

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

By

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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 °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 değiştiricinin 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

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 specificity. 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 $2.7 \times 10^6 \text{ kg y}^{-1}$ 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 $4.1 \times 10^6 \text{ kg y}^{-1}$ during World War 2 and leveled off to about $2.3 \times 10^6 \text{ kg y}^{-1}$. A $90 \times 10^6 \text{ kg y}^{-1}$ 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 stearyl-2-lactylates for the baking industry opened the way for a synthetic route to lactic acid. The 1982 world-wide production of lactic acid is $24\text{-}28 \times 10^6 \text{ kg y}^{-1}$. More than 50% of the lactic acid production is used in food industry as an acidulant and a preservative. The production of stearyl-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 (α -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(+)	52.8-54°C
DL(varies with composition)	16.8-33°C
Boiling point DL	82°C at 0.5 mmHg
Dissociation constant(K_a at 25°C)	1.37×10^{-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 (Børgardts *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 production, 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*,