Chapter 4

Lactic acid production and its recovery
4.1 Synthetic manufacture:

The synthetic manufacture of lactic acid on a commercial scale began around 1963 in Japan and in the United States. These two countries contribute to the 50% production of the world lactic acid. Synthetic lactic acid production is based on the hydrolysis of the lactonitrile by strong acids such as HCl.

\[
\text{MeCH(OH)CN} + 2 \text{H}_2\text{O} + \text{HCl} \rightarrow \text{MeCH(OH)CO}_2\text{H} + \text{NH}_4\text{Cl}
\]

An ammonium salt is formed as a by-product of this reaction. Lactonitrile was obtained along with acetaldehyde as by-product of acetylene based acrylonitrile synthesis, but it is presently made from hydrogen cyanide and acetaldehyde.

\[
\text{HCN} + \text{MeCHO} \rightarrow \text{MeCH(OH)CN}
\]

Hydrogen cyanide may be obtained as by-product from the propylene ammoxidation route to acrylonitrile, or from the reaction of ammonia and light hydrocarbons such as methane. Synthetic lactic acid made substantial gains when it was introduced to the market place because the process used by-products from other synthetic routes and perhaps more importantly, the production of stearoyl-2-lactylates required a high purity, heat stable lactic acid. The synthetic lactic acid contains no residual sugars and does not discolor significantly upon heating. Although by-products were used in synthetic manufacture in the past, lactic acid is now made starting from acetaldehyde and hydrogen cyanide.

The lactic acid is purified by forming methyl lactate, distilling the ester, and then hydrolyzing the methyl lactate. Methanol, hydrogen cyanide and other impurities are then removed by a combination of steaming, carbon treatment and an ion exchange. The acid can also be recovered by solvent extraction. The alkaline degradation of sugars; the synthesis from carbon monoxide; acetaldehyde and water; the hydrolysis of chloropropionic acid; and the nitric acid oxidation of propylene are the other synthetic methods for preparation of lactic acid. Synthetic means of preparation form a racemic DL mixture of the stereoisomers (Murray- Moo- Young, 1985).
4.2 Microbial Fermentation:

Batch fermentation has been the method used industrially for lactic acid production. Fermenters have been constructed of wood or 316 grade stainless steel, and are equipped with heat transfer coils for temperature control. Minimal agitation is provided by top or side mounted stirrers in order to keep the content mixed. Contamination is not a large problem; the most serious contamination problems are due to the growth of butyric acid bacteria at the end of the fermentation. The inoculum size is usually 5-10% of the liquid volume in the fermenter. The inoculum can be propagated in seed tanks or taken from completed large scale fermentation. The acid formed is neutralized by Ca(OH)_2 or CaCO_3. The neutralizing agent can be added in excess as slurry, at the beginning of the fermentation or added intermittently during the fermentation on the basis of pH or acid titration measurements. The fermentation time is 1-2 days for a 5% sugar source such as whey and 2-6 days for a 15% sugar source such as glucose or sucrose. Under optimal laboratory conditions the fermentation takes one or two days. The yield of lactic acid after the fermentation stage is 90-95 wt% based on the initial sugar concentration. The residual sugar concentration is typically less than 0.1%. Cell mass yield can be as large as 30 wt% but generally are less than 15 wt% based on the initial sugar concentration. The yield of cell mass depends heavily on the amount of nitrogenous nutrients used.

The fermentation rate depends primarily on the temperature, pH, concentration of nitrogenous nutrients and the lactic acid concentration. Batch fermentation with controlled pH will proceed quickly at initial stage. Minimum cell mass doubling time is about one hour, but these rates are not achieved under industrial operating conditions where nitrogenous nutrients are suboptimal. As the fermentation proceeds, the rate begins to slow because of the depletion of non-essential but stimulatory growth substances and the accumulation of the lactic acid. The undissociated, electro neutral form of lactic acid rather than lactate appears to be the species which inhibits the fermentation (Murray- Moo- Young, 1985).

The best fermentation conditions are not always the most favourable for the whole processes from an economical point of view, because the cost of substrate and downstream processing are proportionally high. The substrate is usually a question of
geographical availability. Wastes from, for example, agriculture and forestry are preferable to expensive, pure sugars for the low price product.

4.2.1 Continuous and batch fermentations:

Lactic acid is commonly produced in batch fermentation (Bruno-Barcena et al, 1999; Amrane, 2001), but continuous and fed batch productions (Bai et al, 2003) are also used. In comparison of continuous fermentation to batch fermentation, the latter is of choice because of higher lactic acid concentration and yield. This is mainly due to the better utilization of all the substrate in batch fermentation, where as residual concentration of substrates remains in the continuous one. On the other hand, the continuous fermentation generally results in higher productivities probably because these are run at higher dilution rate. Fed batch, semi-continuous and repeated-batch culture gives higher yield than the batch culture (Hofvendahl and Hagerdal, 2000).

De Boer et al (1990) produced lactic acid through anaerobic gas lift bioreactor in a continuous mode and achieved productivity as much as 13 g/l h’ with aggregate forming Bacillus laevolacticus. They showed the effect of growth limiting nutrient on formation of aggregates and lactic acid production. Bruno Barcena et al (1999) used continuous fermentation with adherent variety of Lactobacillus casei in up-flow packed bed bioreactor operated in two stages. They achieved higher productivity in immobilized cells than in free cells. However, yield was the same for immobilized and free cells. Trials have been made to deduce the xylose metabolism in Lactococcus lactis IO-1 from continuous cultures and it has been found that xylose is metabolized by two different pathways namely, the phosphoketolase pathway for mixed acid fermentation and glycolytic pathway for homo lactic acid fermentation (Tanaka et al, 2002).

Several authors have used batch fermentation for the production of lactic acid (Srivastava et al, 1992; Vaccari et al, 1993; Kurbanoglu and Kurbanoglu, 2003; Wee et al, 2004a; Wee et al, 2004b). Fed batch cultures have been used for the hyper production of lactic acid using L. lactis by Bai et al (2003) and were able to produce 210 g/l of lactic acid. Variant of batch culture- cell recycled repeated batch bioreactor was used by Oh et al (2003) whereby they investigated the effect of various nitrogen sources and found yeast extract to be the best suited. They found 20 % yeast extract
dosage optimum to achieve the same amount of lactic acid thereby decreased the cost of production. Batch fermentations of sugarcane bagasse hemicellulose hydrolysates were performed by a natural thermotolerant acidophilus *Bacillus* sp. (Patel et al, 2004). They reported glucose and arabinose to be utilized first followed by xylose. Maximum lactic acid production obtained was 55.5 g/l and observed that increase in sugar concentration does not influence the lactic acid production.

4.2.2 Simultaneous Saccharification and Fermentation (SSF):

A novel approach has been developed wherein organisms are benefited by the action of hydrolytic enzymes that they are lacking and be able to utilize the raw substrate they otherwise can not degrade. Such approach has been termed as simultaneous saccharification and fermentation (SSF). Sreenath et al (2001) used SSF to produce lactic acid from various cellulosic raw agriculture residues using pectinase and cellulase enzymes. Nakasaki and Adachi (2003) used SSF with cellulase to improve substrate utilization by *Lactobacillus paracasei* from waste water sludge. *Lactobacillus coreymiformis* subsp. *torques* was able to utilize cellulose as carbon source in SSF with cellulase and β-glucosidase (Yanez et al, 2003). Milled corn cobs along with spent yeast cells and CSL were used to produce lactic acid by *Lactobacillus rhamnosus* CECT-288 in SSF (Rivas et al, 2004). Tanaka et al (2005) have reported production of D-lactic acid from defatted rice bran using SSF with *Lactobacillus delbrukii* subsp. *delbrukii* IFO-3202. Application of hydrolytic enzymes on agriculture feedstock substrates leads to generation of fermentable sugars and simultaneously lactobacilli ferments sugars to lactic acid. Hence both enzymatic saccharification and fermentation of feedstock substrates proceed at a faster rate and result in higher yield of lactic acid (Sreenath et al, 2001).

Tainiguchi et al (2004) investigated the possibility of co-culture of lactic acid bacteria in a medium containing xylose and glucose. *Enterococcus casseliflavus* and *L. casei* were co-cultured to produce 95 g/l of lactic acid.

4.2.3 Lactic acid production by immobilized cells:

Hollow-fiber, cell recycled and artificially immobilized cell and biofilm reactors maintain high densities (Friedman and Gaden, 1980; Roy et al, 1982; Ohleyer et al, 1985; Demirci et al, 1993a; Demirci et al, 1993b; Hongo et al, 1986) and result
into increased volumetric productivity rates. However, the use of hollow fiber and cell recycled fermenter are limited by high start up costs and membrane fouling during the fermentation (Roy et al, 1982; Ohleyer et al, 1985). In reactors with artificially immobilized cells, production rates and yields are low because of limited diffusion rates, cell leakage and poor cell reproduction in beads (Stenrous et al, 1982). However in biofilm reactors, organisms are immobilized by a natural attachment to solids while they continuously grow. Several materials have been used to produce biofilms for lactic acid fermentation like poly propylene (Demirci et al 1993), polyethylene amine (Senthran et al, 1999), and plastic composite support (Ho et al, 1997a; Ho et al, 1997b; Ho et al, 1997c, Cotton et al, 2001; Velazquez et al, 2001). A study has been conducted using an activated carbon column for simultaneous cell immobilization and lactate adsorption, in a semi batch process with periodical medium replacement (Chen and Ju, 2002). Membrane bioreactor system was used for thermophilic Bacillus strain BS119 in an ultrafiltration membrane to produce 115 g/l lactic acid (Danner et al, 2002).

4.2.4 Extractive Lactic acid fermentation:

Lactic acid production processes suffer from several drawbacks, namely, productivity, process economics and end product inhibition. An undissociated lactic acid (Na-lactate) passes through the bacterial membrane and dissociates inside the cell (Na⁺ and Lactate⁻). The inhibition mechanism of lactic acid is probably related to the insolubility of dissociated lactate ions in the cytoplasm which causes the acidification of cytoplasm and failure of proton motive force. It eventually influences the transmembrane pH gradient and decreases the amount of energy available for cell growth (Goncalves et al, 1997; Yin et al, 1997). To overcome the inhibitory effects of lactic acid, either the lactic acid must be continuously removed from the fermentation broth, or it must be neutralized with alkali during the fermentation to convert lactic acid into its less inhibitory dissociated form. The resultant lactate salt however presents extra downstream costs especially when the free lactic acid is the desired product.

The end product inhibition by lactic acid causes several problems in which the most important ones are low lactate formation rate and its recovery from fermentation broth (Roffler et al, 1984; Roucourt et al, 1989). Moreover, the lactic acid itself
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Esterifies and forms polyactic acid. Therefore, integrated approach with separation of product had gained a lot of interest in this fermentation process. One possible approach is extractive fermentation which involves fermentation with \textit{in situ} separation of lactic acid (Srivastava et al., 1992). Gerhardt and Gallup (1963) reported that lactic acid production by \textit{L. acidophilus} was increased by dialysis. Abbott and Gerhardt (1970) reported that in the production of salicylic acid by \textit{P. fluorescens}, dialysis fermentation was a very effective method. Dialysis fermentation has been proven to be very effective. However, to achieve more effective dialysis fermentation, the reservoir volume or volume of the dialysate must be expanded to more than that of the fermentation vessel or fermentation broth. Therefore, diffusion dialysis fermentation has the limitation of diffusion efficiency (Hongo et al., 1986).

Considerable insight into these problems can be gained via the techniques of extractive lactic acid fermentation. Studies have been carried out by several authors (Friedman and Gaden, 1970; Wardell and King, 1978; Stieber and Gerhardt, 1981; Hongo et al., 1986; Boyaval et al., 1987; Wang et al., 1988; Yao and Toda, 1990) using either liquid-liquid extraction or membrane separation techniques. The solvent extraction technique is not preferred because it causes several physical, chemical and biochemical problems on the catalytic activity of the cells (Wang, 1983; Yabanavar and Wang, 1985).

Lactic acid fermentation with \textit{in situ} separation by ion-exchange resin involves considerably less operational and maintenance cost. In this technique, the system is started as a batch process (Fig. 4.1). When considerable lactic acid production has been achieved (18-19g/l), the separation process is started by switching on the pump that allows the broth containing cells to pass through the Resin Packed Column (RPC). The effluent of the column is recycled back to the fermenter. The anion exchange resin in the column selectively removes the lactate ions from the fermentation broth passing through it. As long as the pump is on, the pH of the broth within the fermenter increases due to continuous removal of lactate ions. On the other hand pH begins to decrease due to the production of lactic acid when the pump is switched off.
Thus recycling through the column permitted the control of pH in the fermenter by automatic switching of the recycling pump connected to a pH controller. The RPC saturated with lactic acid was washed with distilled water to remove the unadsorbed lactic acid from the column. The adsorbed lactic acid was recovered by eluting the column with 2.5 N hydrochloric acid. By this method authors have obtained 80.2 g/l lactic acid. It has been observed that the removal of lactic acid from the broth decreases its inhibition on fermentation. It is evident that extractive fermentation reduces the fermentation time when compared with the conventional batch process. This time reduction is directly reflected in the overall increased lactic acid productivity. In extractive fermentation more substrates can be diverted for lactic acid fermentation. Also it has been observed that the growth is very much affected by the accumulation of lactic acid and this adverse effect can be relieved by extractive fermentation (Srivastava et al, 1992).

4.3 Recovery process:

Lactic acid is sold in three major grades; technical, food and pharmaceutical. The grades are listed in order of increasing purity and more elaborate recovery processes are needed to produce the higher quality material. In addition, heat stable
Lactic acid, which does not discolor significantly upon heating to about 200 °C for a few hours, has a large market. The recovery of lactic acid or lactate salts from the fermentation broth contributes a large part of the total cost of the manufacturing.

The first step in all recovery processes is to raise the fermentation liquor's temperature to 80-100 °C and increase the pH to 10 or 11. This procedure kills the organisms, coagulates the proteins, solubilizes the Ca-lactate, and degrades some of the residual sugars. The liquid is then decanted or filtered. Several methods are available for purification of lactic acid from fermentation media. The classical methods are based on precipitation, extraction or distillation (Smith and Claburn, 1939; Peckham, 1944; Vaccari et al, 1993, Lazarova and Peeva, 1994). Methods based on ion exchange (Srivastava et al, 1992; Vaccari et al, 1993), or electrodialysis (Hongo et al, 1986; Boyaval et al, 1987) have been investigated.

4.3.1 Filtration, carbon treatment and evaporation:

Commercial lactic acid production relies on the fermentation of relatively pure sugars with minimal amounts of nitrogenous nutrients. Thus, by using a pure feedstock the recovery process is simplified. The process may be used to produce technical or food grade acid. After the fermented broth is filtered, activated vegetable carbon is used to bleach the Ca-lactate for production of food grade acid. No carbon treatment is used for the technical grade. The Ca-lactate is evaporated to a 37 % concentration at 70 °C and 0.57 atm. The concentrated lactate is then acidified with 63 % sulfuric acid, and the CaSO₄ precipitate is removed by a continuous filter. The filtered acid is then treated with activated carbon. The lactic acid is then evaporated from 8 % to 52 % or 82 % in 316 grade stainless still evaporators (Murray- Moo-Young, 1985).

4.3.2 Ca-lactate crystallization:

The classical route of recovery of lactic acid from fermentation broth is based on the precipitation of the calcium lactate by adding Ca (OH)₂ to the broth after separation of the microorganisms. The salt is filtered off and the cake is treated with sulfuric acid leading to the free acid and CaSO₄. Technically, this process is complex and additional purification steps are required to produce commercial grade lactic acid. The disadvantages of the process are caused by the high solubility of calcium lactate.
and the formation of CaSO₄, which has to be dumped (Frieling and Schugerl, 1999; Chen and Ju, 2002). Typically the filtered liquor from the fermenters was treated with carbon, first under slightly alkaline and then under slightly acidic conditions. The crude Ca-lactate liquor was then evaporated under vacuum. Technical grade acid was made from this liquor after evaporation, acidification, and filtration of the precipitated CaSO₄, carbon treatment and heavy metals precipitation. To make higher grades of product the liquor was cooled, crystallized and washed. The mother liquor and wash water were also cooled, crystallized and washed. The crystals were redissolved and similarly recrystallized as in earlier steps to create purer grades. These methods provide a product that is low in unfermented carbohydrates but may contain some ash which is mainly CaSO₄. The crystals tend to form clusters and can be difficult to wash. The wash water and the mother liquor contain high amounts of Ca-lactate due to its high solubility, and must be recycled. Important costs in the purification process are related to the energy for water removal, losses in yield and labor. To avoid the formation of CaSO₄, lactic acid can be recovered by solvent extraction.

In other method, lactic acid is precipitated as the Mg salt and reported to have a larger difference between solubilities at 0 °C and 100 °C. Then Mg-lactate was extracted with organic solvent; butanol and back extracted in water. Butanol has been chosen as the solvent for extraction because it is not as hazardous as other solvents, e.g. isopropyl ether (Benthin and Villadsen, 1995).

4.3.3 Liquid-liquid extraction:

Lactic acid can be purified by this method from fermentations using crude raw materials. In all such processes the acid must first be extracted from the crude liquor by the solvent, and then recovered from the solvent by some means such as back extraction into water or distillation of the solvent-lactic acid mixture. The extraction solvent should have low water solubility, a high distribution coefficient for lactic acid, and a low distribution coefficient for impurities such as the residual sugars. The distribution coefficient is defined as the concentration of lactic acid in the solvent phase divided by the concentration of lactic acid in the water phase.

Croda-Bowmans Chemicals Ltd (England) has used a countercurrent extraction with isopropyl ether as the solvent. The fermentation liquor is filtered and
then acidified with sulfuric acid; the CaSO₄ is filtered off. Next the crude lactic acid is decolorized with activated carbon and then heavy metals, calcium and amino acids are removed by ion exchange. The acid is then evaporated under vacuum before it enters the counter current extraction columns. The acid is recovered from the solvent by counter-current extraction into water. Next, the acid is given additional activated carbon and ion exchange treatments as needed. Lastly the acid is evaporated to its final concentration. The solubility of isopropyl ether in water is low and the loss of solvent is tolerable. Lactic acid refined by liquid-liquid extraction is substantially free from ash, but contains other impurities from the raw material and needs additional treatments by activated carbon, oxidation and other means.

Liquid extraction recovery methods have been widely studied (Roffler et al, 1991; Yabannavar and Wang, 1991; Martin et al, 1992; Hano et al, 1993; Lazarova and Peeva, 1994). Liquid extraction system consists of two separate phases of liquid where organic acid transfers from one phase to another phase based on the solubility differences between phases.

With regard to the good solubility of lactic acid in water, its extraction is only possible with carriers dissolved in the organic phase at pH values below pKa value (3.86) of the lactic acid (Frieling and Schugerl, 1999). The transport process can be improved by a carrier compound usually a secondary or tertiary amine soluble in organic solvent. Carrier reacts with lactic acid called carrier-facilitated transport or reactive extraction, which is kept in the organic phase until it encounters a stripping solution such as sodium carbonate solution (Demirci et al, 1999). Several authors investigated its recovery from fermentation broth by liquid-liquid extraction (Seevaratnam et al, 1991; Yabannavar and Wang, 1991, Siebold et al, 1994; Roychoudhry et al, 1996).

4.3.4 Distillation of lactate esters:

High quality lactic acid, substantially free from residual sugars and other impurities can be prepared by the esterification of lactic acid with a low molecular weight alcohol, distillation of the lactic ester, hydrolysis of the distilled lactate esters to yield the alcohol and lactic acid, and the distillation of the alcohol from the degenerated lactic acid. Sulfuric acid was used to catalyze the reaction in an excess of
methanol. Corrosion of the stainless steel columns contaminated the product with the iron. Ceramic equipment was found to be unsuitable because of frequent temperature changes and the strong acid.

4.3.5 Lactic acid extraction from silage:

Silage is fermented, high-moisture forage to be fed to ruminants, cud-chewing animals like cattle and sheep (Fig. 4.2 C). It is fermented and stored in a storage silo, a process called ensilage. Silage is most often made from grass crops, including corn (maize) or sorghum. Silage is made from the entire plant, not just the grain.

Figure: 4.2 A, Sealed silage; B, Concrete silage silo; C, Cows are being fed with silage.

In silage production grass is cut, harvested, stored in a silo and consolidated and sealed. The consolidation and sealing phases are vitally important as the removal of the air from grass allows fermentation rather than oxidation (Fig. 4.2 A). The
naturally occurring microorganisms in the grass use grass sugar as an energy source and produce acids as the by-product. Silage undergoes anaerobic fermentation, typically beginning at about 48 h after the silo is filled (Fig. 4.2 B). Traditionally, the fermentation is caused by indigenous microorganisms; today, some silage is inoculated with specific microorganisms to speed the fermentation or to improve the resulting silage. The process converts sugars to acids and exhausts any oxygen present in the crop material. The fermentation is essentially complete in about two weeks. The major acid in well preserved silage is lactic acid. Badly preserved silage contains large amounts of butyric, acetic and other acids from secondary fermentation with low levels of lactic acid. The level of lactic acid in the silage thus depends on the sugar level of the grass at cutting, the degree of wilting, the quality of sealing and the preservation. High levels reflect dominance of lactobacillus fermentation.

The spontaneous lactic acid fermentation of lignocellulosic residues during the ensiling processes makes the resultant silage a strong potential source of lactic acid. As compared to the conventional lactic acid production systems, the possibility of extracting lactic acid from silage is a proposition that has attracted economical and environmental interests. However, due to the heterogeneous nature of the silage micro-flora, the selective recovery and concentration of lactic acid requires delicate unit operations to produce an end product of acceptable quality. Technical and economic studies have been carried out (Danner et al, 2002) wherein the extraction of lactic acid from silage was investigated by using laboratory and pilot-plant mechanical presses. Supercritical fluid extraction (SFE) technology for simultaneous recovery and purification of lactic acid from cereal and maize silages had been investigated by Danner et al (2000). In general, deionized water at ambient temperature is used as the extracting solvent, at solvent ratio of 2:1 to 3:1. Then the silage is pressed, filtered to separate the crude lactic acid extract from the residual solid pulp. The particulate extract was clarified further by either centrifugation or ultrafiltration before recovery and partial purification of the lactic acid by electrodialysis (ED) (Danner et al, 2002).

De-merits associated with the SFE are the low purity of the lactic acid product due to the co-extraction of fermentation by-products like acetic acid and butyric acids and its high capital cost. Any lactic acid derived from silage must either undergo an
exhaustive extraction and purification regime to render its quality acceptable for pharmaceutical, food and biodegradable plastics application; or to be restricted to low grade applications like animal feeds, and the production of lactate salts used as road-de-icer during the winter in cold climatic area (Danner et al, 2000).

4.3.6 Electrodialysis:

The organic acid recovery processes, such as crystallization, solvent extraction, and electrodialysis (ED), have been extensively studied, revealing certain limitations. The crystallization method is disadvantageous in terms of low yield, high chemical costs and waste production. Solvent extraction is handicapped by unfavorable distribution coefficient and environmental problems associated with hazardous solvent use. However, the third process, electrodialysis, is promising for the downstream processing of organic acids from fermentation broth.

Electrodialysis is an electro membrane process in which ions are transported through ion exchange membranes from one solution to another under the influence of an electric potential. In other words, ED is an electrochemical separation process in which electrically charged membranes and an electric potential difference are used to separate ionic species from an aqueous solution and other uncharged components (Hongo et al, 1986; Choi et al, 2002) (Fig. 4.3).

Typically ED consists of five compartments, two anode, two concentrations and one cathode compartment. Each compartment is separated by and anion exchange membrane or cation exchange membrane. The fermentation broth passes through the cathode compartment (Fig. 4.3 III). As lactic acid in the fermentation broth, is charged negatively, it penetrates the anion-exchange membrane and is attracted to the anode compartment (Fig. 4.3 I and V) but is unable to penetrate the cation exchange membrane. Consequently, lactic acid produced, accumulates in the concentration compartments (Fig. 4.3 II and IV).
Figure: 4.3 Schematic diagram of electrodializer. $L^-$, lactate ion; $C$, cation exchange membrane; $A$, anion exchange membrane; I & V, anode compartment; II & IV, concentration; III, cathode compartment; DC, direct current.

Figure: 4.4 Schematic diagram of Electrodialysis coupled fermentation.
Fermentation process can also be coupled with electrodialysis. When lactic acid is produced and the pH of the broth falls below the set value, the direct power supply connected to pH meter and the pump operate, resulting in the flow of electric current into the ED and the flow of the broth from the fermenter to the ED. Lactate ion penetrates the anion exchange membrane and accumulates in the concentration compartment. As lactate ions move to this compartment the pH of the broth increases above the set value and the direct power supply is switched off by the pH controller. This process continues and the thus the produced lactic acid is continuously excluded outside of the fermentation broth (Fig. 4.4).

The principle of monopolar electrodialysis is depicted in figure 4.5 A. Indicated is the arrangement of the ion exchange membranes to form distinct channels, namely diluting streams (DS) and concentrating stream (CS). In most applications, multiple anion and cation exchange membranes are arranged in an alternating pattern between an anode and a cathode to form a series of concentrating and diluting cells in the stake (between and 500 cell pairs, typically more than 100). A third channel, the electrode rinse streams is located on either ends of the stack. During operation an aqueous electrolyte solution is circulated in the electrode rinse streams to facilitate the transfer of the electric current and, to remove gases produced at the electrodes.

Figure: 4.5 A, Mono-polar electrodialysis; B, Bi-polar electrodialysis unit
Electrodialysis has found large scale application in the food, dairy, pharmaceutical, chemical, textile and water treatment industries for purposes ranging from recovery, concentration and purification, to brackish water desalination and ground water denitrification (Madzingaidzo et al, 2002). Chukwu and Cheryan (1999) described a continuous integrated process where permeate from the membrane bioreactor was directly fed to the online mono polar ED unit with simultaneous recovery, concentration and purification of acetic acid. The residual sugars and nutrients were retained in the diluting stream of the ED and recycled back to the bioreactor to facilitate optimum material utilization with concomitant reduction in waste generation. Bi-polar membrane electrodialysis (Fig. 4.5 B) also referred to as water splitting electrodialysis, can convert aqueous salt solutions into acids and bases. A water splitting stack is similar to a conventional (mono-polar) electrodialysis stack but incorporates a third type of membranes, the bi-polar membrane, which is composed of a cation and an anion membrane, can split water molecules that have diffused at the interface into their hydrogen and hydroxyl ions. The combination of each bi-polar membrane on either side creates a stack with three separate streams namely, base, salt and acid. While color and other chemical impurities are reduced significantly, additional bleaching and de-ionization process steps however be integrated to polish the free lactic acid for high grade application in the biodegradable thermoplastic and pharmaceutical industries (Madzingaidzo et al, 2002).

4.4 Materials and Methods:

4.4.1 Lab Scale Production of Lactic Acid:

The bacterial culture Lactobacillus KCP01 on the basis of lactic acid production studies in flasks was applied for lactic acid production in laboratory scale fermenter. The apparatus used for this study was 5 lit batch fermenter (Bioflo 110, New Brunswick, New Jersey) (Figure 4.6). Four vertical baffles (positioned 90° apart from each other) made from stainless steel were provided to reduce the vortex effect. Provisions were made for mounting pH probe, temperature probe, dissolved oxygen probe, motor (agitation) shaft, sterile additions, to introduce sterile air for aeration, sample collection and decantation ports, on the head plate of the fermenter vessel. pH was maintained at 7.0 by automatic addition of 8 N NaOH or aqueous slurry of CaCO$_3$ through one of the four peristaltic pumps provided with the fermenter controls.
that are run by the microprocessor. Agitation speed was kept to 150-200 just to ensure proper mixing of the contents. Temperature was maintained at 35 °C by circulating the hot water into the outer jacket of the fermenter vessel. Inoculation was done using sterile syringe and needle through the rubber septum provided for inoculation on to the head plate. Samples were removed and analyzed as described earlier except that the growth was measured in terms of cell dry weight.

Figure: 4.6 5.0 ltr Batch type fermenter

4.4.2 Recovery of Lactic Acid:

After completion of fermentation the lactic acid was recovered by different approaches. Cells were removed by centrifugation at 10, 000 rpm for 10 min. The supernatant was subjected to various treatments for lactic acid recovery.
4.4.2.1 Ion-exchange resin, its preparation and extraction of lactic acid:

An anion exchange resin Amberlite IRA-400 was used for lactic acid separation. The resin has chloride ion on its surface, which has high electro negativity as compared to lactic acid. The chloride ion of the resin was replaced with hydroxyl ion by saturating the resin with NaOH solution. First, the resin was thoroughly washed with distilled water. Second, the resin was treated with 2.5 N NaOH solution overnight. Third, the resin was again washed thoroughly with distilled water to remove alkali and packed into the column. Treated resin was then used to determine the optimum resin to lactic acid ratio. Variable amount (1-10 gm) of above treated resin was taken into a series of tubes to which known amount of lactic acid containing broth was added and kept for 1 h with intermittent shaking. The supernatant was analyzed for the lactic acid content and the quantity of resin having minimum lactic acid in the supernatant was selected as the best for binding of maximum lactic acid. This ratio was used for further recovery studies.

Known volume of the fermented broth was passed through the column at slow rate to allow the exchange of ions. Lactate present in the broth replaced –OH ions on the resin and NaOH formed eluted out of the column. The broth was recycled twice through the column and eluent was preserved for further analysis. The column was washed with distilled water to remove unbound lactic acid. The column was eluted with 2.5 N HCl and fractions of 20 ml were collected and subjected to further analysis.

4.4.2.2 Recovery by solvent extraction:

Cell free supernatant was concentrated by heating at 100 °C to 1/4th volume. The resulting solution was extracted with butanol and back extracted to water. For extraction, supernatant was mixed with equal volume of butanol for five minutes. pH of the mixture was adjusted to 2.5 with concentrated HCl and mixing was continued for another five minutes. Butanol was separated from the aqueous phase for back extraction in water. For this, lactic acid containing butanol phase was mixed with equal volume of water for five minutes and pH was adjusted to 6.5 with 4 N NaOH and mixing was continued for another 5-10 minutes. The aqueous phase was separated from butanol and preserved whereas butanol phase was discarded.
These two steps were repeated for three more times using the same water for the back extraction. The final aqueous phase was treated with the activated charcoal to remove the impurities (Benthin and Villadsen, 1995).

4.5 Results and Discussions:

After optimization of cultural and physical parameters at flask level, there comes a scaling up of the process. Lactic acid production from date juice was subjected to scaling up from 100 ml to 3000 ml in 5 lit batch type fermenter.

Batch fermentation of lactic acid was carried out where pH during the fermentation was maintained by automatic addition of 8.0 N NaOH (Fig. 4.5).

Maximum lactic acid production obtained was 21 g/l after 58 h of run. During fermentation process, cell growth, sugar utilization and lactic acid production were in pace with each other. However, lactic acid production was same as obtained at the flask level where pH was not controlled and production in the fermenter (21 g/l) is about half than that obtained in flask (45.59 g/l) with 2 % CaCO₃. Lactic acid yield obtained was 0.73 g of lactic acid / g of date sugar. The reason behind this low production in the fermenter can be the sodium toxicity, as 8 N NaOH is a quite a high ionic strength. Second reason can be the toxicity of the lactate ions. This toxicity effects can be revealed from the Fig.4.5, as cell growth was also inhibited and about
half of the sugar remained unutilized at the end of the fermentation. Thus, pH control alone is not enough for optimal lactic acid production. A mechanism to prevent the accumulation of lactate ions has to be included (Chen and Ju, 2002).

Subsequently, CaCO$_3$ was used to neutralize the lactic acid. It reacts with lactic acid to form Ca(La)$_2$ and precipitates and the carbonate released neutralizes the hydrogen ions.

$$
CaCO_3 + 2HLa \leftrightarrow Ca(La)_2 + H_2O + CO_2
$$

Thus, the inhibition caused by both free acid and lactate ions may be alleviated with the use of CaCO$_3$ (Chen and Ju, 2002). This is evident from Fig. 4.6 when pH neutralization was carried out using 2% CaCO$_3$ instead of 8.0 N NaOH.

Lactic acid production was significantly improved and maximum lactic acid obtained was 30.16 g/l with productivity of 0.65 g/l/h. This has been the case in flask level where addition of CaCO$_3$ supported the maximum lactic acid production of 45.59 g/l (53 g/l, initial sugar) with 93% substrate conversion; otherwise, lactic acid production was only 23.7 g/l without pH control (Chapter 3, table 3.6). It is also evident that there was a significant difference between final pH in both the flasks and sugar utilization was more in case of flask containing 2% CaCO$_3$ leading to increased lactic acid production. Thus, these findings confirm the need for combining pH control with a better method for lactate removal. During further studies, effect of initial sugar concentration on lactic acid production was investigated (table 4.1).

**Figure: 4.6 Effect of addition of CaCO$_3$ on lactic acid production**

Lactic acid production was significantly improved and maximum lactic acid obtained was 30.16 g/l with productivity of 0.65 g/l/h. This has been the case in flask level where addition of CaCO$_3$ supported the maximum lactic acid production of 45.59 g/l (53 g/l, initial sugar) with 93% substrate conversion; otherwise, lactic acid production was only 23.7 g/l without pH control (Chapter 3, table 3.6). It is also evident that there was a significant difference between final pH in both the flasks and sugar utilization was more in case of flask containing 2% CaCO$_3$ leading to increased lactic acid production. Thus, these findings confirm the need for combining pH control with a better method for lactate removal. During further studies, effect of initial sugar concentration on lactic acid production was investigated (table 4.1).
Lactic acid production and recovery

<table>
<thead>
<tr>
<th>Initial sugar concentration g/l</th>
<th>Lactic acid g/l</th>
<th>Yield g/g</th>
<th>Volumetric productivity g/l/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>35</td>
<td>30.16</td>
<td>86.17</td>
<td>0.65</td>
</tr>
<tr>
<td>50</td>
<td>46.25</td>
<td>92.5</td>
<td>0.79</td>
</tr>
<tr>
<td>75</td>
<td>69.43</td>
<td>92.57</td>
<td>1.19</td>
</tr>
</tbody>
</table>

Table: 4.1 Effect of initial sugar concentration on lactic acid production

It was observed that lactic acid production increased with increase in the initial sugar concentration and maximum lactic acid production obtained was 69.43 g/l. Moreover, volumetric productivity was also increased with the yield remained same for 50 and 75 g/l sugar concentration. Table 4.2 shows the comprehensive data of lactic acid production reported in other studies. Both the strain KCP01 and date juice as substrate seems to be promising for large scale industrial level lactic acid production.

<table>
<thead>
<tr>
<th>Organism</th>
<th>Lactic acid g/l</th>
<th>Productivity g/l/h</th>
<th>reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enterococcus faecalis RKY1</td>
<td>47.00</td>
<td>2.0</td>
<td>Oh et al, 2003</td>
</tr>
<tr>
<td>Lactobacillus rhamnosus ATCC 10863</td>
<td>120.00</td>
<td>2.1</td>
<td>Kwon et al, 2001</td>
</tr>
<tr>
<td>Lactobacillus lactis IO-1 JCM 7638</td>
<td>39.00</td>
<td>0.9</td>
<td>Nomura et al, 1998</td>
</tr>
<tr>
<td>Lactobacillus rhamnosus IFO 3863</td>
<td>98.00</td>
<td>1.9</td>
<td>Min-Tian et al, 2005</td>
</tr>
<tr>
<td>Lactococcus lactis IFO 12007</td>
<td>25.00</td>
<td>0.72</td>
<td>Hofvendahl and Hagerdal, 2000</td>
</tr>
<tr>
<td>Lactobacillus delbrukii sp. Bulgaricus ATCC 11842</td>
<td>50.00</td>
<td>0.62</td>
<td>Hofvendahl and Hagerdal, 2000</td>
</tr>
<tr>
<td>Lactobacillus helveticus ATCC 15009</td>
<td>49.00</td>
<td>1.3</td>
<td>Hofvendahl and Hagerdal, 2000</td>
</tr>
<tr>
<td>Lactobacillus KCP01</td>
<td>69.43</td>
<td>1.19</td>
<td>Present study</td>
</tr>
</tbody>
</table>

Table: 4.2 Reports in literature of lactic acid production by batch fermentation

4.5.1 Recovery of lactic acid by Ion-exchange resin:

For any ion-exchange chromatography, it is necessary that compound to be separated does not exceed the binding and exchange capacity of the resin. Thus, in the present study, amount of resin required for maximum binding of lactic acid was determined and the results are presented in figure 4.7.
Figure: 4.7 Amount of ion-exchange resin required for maximum binding of lactic acid

It can be seen from the figure that as amount of resin increases, free lactic acid in the supernatant decreases. For maximum of lactic acid binding (450 mg), 9 gm of pretreated ion exchange resin was required. Thus, this proportion was followed for subsequent experiments.

Figure: 4.8 Lactic acid recovery using ion-exchange resin

In the next experiment, 45 g of resin was used to recover 2.23 g of lactic acid from 150 ml of fermented broth. Elution in form of fraction, 20 ml each, was carried out with 2.5 N HCl (Fig. 4.8). Lactic acid elution in first fraction was only 16 mg and in remaining fractions elution was very low revealing that higher concentration of HCl might be required for better displacement of lactate ions from the resin (table 4.3).
Lactic acid production and recovery

<table>
<thead>
<tr>
<th>Eluent type</th>
<th>Lactic acid (mg)</th>
<th>Lactic acid Lost/recovered (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude</td>
<td>1217</td>
<td></td>
</tr>
<tr>
<td>Eluent After binding</td>
<td>85</td>
<td>85</td>
</tr>
<tr>
<td>I wash</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>II wash</td>
<td>152</td>
<td>220</td>
</tr>
<tr>
<td>III wash</td>
<td>56</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>79</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>84</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>43</td>
<td>431</td>
</tr>
<tr>
<td>4</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>30</td>
<td></td>
</tr>
</tbody>
</table>

Table: 4.3 Recovery profile of lactic acid using ion exchange resin

It was observed that binding of lactic acid to the resin is very efficient but at the same time elution with concentrated HCl was insufficient. Lactic acid recovered in the fractions was only 35.41%, whereas 25.06% was lost during washing steps and 61% remained bound in the resin. For better elution of this tightly bound lactic acid, strong acids other than HCl must be used e.g. phosphoric and nitric acid and in that case resin will be rendered unuseful.

4.5.2 Lactic acid recovery by organic solvent:

Though there are only few reports on recovery of lactic acid using ion-exchange resin, solvent recovery method has been of choice and is widely used. The purification procedure employed in the present study is very simple as it requires only standard laboratory equipments (Benthin and Villadsen, 1995). As seen in the figure 4.9, lactic acid was maximally extracted in the second fraction and decreased then after. Lactic acid recovery was 72.86%, with 4% lost in the spent broth and 24% in the intermediate steps. The probable reason may be the use of the same water fraction for back extraction. Each time the water is used to back extract the lactic acid from a fresh butanol phase, butanol takes some water for its saturation and there by may decrease the proportion of aqueous phase leading to loss in the total lactic acid extracted.
Figure: 4.9 Lactic acid recovery in fractions by organic solvent using same water phase for back extraction

Therefore, in subsequent studies, fresh water was used for back extraction and lactic acid recovered was 96.66 % (Fig. 4.10). Thus, lactic acid recovery by ion-exchange chromatography was unsuccessful but better recovery was obtained with organic solvent.

Figure: 4.10 Lactic acid recovery in fractions by organic solvent using fresh water phase for back extraction

4.6 Conclusion:

Lactic acid production was successfully scaled up to the batch type laboratory scale fermenter with maximum production of 69.43 g/l using date juice by \textit{Lactobacillus} KCP01. Comparison of the lactic acid production, by \textit{Lactobacillus} KCP01 from date juice, throughout the study at different stages was made and presented in table 4.4.
## Lactic acid production and recovery

<table>
<thead>
<tr>
<th>Type of medium</th>
<th>Lactic acid production</th>
<th>Fold increase in the production</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date juice alone</td>
<td>2.65</td>
<td>1.00</td>
</tr>
<tr>
<td>Date juice + MRS medium</td>
<td>10.30</td>
<td>3.88</td>
</tr>
<tr>
<td>Medium after PB design</td>
<td>16.86</td>
<td>6.36</td>
</tr>
<tr>
<td>Medium after RSM</td>
<td>22.60</td>
<td>8.52</td>
</tr>
<tr>
<td>Fermentor</td>
<td>21.50</td>
<td>8.11</td>
</tr>
<tr>
<td>Fermenter</td>
<td>69.43</td>
<td>26.20</td>
</tr>
</tbody>
</table>

Table: 4.4 Comparison of the lactic acid production by *Lactobacillus* KCP01 from date juice

What are \( a + b \)?
4.7 References:


Demirci A, Pometto III AL and Harkins KR. (1999) Rapad screening of solvents and carrier compounds for lactic acid recovery by emulsion liquid extraction and toxicity on Lactobacillus casei (ATCC 11443). Biosep. 7 297-308.


