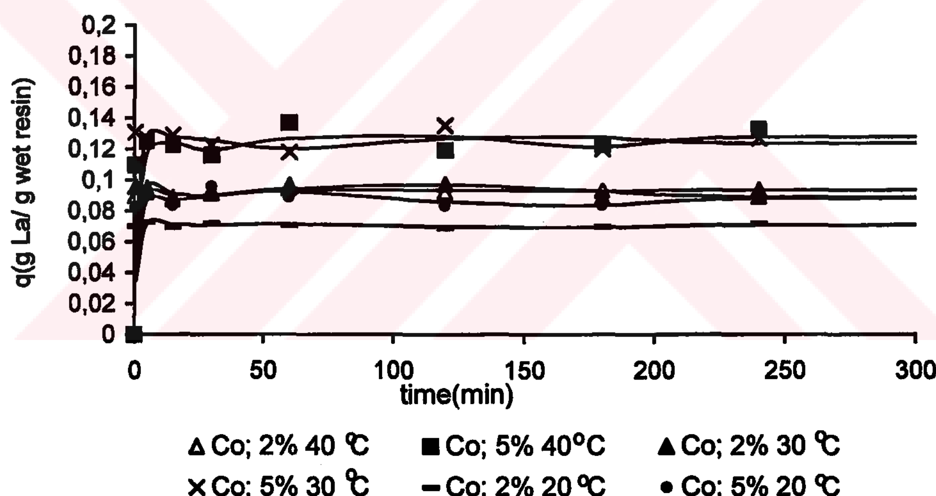


Figure 5.14. Uptake of Lactic Acid from model solution by Amberlite IRA-400 at pH≅7

The temperature values selected for investigating the effect on sorption kinetics for Amberlite IRA-402 were 20 °C, 30 °C, 40 °C. Lactic acid solutions at two different concentrations about 2%-5% (w/v) were prepared. As it is seen from Figure 5.15, there were not significant changes due to the alteration of the temperature. The amount of sorbed lactic acid was similar for three different temperatures. The uptake of lactic acid values on Amberlite IRA-402 at three different temperatures were in between the ranges of 0.12 g La /g wet resin 0.16 g La /g wet resin.

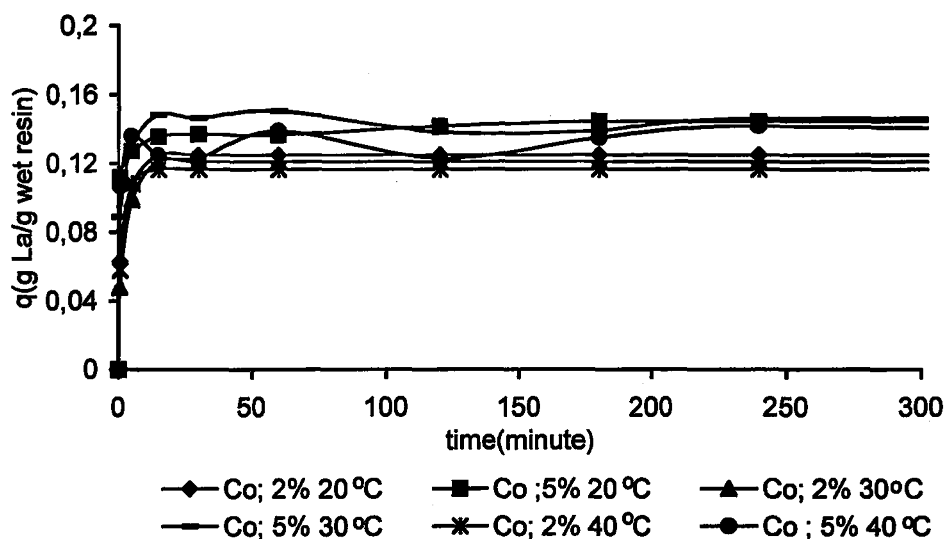


Figure 5.15. Uptake of Lactic Acid from model solution by Amberlite IRA-402 at pH≅7

Figure 5.16 illustrates the uptake behavior of Dowex Marathon WBA resin at temperature values of 20 °C, 30 °C and 40 °C. After the preparation of samples, the solutions were stored at the studied temperature until contacting with this resin. The total contact time was 20 hours and samples were collected at various time intervals. The uptake of lactic acid from model solution did not changed due to the temperature alteration. The adsorbed amount was around 0.2 g La/ g wet resin.

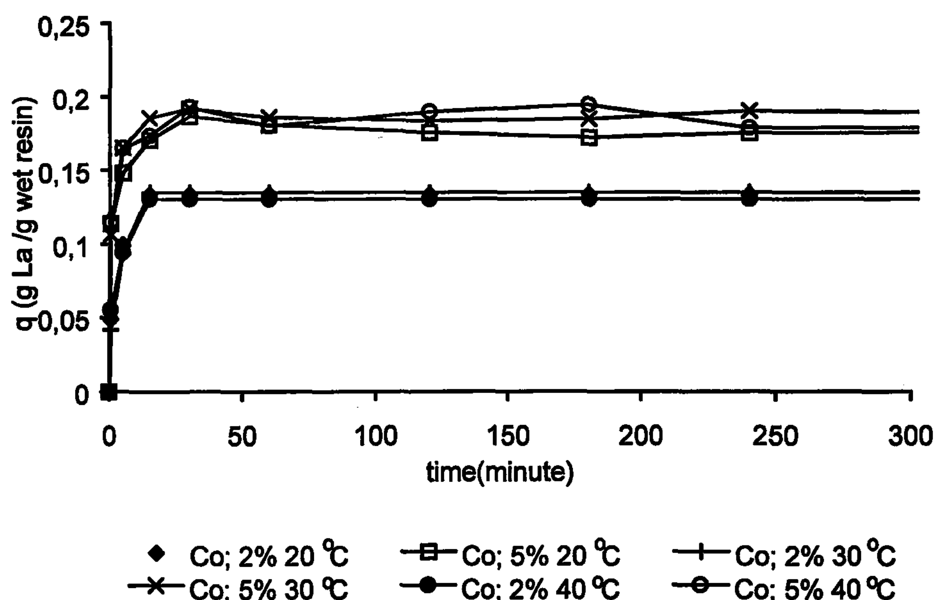


Figure 5.16 Uptake of Lactic Acid from model solution by Dowex Marathon WBA at pH≅7 by shaking at 180 rpm at 30 °C

5.2.1.3. Effect of pH

In Figures 5.17 and 5.18 uptake of lactic acid by Amberlite I-6766 for two different concentrations at six different pH values are given. The uptake experiments were carried out at 30 °C by agitating at 180 rpm. The uptake experiments of lactic acid in biomass free fermentation broth were held with a weak base anion exchanger, Dowex marathon WBA. The results of fermentation broth Dowex marathon WBA system are plotted in Figure 5.19. The pH effect on uptake of lactic acid inside the fermentation broth by Dowex marathon WBA was investigated because it was the selected resin to purify lactic acid.

The effect of pH was investigated by washing the ion exchanger several times and measuring the pH of the eluting water. In Figure 5.17 the amount of lactic acid sorbed by Amberlite I-6766 was plotted versus time. The initial concentration of the model lactic acid solution was 2%(w/v). Each curve plotted in the figure represents the different pH values of the eluting water. It is obviously seen that the amount of sorbed lactic acid did not change significantly due to the diferences in pH. The lactic acid uptake values were almost same for each pH value. The equilibrium lactic acid uptake values at each pH were around 0.08-0.1 g La / g wet resin. The effect of pH on model lactic acid anion exchanger system was investigated by adjusting the pH of hydrated resin. The adjustment was done by measuring the pH of eluting water.

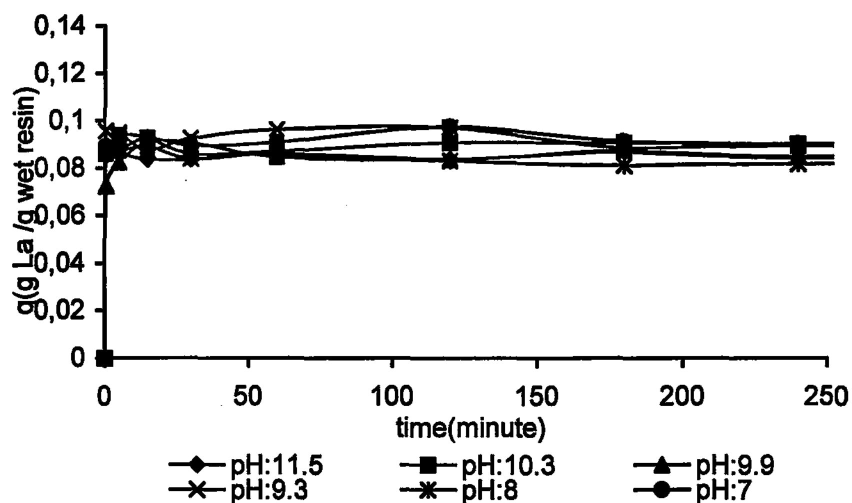


Figure 5.17. The effect of pH on the uptake of model lactic acid by Amberlite I-6766 at T=30°C stirring speed 180 rpm with the initial lactic acid concentration 2 %(w/v)

The pH effect on the sorbed amount of lactic acid is presented in Figure 5.18. In this figure the initial concentration of model lactic acid concentration is 50 g/l. These experiments were conducted for 20 hours and Figure 5.18 shows the first 250 minutes of the experiments. The experiments in both Figure 5.17 and Figure 5.18 were done in the same way; and, it is easily seen that the amount of lactic acid sorbed per gram of resin did not change due to the differences in pH. Nevertheless, small effect of pH can be seen in Figure 5.18 while the effect is minor in Figure 5.17. The main difference between them is the initial concentrations of the experiments. The pH affected the uptake amount of lactic acid in the range of 0.1-0.16 g La/g wet resin. This value was smaller for the lactic acid with the initial concentration of 20 g/l. It can be concluded that the uptake amount of lactic acid by Amberlite I-6766 did not change within the same initial concentration value.

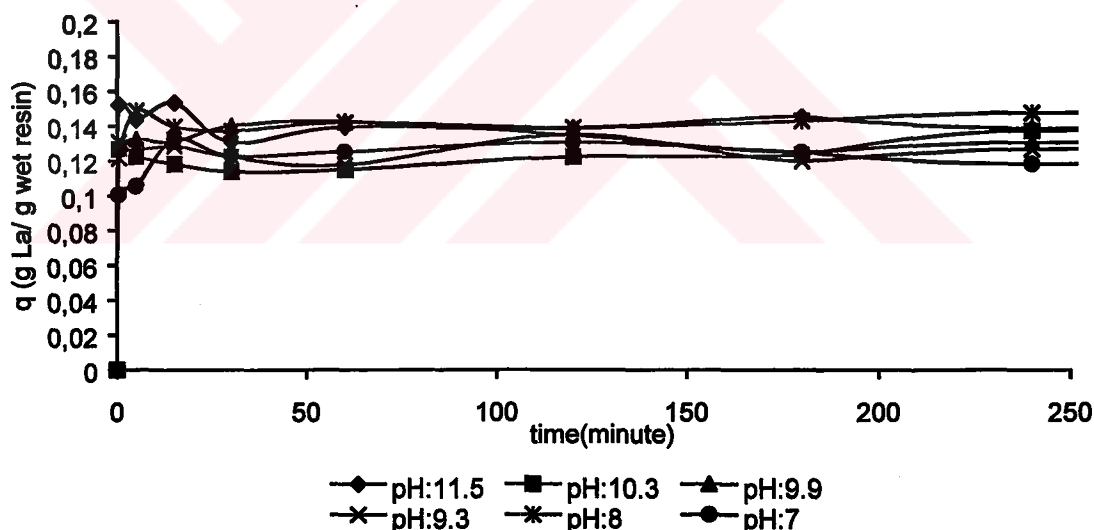


Figure 5.18. The effect of pH on the uptake of model lactic acid by Amberlite I-6766 at T=30°C, stirring speed 180 rpm with the initial lactic acid concentration 5 %(w/v)

The pH of the fermentation broth was 5.5 at the end of the process. The lactic acid content of the fermentation broth was 50 g/l. The fermentation broth was centrifuged and the biomass was separated by centrifugation. The supernatant free of cells was used for investigating the pH effect on the fermentation broth/Dowex

marathon WBA system. The pH adjustments of lactic acid solutions were done by dropwise addition of 1.25 M NaOH and 1 M H_2SO_4 to pH 2.2 and pH 8.3. The pH effect was investigated for three different pH values. Besides the original pH of fermentation broth which was 5.5 at the end of the process, two more pH values were selected to observe the lactic acid uptake behavior. The pH value of 2.2 was selected, which is a value lower than the pH of the fermentation broth and the pH 8.3 that is above the pH of fermentation broth. As seen from Figure 5.19 equilibrium uptake amount of lactic acid was stable regardless of the change in pH.

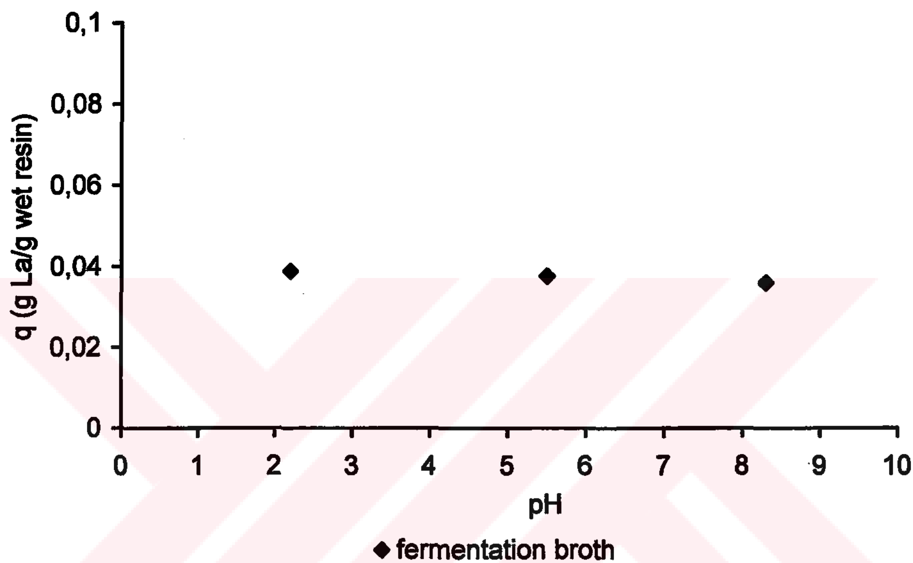


Figure 5.19. The effect of pH on the uptake of lactic acid in fermentation broth by Dowex marathon WBA at T=30°C, stirring speed 180 rpm with the initial lactic acid concentration 5 %(w/v)

The sorption curves for a strongly basic Amberlite I- 6766 and for weakly basic Dowex WBA anion exchangers were generated with lactic acid solutions of different starting pHs. The sorption capacities did not change markedly by varying pH.

In a study carried out by Raya-Tonetti et al, the culture broth was conditioned to adjusting the pH to 8.0 with 1.25 M NaOH solution and heating the culture broth at 100 °C. To evaluate resin adsorption with respect to L(+) and D(-) lactic acid isomers, a racemic mixture was used in the purification procedure. The resin adsorbed approximately 0.18 g La/ g resin. When the culture broth was heated and adjusted the pH to 8.0; the adsorbed amount was 0.4 g La/g resin (Raya-Tonetti *et al.,* 1999).

Little influences of the initial lactic acid concentration, pH and T on selection of resins were found in Zihao and Kefeng study (Zihao and Kefeng, 1995).

The effect of pH on sorption capacities of weak base VI and moderate base MWA- 1 were investigated by Evangelista et al, The pH values were pH: 2.8–3.8– 4.8–5.8. They observed that the sorption capacities of VI–15 and MWA-1 decreased markedly with increasing pH of lactic acid solution (Evangelista *et al.,* 1994).

5.2.1.4. Effect of Lactose Concentration

To investigate the effect of lactose concentration, experiments were held with fermentation broth which was still containing lactose besides lactic acid. Samples were taken before lactose has been converted to lactic acid completely. The samples were then centrifuged in order to remove the biomass and some insoluble large molecules. The supernatant was used to prepare solutions at desired concentrations. Ion exchanger and the lactic acid-lactose solutions were treated together. The results showed that lactose inside the fermentation broth made interferences with lactic acid on the ion exchange reaction. Because of that, equilibrium phase cannot be seen in this case (Figure 5.20). In other words, the negative effect for presence of lactose on lactic acid uptake was seen.

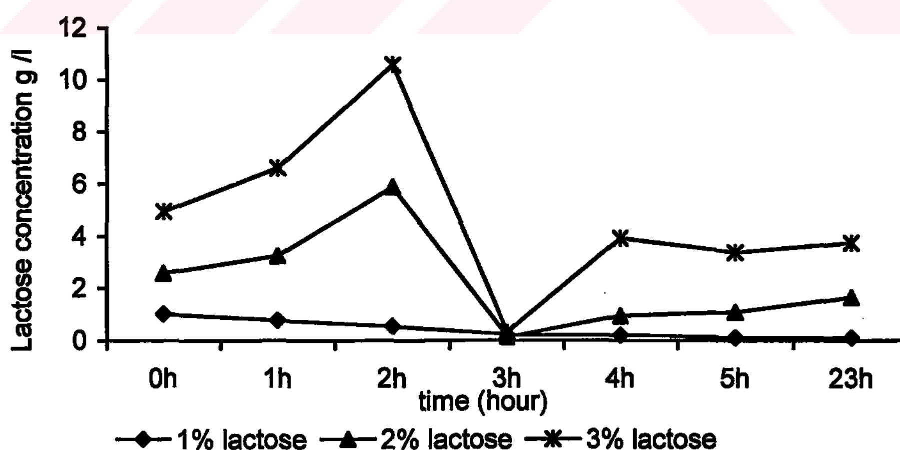


Figure 5.20. Uptake of lactose by Amberlite I-6766 at pH≅7 by shaking at 180 rpm at 30 °C

In the literature there wasn`t much study about the utilization of whey as substrate in a fermentation system which is combined with an ion exchange recovery

process. One of them was done by Rincon et al, in 1997. In their study, whey fermentation was carried out. At the end of the fermentation Amberlite IRA-120, a strong cationic exchanger, was used to remove Na^+ from lactate solutions with an acid concentration of 40 g/l. That was, however, not an actual lactic acid recovery process because they obtained lactic acid solutions containing lactose, proteins, phosphates and salts.

5.2.2. Ion Exchange Equilibria

One of the controlling factors governing the use of ion exchangers is the equilibrium distribution of ions between the resin and solution phases which can be achieved in any given system. In this study, the ion exchange equilibrium was determined for two preselected resins since the equilibrium had been reached in about 1 minute and 15 minutes, the Amberlite I-6766 and Dowex marathon WBA anion exchangers, respectively. From the equilibrium studies the capacities and affinity of the ion exchange resins for lactic acid have been determined.

The ion exchange isotherms for lactic acid in model solutions and fermentation broth at pH 7.0 and temperature of 30 °C are shown in Figures 5.21, 5.22, 5.23, and 5.24. The equilibrium data were analyzed by using the Langmuir and Freundlich equations. The experimental data were exposed to linear transformation to see the deviations from the theoretical model line. For linear transformations of data, Langmuir equations result in linear trendlines. Since the linear transformation of data did not result in linear trendlines for Freundlich equations, the parameter values could not be obtained. Therefore, the Langmuir isotherm best correlates the data obtained for the ion exchange of lactic acid solutions on Amberlite I-6766 and Dowex marathon WBA.

The linearization of Langmuir equation was achieved by converting q vs C data into C/q vs C as shown in Table 5.2. When the transformed linear equation values were plotted, the good linearity was obtained with the regression coefficient values around 0.9 which indicates the validity of the model. The parameters q_m (maximum exchange capacity) and b (affinity constant) were calculated from the slope and intersection of the linear plots. These linearized plots are given in Appendix C. By using these coefficients, the corresponding Langmuir isotherm model lines were drawn and shown in Figures 5.21-5.24.

Table 5.2. Isotherm Equations and Linear Transformation Expressions

	Freundlich Equation	Langmuir Equation
Equation	$q = K_F C^n$	$q / q_m = bC /(1+bC)$
Linear Transformation Expression	$\ln q = \ln K_F + n \ln C$	$C / q_m = 1/(q_m b) + (1/q_m) C)$

Isotherms for the model lactic acid Amberlite I-6766 and fermentation broth Amberlite I-6766 at pH ≅7, temperature 30 ^{0}C are given in Figures 5.21 and 5.22 respectively. Solid lines show the Langmuir möodel, calculated model parameters and the regression coefficients are tabulated in Table 5.3.

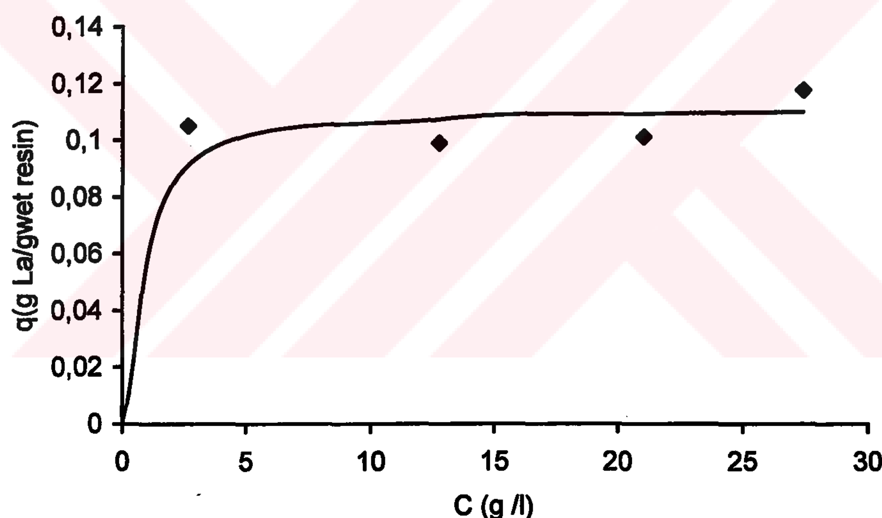


Figure 5.21 Ion exchange isotherms for model lactic acid/Amberlite I-6766 system at pH≅7; T=30°C; agitation speed=180 rpm

As seen from Table 5.3, the maximum amount of lactic acid sorbed (q) in model solution is higher than the maximum sorbed amount of lactic acid in fermentation broth. The q_m value for model lactic acid- Amberlite I-6766 system is 0.1125 g La/g wet resin. This maximum exchange capacity value decreases significantly when the system consists of fermentation broth. The difference between the maximum capacity of the ion exchanger is due to the presence of competing ions in the fermentation broth. The fermentation broth used for the lactic acid Amberlite I-6766 system was subjected to

centrifugation and the supernatant was used for the analyses. The fermentation broth consists of various minerals as given in Table 4.5. Besides the supplemented minerals, whey powder contains different elements. The rich composition of fermentation broth reduces the available exchanging sites of the ion exchanger with lactic acid.

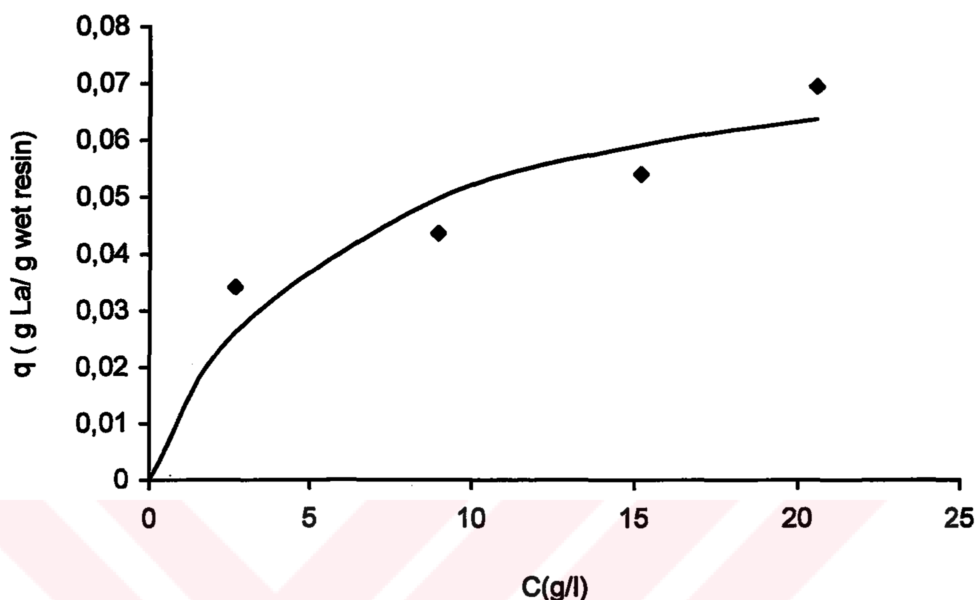


Figure 5.22. Ion exchange isotherms for lactic acid in fermentation broth/Amberlite I-6766 system at pH≅7; T=30°C; agitation speed=180rpm

Similarly, the isotherms for the model lactic acid Dowex marathon WBA system at pH ≅7, temperature 30 ^{0}C are given in Figure 5.23. The uptake of lactic acid in fermentation broth on Dowex marathon WBA at pH ≅7, temperature 30 ^{0}C as a function of equilibrium lactic acid concentration is given in Figure 5.24. The experimental data could be explained by the Langmuir model. As seen from Table 5.3, the maximum amount of lactic acid sorbed (q_m) in model solution was higher than the maximum sorbed amount of lactic acid in fermentation broth. The q_m value for model lactic acid Dowex marathon WBA system was 0.2526 g La/g wet resin where the q_m value for fermentation broth Dowex marathon WBA system was 0.0423 g La/g wet resin. The calculated model parameters showed that model lactic acid-Dowex marathon WBA system had the highest regression coefficient value as 0.9926. The model equation predicted the experimental data very well.

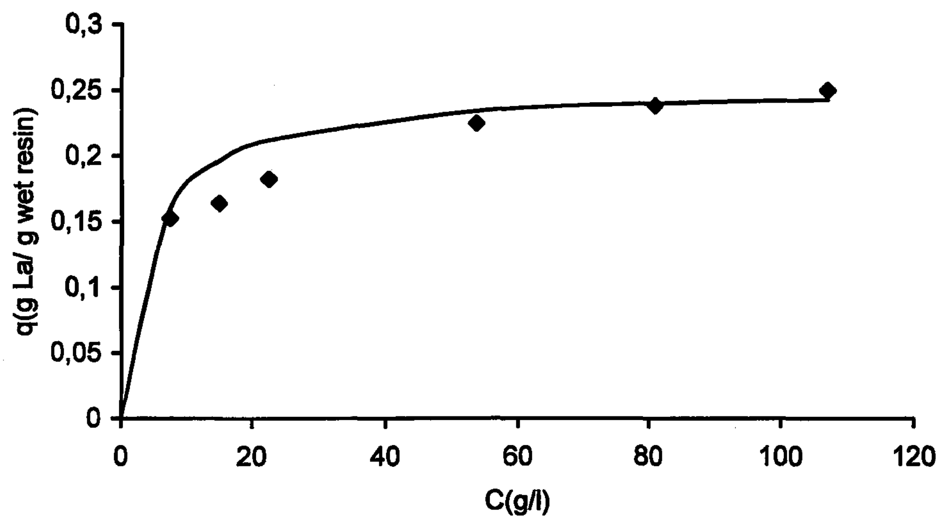


Figure 5.23. Ion exchange isotherm for model lactic acid/Dowex marathon WBA system at pH≅7; T=30°C; agitation speed=180rpm

The ion exchange isotherms obtained at pH ≅7 are shown in Figure 5.23. The highest initial concentration of model lactic acid solution contacted with Dowex marathon WBA was 145 g/l. The curve was fitted by Langmuir equation and parameters were calculated. The maximum capacity was 0.25 g La/g wet resin. Cao and his co-workers have studied at pH 2 and at pH 5 and they have found q_m to estimate the amount of loaded lactic acid during column separation as 217.46 mg/ g wet resin when Amberlite IRA-400 was used (Cao *et al.*, 2002).

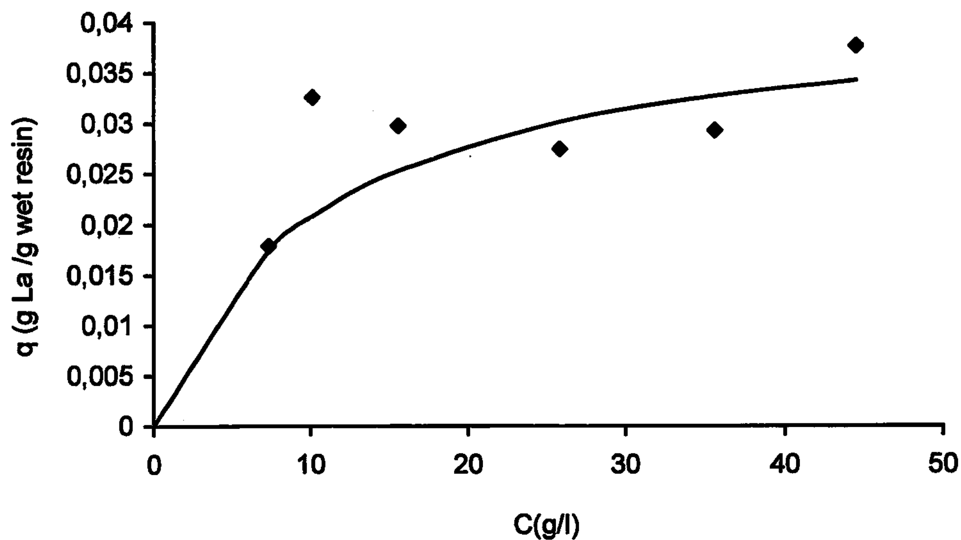


Figure 5.24. Ion exchange isotherm for lactic acid in fermentation broth/Dowex marathon WBA system at pH≅7; T=30°C; agitation speed=180 rpm

Among the literature Sosa and her colleagues have studied the adsorption equilibrium of the lactic acid solution with an anion exchanger. Amberlite IRA-400 characterization was performed by preparing solutions of the unclarified culture broth at different concentrations (ranging from 10% to 100%) diluted with distilled water. A period of 60 minutes was required until adsorption equilibrium was reached. Static adsorption of lactic acid on the resin was conducted and the maximum capacity (q_m) and K_d coefficients, based on the Langmuir equation were calculated. The batch adsorption experiments gave a q_m of 0.342 g La/ g resin while the K_d coefficient value was 0.0207 g/l (Sosa *et al,* 2001). In previous works of Cordoba et al, working with different source of lactic acid found that q_m=0.125g La/ g resin and a K_d value of 0.00097 g/l (Cordoba *et al.,* 1999). The relation between the K_d values and the b values are given as; $K_d = 1/b$ where b is equal to the proportion of adsorption rate to desorption rate (k_a/k_d).

Rincon and his colleagues studied with 5 resins. The data they obtained for the equilibrium studies were correlated well by Langmuir equation (Rincon *et al.,* 1997). As in the latter study, the equilibrium obtained for Dowex marathon WBA and Amberlite I- 6766 were explained by Langmuir equation in this study. Table 5.3 lists the parameters of Langmuir equation. The saturation capacity (q_m) and the equilibrium constants (b) which is equal to 1/ K and the regression coefficients are tabulated. The highest exchange capacity was observed for Dowex marathon WBA. Therefore, Dowex marathon WBA seems to be the most promising candidate for carrying out the process.

The curves in Figure 5.22 and Figure 5.24 show the experimental equilibrium isotherm for ion exchange of lactate from biomass-free fermentation broth on Amberlite I- 6766 and Dowex WBA. From these figures it is evident that the equilibrium is very favourable for these resins.

As can be seen in such figures the data are correlated quite well by the Langmuir equation. The isotherms had convex upward shapes meaning that the equilibrium is favorable. Table 5.3 lists both parameters of Langmuir equation, the equilibrium constant (1/b) and the saturation capacity (q_m), as well.

The values of the equilibrium constants, (1/b) confirm that the isotherms are very favourable for both resins. On the other hand, resins showed high values of exchange capacity q_m if compared to the values in the literature (Monteagudo and Aldavero, 1999).

Table 5.3 The Langmuir parameters of the equilibrium data for lactic acid/ion-exchanger system

Lactic acid/ion exchanger system	Maximum exchange capacity q_m (g La /g wet resin)	b (l /g)	Regression coefficient (R^2)
Model lactic acid/ Amberlite I-6766	0.1125	1.62	0.9851
Fermentation Broth / Amberlite I-6766	0.0813	0.176	0.915
Model Lactic acid/ Dowex marathon WBA	0.2526	0.234	0.9926
Fermentation broth/ Dowex marathon WBA	0.0423	0.096	0.9193

5.3. Fixed Bed Column Operations

5.3.1. Breakthrough Curves

The breakthrough curves are illustrated in terms of effluent concentrations against time or volume. The shape of the breakthrough curve and the time scale are two important properties to make comments about the performance of column application. In breakthrough studies the operation is stopped at a time, breakthrough time, when the effluent concentration of the ion being absorbed from the solution rises above a specified limit, termed the breakthrough concentration. The area above the breakthrough curve up to breakthrough time gives the operating resin capacity or breakthrough capacity. The relation between the shape of breakthrough curve and the degree of column utilization is that breakthrough being sharp, the difference between breakthrough curve, breakthrough capacity and overall capacity will be low, leading to a high utilization degree (Helfferich, 1962).

In order to characterize the breakthrough curves two different times are selected. The first one is the breakthrough time, t_B, represents the time at which the exiting breakthrough concentration was approached and we can afford to discard. The second, an exhaustion time, t_E, corresponds to a time at which the bed is judged ineffective (Zihao and Kefeng., 1995).

Other effects such as flow rate and column properties like particle size, crosslinking, volume capacity that affect the rate being constant for all the sets during the breakthrough studies, feed concentration is the second factor that affects the shape of the breakthrough curves.

The breakthrough curves of L(+) lactic acid in biomass free fermentation broth and model lactic acid solution are shown in Figure 5.25 and in Figure 5.26. The breakthrough curves during ion exchange of lactic acid by Dowex marathon WBA were determined at room temperature since the effect of temperature on the uptake of lactic acid by Dowex marathon WBA was insignificant. The feed lactic acid solution was prepared from biomass free fermentation broth. The typical breakthrough curve was determined at a flow rate 1 ml/min at room temperature. In this experiment, the effluent concentration was measured as a function of time. The curve was plotted in terms of the ratio of outlet lactic acid concentration to feed lactic acid concentration versus time. Fractions of the eluate (100 ml) were collected and analyzed for their lactic acid

contents. Lactic acid was first appeared in the effluent at a time around 10 minutes. The concentration of L(+) lactic acid in effluent increased rapidly during elution time between 10 and 20 minutes. The lactic acid levels increased slightly to increase in the following 10 minutes and reached a plateau after 30 minutes. In Figure 5.25 it is shown that the column became saturated after approximately 30 minutes by feeding with L(+)lactic acid in fermentation broth at a flow rate of 1 ml/min. The retention time of lactic acid in column was around 10 minutes. From the figure it is seen that the breakthrough time is around 10 minutes whereas the exhaustion time was around 30 minutes.

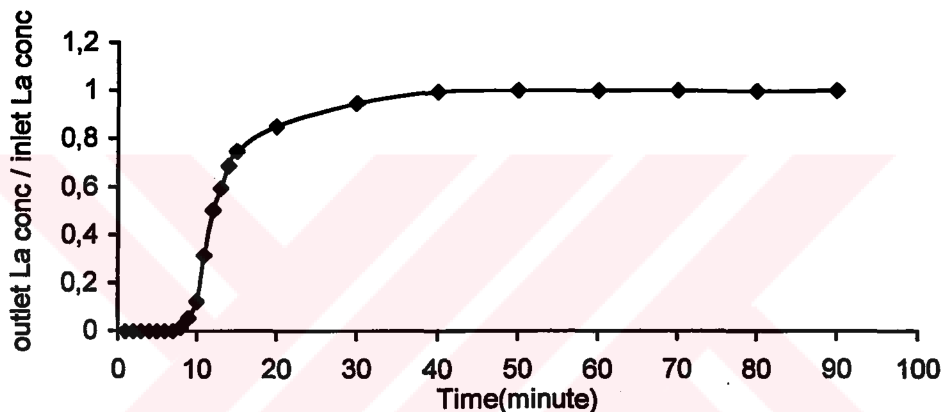


Figure 5.25. Breakthrough curve of lactic acid in biomass free fermentation broth on Dowex marathon WBA

Batch ion exchange of lactic acid from aqueous lactic acid solution was carried out in a fixed bed with Dowex marathon WBA at room temperature. The concentration of lactic acid prepared was the same as the amount of lactic acid in the fermentation broth at the end of the fermentation. The breakthrough curve during this process is given in Figure 5.26. As seen from the figure, the breakthrough time is around 20 minutes and the exhaustion time is around 60 minutes. These time values measured for model solution are greater than that for fermentation broth. The possible reason for that might be the competition of other species present in the fermentation broth. All the operating conditions for both of these studies were identical.

The breakthrough curves are measured by analyzing the effluent concentrations of lactic acid from fixed bed as a function of the contact time.

It is obvious that the exchange of lactic acid from fermentation broth did not approach the levels achieved in the model solutions.

Zihao and Kefeng conducted a similar study. They have plotted the breakthrough curves of lactic acid solution and fermentation broth. Consequently, they have obtained shorter breakthrough time and exhaustion time than those for model solutions (Zihao and Kefeng., 1995).

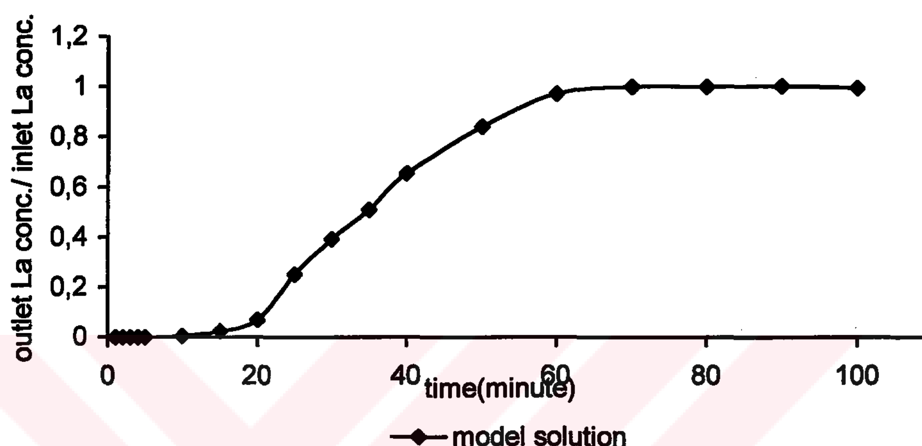


Figure 5.26. Breakthrough curve of lactic acid solution on Dowex marathon WBA

5.3.2. Selection of Elution Conditions

Following a batch or column sorption procedure washing is necessary to remove unbound substances from the ion exchanger before elution proceeds. The washing conditions are chosen to avoid the elution of bound analytes; this means using the adequate buffer solution or distilled water (Addo, 1988). In this study, washing was applied by deionized water which had a conductivity value smaller than 1.0 µS.

After sample application and washing, the following step is elution in ion exchange chromatography. The elution results of various eluants for lactic acid adsorbed on Dowex marathon WBA are shown in Figure 5.27. Selection of the eluting agents is of great importance from the point of efficient product recovery. Eluants mentioned were chosen according to the information regarding to the similar ion exchange resins described in the literature. Figure 5.27 shows the average equilibrium lactic acid concentrations in different test tubes containing 1 g of lactic acid loaded resin with 10 ml of several eluting agents with various ionic strengths. It is obvious that the

eluting performance of methanol was significantly lower than those of HCl and H_2SO_4. As seen from the figure, the increase in ionic strength of HCl increased the eluted amount of lactic acid. However, H_2SO_4 solutions with different ionic strengths did not change the lactic acid concentration in tubes. The highest elution recovery was obtained with 1.5 M HCl. Methanol as an eluting agent did not show a good performance in the elution of lactic acid. However, methanol could be a good solvent for washing step and it could reduce the product loss during the washing step.

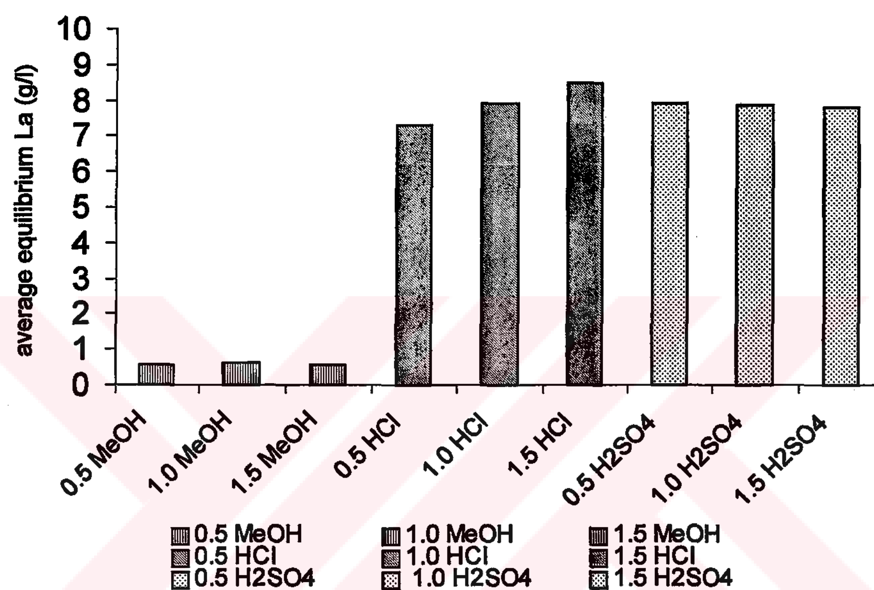


Figure 5.27. Model solution treated with 10 ml eluting agent (Lactic acid concentraton: 50 g/l in 50 ml solution loaded on 10 g.resin

Batch studies on elution behavior of lactic acid was applied to Dowex marathon WBA that was loaded with biomass free fermentation broth. Figure 5.28 shows the effect of eluting agents on lactic acid loaded on Dowex marathon WBA. The results of the elution of L(+)lactic acid in fermentation broth sorbed on Dowex marathon WBA at room temperature are illustrated in Figure 5.28. Lactic acid concentration in eluate increased slightly with increasing concentrations of H_2SO_4 and HCl.

Cao and his team studied various eluants for the recovery of lactic acid in fermentation broth. Proper elution and washing conditions were sought by using H_2SO_4, methanol, ammonia, HCl, NaCl or mixtures of them as eluants. They have found that the elution of lactic acid by H_2SO_4, was more effective than others. Washing the column

with water also resulted in high poduct loss (40%). Washing column with 50% methanol showed much lower product loss (10%) (Cao *et al.*, 2002).

To see the effect of washing step on ion exchange reactions, 1 g of Dowex marathon WBA was delivered to each test tubes and contacted with 0.5 M methanol,1.0 M methanol, 1.5 M methanol 0.5 M HCl, 1.0 M HCl, 1.5 M HCl, 0.5 M H_2SO_4,1.0 M H_2SO_4 and 1.5 M H_2SO_4. Deionized water was used to see its elution property. The lactic acid loaded on Dowex marathon WBA was treated with elution agents without applying a prewashing step. As seen from the Figure 5.28 methanol and deionized water had almost the same lactic acid concentrations whereas HCl and H_2SO_4 had higher concentration values compared to them. When Figures 5.27 and 5.28 are compared the requirement for washing step is obviously seen. The results of experiments for selection of elution agent with a washing step is more valid for determining the most effective eluting agent. In this case, the elution of lactic acid by HCl was found to be more effective than others as shown in Figure 5.27. It seems that methanol was not a good eluent for lactic acid sorbed on Dowex marathon WBA.

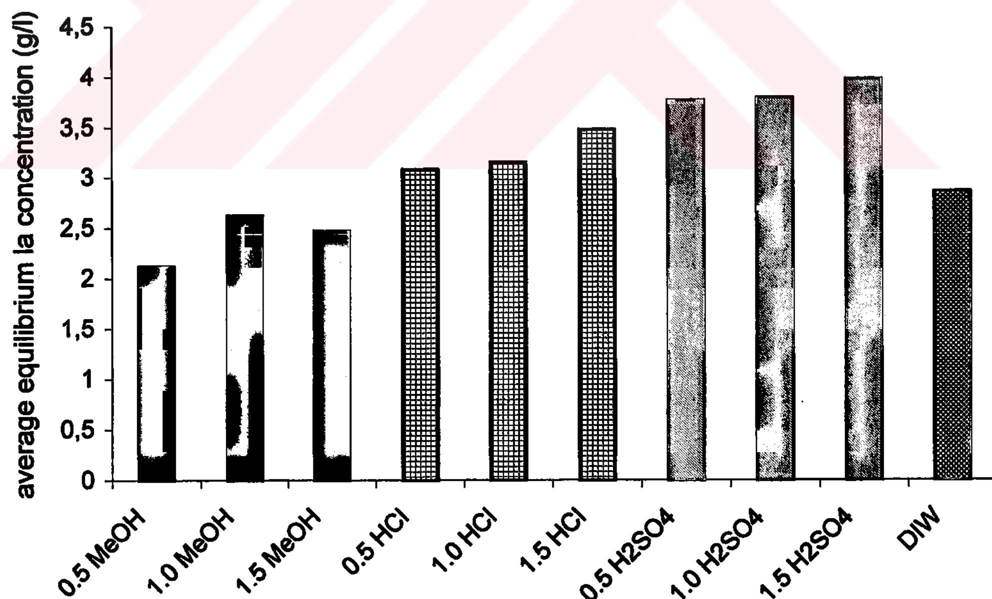


Figure 5.28. Fermentation broth treated with 10 ml eluting agent (Lactic acid concentration:50g/l in 50 ml solution loaded on 10 g resin)

5.3.3. Elution of Lactic Acid in a Fixed-Bed Column Operation

The eluting effects of various chemicals with different ionic strengths were studied. Methanol showed low elution performance of lactic acid from Dowex marathon WBA. On the other hand HCl and H_2SO_4 showed higher elution degree. The elution behavior of latter chemicals was examined in column applications due to their high yield of lactic acid in batch studies.

Column separation of lactic acid by H_2SO_4 and HCl at varying concentrations at room temperature are shown as column separation curves. In Figure 5.29 the column separation of lactic acid loaded on Dowex marathon WBA at room temperature by 0.5 M H_2SO_4 is given. In all column studies the flow rate was adjusted as 1 ml/min. Figure 5.30 shows the column separation curve of lactic acid with 1.0 M H_2SO_4 and the elution results of 2.0 M H_2SO_4 are given in Figure 5.31. Fractions of 250 ml eluants are collected for column studies. In Figure 5.32, Figure 5.33 and Figure 5.34 the concentration of eluents were 0.5 M HCl, 1.0 M HCl, 2.0 M HCl. Besides the column separation curve of lactic acid, the elution curve of L(+) lactic acid with 2.0 M HCl obtained at room temperature is given in Figure 5.35.

The flow rate of the feed lactic acid solution was 1 ml/min. The column application was done at room temperature. The weight of the wet resin inside the column was about 14 g. 50 ml lactic acid solution at a concentration of 50 g/l was fed to the column. The column was washed with 2 bed volumes of deionized water to remove the unbound substances. Then 0.5 M H_2SO_4 at a volume of 250 ml was given to the column (Figure 5.29). Fractions of 1 ml were collected at various time intervals. The high percent of recovery occured in 30 minutes whereas the recovery continued until the 80 minutes of the experiment.

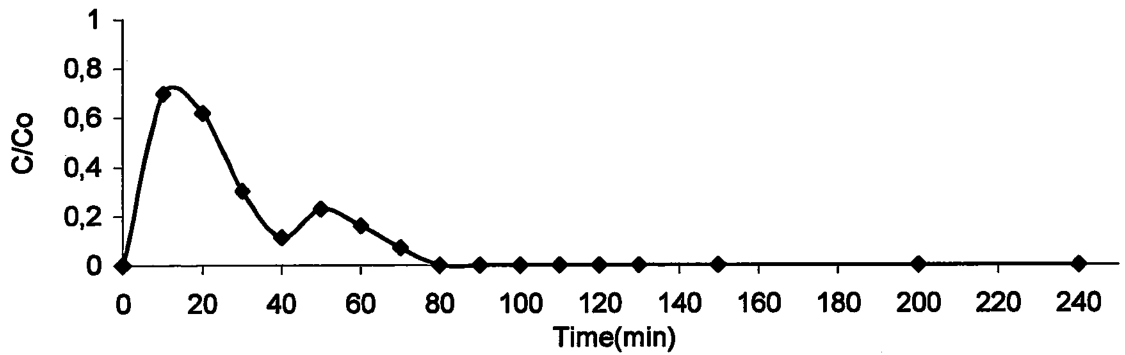


Figure 5.29. Elution curves of lactic acid on Dowex marathon WBA, the eluent used is 0.5 M H_2SO_4, at a flow rate of 1 ml/min

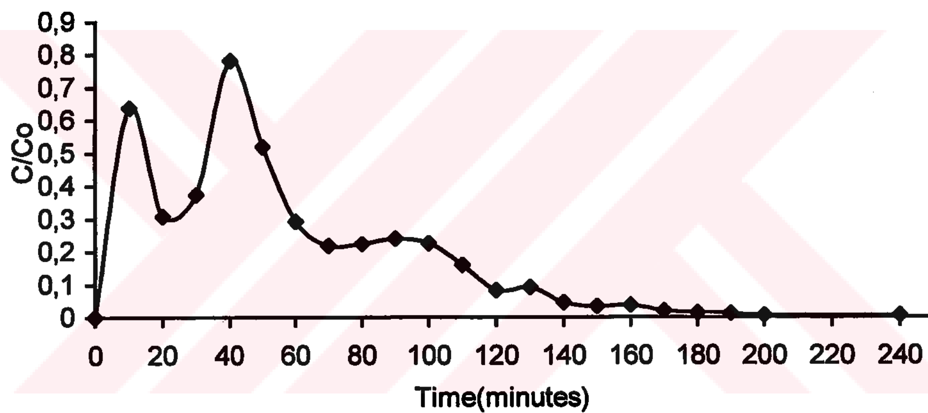


Figure 5.30. Elution curves of lactic acid on Dowex marathon WBA. the eluent used is 1.0 M H_2SO_4, at a flow rate of 1 ml/min

The elution performance of 1 M H_2SO_4 was investigated. It is seen from the Figure 5.30 that the lactic acid concentration in the eluent started to increase in the first 10 minutes than the C/C_0 value decreased and it started to increase during 40 minutes. And in the subsequent time period the lactic acid concentration started to decrease slightly. The lactic acid elution continued for about 150 minutes and 80% of lactic acid was recovered from Dowex marathon WBA.

In Figure 5.29 and Figure 5.30 fluctuations in the elution curves are seen. The possible reason for that can be the differences occurred in flow rate during elution.

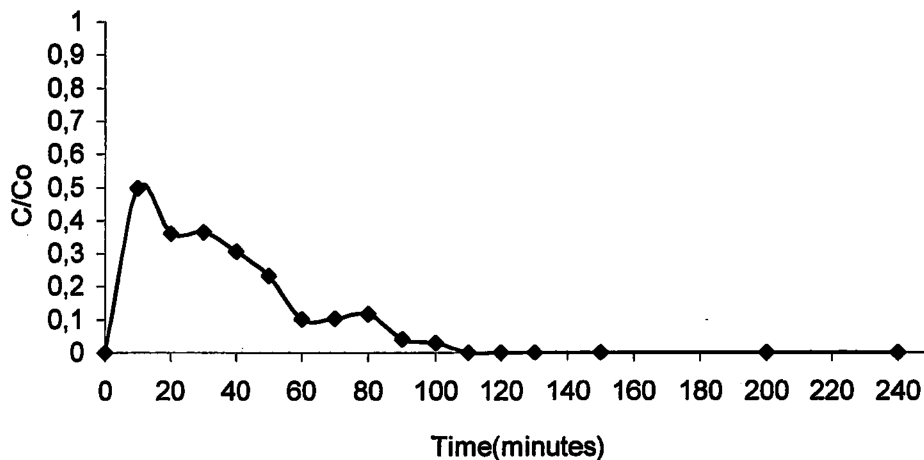


Figure 5.31. Elution curves of lactic acid on Dowex marathon WBA Eluent: 2 M H_2SO_4, flow rate:1 ml/min

2 M H_2SO_4 was also studied to check its elution performance as shown in Figure 5.31. Most of the lactic acid was recovered within 60 minutes. About 50 % of lactic acid was recovered using 2 M H_2SO_4. After 90 minutes there was not significant amount of lactic acid in the eluent.

The effect of concentration on elution profile of lactic acid from Dowex marathon WBA was investigated by alteration of HCl concentrations. 0.5 M HCl was prepared at a volume of 250 ml and fed to the column. The flow rate was adjusted as 1 ml/min. Around 40 minutes of elution step, 44g/l lactic acid concentration in the analyte was obtained. At about 60 minutes of elution lactic acid was not present in the eluate anymore (Figure 5.32).

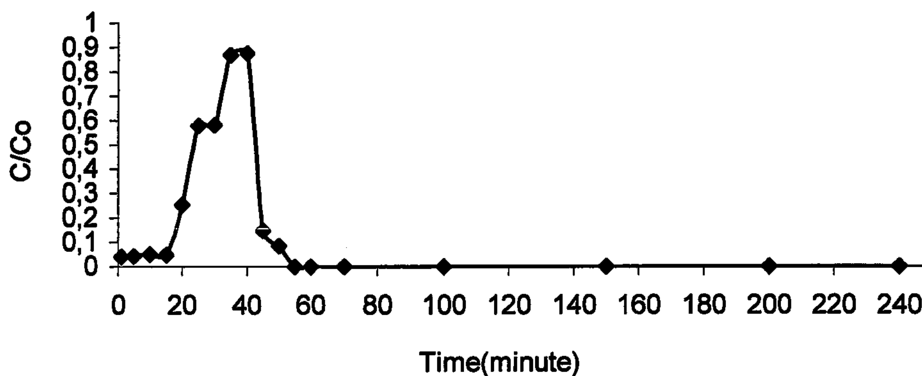


Figure 5.32. Recovery of lactic acid by eluting with 0.5 M HCl operating at room temperature and a flow rate of 1 ml/min

1.0 M HCl solution was prepared and used as the eluting agent of lactic acid loaded Dowex marathon WBA. As seen from Figure 5.33, lactic acid concentration in the eluate showed increments in the first 40 minutes and reached a recovery value almost 100%. In the following time period lactic acid concentration started to decrease. And lactic acid concentration levelled off after 70 minutes.

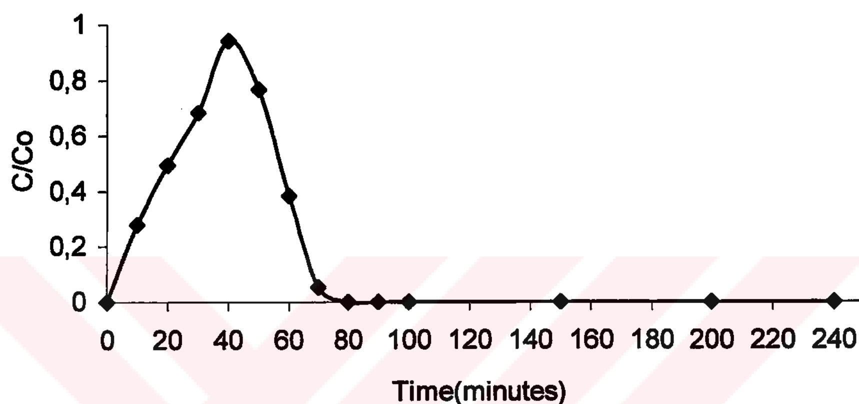


Figure 5.33. Elution curve of lactic acid by eluting with 1.0 M HCl operating at room temperature at a flow rate of 1 ml/min

In Figure 5.34 and Figure 5.35, 100 ml volume of 50 g/l lactic acid solution and L(+)lactic acid in fermentation broth were fed to the column. For model lactic acid solution 100 % recovery was observed whereas depending on the maximum exchange capacity of Dowex marathon WBA more than 95% of bound lactic acid was recovered.

The column separation curve of lactic acid with 2.0 M HCl at room temperature is given in Figure 5.35. Elution peak changed rapidly after elution began. It demonstrates that elution of lactic acid was adequately performed. About 110 ml of eluting agent could recover almost the whole lactic acid from loaded column.

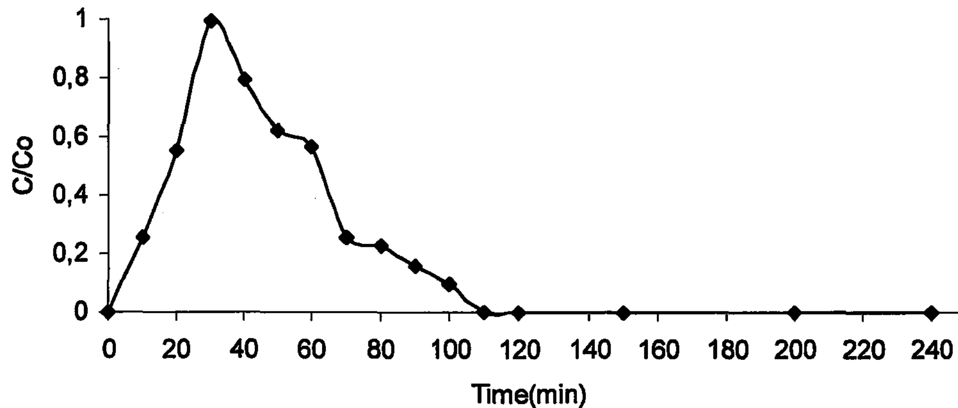


Figure 5.34. Elution curve of lactic acid by eluting with 2.0 M HCl operating at room temperature at a flow rate of 1 ml/min

In similar studies among the literature, the researchers preferred to use HCl as an eluting agent. HCl was used with a concentration of 2.5 M to elute lactic acid at room temperature (Van`t Hul and Gibbons, 1997). Raya-Tonetti et al. carried out a study with fermentation broths and 0.3 g La/g resin were bound to the resin when 2.56 g of lactic acid were fed to the column giving 92% of recovery after elution using a 4M HCl. The study was carried out in a fluidized bed (Raya-Tonetti *et al.,* 1999). The results of HCl elution were satisfactory to obtain lactic acid at high concentrations. Thus it was selected to be used in column elution of L(+) lactic acid in fermentation broth loaded on Dowex marathon WBA.

The fermentation broth with a stable lactic acid concentration of 50 g/l was pumped through a column filled with fresh resin. The column was then flushed with water, contacted with 2.0 M HCl. The fractions were analysed to determine the L(+) lactic acid concentration. As seen from the elution curve, the elution was completed within almost 30 minutes (Figure 5.35). Besides the results obtained for the column containing Dowex marathon WBA saturated with lactic acid solutions, the eluted lactic acid concentration in the eluent was lower than those results. The results showed that the equilibrium loading of Dowex marathon WBA resin was found to be reduced during an ion exchange process of lactic acid in the fermentation broth when compared to the higher equilibrium loading obtained from the model solutions. It is likely that in this case the anions of strong mineral acids have strong affinities for Dowex marathon WBA as noted above the other anions in the fermentation broth may change the equilibrium

loading of lactate ions and introduce competition with the lactic acid for ion exchange (Zihao and Kefeng, 1995).

The sorbed lactic acid on the resin surface was estimated by the following procedure. The Resin Packed Column saturated with lactic acid was washed by distilled water to remove the unadsorbed lactic acid from the column. The sorbed lactic acid was recovered by eluting the column with 2.5 N HCl. The eluted lactic acid was estimated by HPLC after dilution (Srivastava *et al*,1992).

Senthuran et al. observed the same trend in their study of treatment with 2 N HCl. They were succeeded to elute 98 % of the bound acid (Senthuran *et al.,* 1996).

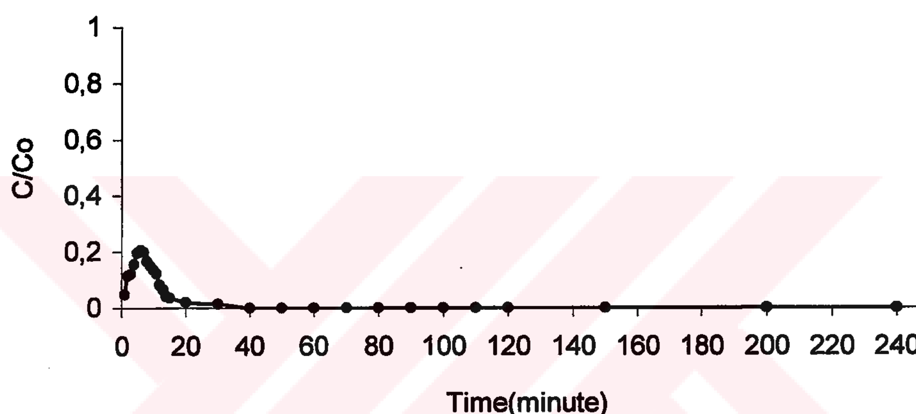


Figure 5.35. Elution curve of fermentation broth from saturated Dowex marathon WBA column by elution with 2.0 M HCl operating at room temperature at a flow rate of 1 ml/min

The reduction in the recovery of lactic acid in fermentation broth from Dowex marathon WBA revealed the idea of competition of ions inside the column. The major mineral content of fermentation broth was investigated by using Inductively Coupled Plasma(ICP). As it is known that not only the mineral content of whey already present, but the addition of minerals for the growth of microorganism and lactic acid production enriched the fermentation media. The samples were analysed before and after the column operation and the ratio of outlet mineral concentration to inlet mineral concentration are given in Figure 5.36. It is obviously seen that most of the minerals retained inside the column. Especially calcium and manganese content of the broth significantly changed. The difference between the feed and effluent concentration of $Ca^{2+}$and Mn^{2+} exceeds almost 80%. Potassium, magnesium, sodium and zinc contents

were decreased about 60% in concentration. The nickel content was diminished about 30% whereas cupper and iron concentrations did not significantly changed. It should be remembered that fermentation media contains various elements and whey proteins as well.

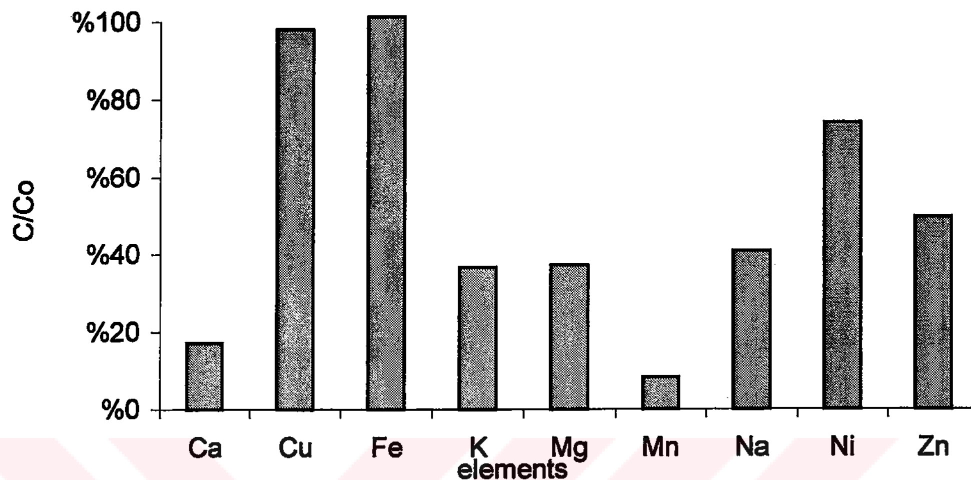


Figure 5.36. The change in the concentration of major elements in the fermentation broth after column separation at room temperature.

Resin sorption is a crude separation method and it is impossible to obtain high product purity. As a result it is important to decide how to arrange following refining steps (Cao et al, 2002).

The repeated use of spent broth may be limited because of the accumulation of inhibitory substances. The use of a mixed bed resin column might be expected to remove these inhibitors but it increases the complexity and cost of the system. It is feasible that the use of several ion-exchange resin columns in series would enable continuous microbial cell recycle and broth reuse (Ye *et al.,* 1996).

CHAPTER 6

CONCLUSION

In this study, the aim was to produce lactic acid from whey and to recover the product by an inexpensive and feasible method. Ion exchange chromatography was selected for the downstream processing technique due to the fulfillment of those requirements.

The production of lactic acid from whey was carried out at 37 ^{o}C, pH 5.5 using *Lactobacillus casei* NRRL B-441. At the end of fermentation 50 g/l lactic acid was produced within 12 hours. The fermentation broth was analysed to determine the isomer distribution of lactic acid. It was obtained that L(+) lactic acid was present inside the broth at 100%.

A variety of commercially available ion exchangers have been screened for the separation of lactic acid. The weak base sorbent, Dowex marathon WBA, exhibited significantly greater sorption capacity and rapid equilibrium state for lactic acid than the others. The sorption process was quite rapid for Dowex marathon WBA with the majority of uptake occurring in 15 minutes. It was found that pH and temperature did not affect the phase equilibria.

The ion exchange equilibrium behaviors of lactic acid as well as L(+)-lactic acid in fermentation broth on the OH^- form Dowex marathon WBA were expressed by the Langmuir isotherm. The maximum exchange capacity (q_m) of model lactic acid was 0.25 g La/g wet resin, while L(+)-lactic acid in fermentation broth has a q_m value of 0.04 g La/g wet resin.

The equilibrium data obtained for biomass free fermentation media, with all the components present, show significant differences in the exchange capacity of the resin for lactic acid. The presence of salts, phosphate ions and whey proteins inside the broth inhibit the lactic acid binding with avaliable sites on the ion exchanger.

Several eluting agents at different concentrations were investigated and 2.0 M HCl was found to be appropriate for the elution of lactic acid with high recovery. The culture broth fed to the column yielded a final product which is L(+) isomer at high purity. The yield of recovered lactic acid was as high as 95%.

Therefore, it is concluded that pure L(+)-lactic acid can be recovered by the ion exchange process with high recovery yield.

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APPENDIX A

Chemicals Used During Study

Table A1. Chemicals Used During Study

Chemical	Features	Source
L(+)-Lactic acid 30%,	MW: 90.08 g/mole, d=1.21 kg/cm^3	Sigma
D(-)-Lactic acid 95%,	MW: 90.08 g/mole	Sigma
α-Lactose	FW: 360.3 g/mole	Sigma
Calcium carbonate	FW: 100.1 g/mole	Fluka
Sodium Hydroxide	FW: 40.00 g/mole	Sigma
Sulfuric acid 95-98%	FW: 98.08 g/mole d=1.84 kg/cm^3	Merck
Litmus Milk	pH=5-6	Difco
Magnesium sulfate	99.5% FW: 120.4 g/mole	Sigma
Hydrocloric acid	37% FW: 36.5 g/mole	Merck
Manganese sulfate monohydrate	FW: 138 g/mole	Merck
Potassium dihydrogen phosphate	99.8% MW: 136.09 g/mole	Merck
Potassium phosphate(dibasic)	99% FW:174.2 g/mole	Sigma
Cupper sulfate pentahydrate	FW: 250 g/mole	Merck
Yeast extract	99.5%	Oxoid
Amberlite I-6766	anion exchanger	Sigma
Amberlite IRA-400	anion exchanger	Sigma
Dowex 1	anion exchanger	Sigma
Amberlite IRA-67	anion exchanger	Sigma
Amberlite IRA-402	anion exchanger	Sigma
Dowex Marathon	anion exchanger	Supelco

APPENDIX B

Calibration Curves

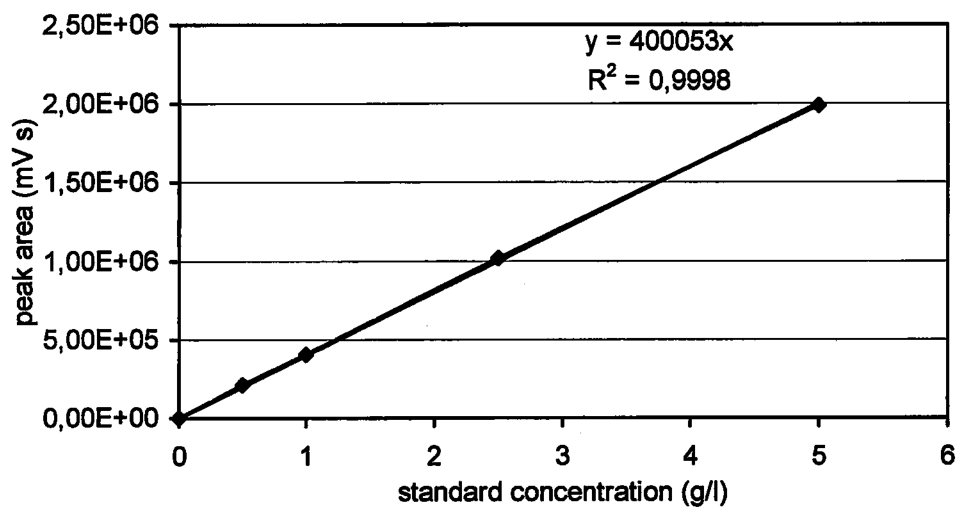

Figure B.1. Calibration curve for Lactic acid standard (HPLC)

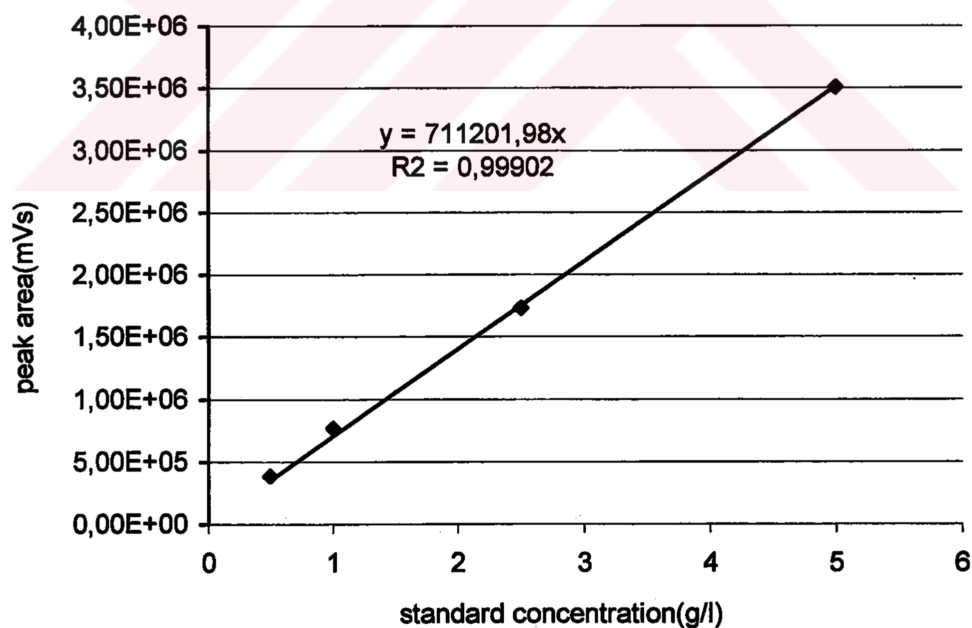

Figure B.2. Calibration curve for Lactose standard (HPLC)

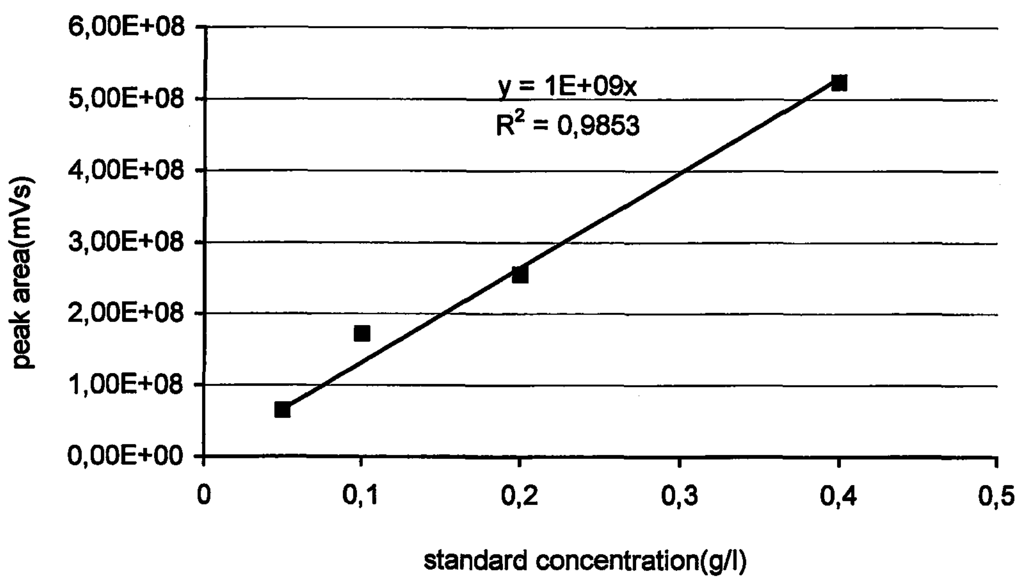

Figure B. 3. Calibration curve for L(+)-Lactic acid standard (HPLC)

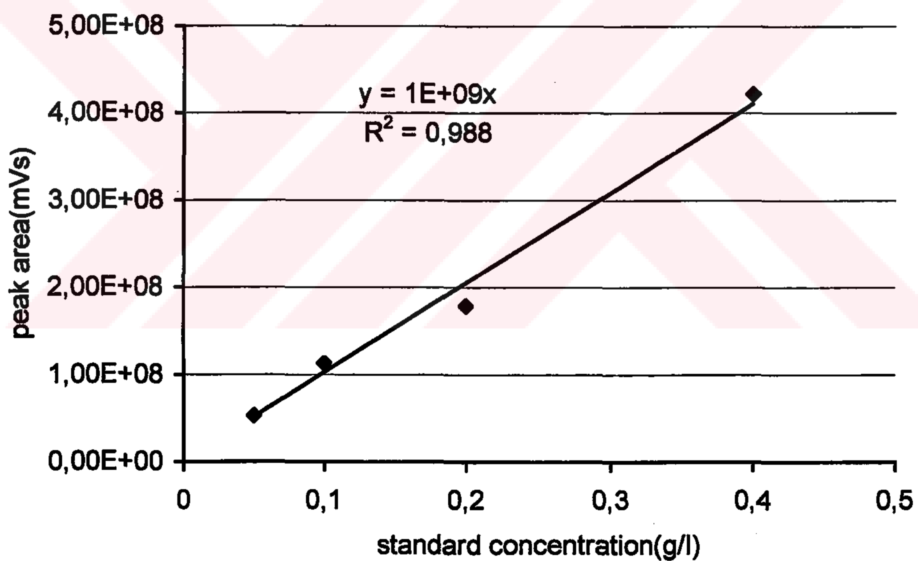

Figure B. 4. Calibration curve for D(-)-Lactic acid standard (HPLC)

APPENDIX C

Linear Transformations

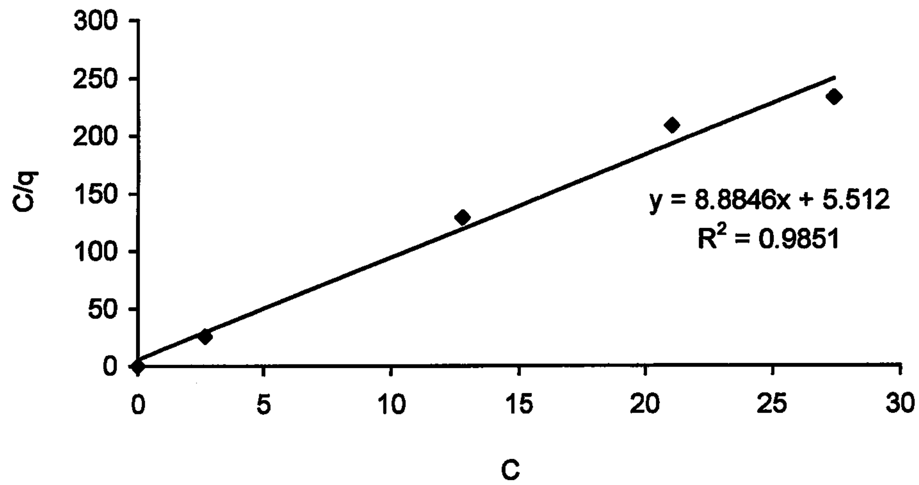

Figure C.1. Linear transformations of model lactic acid with Amberlite I-6766 at pH≅7; 30°C

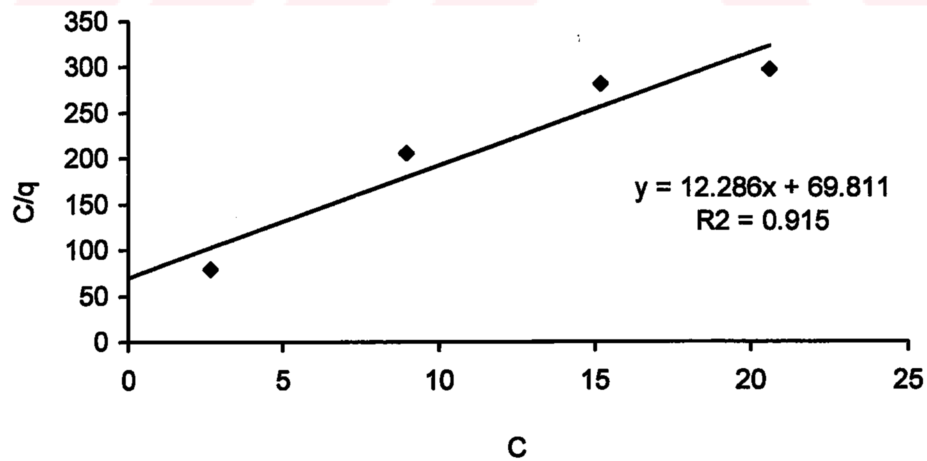

Figure C.2. Linear transformations of lactic acid in fermentation broth with Amberlite I-6766 pH≅7 T=30°C

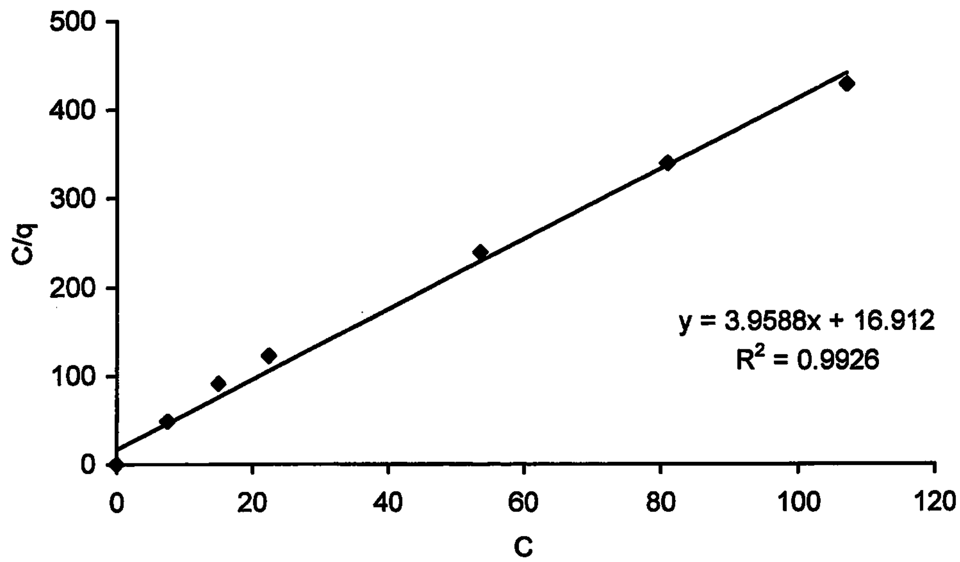

Figure C.3. Linear transformations of model lactic acid solution / Dowex marathon WBA system at pH≅7 ;T=30°C

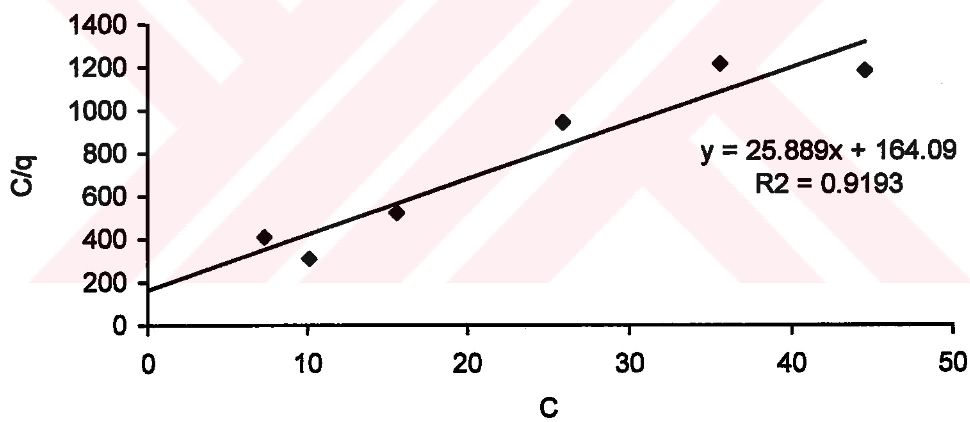

Figure C.4. Linear transformations of lactic acid in fermentation broth/Dowex marathon WBA system at pH≅7 ;T=30°C

APPENDIX D

D.1. Resin Properties

The arithmetic average particle diameter of Dowex marathon WBA was determined by using Scanning Electron Microscopy (SEM). The results of SEM are shown in Figure D.1. The average particle diameter of this anion exchanger was measured as about 520 μm . The results of the circle area values are given in Table 5.6. Particles were examined to observe the size distribution of the particles. The spherical particles showed uniformity. Besides the particle diameters circle areas were measured for 11 samples arbitrary selected.

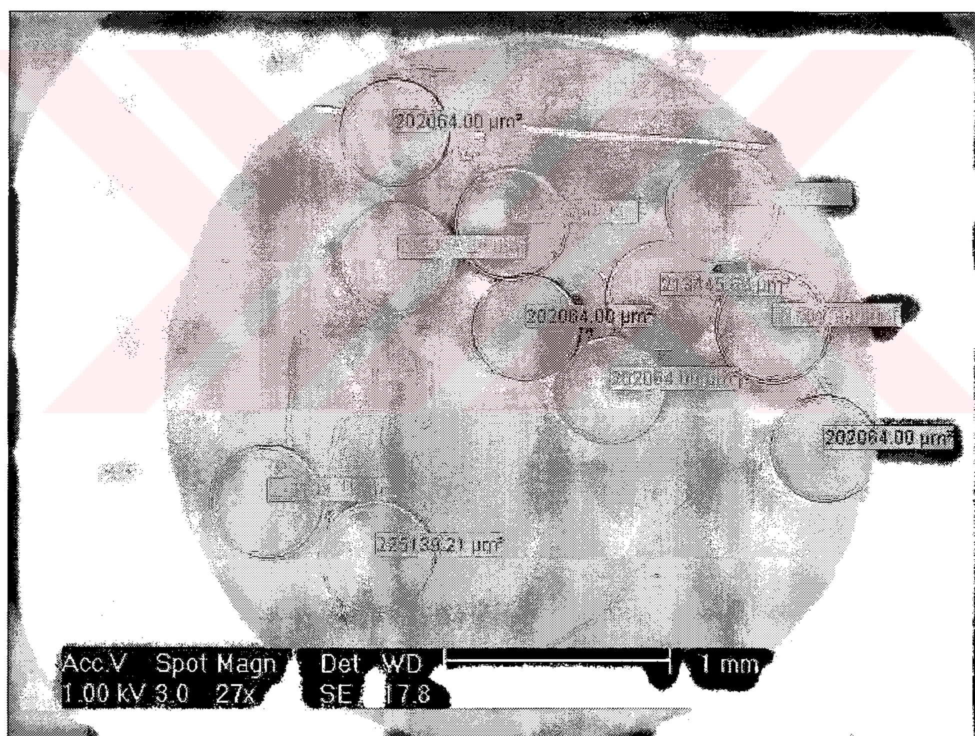

Figure D.1. Circle areas of anion exchanger (Dowex marathon WBA)

Table D.1. Circle areas of Dowex marathon particles

Statistical function	Circle area
Base unit	μm^2
Count	11
Mean	212496,04
Minimum	202064,00
Maximum	225139,21

D.2. The microorganism: *Lactobacillus casei* NRRL B-441

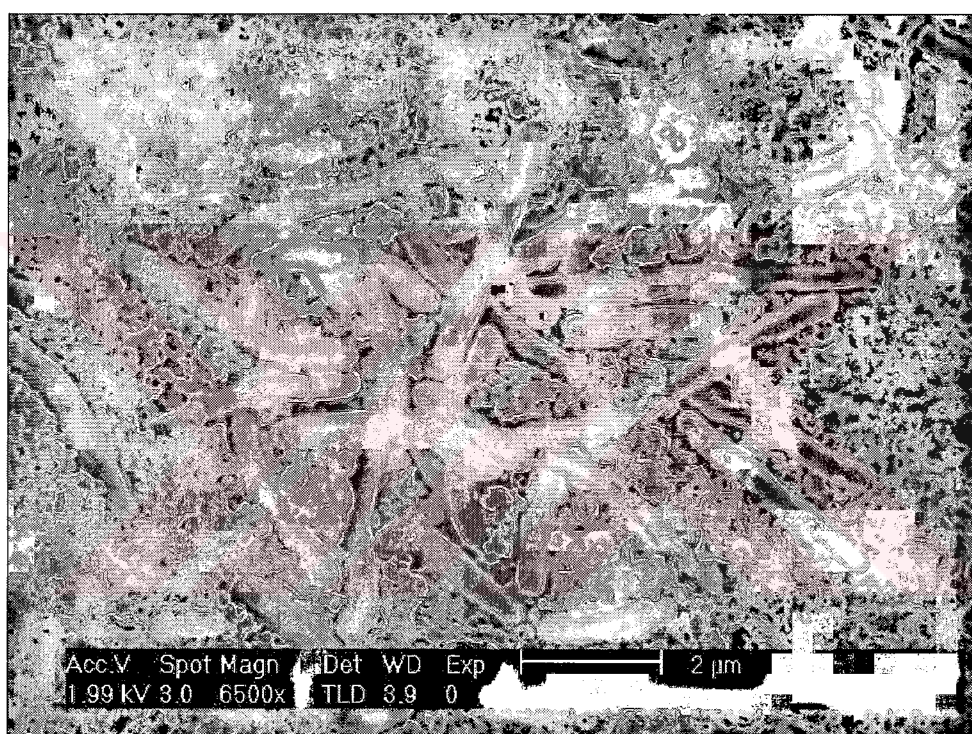

Figure D.2. SEM picture of *Lactobacillus casei* NRRL B-441

T.C. YÜKSEKÖĞRETİM KURULU DOKÜMANTASYON MERKEZİ