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Lactic Acid Production from Lignocellulosic Materials

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ABSTRACT: Lactic is one of the rising products for industrial use, and it has many functions in different fields. For economical production by fermentation, it has many factors, one of the essential factors is cost-effective raw material. The feedstocks sugars like starch, sucrose, glucose used for lactic acid production are very expensive. Thus, in place of expensive sugar feedstock, lignocellulosic biomass is used for the manufacturing of lactic acid considering its low cost, sustainability, availability in contrast to processed sugars. In spite of these benefits, commercial utilization of lignocellulosic material for lactic acid production still imposes challenge. This review describes the conventional method and different processing stages involved during production of lactic acid. Additionally, the use of lignocellulosic raw material, different microbial strains, and fermentation methods for lactic acid production and highlighted the numerous key points have been pointed out, which could be targeted for advancing fermentation processes.

Keywords: Lactic acid, Lignocellulosic raw material, Fermentation method.

INTRODUCTION

Naturally occurring organic acid, lactic acid (2-hydroxypropanoic acid, CH3-CH (OH)-COOH), is in great demand due to its applications in different fields like textile, pharmaceutical, food, biomedical and chemical industries (Lopes *et al.,* 2012). In recent times, the global demand for lactic acid has increased due to the production of PLA (Poly Lactic Acid), which is biodegradable, biocompatible and eco-friendly (Eş *et al.,* 2018; de Albuquerque *et al.,* 2021). Lactic acid is produced by two methods: chemical synthesis and microbial fermentation (Thygesen *et al.,* 2021; Anagnostopoulou *et al.,* 2022; Sudhakar and Dharani, 2022). Chemical production has less advantage over microbial production. Chemical production always gives the racemic mixture L (+)- or D (-) lactic acid, which is one of the most significant disadvantages. In contrast, microbial production gives optically pure lactic acid (Boonpan *et al.,* 2013). In 1780, Swedish chemist Scheele discovered lactic acid from sour milk. Lactic acid fermentation with numerous carbohydrates like starch, sugar, milk and dextrin was done by Fermy in 1839. Lactic acid is a metabolite and not a milk component produced by fermentation by some of the microorganisms; Pasteur discovered this fact in 1857 (Ghaffar *et al.,* 2014).

For lactic acid production, one of the biggest obstacles is the overpriced raw materials like starch and sugar; this results in one of the highest production costs (Datta *et al.,* 1995; Pratush *et al*., 2011). To overcome this obstacle cheap raw material should be needed which does not compete with the food chain. One of the best alternatives is lignocellulosic material from forestry, agro-industry and agriculture (Abedi and Hashemi, 2020). It is a low-priced, abundant and renewable raw material for producing lactic acid in large amounts. Lignocellulosic material consists of lignin, hemicellulose and cellulose (Abdel-Rahman *et al.,* 2011). Pretreatment is required to break the lignocellulosic structure. For the manufacture of lactic acid, lignocellulosic material would help acquire sugar substrates. Pretreatment will be followed by an enzymatic hydrolysis process where the obtained sugar will be further utilized for the production of lactic acid (Tayeh *et al*., 2016, Mora-Villalobos *et al.,* 2020). The bioconversion of lignocellulosic material into lactic acid is an essential and efficient process for utilizing lactic acid in the food, pharmaceutical and cosmetic industry (De Oliveria *et al.,* 2018).

Substrates required for lactic acid production are sucrose, fructose, glucose, and starch; this all can be obtained from the lignocellulosic feedstock, which is rich in carbohydrates. Lignocellulosic feedstock like banana, sugarcane molasses, whey, corn stover, agriculture waste, aloe vera byproduct and much organic waste. These raw materials are a great alternative to the high cost of raw material that competes in the food industry (Neureiter *et al*., 2004; Gupta *et al*., 2016). Banana is abundant in nature, and its peduncle leftover part of the banana is rich in fermentable sugar as molasses leftover from sugarcane is highly rich in sugar substrate; therefore, the banana peduncle can also be used as lignocellulosic feedstock (Azaizeh *et al.,* 2020). Aloe vera leftover part leaves are good raw material because it has a high amount of fermentable sugar like mannose, glucose, xylose, arabinose and galactose (Sharrif Moghaddasi *et al*., 2011).

The present review focusses on the conventional method involved in the lactic acid production, the different fermentation processes and technology used for lactic acid production. Moreover, the application of lignocellulosic biomass and its derived sugars in lactic acid production has also been discussed.

INDUSTRIAL APPLICATION OF LACTIC ACID

Lactic acid has vast applications in different fields like food, pharmaceutical, textile, cosmetic and chemical industry (Krishna *et al.,* 2018). In the chemical industry, it is used for the manufacture of propionic acid, calcium lactate, ester, propylene glycol, 2-3 pentanedione, polylactic acid, lactide, ethyl lactate, acrylic acid, acetaldehyde (Wasewar *et al*., 2005). It is also converted into ethanol, acrylic polymers and propylene glycol. As solvents, emulsifiers and plasticizers, lactic acid derivatives, salts and esters are utilized. In the cosmetic industry, it is used to prepare soap and creams as humectants. It is used in the production of oral sterility products. Due to its antimicrobial, rejuvenating and moisturizing effects on the skin, it is used to produce hygiene and aesthetic products (Bouwstra *et al.*, 2006).

In the food industry, lactic acid has extensive uses, such as being used as a repressor of residual bacteria in food processing for bread, soft drinks, beers, sweets and more products. It is also used as a ph regulator and flavouring agent. Due to its mild acidic taste compared to other acids used in food, it is used as an acidulant for flavouring and preserving pickled vegetables. For fermented food, it is an essential item like curd, canned vegetables, butter, and many more (Krishna *et al.,* 2018). Lactic acid is also pre-owned in the leather tanning industry in the descaling process. As a mordant (fixative) for dyeing purposes, it is used in the textile industry, and it will restore ethylene glycol in antifreeze which benefits in higher efficiency and lower cost. Lactic acid is used in implants, dialysis, controlled and drug release systems, pills and surgical sutures. It produces parenteral solutions and dermatological and osteoporosis drugs (Krishna *et al.,* 2018).

In recent years, the demand for lactic acid has increased due to its use in producing poly-lactic acid (PLA). It is also biodegradable, biocompatible, and environment-friendly, which can be the best alternative for the plastic derived from petroleum resources. It reduces the severe problem of disposing of the material used in medical treatment. It can be used in the drug delivery system, orthopaedic implants and surgical sutures (Adnan *et al*., 2007). High chemical and optimal purity are essential for producing polylactic acid. Attain the high purity and polymer properties D- and L- lactic acid is necessary. Due to its industrial function of pure isomers, L- and D-lactic acid is more valuable than the racemic DL form of lactic acid. L-lactic acid is used for the manufacture of poly L lactic acid, which is a semi-crystalline biodegradable and thermostable polymer. It has significant potential in the packaging market. It is suitable for medical products due to its high tensile strength and low elongation with high modulation property. It is used in medical products like dental application, orthopaedic fixation, cardiovascular application, intestinal application and sutures (John *et al*., 2006). Poly D- lactic acid is produced by d-lactic acid. The pure polymers are more heat-sensitive, while the complex of PLLA and PDLA have a 50° higher melting temperature than the pure polymers (Ikada *et al.,* 1987, Tsuji *et al*., 2003). The properties and degradability of PLA have been influenced by the L- and D- lactic acid ratio. From PLA it is expected to replace the fossil fuel-based plastic in many functions, but to compete with fossil fuel-based plastic, the production cost of PLA has to decrease by its half (Kharas *et al.,* 1994).

COMMON SUBSTRATES FOR LACTIC ACID PRODUCTION

Lactic acid is manufactured from food substances with large amounts of sugar such as glucose, fructose, lactose and starch. These substrates have the most significant advantage because they don't need pretreatment and minimize recovery costs. However, these substrates have to compete with human food, making it expensive to produce lactic acid. Thus, a cheap and alternative substrate is discovered lignocellulosic biomass. It is eco-friendly, non-food and affordable (Abdel-Rahman *et al*., 2013). Lignocellulosic biomass is an organic material primarily utilized as a global source of biomass for lactic acid production (Lin *et al.,* 2006). It consists of hemicellulose, lignin, and cellulose (Balat *et al.,* 2011). It has significantly less oil, components and minerals while carrying more than 90% of dry matter. Wood waste, industrial waste, municipal solid waste, waste paper, industrial food waste, and forest and crop residues all come under lignocellulosic biomass (Linko *et al.,* 1984). The proportion of biomass is different for different species; it's different for hardwood and other for softwood. The ratio of lignin is higher in softwood, 29.2% than hardwood, 21.7%, while the proportion of cellulose and hemicellulose is much more in hardwood, 78.8% than softwood, 70.3% (Balat *et al.,* 2009). The amount of cellulose, hemicellulose, and lignin depends on the lignocellulosic biomass type. The major component of plant biomass is cellulose, a homopolysaccharide composed of beta d-glucopyranose and linked by beta (1-4) glycosidic bond. It is 30-60% of total feedstock dry matter. Hemicellulose consists of hexos, pentose and acid sugars. It is a tiny and thoroughly branched heterogeneous polymer. It contains 20-40% feedstock of total dry matter (Saha *et al.,* 2000). Xylose is commanding in hardwood and agriculture residue, while mannose rules over the softwood. Lignin is an aromatic polymer which is blended by phenylpropanoid precursor. It contains 15-25 % feedstock of total dry matter. For the use as a fermentation substrate, lignin is considered as difficult as it makes the biomass immune to biological and chemical degradation (Demirbas *et al*., 2007).

LIGNOCELLULOSIC RAW MATERIAL FOR LACTIC ACID PRODUCTION

For large lactic acid production, inexpensive products such as lignocellulosic biomass should be used. Besides being a cheap raw material, it should also produce a large amount of product; there should not be a byproduct and less contamination so that no amount is used for pretreatment (John *et al*., 2007). Some of the cheap lignocellulosic material sources are starchy materials, corn stover, wheat starch, etc. and cellulose material contains wood waste, forest and crop residue, etc., cellulose is present in large amounts and cheap, so it's mainly used as raw material (Richter *et al.,* 1998; Anuradha *et al.,* 1999; Akerberg *et al.,* 2000). Renewable sources such as agriculture residue, which are ample in carbohydrates, are used aside from starchy and cellulose material. Apart from a large amount of agricultural residue present in the environment, the presence of cellulose is minor and has poor digestibility. So, the utilization of agriculture residue is significantly less (John *et al.,* 2007). Some agricultural residues are corn cob, beet molasses, sugar molasses, banana peduncle, aloe vera leftover, carrot waste (Vickroy *et al.,* 1985, Kotzamanidis *et al.,* 2002). Industrial unwanted waste such as whey and molasses are also used for the production of lactic acid. Whey is the byproduct of the dairy industry; it contains protein, lactose, fat and minerals. Food industrial waste is rich in carbon sources and high in moisture. The waste material of the sugar industry that is molasses is rich in sugar which is 46%. It is rich in sucrose, carbohydrate, glucose and fructose (John *et al*., 2009).

Banana is cultivated worldwide, and its peduncle is discarded as waste which contains sucrose, fructose and glucose. It is rich in cellulose which is 42-63%. It is used as lignocellulosic biomass to produce lactic acid (Idrees *et al*., 2013; Mendez *et al.,* 2019). The Leftover part of the aloe vera leaves contains fermentable sugar such as mannose, glucose, xylose and galactose. They are low in lignin and hemicelluloses (Cui *et al*., 2011).

MICROBIAL STRAINS USED FOR THE PRODUCTION OF LACTIC ACID

Lactic acid is produced from various microorganisms such as bacteria, fungi, yeast, algae and cyanobacteria. Mainly lactic acid is made by using lactic acid bacteria (Nancib *et al*., 2009; Yin *et al.,* 1997). Fermentation of lactic acid depends on the type of lactic acid bacteria. Lactic acid bacteria are of two kinds: homofermentative and heterofermentative. Fungal species of Rhizopus have the advantage of producing lactic acid but are low in fermentation due to mass transfer limitations. Genetic engineering approaches refine optical purity and lactic acid production (Okano *et al*., 2010). The extensive list of different microbial strains used for lactic acid production with their yield is shown in Table 1.

Bacteria. Bacteria used for the production of lactic acid are divided into four categories: Escherichia coli, Lactic acid bacteria, Bacillus strains, and *Corynebacterium glutamicum*. Out of all, lactic acid bacteria are used mainly to produce lactic acid (Budhavaram *et al.,* 2009; Krishna *et al*., 2018). Choosing a good quality strain is necessary for better purity, high yield, nutritional requirements, and productivity (Yun *et al*., 2003). A few of the disadvantages of using bacteria for lactic acid production are low yield production, formation of byproducts, the requirement of mixed strains for the development of phage resistant strain, the medium should be full of nutrient-rich and the chances of cell lysis are high. By anaerobic glycolysis, bacteria can usually produce lactic acid with high yield and productivity; bacterial growth occurs from the temperature 5 - 45 and pH range from 3.9-9.6 (John et al., 2007). Lactic acid bacteria are classified into homofermentative and heterofermentative (Cui *et al.,* 2011). For commercial production of lactic acid, homofermentative bacteria are used. Some examples of homofermentative bacteria used for the production of bacteria are *Lactobacillus acidophilus, Lactobacillus delbureckii, Lactobacillus casei, Lactobacillus lactis, Lactobacillus rhamnosus* and *Lactobacillus helveticus*. Heterofermentative lactic acid bacteria form byproducts because lactic acid yield is reduced (John *et al.,* 2007). Some examples of heterofermentative lactic acid bacteria are: *Lactobacillus brevis* (Cui *et al.,* 2011), *Lactobacillus bifermentans, Lactobacillus pentosus (*Bustos *et al*., 2004).

With the help of the homofermentative process, *Enterococcus mundit* (Abdel-Rahman *et al.,* 2013) and genetically engineered *Lactobacillus plantarum* can convert pentose sugar into lactic acid. As lactic acid bacteria do not cause any adverse health effects, they are safe to use in the industrial production of lactic acid (Abdel-Rahman et al., 2013). Lactic acid bacteria show high acid forbearance and can be easily engineered for the production of specific D-or L- lactic acid to make these bacteria more commercially valuable (John *et al.,* 2007).

Fungi. Even though most of the lactic acid bacteria are utilized for the production of lactic acid, some of the fungal species can produce lactic acid as well. Examples include *Rhizopus,* which can turn starch into L (+) lacticacid (Wee *et al.,* 2006). A few assets of the fungal fermentation over bacterial fermentation for lactic acid production are a low requirement of nutrients, economic downstream processing, and evolution of fungal biomass, which is a critical byproduct (Zhang *et al.,* 2007). A chemically defined medium is used for fungal fermentation, so the purification process is more straightforward, one of the essential advantages (John *et al*., 2007). Standard fungal fermentation products are ethanol and fumaric acid (Litchfield *et al.,* 2009, Vink *et al.,* 2010). Fungi other than *Rhizopus* for lactic acid production are *Monilia* and *Mucor (*John *et al*., 2007). One of the crucial disadvantages of using fungi to produce lactic acid is that lactic acid yield is lessbecause of the production of byproducts that utilizes the carbon. Another drawback is massive transfer, resulting in less production rate and requirement of various aeration as it is an anaerobic process (Wee *et al*., 2006; Krishna *et al*., 2018).

Yeast. Fermentation with wild type yeast as a nutrient source makes the lactic acid yield low (Abdel-

Rahman *et al.*, 2013). With the help of genetic engineering, yeast has been created to produce high yield products (Bianchi *et al.,* 2001). The genetically modified yeast for lactic acid production is *Candida, Saccharomyces, Zygosaccharomyces,* and *Pichia* (Sahay *et al*., 2021). One of the significant drawbacks of using yeast as a nutrient source for lactic acid production is that it has increased the amount of output. Some of the yeast alternatives include arerice bran, wheat bran and corn steep liquor (Oh *et al.,* 2005).

Table 1: Enlist of Different Microbial strains used for Lactic acid production with their yield

Microorganisms Name	Homofermentative/Heterofermentative	Substrate used	Yield of lacticacid	Reference
Escherichia coli	Heterofermentative	Molasses	75g/L	(Wang *et al*., 2013)
Lactic acidbacteria	Both	Glucose	1.1 g/g	(Martinez et al., 2013)
Lactobacillus rhamnosus	Homofermentative	Cassava wastewater	41.65g/l	(Coelho et al., 2010)
Lactobacillus acidophilusR	Homofermentative	Paneerwhey	8.6g/l	(Abdel-Rahman et al., 2013, 59)
Lactobacillus delbrueckii	Homofermentative	Soya fiber	44g/100g	(Sreenath *et al.,* 2001)
Lactobacillus delbrueckii	Homofermentative	Alfalfafiber	32g/100g	(Sreenath *et al.,* 2001)
Lactobacillus delbrueckii	Homofermentative	Molasses	88g/l	(Kotzamanidis *et al.,* 2002)
Lactobacillus casei	Homofermentative	Sugarcane bagasses	21.3g/l	(Oonkhanond *et al.,* 2017)
Lactobacillus casei	Homofermentative	Whey	22g/l	(Abdel-Rahman *et al*., 2013, Abdel-Rahman *et al.*, 2011)
Lactobacillus casei	Homofermentative	Glucose	210g/l	(Ding et al., 2006)
Lactococcus lactis	Homofermentative	-	-	(Abdel-Rahman et al., 2013, Abdel-Rahman et al., 2011)
Lactobacillus brevis	Heterofermentative	Cottonseed	15g/l	(Grewal et al., 2018)
Lactobacillus bifermentans	Heterofermentative	Wheatbransyrup	0.83g/g	(Givry *et al.,* 2008)
Lactobacillus pentosus	Heterofermentative	Sugarcane baggases	65g/l	(Wischral *et al.,* 2019)
Lactobacillus pentosus	Heterofermentative	Wood extract hydrolysate	43.2g/lto65.02g/l	(Buyondo *et al*., 2011)
Lactobacillus plantarum	Heterofermentative	Soya fiber	44g/100g	(Sreenath et al., 2001)
Lactobacillus plantarum	Heterofermentative	Corn syrup	17g/l	(Abdel-Rahman *et al.,* 2013, Abdel-Raham *et al.,* 2011)
Lactobacillus plantarum	Heterofermentative	Hydrolysate soluble starch	15g/l	(Abdel-Rahman *et al.,* 2013, Abdel-Rahman *et al.,* 2011)
Lactobacillus plantarum	Heterofermentative	Alfalfa fiber	46g/100g	(Abdel-Rahman et al., 2013, Abdel-Rahman *et al.,* 2011)
Rhizopus oryzae	-	Myceliaflocs	113g/l	(Abdel-Rahman *et al.,* 2013, Abdel-Rahman *et al.,* 2011)
Rhizopus oryzae	-	glucose	103.7g/l	(Abdel-Rahman *et al.,* 2013, Abdel-Rahman *et al.,* 2011)
Saccharomyces cerevisiae	-	Glucose	0.22g/g	(Turner *et al.,* 2015)
Saccharomyces cerevisiae	-	Xylose	0.69g/g	(Turner *et al.,* 2015)

FERMENTATION METHODS FOR LACTIC ACID PRODUCTION

Like other fermentation processes, lactic acid fermentation also depends on raw material use4d, nutrients required in the media and microorganisms used. Three fermentation methods have been practised: batch fermentation, fed-batch fermentation, and continuous fermentation.

Batch Fermentation. At the beginning of the batch fermentation, all nutrients are added, such as carbon source, nitrogen source, and other components. It is one of the exceedingly common fermentation processes because it is too simple to perform. It produced a high lactic acid concentration as it avoided contamination to a reasonable extent compared to other fermentation (Kumar *et al.,* 2016; Abdel- Rahman *et al.,* 2013). The drawback of batch fermentation is that poor cell concentration is observed due to a low concentration of nutrients. It also includes poor productivity due to substrate inhibition or product inhibition (Kadam *et al.,* 2006; Yun *et al.,* 2003). Batch fermentation is categorized into two categories that are: separate hydrolysis fermentation and solid-state fermentation.

No water is required in the solid-state batch fermentation. In this process, natural materials are needed, such as wheat bran, sugarcane baggases, rice bran and food pulps (Krishna *et al.,* 2018). This process is used to manufacture industrial chemicals, pharmaceuticals products, feed and fuels (Pandey *et al.*, 2000). In solid-state fermentation, by using *Rhizopus oryzae,* lactic acid can be produced. Using the solid-state fermentation method 0.97 g/g of lactic acid can be originated from recycled paper (Marques *et al.,* 2008). This fermentation method provides high productivity, needs less enzyme load, and has a rapid processing time and single reaction vessels (Tsuji *et al.,* 2003). While using lignocellulosic biomass in this fermentation method requires the first pretreatment of biomass, and unnecessary compounds, such as lignin, will be removed. After that, raw material will be put through enzymatic saccharification, and hydrolysate, which is formed, is subjected to fermentation (Choudhary *et al*., 2016).

The real productivity is decreased after the pretreatment method in separate hydrolysis fermentation. It was observed that the same *Lactobacillus rhamnosus,* which produces lactic acid of 0.97 g/g of recycled paper by using the solid-state fermentation, will decrease the productivity rate in the separate hydrolysis fermentation by 0.81 g/g (Marques *et al.,* 2008).

Fed-Batch Fermentation. In this fermentation process, nutrients are added after a small interval at the appropriate time without removing the fermentation broth (Ding *et al.,* 2006). This fermentation method is used to keep up the low substrate concentration by adding the nutrients into the fermentation process, reducing the substrate inhibition (Tsuji *et al.,* 2003). Using *Lactobacillus casei* in fed batch fermentation production of lactic acid is more efficient than other methods. By using 1% yeast and glucose as raw material, 90.3% of L-lactic acid can be obtained. Maximum production of L- lactic acid of 180 g/l at the 2.14 g/l/h is obtained. 97% of l-lactic acid can be obtained by using *Lactobacillus lactis* at 2.2 g/l/h (Bai *et al.,* 2003).

Continuous Fermentation. In this fermentation method, the introduction of fresh medium in the fermenter will be done after removing the present broth at the same rate. Due to these steps, the substrate and product balance is maintained properly (Tsuji *et al.,* 2003). There are many advantages of using this fermentation method. Some of the benefits are: there will be no end product inhibition, during the lag phase, there will be less productivity. It has some drawbacks, too, like it is expensive to perform, contamination problems, and a need for a field operator with expertise. Using *Enterococcus faecium* in this fermentation method, the lactic acid production will be 1.56 g/l/h (Shibata *et al*., 2007). This fermentation method observes the production of lactic acid at 3.69 g/l/h by using *Bacillus coagulans.*

In continuous fermentation, a rich amount of lactic acid production is achieved, while in the fed-batch and batch, the fermentation concentration of lactic acid is higher from continuous fermentation. While batch fermentation is used more for the production of lactic acid, it has problems like low productivity and low cell concentration due to its long fermentation duration; end-product inhibition is also seen. Compared to continuous fermentation, batch fermentation has higher lactic acid concentration and yield, while continuous fermentation has lower lactic acid productivity. Results can be improved by changing the fermentation system. Example like: higher cell density can be achieved by integrating a cell recycling system with continuous fermentation. This strategy can affect lactic acid productivity in a more significant way. Continuous fermentation can run for longer duration; this is one of the advantages of continuous fermentation over batch fermentation (Krishna *et al.,* 2018).

PRODUCTION OF LACTIC ACID

Lactic acid can be produced by two methods such as chemical synthesis and biotechnological processes (Fig. 1). Chemical synthesis produces lactic acid, which combines L- and D-lactic acid. In chemical synthesis lactonitrile hydrolyzed with strong acids is used. Many other chemical pathways are responsible for lactic acid production, but they are not economically beneficial (Gao *et al.,* 2011). Biotechnology processes provide advantages like using different types of raw materials for substrates. The natural utilization of biomass and byproducts of agriculture and food industries is considered one of the best alternatives of the substrate and renewable source for lactic acid production. Biotechnological processes produce optically pure L- or D- lactic acid (Tsuji *et al*., 2003).

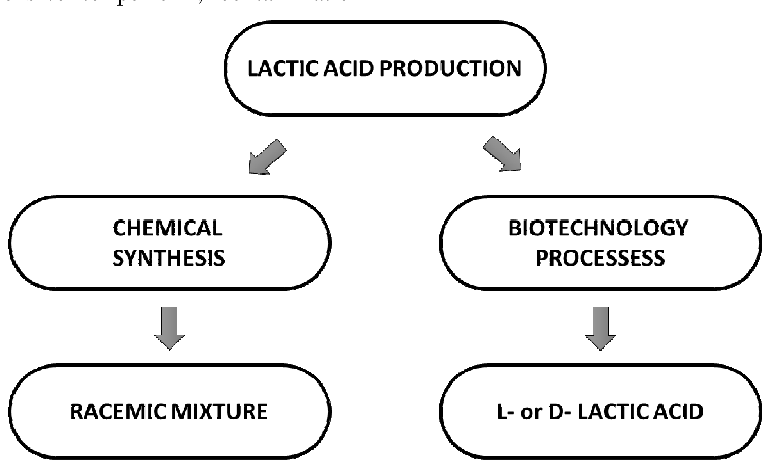

Fig. 1. Scheme for the production of lactic acid.

LACTIC ACID PRODUCTION FROM LIGNOCELLULOSIC BIOMASS BY USING LACTIC ACID BACTERIA

Though the lignocellulosic biomass is accessible in large quantities and cheap, it is problematic because of its complexity. Converting biochemically lignocellulosic biomass requires a few steps to turn structural carbohydrate into monomeric sugars, e.g., mannose, glucose, xylose, and arabinose. There are four steps for the production of lactic acid from lignocellulosic biomass. They are:

- Pretreatment- in this, the breakdown of lignocellulosic structure is done.
- Enzyme Hydrolysis-depolarization of lignocellulosic biomass into fermentative sugar is done in this step with the help of hydrolytic enzymes.
- Fermentation-with the help of lactic acid bacteria, metabolization of sugars into lactic acid is done.
- Separation and purification of lactic acid-purification of lactic acid is done for commercial application (59).

Pretreatment method. Due to the linkage of hemicellulose and cellulose with lignin, the enzymatic activity with native lignocellulosic is quite hard and slow (Schmidt *et al*., 1998). The chemical linkage between lignin and cellulose or hemicellulose makes it difficult for the enzymes to break down complex substrates. Therefore, there is a need for pretreatment of the lignocellulosic material to enhance the rate of enzyme activity. The main goal of the pretreatment method is to deduct the lignin, partially remove the polarization of cellulose and increase the attainable surface area (Ch *et al*., 2007; Hendriks *et al.,* 2009, Kumar *et al.,* 2009). Without declination of high sugar yield, the hemicellulose should be deducted (Mosier *et al.,* 2005). Pretreatment includes four methods, they are:

1. Physical method (milling and grinding process)
2. Biological method
3. Chemical method (organic solvents, dilute acid, oxidizing agents and alkalis)
4. Physico-chemical method (wet-oxidation, hydro-thermolysis, steam explosions) (Sun *et al.,* 1995)

There are many different pre-treatment methods, such as alkaline pre-treatment (Lau *et al*., 2008), liquid hot water (Antal Jr *et al.,* 1996), dilute and acid steam explosion (Laser *et al.,* 2002; Mosier *et al.,* 2005), ammonia fibre explosion, ammonia recycle percolation (Jorgensen *et al.,* 2007) and enzymatic treatment (Peterson *et al.,* 2005). A mild acid is required for the beginning of pre-treatment action, which catalyzes the hydrolysis of the glycosidic bonds of hemicellulose with the ether linkages in lignin (Fengel *et al.,* 2011). Organic acid set up by the cleavage of the labile ester group catalyzes the hydrolysis of hemicellulose. By the enlargement of the inner surface, fractionation will be achieved. There are different effects of pre-treatment methods on other lignocellulosic materials. The Lignocellulose bioconversion stage is the most critical stage for the pre-treatment process. Hydrolytic enzymes do not saccharify the resultant residue if pre-treatment is insufficient. At the same time, toxic compounds can be produced that will inhibit microbial growth and metabolism if it is too severe (Kodali et al., 2006). The downstream cost is affected by many processes like waste treatment demands, product purification, enzyme loading, determination of fermentation toxicity, enzymatic hydrolysis rate, power generation, and many other functions. (Galbe *et al*., 2007; Sun *et al.,* 2002; Wu *et al.,* 2011; Yang *et al.,* 2008).

Enzymatic Hydrolysis. This process allows lactic acid bacteria to consume polysaccharides as a carbon source. The main focus of this process is to depolymerize polysaccharides left in the water-insoluble fraction after the pretreatment step. The mixture of enzymes will increase the hemicellulose hydrolysis, and it also increases the access to cellulase, and it also decreases the hydrolysis timing and cost of the process (Ohgren *et al*., 2007; Tu *et al*., 2010; Zhang *et al*., 2010). Due to its degree of polymerization, the rate of enzymatic hydrolysis of cellulose is greatly affected (Chang *et al.,* 2000; Cohen *et al.,* 2005; Kumar *et al.,* 2008). A synergistic reaction of 3 classes of cellulolytic enzymes required efficient saccharification and degradation of cellulose. They are:

- Endo-beta-1, 4-glucanases: it inserts a water molecule in the beta (1,4) and hydrolyzes beta (1,4) glycosidic cellulose bond and is also a new reducing non-reducing chain end pair.
- Exo-beta-1, 4-glucanases or Cellobiohydrolase: at the end of the polymer, it cleaves the cellulose chains by releasing soluble cellobiose or glucose.
- Beta-glucosidases relieve the system from the end-product inhibition, and by cleaving the cellobiose into two glucose molecules, complete the hydrolysis (Lynd *et al*., 2002). It is also active on Cellobiohydrolase (Kumar *et al.,* 2008).

A mixture of cellulases exhibits the synergistic effect; therefore, individual cellulase has very little hydrolytic activity (Nidetzky et al., 1994).In lignocelluloses hydrolysis, a multi-enzyme mixture's hydrolytic efficiency depends on every unique enzyme and their ratio in multi-enzyme (Irwin *et al.,* 1993; Zhou *et al.,* 2009). One of the recently manufactured cellulase cocktails is made up by using three enzymes: 2 cellulases and xylanase. It is one of the more efficient enzymatic hydrolysis of lignocellulose.

Fermentation process with LAB

Lignocellulosic biomass is a combination of hexoses and pentose. For lactic acid, fermentation lignin cannot be used. 25% of the production cost of biomaterials is reduced by using cellulose and hemicellulose derived sugar (Hinman et al., 1989). The fermentation process requires much cost, so there will be competition between the chemical synthesis process and the biotechnology process for industrial use (Melzoch et al., 1997). A cost-effective fermentation procedure gives high product yields, productivity and the concentration of product formed, which will affect the product recovery costs. To achieve maximum lactic acid yield and productivity, the fermentation process is done using lactic acid bacteria from lignocellulosic biomass. For lactic acid production by using lactic acid bacteria, biomass materials are used as a substrate, including hemicellulose/lignocelluloses hydrolysate (Melzoch *et al.,* 1997), wheat bran (John *et al.,* 2006, Naveena *et al.,* 2005; Naveena *et al.,* 2005), corncob (Guo *et al.,* 2010, Moldes et al., 2006), paper sludge (Marques *et al.,* 2008), carrot processing waste (Soccol *et al.,* 2008), cellulose (Venkatesh *et al.,* 1997), cassava bagasse (Singhvi et al., 2010, John et al., 2006), sugarcane press mud and bagasse (Xavier *et al.,* 1994), beet molasses (Goksungur *et al.,* 1999, Kotzamanidis) and many more.

Separation and purification of lactic acid. The fermentation broth is neutralized first by calcium carbonate for the chemical separation procedure. Then calcium lactate will be formed and filtered to remove cells. To produce lactic acid and insoluble calcium lactate, the carbon will be decolorized, evaporated and acidified with sulfuric acid (Datta *et al.,* 2006). By esterification, hydrolysis and distillation, pure lactic acid will be obtained. Consumption of high amounts of sulphuric acid and the production of vast quantities of gypsum as a byproduct is the principal disadvantage of this process (Qin et al., 2010). There are many other lactic acid separation methods; they are ultrafiltration (Choi *et al*., 2002; Datta *et al*., 2006), reactive distillation (Kumar *et al.,* 2006), adsorption (Chen *et al.,* 1998), electrodialysis and nanofiltration (Gonzalez *et al.,* 2008). Compared with traditional chemical separation processes, these are more efficient in cost and energy. They have some advantages like more minor energy-intensive phase changes; they have potentially expensive solvents or adsorbents, and they have the potential for simultaneous separation and concentration of lactic acid (Kotazamanidis *et al.,* 2002).

CONVERSION OF LACTIC ACID TO CALCIUM LACTATE

For the production of calcium lactate, a calcium source is usually added to the fermentation media. Commonly used sources include calcium carbonate and calcium hydroxide. Calcium carbonate is added to the lactic acid solution to maintain the solution's pH (Kotzamanidis *et al.,* 2002, Taherzadeh *et al.,* 2008). The fermentation process generally takes 48 hours in the case of monocultures, and in the case of mixture culture, it takes 96 hours. The solubility of calcium lactate at the fermentation temperature will range between 12 to 15%. The calcium lactate is also obtained from the molasses-based fermentation broth (used for lactic acid production) by the centrifuge tube; after centrifugation, a minimal amount will be formed in brown precipitate (Thitiprasert *et al.,* 2011). During lactic acid fermentation, calcium hydroxide, which is used as a neutralizing agent, will produce calcium lactate as an end product.

CONCLUSION AND FUTURE SCOPE

In recent times, lactic acid use has increased due to its applications in different fields such as pharmaceutical, food, cosmetic, and chemical industries. For commercial purposes, batch and continuous fermentation methods are used to produce lactic acid. The continuous fermentation method gives more productivity, while the batch fermentation method gives high concentration. In batch fermentation, the bacterial genus Lactobacillus and fungal genus Rhizopus are used to produce lactic acid. Yeast species like *Candida, Saccharomyces*, and *Pichia* are established to produce a high yield of lactic acid, but their production cost is relatively high. Batch fermentation is classified into two Solid State Fermentation and Separate Hydrolysis and Fermentation. Solid-State Fermentation is more effective in comparison with Separate Hydrolysis and Fermentation. Raw material for lactic acid production, generally refined starch and cellulose materials, is used. However, the present trend of research is the production of lactic acid by using lignocellulosic materials such as agricultural waste like corn cob, sugarcane molasses, banana peduncle, beet molasses and many more. There is ample range for the production of lactic acid using lignocellulosic material as raw materials. Moreover, the progressive development in the field of molecular biology has open new opportunities to modify the genetic code of microbes for improving the production and activity of these enzymes synthesized by them.

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