STUDIES REGARDING THE PROCURANCE OF THE LACTIC ACID THROUGH FERMENTATION

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Abstract: In this study one presents several modern methods for the procurement of the lactic acid. The lactic acid can be obtained through fermentation with selected bacteria using: corn flour, potatoes, molasses, and milk whey. The lactic acid resulting from this process of fermentation is afterwards recovered or extracted and the purified.

Keywords: biotechnology, lactic fermentation, lactic bacteria, lactic acid, purification.

1. INTRODUCTION

Lactic fermentation is an anaerobic process in which fermentable glucides are transformed under the action of the enzymes from the micro organisms found in the lactic acid as a product. The lactic fermentation goes following the chemical reaction:

$$\underset{\text{Glucose}}{C_6H_{12}0_6} \rightarrow \underset{\text{Lactic acid}}{2C_3H_60_3}$$

The lactic acid is an organic natural hydroxide that can exist in two states: levorotary and clockwise D (-). Lockwood and Co. showed that esters and salts derived from lactic acid are levorotary. Both isomers are found in the biological system and the lactic acid resulted after the fermentation process is generally found as a racemic mixture. Soccol and Co. showed that the L(+) isomer is by far the most important for the alimentary industry, being the only one that the human body can assimilate under the action of the dehydrogenises of lactic L(+) [8,9].

In order to produce lactic acid using the fermentation process one can use standard fermentation technologies. Although discontinuous processes have generally been used, studies have also indicated continuous processes as fit for the creation of lactic acid.

Lactic fermentation has a very important role in the biodegradation and in the bioconversion of some produces [11]. One of lactic acid applications, which have a remarkable ecological and biotechnological impact, is its uses in the production of biodegraded plastic material. Lactic acid can be transformed in important chemical products like: ethanol, glycol, propylene, polyesters and acrylic polymers. Some features for the fermentation process are showed in table 1[9,12].

2. PROCESSES USED IN ORDER TO OBTAIN LACTIC ACID

The literature regarding the lactic acid procurement (involving its salts also) is very rich and describes several alternatives used in the amelioration of the classic process.

Features of the process used in order to produce lactic acid. Table 1.

Features	*Values and quantities*	*References*
Temperature, C^0	43	Burton (1937)
	49	Inskeep and co. (l956)
	30, 45 ... 50	Prescott & Dunn (1959)
	30 ... 50	Snell & Lowery (1964)
pH	6-7	Burton (1937)
	5,8-6	Inskeep and co. (1956)
	<6	Snell & Lowery (1964)
Neutralizing agent	NH_3, K_2C0_3,Na_2C0_3	Kempe and co. (1950)
	$Ca(OH)_2$, $CaC0_3$	Prescott & Dunn (1959)
	$Zn(OH)_2$, $Zn\ C0_3$	Lockwood (1979)
		Prescott & Dunn (1959)
Glucides initial concentration, %	5-20	Prescott & Dunn (1959)
Product's final concentration, %	< 12-15	Peckham (1944)
Effective power, %	85-90	Prescott & Dunn (1959)
	90-95	Vick Roy (1985)
Waste gluciedes' concentration , %	<0,1	Daly şi colab. (1939)
Inoculating product concentration, %	5-10	Vick Roy (1985)
Fermentation time, days	1-6	Vick Roy (1985)
Reactor productivity, $Kgm^{-3}\ h^{-1}$	1-3	Vick Roy (1985)
Bioreactor construction material	Wood	Lockwood (1979)
	Stainless steel	Vick Roy (1985)

Tyagi & Co. analyzed several discontinuous and continuous fermentation systems involved in the procurement of lactic acid and showed different comparisons between different bioreactors types. Generally speaking, one shows modern methods used for the production of lactic acid in a filtering bioreactor. These are equipped with filtering membranes that immobilize Streptococcus Faecalis cells, during a two week time period [1,6,11,15].

The procurement of lactic acid using immobilized Lactobacillus spp. is inhibited by the concentration of the layer (glucose), by the lactic acid concentration, the pH level of the concentration, and by some salts to be found in the lactic acid. When the concentration of the layer is greater than 10%, the pH is 4.0, and the concentration of the lactic acid is 6-7 mg/ml, the production of lactic acid is inhibited. Among the salts, calcium lactate is not considered to inhibit, compared to sodium lactate; when the ion is NH_4, the effect is quite variable, depending on the used root.

Conventional methods used during the fermentation discontinuous and continuous processes need the separating of the environment cells at the end of each process. On the other hand, the immobilization of the cells on a solid support or on a gel matrix leads to the apparition of the resistance to diffusion and to waste associated with the immobilizing process[10,18]. In order to prevent these inhibitions, one went to studying recyclable membranes and hollow fibers membranes reactors [5,14].

The inhibition realized through the final product during the fermentation process of the lactic acid and the effects on the productivity of lactic acid have been studied by different authors. One has established that, due to the inhibition, the advantages of continuous fermentation are not available as far as the procurement of lactic acid is concerned and excepting the very low concentrations of the layer, the ratio of the growth of micro organisms becomes more and more dependent on the concentration of the product than on layer concentration. Thus, the processes that separate the product from the fermentation environment can improve the efficiency of the system.

Friedman and Saden (1970) used culture dialysis systems that removed the lactic acid from the environment. The system helped maintaining a low lactate concentration after a lag step, leading to the improving of the ratio of growth and of the lactic acid production. The authors claim that these results confirm the inhibiting effect of the final product during lactic fermentation. Stieber & Co. showed that, in order to produce lactic acid, the continuous fermentation of the lactic acid in the bioreactor using dialysis membranes, as opposed to the discontinuous and continuous processes, permits the use of a layer that is more concentrated and also allows the increase of the efficiency during the layer conversion [2,11]. The monitoring of the process variables is of great importance during the fermentation process.

A semi-on-line system using a discontinuous flux, studying the lactic fermentation was imagined by Nielsen & Co., (1989). In fermentation, computer controlled device, one measured the concentrations and the optical density of glucose, lactic acid, and proteins. The authors showed that the answer of the system was rapid and sure; it could be used in order to study the mathematical methods of fermentation [12].

The lactic acid produced during the fermentation process must be recovered or extracted and purified. Some fermentation problems actually can slow down this process. For instance, in order to control the pH, one usually adds to the lactic fermentation ammonia compounds, calcium carbonate and sodium hydroxide. The calcium carbonate helps precipitating the calcium lactate that can slow down the producing of lactic acid of a material that has a certain degree of polymerization [2,4,17]. The removal of lactic acid from the fermentation environment can be a solution of this problem, by the decreasing of the pH of the environment. Through a known liquid-liquid extraction system when one uses amines, one can realise the removal of the lactic acid from thee fermentation environment [San Martin & Co., 1992]. Yabannavar & Wang (1991) studied the extractive fermentation production of lactic acid presented by *Lb. debrueckii,* using third amines (Alamim 336) and oleic alcohol having acid pH.

As raw materials, during the lactic fermentation, one uses different kinds of glucose as well as raw materials containing starch, such as corn flour, potatoes porridge. Besides the corn flour, as raw material one also uses fermented molasses with Lactobacillus salivarius. The optimum parameters for the lactic fermentation in this case were established ph=5.6, 46C degrees. The adding of 0.5 % yeast reduces the time of fermentation and increases the production of lactic acid by 30%.

Whey is also used in the production of lactic acid using as micro organism Lactobacillus bulgaricus. In the production environment one also adds yeast extract and mineral salts. The lactic acid separates from the fermentation environment through ion exchange chromatography and it purifies through ultra filtering [8,20]. The production of calcium lactate for medical and dietary purposes can also be obtained through enzyme hydrolysis and through rice flour fermentation.

The lactic acid, obtained by bio synthesis, can be found in the final fermentation environment having a concentration of 10-15%; there are several processes of isolating and purifying the lactic acid and its salts from the fermentation environment, processes that lead to their procurement under the for of compounds having different purity degrees.

Some processes recommend a summary purification and then their use in the alimentary industry. In order to use them in the pharmaceuticals industry, one needs to purify them further more. Thus, the lactic acid is being isolated from the fermentation environment, by its being treated with organic flocculents, the optimum flocculating conditions of the bacteria cells and of the colloidal substances being often pH=7, 65^0C, flocculent concentration 0.08 g/l. The purified lactic acid is recovered from the clear solution by centrifuging; then it is being used as such [2,8,17].

O. Hitomi (1989) describes an advanced lactic acid fermentation purification method. The lactic acid is being cheaply and continuously purified from the fermentation environment containing alkaline salts of lactic acid that come as a result of the removal of bacteria, as a result of a chromatography of ions exchange resins, lactic acid concentration of up to 80% and the distilling of the concentrate using small pressures [20]. The fermentation environment contains sodium lactate (obtained in a bioreactor) and it is treated with DIAIONI, SK IB (ions exchangers); the concentration rising to up to 90% and the distilling at 130^0C lead to the obtaining of highly purified lactic acid.

The same authors describe a purifying process of the lactic acid from the fermentation environment, as well as the needed devices [9,4,7,8]. The process has several steps: the changing of the fermentation environment to a ion exchange resin in order to obtain the desalination; the absorption of lactic acid on a ion exchange resin; the replacing of the lactic acid from the javelin by a powerful acid.

The lactic acid from the fermentation liquid has neutralized with NH_3, thus obtaining ammonia lactate; the liquid lost color, and then it was filtered through the ion exchange resin. The cationite was regenerated using H_2SO_4 In solution, and then the anionite was regenerated with NaOH 1n. A version of modeling of the fermentation environment has the following steps: the reaction of Maillard in the presence of Mg salts ($MgSO_4$, $MgCl_2$ or $MgCO_3$); flocculation with Mg or Fe salts or with compounds; for the absorption with active char coal or oil; the second absorption with PAM (ion exchange resin)[7,12,20].

The purification of the lactic acid from the fermentation environment using corn flour is realized by removing the protein impurities, waste glucides and of other contaminating substances through the precipitation of calcium lactate. The method uses absorbents, precipitation adjuvant such as lime $MgCl_2$, $(NH_4)_2SO_4$. The efficiency can be increased by 10-20%.

A process of purification of lactic acid without crystallisation is applied in China (Wang Zhang) and has the next steps: the reaction with hydroxylamine (the Maillard reaction) in the presence of alkaline metal salts acting as crystallisers; the discolorations on active charcoal (1%); flocculation with a mixture: Mg salt-CaO; the discolorations on active charcoal; conventional processes: evaporation, concentration, filtering, and ion exchanger. This method reduces the duration of isolation and increases the quality and the quantity of the purified lactic acid.

Komori Akiyoshi describes a process of separation and of purification of lactic acid by the crystallization of the Mg lactate from the raw solutions, the dissolving in water and the treatment with H_2SO_4, the cooling, the extraction of the resulted $MgSO_4$ and the removal of residual $MgSO_4$ using ion exchange resins; thus, the Mg lactate was dissolved in the water, was mixed with 75% H_2SO_4, 30minutes at 30^0C, and then cooled, filtered at 25^0C and treated with Amberlite 120B and was concentrated up to 90.5% lactic acid.

Another version of recovering the lactic acid from the fermentation environmental, described by the same authors, consists in the total conveying of the lactic acid in its Mg salt and in the evaporation of the solution at 50^0C. The crystallised Mg lactate is retransformed into lactic acid using an ion exchange resin. Thus, 5 liters of filtered culture liquid containing 8.3mg/ml lactic acid obtained by the using of Rhisopus oryzae in the presence of MgO was concentrated by evaporation to 1l, at a temperature of 80-90^0C, resulting 87% lactate crystals having particles of 30-200 μ. The salt was dissolved in hot water and one went on using Amberlite IR 120B (H+) in order to obtain lactic acid [20].

The increase of the production of calcium lactate was realized by Maslowski (1989) by the increase in the concentration of the sub-layer and by the precipitation of calcium lactate during the fermentation, thus the equilibrium being oriented in the direction of the final product. Thus, the fermentation of 26% saccharose with Cthermobacterium cereale at 50^0C, pH=6.3 maintained by the adding of CaO, lead to the continuous precipitation of the calcium lactate. The conversion efficiency was 99.6% after three days.

In order to extract the lactic acid from the fermentation environment, one also uses a combined technology of electrodialysis using ion exchangers [2]. Using this method the lactic acid efficiency can reach a value of 85%, compared to 50% obtained by the technology using the method of calcium lactate precipitation.

Thus, Heriban V. recommends the use on a large scale of electro dialysis, during the purification of lactic acid and of sodium lactate [Heriban V & Co. 1993]; in both cases one realizes a concentration 4 times greater for the lactic acid, even during the fermentation. In order to obtain a highly purified product, one recommends discolorations, followed by a double exchange reaction.

Another team of authors (Lee & Co.) describes the production of lactic acid and sodium lactate by the fermentation with Lactobacillus delbrueckii in an environment containing glucose, yeast extract, mineral salts, 42^0C and the maintaining of pH at 7.2 by the adding of NaOH, thus obtaining 24.8 g/l sodium lactate. At this point, one ceased to add NaOH, and the pH of the environment decreased to 4.22. The environment was filtered and then one used ion exchange, thus the speed of recovering of the lactic acid from the environment was higher than its forming speed; the lactic acid recovered in the javelin indicates an efficiency of 0.98 g/l/h.

Another method used in order to recover the lactic acid by ion exchange chromatography from the fermentation environment [Mantovani & Co. 1993] consists of the passage of the filtered fermentation liquid on a anion exchange javelin in the form of carbonate and the replacing of the lactic acid with $(NH_4)_2CO_3$. Later $(NH_4)_2CO_3$ is removed from the solution through heating [4,6,7]. Then the solution is passed on to a highly acid ion exchange, in the form of H+. Considering the concentration of the substance from the second resin, one obtained a watery solution containing 90% lactic acid.

In order to concentrate the lactic acid one uses the phenomenon of reversed osmoses, using a tubular thin membrane concentrator, specially designed for reversed osmoses. The flux grows linearly, pressure is applied on the membrane and it is relatively unaffected by the debit. The osmotic pressure of a solution of 1% lactate was of 280-560 kPa, depending on the pH or on the associating degree. The rejection grows when pressure is applied and the high pH leads to a decreasing of the flux (due to the osmotic great pressure) and a significant increase of the rejection. Should we apply pH over 5.6, the rejection of the lactate and of the residual glucides was 97%; having cellulose acetate membranes, the flux was generally lower and the rejection of the lactate was proportional to the degree of dissociation when the pressure is low.

3. CONCLUSIONS

With the nowadays development of technique, the modern methods of procurement of lactic acid are being used more and more, thus, simplifying the processes of fabrication, diminishing the expenses of investment and exploitation, increasing the capacity of production of the assembly lines; at the same time the productivity of work, the procurement of superior, cheaper lactic acid are easily obtained.

The lactic acid is used in alimentary processes, chemical, pharmaceutical and industrial ones. Approximately half of the produced lactic acid is being used in the alimentary industry as an acidulating agent and approximately half of the lactic acid produced in the entire world is obtained by fermentation processes.

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