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Investigating the Impacts of Various Parameters on Lactic Acid Production; A Review

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Abstract – This review examines the effects of different process parameters on the production of lactic acid. Especially focusing on the factors such as, pH, temperature, utilization of fungi, viz., Rhizopus species and selection of carbon and nitrogen sources. The development of lactic acid synthesis is promoted by acidic environment, usually falling within pH < 3.5, which allows optimal lactic acid synthesis. Another important factor is temperature. Strains such as lactobacillus rhamnosus DUT1908, have a high tolerance to temperature as high as 50 ℃, which allows for effective substrate utilization and high lactic acid yield. This review highlights the need of tailoring these process parameters to the specific characteristics of the biomass and the metabolic pathways of the microorganisms to achieve increased lactic acid production.

Key words: Fermentation, Thermophilic microorganisms, Yeast, Fungi, Enzymes

1. Introduction

Lactic acid (2-hydroxypropionic acid), a naturally occurring organic acid, first found in sour milk by Scheele in 1780 [1]. The fermentation process that Fremi invented in 1881 marked the beginning of its industrial production and opened the door for its extensive application in a variety of industries [2]. Lactic acid is now a versatile chemical with a wide range of uses and have been approved as generally regarded as safe (GRAS) by the United States FDA [3]. Lactic acid plays a major role in two sectors; first, with food-related applications accounting for around 85% of the demand and non-food uses making up the remaining 15% [2]. It is used as an acidulant, decontaminant, food preservative, fermentation agent, and flavor enhancer in the food industry. Lactic acid is used in the chemical industry as a mosquito repellant, green solvent, and precursor to long-lasting polymers like poly-lactic acid (PLA). Lactic acid is also used in medicines, technical procedures, and cosmetics. It is a component of moisturizers, medication delivery systems, tanning leather, and many other products.

The worldwide lactic acid market is expanding rapidly, reaching USD 3.46 billion in 2022 and expected to exceed USD 7.93 billion by 2032, reflecting an 8.70% compound annual growth rate (CAGR) throughout the forecast period. Sugarcane emerges as the dominant raw material for lactic acid production, accounting for 41% of the market share, followed by maize. In terms of application, the polylactic acid (PLA) category has a 30% market share, indicating a rising need for sustainable polymers. Geographically, the Asia Pacific area comes at second with 20% of the market share, with North America leading the way with a revenue share of 46% in 2022. Lactic acid market size is shown below in Fig. 1.

There are several challenges in the way of producing lactic acid (LA), especially when it comes to the long-term viability and economic viability of conventional processes. Since starch-derived glucose is now the main source of LA, there are worries regarding the environment and economy as well as increased production costs and disruptions to the food supply chain [4]. Due to its expensive raw material requirements, including refined sugars, LA's manufacturing costs are considerably high, making it less competitive than its competitors that are chemically synthesized. In an effort to address these issues, industry interest has been driven by exploring the possibility of alternate, renewable substrates. Due to its widespread availability, affordability, and renewability, lignocellulosic biomass of which over 10^{10} MT are produced globally each year becomes a potential alternative [5]. Furthermore, in certain nations without sustainable management standards, agricultural waste is frequently burnt, producing excessive gases and aerosol emissions that lead to air pollution that may be harmful to human health. Since inexpensive agricultural leftovers provide a sustainable option while lowering manufacturing costs, integrating them as alternative feedstocks might reduce environmental concerns connected with conventional raw materials. Despite its abundance, the structural complexity of lignocellulosic biomass (LCB) presents major problems in lactic acid synthesis. Owing to the LCB's tightly bonded structure, fermentable sugars are not easily accessible and must be pretreated before being used to produce LA. By degrading the lignocellulosic biomass's complex structure, this crucial pretreatment

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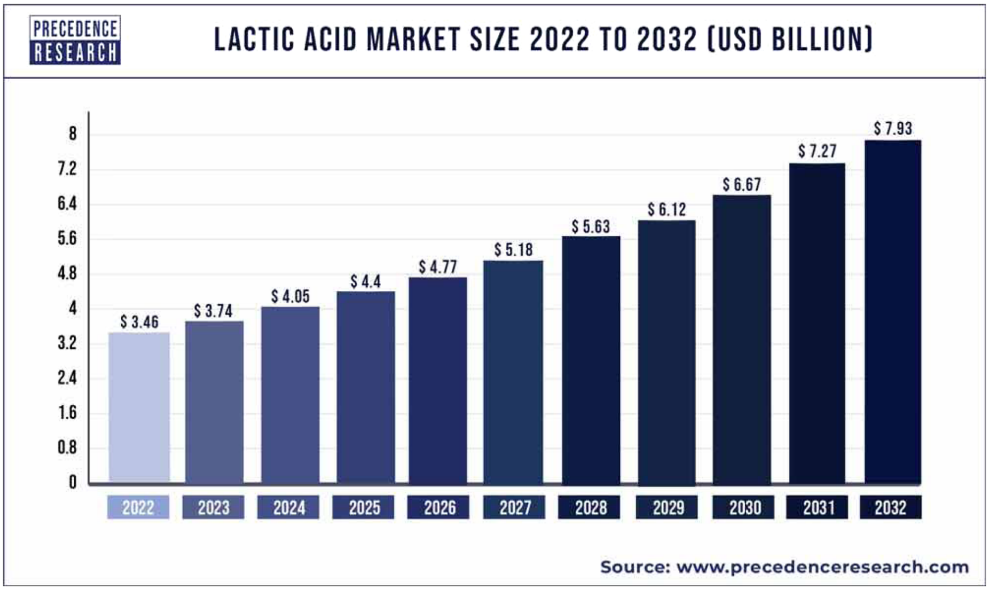

Fig. 1. Lactic acid market size from 2022 to 2032.

step exposes cellulose and hemicellulose to enzymatic activity [6,7]. On the other hand, the pretreatment process may cause the generation of inhibitors that result from the breakdown of lignocellulose biomass, such as weak acids, phenolic compounds, and furan derivatives [7,8]. LA fermentation from lignocellulosic biomass is significantly hampered by these inhibitors, which also negatively impact microbial cell viability, reduce feedstock conversion efficiency, and raise production costs.

There are several challenges in the way of producing lactic acid (LA), especially when it comes to process that involve both fermentation and saccharification simultaneously (SSF). The incompatibility of the pH and temperature conditions optimal for the hydrolysis and fermentation stages is a primary drawback of SSF systems. This mismatch forces compromise in optimizing conditions for saccharification and LA fermentation, leading to inferior performance. Another challenge is that optimal conditions for LA fermentation and lignocellulose saccharification are different. Specifically, cellulases and other degrading enzymes demonstrate their optimum catalytic activity at temperature between 45 and 50 ℃ [9,10].

To overcome the intrinsic temperature and pH incompatibility issue, use of thermophilic enzymes appears as viable solution. The ability to operate at high temperatures throughout the entire production process is made possible by the use of thermophilic microorganisms and enzymes. This has a number of benefits, including: (i) improved solubility of the substrate and product; (ii) increased reaction rates;

(iii) reduced requirements for enzymes, (iv) easier mixing due to decreased medium viscosity and, (v) decreased risk of contamination [11]. Thermophiles, as opposed to their mesophilic counterparts are best suited for higher temperatures, which is closer to the optimal operating temperature of the hydrolytic enzymes required for saccharification, which is between 50 and 55 ℃.

Recent research on thermophilic microbes, such as Thermus thermophilus, has revealed their potential as a abundant source of enzymes that break down polymers, such as pullulanases, α-amylases, xylanases, esterases, lipases, and proteases [12]. Thermophiles ensure effective saccharification enzyme activity between 50 to 110 ℃, which eliminates the need to compromise enzyme performance and lowers the need for higher enzyme loading [11]. Industry can overcome temperature and pH incompatibility constraints and produce lactic acid from renewable substrates like lignocellulosic biomass with greater efficiency, lower costs, and greater sustainability by incorporating thermophilic biocatalysts into SSF processes.

Several strategies can be used to handle particular problems that may arise during lactic acid production at various temperature ranges. The slower growth rate of lactic acid bacteria (LAB) during low temperature fermentation raises serious concerns about contamination risk because this allows opportunistic germs to thrive. Strict aseptic conditions and use of antibacterial agents can reduce this. Furthermore, it is possible to shorten the fermentation period and lower the risk of contamination by utilizing LAB strains that grow faster at lower temperature by optimizing the amount of inoculum. Energy consumption becomes an important consideration in mesophilic fermentation. Although mesophilic temperatures are typically energy efficient, it might be difficult to sustain ideal temperature for long period of time. To reduce energy losses, this may be controlled by utilizing insulation. Additionally, by leveraging the synergetic effects of various microorganisms, the utilization of mixed cultures can improve fermentation efficiency and lower the energy requirement [13]. Thermophilic microorganisms lower the risk of contamination because of its high operating temperature but issue of enzyme stability and substrate degradation must be addressed. A viable solution is to employ thermophilic enzymes, which are stable and active at high temperature [14]. Furthermore, employing strong thermophilic bacteria and optimizing the fermentation process to maintain constant pH levels will further

increase the efficiency of lactic acid production.

To circumvent the inhibitory effects of lignocellulosic biomass breakdown during the pretreatment stage and enable effective fermentation, several techniques have been proposed. Pretreatment processes must be improved to reduce the formation of inhibitory compounds, which may be accomplished by better integrating with enzymatic hydrolysis development [15]. Improved enzyme combinations can result in milder pretreatment conditions, lowering costs and inhibiting compound formation. Furthermore, the development of low-cost methods and microorganisms that can tolerate inhibitors or detoxify biomass offers promising results for the commercialization and expansion of lignocellulosic processes.

Genetic engineering and adaptive evolution provide viable paths for improving substrate utilization and tolerance to toxic byproducts, even though natural microbial solutions are less common [16]. Moreover, the risk of acetic acid inhibitory doses may be decreased by the engineering or selection of plants with lower acetyl content [17]. Additional approaches to decrease inhibition issues include strategies aimed against fermenting microorganisms, such as the use of large inocula or the selection of resistant. Ultimately, lignocellulosic hydrolysates can be detoxified or conditioned using methods like chemical additives or polymers, which is a potent way to overcome inhibition and facilitate effective fermentation processes.

To address these issues, research focuses on developing the pretreatment strategies that can reduce the inhibitor formation while increasing sugar release. Furthermore, strain engineering and selection are used to create microbial strains that can effectively convert carbohydrates to lactic acid and tolerate inhibitors.

This paper aims to provide a thorough analysis of the impact of several parameters of fermentation process that produces lactic acid. This research determines the optimum conditions for maximizing lactic acid yield by examining factors such as temperature, pH, selection of microorganisms etc. Moreover, study will investigate developments in fermentation methods and the possibility of using genetic engineering to improve microbial activity in the production of lactic acid.

2. Processes for Producing Lactic Acid

Microbial fermentation and chemical synthesis are two methods for producing lactic acid, each having unique benefits and procedures. String acids hydrolyze lactonitrile, producing a racemic mixture of D-, L- lactic acid, which is the main process used in chemical synthesis. Alternative chemical pathways, like breakdown of sugars the oxidation of propylene glycol, the hydrolysis of chloropropionic acid, the oxidation of propylene by nitric acid , and the reaction of acetaldehyde, carbon monoxide, and water at high pressure and high temperature are not practical from technical and financial standpoint. Chemical synthesis is costly, dependent on fossil fuel byproducts and results in racemic combination that is unsuitable for various specific uses [18]. Fig. 2 illustrated microbial fermentation and chemical synthesis process.

However, there are number of benefits associated with microbial fermentation. It employs bacteria that produce lactic acid by using pyruvic acid as precursor. This process produces optically pure D (-), L (+) lactic acid, which is necessary for numerous purposes [19]. Microbial fermentation uses less energy, operates at lower production temperature and is less expensive when it comes to the substrate. Various inexpensive, renewable raw resources including starch, lignocellulose, molasses, and leftovers from agriculture and agro-industrial processes can be used in this process [20]. These microbes' effectiveness can be further increased by gene manipulation. Microbial fermentation offers several benefits such as producing optically pure L-lactic acid, using renewable feedstocks to reduce reliance on non-renewable petrochemical sources and encourage sustainability, operating under milder conditions (lower temperature and pressure) to save

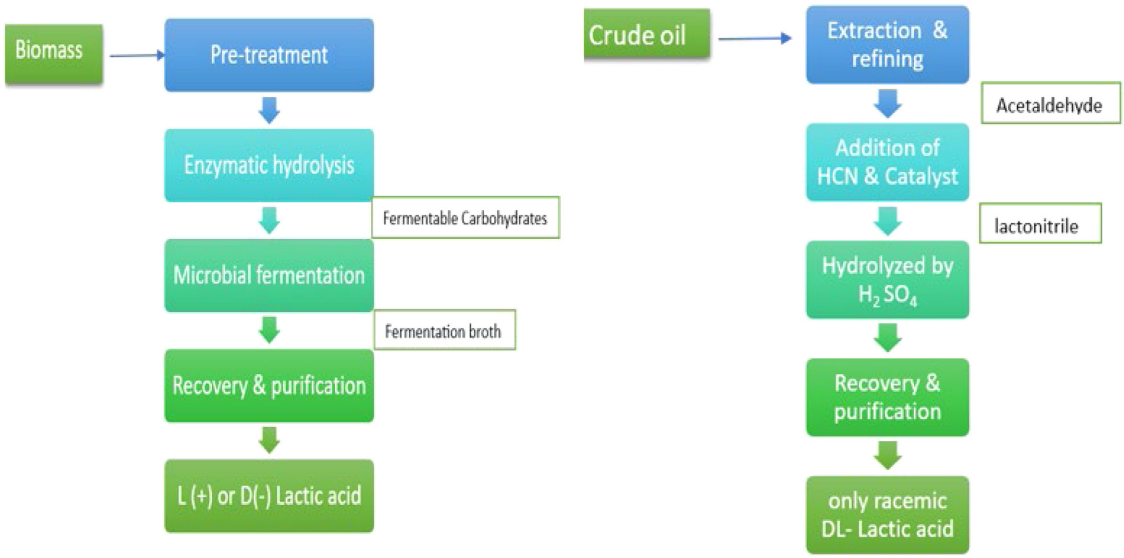

Fig. 2. Microbial fermentation and chemical synthesis process for production of lactic acid.

energy costs and consumption, and substrate flexibility, which enables a more economical and environmentally benign method.

Two extensively research methods for producing second generation lactic acid (2G-LA) are simultaneous saccharification and fermentation (SSF) and separate hydrolysis and fermentation (SHF). Enzymatic breakdown of polysaccharides into fermentable sugars and subsequent fermentation of these sugars into lactic acid are two different steps of SHF. It is possible to optimize each step in this sequential process. The accumulation of sugars can impede hydrolytic enzymes, reducing the effectiveness of hydrolysis process and potentially leading to the reduction of total yields [9]. This is the major drawback of SHF. SSF, on the other hand integrates the fermentation and hydrolysis processes in single unit. Since microbes quickly utilize the sugars generated during hydrolysis for fermentation, this integration overcomes the problem of enzyme inhibition seen in SHF and maintains lower sugar concentration while increasing enzyme activity [8]. In addition to lowering equipment costs (only one reactor needed), this simultaneous process also shortens processing time, which increases the production rates. Furthermore, an SSF doesn't require separate and usually energy intensive saccharification stages which can save energy usage.

Notwithstanding these advantages, SSF efficiency is halted by the incompatibility of optimal pH and temperature for hydrolytic enzymes and microbial development, which means that larger enzymes loads are required for efficient saccharification [10]. Process of SSF and SHF is shown in Fig. 3.

3. Effect of Process Factors on Lactic Acid Production

3-1. Effect of microorganisms

When selecting microorganisms for lactic acid fermentation, the following aspects are considered: substrate compatibility, metabolic pathways, environmental resilience, and product specificity. Several microorganisms, including bacilli, fungus, lactic acid bacteria, and genetically engineered strains, have been studied for their potential in lactic acid fermentation. Wang et al. (2013) were able to exemplify a chemical method which employed plant biomass fractions with crystalline cellulose and water to produce lactic acid with 60% yield even though the racemic mixture was formed in the process in contrast; microbial fermentation has several advantages, including the use of low-cost substrates and lower energy consumption due to moderate fermentation. Additionally, certain enantiomers, such as the L-isomer of lactic acid, can be produced by microbial fermentation [3]. Therefore, the selection of microorganisms significantly impacts the effectiveness and specificity of acid production.

3-1-1. Bacteria

Lactic acid bacteria, known as LAB are a group of organisms that play important roles in different fermentation processes and have significant impacts, on industries like food, pharmaceuticals and biotechnology. These bacteria are recognized for their ability to produce lactic acid as significant fermentation byproduct. It is

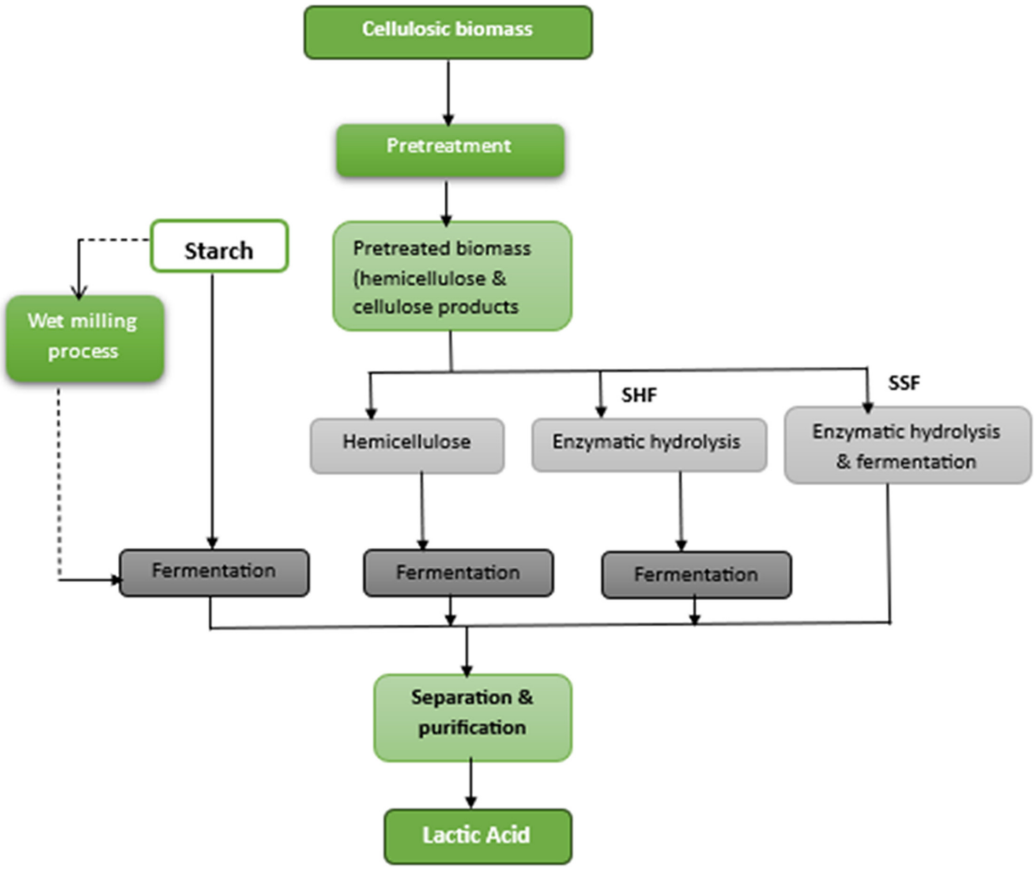

Fig. 3. SHF and SSF process for production of lactic acid.

common for LAB to be non-motile, facultative anaerobes that do not generate spores [2]. Their ideal growth rangs between 20 to 45 ℃, whereas their ideal pH range is between 5.5-6.5, depending on the species. They can grow in both anaerobic and micro aerophilic environment [11,12]. A variety of nutrients including vitamins, minerals, fatty acids, carbohydrates, amino acids, peptides, and nucleotide bases are necessary for the development and metabolism of these bacteria. LAB is classified under several genera, which include Carnobacterium, Weisella, Leuconostoc, Oenococcus, Aerococcus, Tetragenococcus, Enterococcus, Pediococcus, Lactobacillus, and Lactococcus. Anaerobic, carbohydrate-containing environments with an acidic pH and enough of available chemicals for their anabolism are favorable conditions for LAB to proliferate. The Bacilli class includes most investigated LAB species such Lactobacillus, Bacillus, Lactococcus, and Streptococcus. When these bacteria are given enough B vitamins and peptides, they grow rapidly. Bacilli are facultative anaerobic bacteria that are Gram-negative, motile, sporulating, and offer various benefits over traditional LAB in terms of lower lactic acid (LA) production cost. They don't require media sterilization prior to fermentation since they may grow in simpler mineral media with less expensive sources of nitrogen and ferment at 50 ℃. Furthermore, Bacilli also have the ability to metabolize hexose and pentose sugars, providing a way to use all of the sugars found in lignocellulose [10].

During the fermentation of lignocellulosic hydrolysate, harmful substances including 5-hydroxymethyl furfural (HMF) and furfural (FF) may be produced, which might impede the development of microorganisms. Detoxification process is frequently needed which can make fermentation more difficult and raise production costs. Consequently, these problems can be reduced or avoided by using inhibitor tolerant microorganisms, especially FF and HMF-tolerant strains. It also eliminates the need for expensive detoxification procedures. Promising results were obtained in recent research when L. rhamnosus SCJ9 (a glucose-utilizing LAB) and E. mundtii WX1 (a xylose-utilizing LAB) were co-cultured to produce L-lactic acid. With 2 g/L furfural present, this co-culture was able to create L-LA, demonstrating the ability of specially designed microbial consortia to overcome inhibitor difficulties in fermentation processes [1].

Based on the products that they generate during the fermentation of glucose, lactic acid bacteria (LAB) may be divided in to two types. The sole byproduct of fermentation in homofermentative organisms is lactic acid (>85%), which produces two ATP for every mole of glucose metabolized. Conversely, the heterogenous organisms yield CO_2, lactic acid, and either acetate or ethanol from glucose. Compared to homofermentative bacteria, this route produces less growth per mole of glucose metabolized since it only produces one ATP per mole of glucose. Heterofermentative LAB employ the pentose phosphate route, which is sometimes referred to as the phosphogluconate or phosphoketolase pathway. Hexoses can be effectively converted to lactic acid (LA) by homofermentive lactic acid bacteria, mostly through the glycolysis process. These bacteria can effectively use both hexose and pentose carbohydrates through the Embden–Meyerhof route because the aldolase enzyme enables complete glucose conversion feasible. This means two molecules of lactic acid per mole of glucose consumed, with a potential yield of 1 g/g.. However, actual yields might differ based on the sort of carbon source that is used. The particular enzyme involved intimately linked to the efficiency of various fermentation pathways in formation of lactic acid. In homofermentive LAB, lactate dehydrogenase is an essential enzyme that catalyzes the conversion of pyruvate to lactic acid. The stereospecificity of the enzyme is essential in deciding whether D or L- lactic acid is generated, which in turn impacts the overall quality and industry applications lactic acid.

Especially in industrial contexts, the enzymatic conversion of complex substrates, including lignocellulosic biomass into fermentable sugars is crucial for optimizing lactic acid production. In this process, synergistic action of many enzymes including hemicellulases and cellulases takes place. A class of enzymes known as cellulases, a significant part of lignocellulosic biomass, into glucose monomers. Three primary types of enzymes are involved in the mechanism of action of cellulases: Endo-β-1, glucanases, which cleave internal β-1, 4-glycosidic bonds within the cellulose chain at random, generating new end chains; Exo- β-1,4-gluanases, which gradually remove cellobiose units from the non-reducing ends of the cellulose chains; and β- glucosidases, which hydrolyses cellobiose into two glucose molecules preventing product inhibition and promoting the reaction [21].

Conversely, hemicellulose, a heterogeneous polymer present in plant cell walls, is the target of hemicellulases. Owing to its complex structure, hemicellulose requires numerous enzymes to break down. Important enzymes in this process include β-xylosidase, which hydrolyses xylo-oligosaccharides into xylose monomers and endo-1,4-β-xylanase, which cleaves the xylan backbone. Extra auxiliary enzymes that eliminate side groups from the xylan backbone, including α-L- arabinofuranosidase and acetylxylan esterase, improve the main chains accessibility to the key enzymes. The choice of these enzymes takes several factors into account such as substrate specificity, enzyme activity a stability during the process, and their capacity to cooperate with main enzymes, directly affects the efficiency of lactic acid synthesis [22]. Effect of different microorganisms on lactic acid concentration is shown in Table 1.

In an experiment, four different microorganisms Lactobacillus delbrueckii, Lactobacillus casei, Lactobacillus bulgaricus, and Lactococcus lactis were studied having different efficiency and yield of lactic acid as shown in Fig. 4. It was observed that the most lactic acid throughout testing were produced by L. bulgaricus and L. casei whereas maximum concentration achieved was 21.5 g/l and 23.3 g/l, respectively. This suggested that some strains were better at metabolizing whey into lactic acid. These strains were very efficient because of their capacity to adapt to fermentation conditions and their strong enzyme systems, which efficiently catalyzed the fermentation of lactose to lactic acid. Since L. bulgaricus proved most effective strain for producing lactic

Table 1. Effect of microorganisms on yield of Lactic acid

Strain	Substrate	Temp (℃)	Time hr, days	LA (g/l)	Yield (g/g)	Ref
Lactobacillus casei	Cane molasses	37	-	83	0.57	[23]
Lactobacillus sp. MKT878	Cane molasses	37	-	68	0.76	[23]
Lactobacillus plantarum	Molasses	37	6 days	19.67	0.19	[24]
Lactobacillus delbrucckii	molasses	37	8 days	20.60	0.21	[24]
Lactobacillus acidophilus	Molasses	-	3 days	23.1		[25]
L.delbrueckii subsp. Delbrueckii NBRC 3202	Millet bran hydrolysate	37	-	25.38	0.60	[26]
Lactobacillus rhamnosus PCM 489	Whey	44	1 day	27.5	0.27	[27]
Lactobacillus rhamnosus ATCC 7469	Bewer spent grain	37	1 day	58.01		[28]
L. amylovorus	Cassava bagasse	37	6 days	29.69	0.14	[29]
L. acidophilus	Cassava bagasse	37	6 days	15.52	0.07	[29]
Lactobacillus rhamnosus SCJ9, E. mundtii WX	Glucose, xylose	37	2 days	19.82	0.97, 0.68	[30]
Bacillus coagulans A107	carob biomass	52	52hr	48.7	0.84	[31]
Lactobacillus plantarum	Carob syrup	37	12 hr.	49.34		[32]
Lb. delbrueckii spp. Delbrueckii	Orange peel wastes hydrolysates	40	8hr	6.72	0.90	[33]
Lb. delbrueckii	Cassava fibrous waste hydrolysis	37	-	16.15	0.5	[34]
Lb. rhamnosus ATCC 7469	Recycled paper sludge	37	8hr	63.5	0.74	[35]
Lb. rhamnosus	Solid carob waste	37	1 day	22	0.76	[36]
Lb. rhamnosus B103	Dairy industry waste	37	3 days	143.7	0.28	[37]
Lb. bulgaricus CGMCC 1.6970	Cheese whey powder	42	72 hr	113.18	0.41	[38]
Lb. casei Shirota	Mixed food waste bakery waste	37	36 hr	94	0.94	[39]
Lb.casei CICC 6056	Sophora flavescens residues	37	-	55.1	0.835	[40]
Lb. casei	Sugarcane bagasse	37	-	21.3	0.21	[41]
Lb. casei A-8	Potato starch	37	7 days	130	0.32	[42]
Lb. lactis NCIM 2368	Glucose	42	30hr	17.01 72.24	0.17 0.72	[43]
Lb. plantarum	Glucose Hydrolysate of microalga Chlorella vulgaris ESP-31	30	2 hr 4 hr 4 hr	28.45 31.75 39.72	0.94 0.93 0.99	[44]
Lb. plantarum	Brown rice	37	144 hr	117.1	0.58	[45]
Str. Thermophilus	Magazine and office paper	-		24.18–39.71		[46]
E. faecium strain FW26	Banana peels and food wastes mixture	50	5 days	33.3	0.84	[47]
Lb. pentosus CECT4023T	Gardening lignocellulosic residues	32	2 days	21	0.60	[48]
Lb. paracasei ATCC 334	Chlorella	37	1 day	1.2	0.004	[49]
Lb. rhamnosus & B. coagulans	Cassava bagasse	50,42	41	112.5	0.88	[50]
Lb. sanfranciscensis MR29	Wheat straw	25	72	57	0.057	[51]
Lb. rossiae GL14	Wheat straw	37	72	18.6	0.018	[51]
L. crustorum W19	Wheat straw	25	72	58.8	0.058	[51]
Bulgaricus MI Lb. delbrueckii	Wheat straw	42	72	94.8	0.94	[51]
subsp. Bulgaricus DSM 20081	Wheat straw	42	72	96.2	0.096	[51]
Engineered Pediococcus acidilactici	Wheat straw	28	72	130.8	0.67	[52]
Streptococcus sp.(indigenous consortium)	Highly viscous food waste	35	2 days	69	0.86	[53]
B. coagulans BCS13002	Gelatinized corn starch	45	72 hr	11.75		[54]
B. coagulans L-LA 1507	Corn stover	50	20 hr	97.5	0.406	[55]
B. coagulans	Dilute ethylediamine pre-treated rice straw	-	20 hr	92.5	0.57	[56]

acid, more research was directed towards this microbe.

For Lactococcus lactis, Lactobacillus bulgaricus, and Lactobacillus casei, output of lactic acid based on total sugar was between 70 and 80%. Conversely, out of all the strains that were examined, L. delbrueckii produced the least quantity of lactic acid [57].

3-1-2. Fungi

Numerous substantial obstacles are encountered throughout the process of lactic acid synthesis by lactic acid bacteria (LAB). In comparison to other microbes, LAB requires complex nutrients fermentation temperatures that are slightly lower typically below 45 ℃. As a result of this necessity there is a greater chance of contamination and increase in cost. Furthermore, first stage of amylase synthesis has long lag phase and low productivity, which means partly hydrolyzed substrates must be used.

On the other hand, some fungi viz., those in the genus Rhizopus,

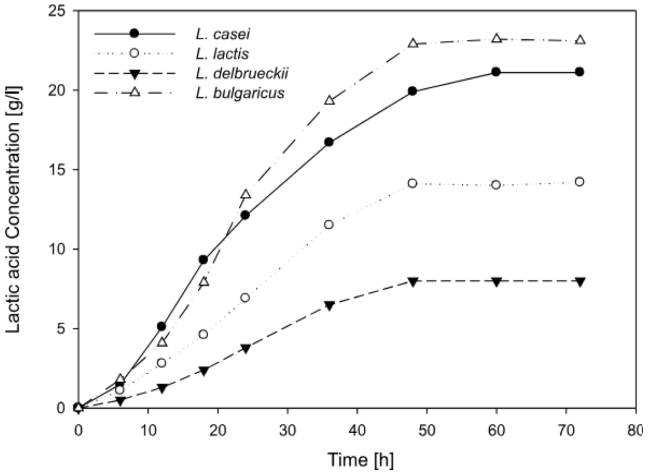

Fig. 4. Lactic acid production by selected microorganisms [57].

have the ability to create large amount of lactic acid and have a number of benefits over bacterial processes. These fungi have the ability to use chemically specified media, which includes inorganic nitrogen sources [3]. This simplifies the manner of separating and purifying the products. Additionally, they have the ability to metabolize both complex carbohydrates and pentose sugars.

There are both several benefits and drawbacks of using Rhizopus strains to produce LA. There amylolytic characteristics enables them to directly convert different starch containing biomasses to L-lactic acid without saccharification, which is one of the main advantages [3]. In addition to less complex medium requirement, they grow in a filamentous or pellet-like fashion in the fermentation medium, which makes separation from the broth easier and ultimately results in a cheaper downstream process. R. oryzae, on the other hand is an obligate aerobe that needs intense aeration, usually at a rate more than 0.3 g O_2/L/h [3,58]. Their morphological development forms during fermentation can vary greatly, including long filamentous appearances, pellets, mycelial mats, and clumps. This heterogeneity can have a major impact on LA yield and the rheology of the fermentation broth, which is another drawback [58]. There have been a number of studies that have been looked into immobilization methodologies for the production of L-lactic acid using R. oryzae. However, this method is challenging since fungal cells need to be trapped on matrices and it contains volume limitations.

3-1-3. Yeast

Conventionally, lactic acid bacteria are used to produce lactic acid. Even though they work, LAB can't handle acidic conditions well, thus neutralizing agents like $CaCO_3$ are used in large quantities which causes gypsum to be formed during fermentation. This causes issues with the environment in addition to complicating the purifying process. On the other hand, yeast and specially modified strain present a potential substitute because of their increased acid tolerance which lessens the necessity for neutralizing agents and down-stream cost [3]. Nevertheless, LA yields are lower when using wild type yeasts. Insertion of heterologous L (+)-LDH genes and deletion of pyruvate decarboxylase and pyruvate dehydrogenase activities are examples of genetic modifications that have been used to efficiently increase LA production in yeast strains.

The BK01 strain was developed using adaptive laboratory evolution (ALE) to enhance lactic acid tolerance of the S. cerevisiae SR8LDH strain. Under conditions of extreme lactic acid stress, BK01 showed better growth and LA production. Although initial culture showed long lag phase of 216 hrs., successive cultures show dramatic reduction in this phase. In contrast to its parent strain SR8LDH, BK01 showed the better tolerance to 8% lactic acid among the six isolated colonies. Two particular SNPS (mYPT7 and mYOL159C-A) have been confirmed as attributors to this tolerance [26]. SNPS were found to be connected with this tolerance using sing genome sequencing. Without pH control, fermentation tests showed that BK01 produced up to 119.1 g/L of lactic acid, 17 times higher than SR8LDH [59]. Furthermore, while utilizing buckwheat husk hydrolysates to produce cellulose lactic acid, BK01 shown greater resistance to fermentation inhibitors such as acetic acid, indicating its potential to use in large scale processes. Another study show that, to increase lactic acid production, Saccharomyces cerevisiae BTCC3 was engineered with the LDH gene. The BTCC3LA2 engineered strain reached a maximum concentration of 43.23 g/L when exposed to near-neutral conditions. While boosting the buildup of lactic acid and decreasing the formation of byproducts, pathway engineering decreased ethanol production [60]. These strains showed great promise for large scale fermentation because of their resilience to their chemical inhibitors and higher lactic acid production capabilities, especially the LA_2 derivative.

3-2. Effect of temperature

Temperature has a major effect on the speed and accuracy of lactic acid fermentation of biomass. Lactic acid bacteria (LAB), such as Lactobacillus and Streptococcus species, are the main agents of lactic acid fermentation. The ideal fermentation temperature varies depending on the strain of LAB. Effect of different temperatures on production of lactic acid is shown in Table 2.

For the most part, fermentation can be done at ambient temperatures (15–27 ℃), mesophilic (growing best at moderate temperatures, 25–40 ℃), thermophilic (growing best at relatively high temperatures, 40–65 ℃), extreme thermophilic (growing best at higher temperatures, 65–80 ℃), and hyper-thermophilic (growing best at temperatures greater than 80 ℃) conditions [61]. An increase in tolerance to high temperatures will be advantageous for increased productivity and decreased contamination. An increase in temperature also increases substrate hydrolysis. Most strains have the best results between 35 ℃ and 40 ℃. Within this range, LAB metabolic activity is at its highest, resulting in faster fermentation rates and higher production of lactic acid. Some strains, like Lactobacillus rhamnosus DUT1908, have been found to be thermotolerant, meaning they can withstand high temperatures up to 50 ℃. This is advantageous because it enables efficient substrate utilization and high lactic acid production. This

Table 2. Effect of temperature

Organism	Substrate	Fermentation Temperature (℃)	Lactic Acid			Reference
			Tilter (g/L)	Productivity (g)	Yield (g/g)	
Lactobacillus rhamnosus DUT1908	Aging paddy rice with hull	47 ℃	91.68	1.95	0.92	[62]
		50	107.8	3.4	0.89	
Lb. acidophilus BCRC 10695	Seaweed Hydrolysates	30	14.21	-	0.19	[63]
Lactobacillus caseishirota	Confectionery waste	37	82.6	2.50	0.94	[64]
Mixed culture from compost	Food waste	50	39.2	-	1.38	[65]
Indigenous microorganisms	Biowaste	37	40.6	1.69	1.04	[61]
Lactobacillus casei	Sugarcane molasses	38	120.23	-	0.91	[66]
Lactobacillus caseishirota	Food waste	37	94	2.50	0.90	[67]

strain could tolerate a rather high temperature up to 50 ℃, which was the highest tolerant temperature currently reported. To get 107.8 g/L of LA titer with a yield to theoretical glucose of 0.89 g/g and productivity of 3.4 g/(L.h), a one-step open SLSF method employing inedible APRH was developed [62].

It has been noted that Lb. acidophilus BCRC 10695 thrives effectively at temperatures of 30, 34, and 37 ℃. The concentration of lactic acid showed rise with temperature up to 37 ℃ however, when bacterial growth slows down beyond this point, the concentration of lactic acid was found to decrease. When the temperature raised from 26 ℃ to 37 ℃, the pH value of the product was likewise seen to increase in the case of Lb. acidophilus [63].

In an experiment, it was shown that 42 ℃ was the optimal temperature for achieving maximal lactic acid concentration of 24.3 g/L. This temperature seemed to be optimum for synthesis of lactic acid indicating that L. bulgaricus's enzymes activity and metabolic processes function best at this particular temperature. As the temperature increased from 32 ℃ to 42 ℃, lactic acid production was also increased [57]. This trend can be explained by the fact that L. bulgaricus's has higher metabolic activity in this temperature range which made lactic acid fermentation- related enzymes function better. A greater rate of substrate conversion to lactic acid was the outcome of increased enzyme activity as shown in Fig. 5.

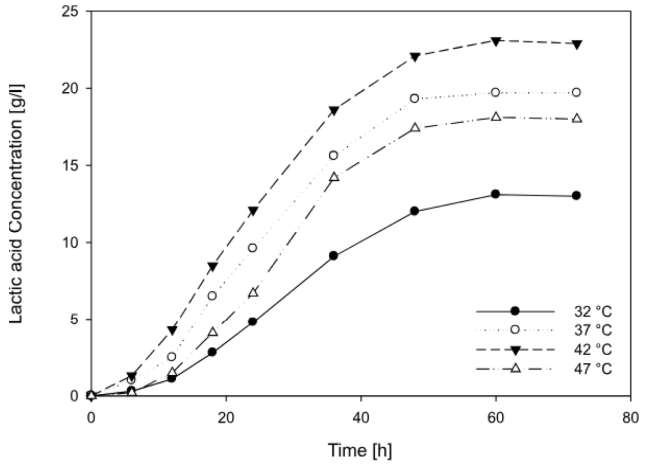

Fig. 5. Effect of temperature on lactic acid production [57].

3-3. Effect of fermentation mode

Fermentation mode is selected on the basis of nature of substrate, fermentation broth's viscosity and the microorganisms involved. Some commonly used modes of fermentation are batch, fed-batch, continuous fermentation. Some of the processes used in these fermentation modes are simultaneous saccharification & fermentation and separate hydrolysis & fermentation. These modes have their own distinct advantages and disadvantages. Effect of different fermentation mode is shown in Table 3.

3-3-1. Batch fermentation

Batch fermentation is the most commonly used fermentation mode currently. As it is a closed system, the risk of contamination is reduced. One of the highest productions has been reported by batch fermentation by Abdel-Rahman et al. [68] reporting 119 $g \cdot L^{-1}$ L-lactate production during batch fermentation of cellobiose using LAB, whereas 80 $g \cdot L^{-1}$ D-lactate was produced using hydrolyzed cane sugar in the fermentation medium. Another researcher Liang et al. [69] reported a production yield of 0.25 $g \cdot g^{-1}$ and productivity of 125 $mg \cdot (g \cdot d)^{-1}$ using potato peel waste in a similar batch fermentation mode. Although high lactic acid production was achieved in batch fermentation but it was observed that as fermentation proceeds the production decreased due to the buildup of lactic acid in the system which further caused pH to drop for which pH controlling agents like: ($CaCO_3$, NaOH, or NH_4OH) were added. Moreover, due to nutrients depletion as fermentation proceeded forward the microbial cell growth decreased reducing the productivity.

3-3-2. Fed-batch fermentation

To overcome the hindrances of batch process i.e., limitation of nutrients fed batch fermentation was investigated. Fed batch process was very similar to batch as in it similar to batch all the raw material, nutrients, nitrogen and microorganisms were added in the system and the system was closed. The only difference was the gradual addition of limiting substances like the nutrients (Carbon and nitrogen). This led to increased microbial growth and in turn higher yield, moreover by gradually adding nutrients more control over the reaction rates were observed. Ding et al. [70] reported LA concentration and production rate of 180 $g \cdot L^{-1}$ and 2.14 $g \cdot (L \cdot h)^{-1}$, respectively by gradually adding

Table 3. Effect of fermentation mode

Fermentation mode	Microorganisms	Substrate	Y_{LA}[e] ($g \cdot g^{-1}$)	C_{LA}[c] ($g \cdot L^{-1}$)	P_{LA}[d] ($g \cdot L^{-1} \cdot h^{-1}$)	References
Batch	*Lactobacillus* sp. RKY2	Cheese whey	0.980	94.06	1.060	[73]
Batch	Lb. casei SU No 22	Whey	0.44	22		[74]
Batch	Lb. delbrueckii IFO 3534	Glucose	0.83	83	1.5	[75]
Fed-Batch	Str. Thermophilus	Lactose		39	1.4	[76]
Fed-batch	Lactobacillus casei	Chicken hydrolysate	0.984	116.50	4.000	[77]
Fed-batch	*Lactobacillus Rhamnosus* CECT-288	Cellulosic bio sludge	0.378	42.00	0.87	[78]
Continuous	Lb. rhamnosus ATCC 10863	Sucrose	0.74	80	8.0	[79]
Continuous	*Lactobacillus Helveticus* R211	Whey permeate		42.00	21.00	[72]
Continuous	Enterococcus faecalis	Sago starch	0.93 ± 0.20	16.60 ± 0.80	1.10	[80]

glucose and yeast extracts. These values were 56.5% and 59.7 higher than the batch fermentation. An increase in yield was observed but still due to the buildup of product the inhibition of substrate occurs.

3-3-3. Continuous fermentation

In continuous fermentation, feed is continuously fed into the system and the product is also continuously removed and a constant volume of broth is maintained in the system. This system is advantageous over others as there is no buildup of lactic acid in spite the inhibition of lactic acid is decreased. Moreover, by this technique the microbials, substrate and product levels are kept steady. This technique also eliminates the down time as the feed is continuously supplied and product is obtained continuously. No separate cleaning and sterilization after every batch is required making this process more efficient as compared to batch process. Ahring et al. [71] observed lactic acid production of 3.69 $g \cdot L^{-1} \cdot h^{-1}$ by *Bacillus coagulans* microorganisms using continuous fermentation. Similarly, Schepers et al. [72] also reported high lactic acid productivity (19–22 $g \cdot l^{-1} \cdot h^{-1}$) and low residual sugar concentration. In this way, continuous fermentation proved to be more efficient, but currently it's a bit hectic to do it at large scale due to expensive equipment and difficult control.

3-4. Effect of pH

Fermentation is caused by lactic acid bacteria, which grow best in an acidic environment. Most strains peaked at 5.8, however the ideal pH range for lactic acid production is normally between 5.0 to 6.0 [81]. After testing five different pH-regulating techniques, it was found that addition of dimethylamine or trimethylamine produced the best productivity. The best alkali (base) choice was ammonium hydroxide as it didn't significantly deplete the soup. The use of calcium carbonate buffering was not recommended for high sugar concentrations. Due to product inhibition and broth flocculation, it resulted in inadequate fermentation. The target pH was 5.8 during experimentation. As it was difficult to maintain pH, certain agents were added to maintain the pH. Some of the agents used and the concentration in which they were used are as follow: dimethylamine (20%), ammonium hydroxide (20%), trimethylamine (20%) and sodium hydroxide (20%) [81].

Too acidic (below pH 5): In an extensively acidic environment due to lactic acid buildup or any other reason as the pH decreased below 5, bacterial growths may get inhibited. Despite the presence of bacteria, decline in the generation of lactic acid can be seen as the bacteria's growth gets inhibited.

Too basic (pH >6): Lactic acid making bacteria's (LAB) were less competitive than other microorganisms. They require specific pH conditions to make efficient amount of lactic acid as compared to their counterparts that produce lactic acid at higher pH levels but are less efficient at producing lactic acid at higher pH levels.

For specific fermentation processes, in order to properly maintain the lactic acid formation range in an efficient way, pH management was required. During creation of lactic acid, a fall in pH was observed and this may increase as lactic acid accumulated. Hence, proper removal of lactic acid from system was required and to counteract the fall in pH some buffers of neutralizing agents were introduced in the system. When fermentation was conducted at a higher initial pH (such as pH 6.7) as opposed to a lower initial pH (such as pH 4.5 or 5.5), lactic acid generation was higher. This was because, the higher pH encouraged the bacteria to produce and accumulate lactic acid. PH was a crucial factor that affected the formation of lactic acid, yet other factors such as, availability of nutrients, temperature and presence of lactic acid bacteria (LAB) were also crucial factors to consider [82]. During fermentation, optimal pH levels (for example, pH 6.5 for Streptococcus thermophilus, pH 5.8–6 for Lactobacillus bulgaricus, and pH 6.3–6.9 for Lacto-coccus lactis) resulted in enhanced growth rates and production of lactic acid bacteria. This was in contrast to the situation in which acidic circumstances were present [83]. The high concentrations of undissociated lactic acid that were present at low pH values (pH 5 or lower) have the potential to cause damage to cell membranes and reduce the efficiency of enzyme activity, nutrition transfer, and cell survival. Certain lactic acid bacteria, such as Lactobacillus, were able to maintain a significant difference in pH between their cell membrane and the surrounding environment, which helped to maintain their intracellular pH. This ability allowed them to survive in acidic circumstances.

The fact that the formation of lactic acid was controlled by other elements in addition to pH was extremely important to keep in mind. Temperature, the availability of nutrients, and the particular species of lactic acid bacteria were also other aspects to be considered [82].

During fermentation, optimal pH levels (for example, pH 6.5 for Streptococcus thermophilus, pH 5.8–6 for Lactobacillus bulgaricus, and pH 6.3–6.9 for Lacto-coccus lactis) resulted in enhanced growth rates and production of lactic acid bacteria. This was in contrast to the situation in which acidic circumstances were there [83]. Even at acidic pH levels (pH less than 3.5), lactic acid fermentation can occur. In a study that used food waste as a substrate, the maximum lactic acid concentration of 8.72 g/L was recorded at pH 3.11. The Lactobacillus bacteria that produced the majority of lactic acid seemed to grow better in acidic environments [84].

3-5. Effect of carbon source

It is well known that glucose is the most easily metabolized carbohydrate of many organisms. Therefore, the effects of different glucose concentrations between 25 and 250 g/l were measured. Lactic acid increased with increasing glucose up to 150 g/l. Each increase in glucose levels led to a decrease in lactic acid. In the presence of high glucose, glucose could not be used efficiently and approximately 40-50% of the original glucose remained unused in the fermentation process. It was well reported that the processing of lactic acid was limited by product inhibition. It can cause a decrease in the concentration of the initial glucose concentration due to an increase in lactic acid. This might also be due to the suppressive effect of glucose on lactic acid fermentation. Restricted products can be partially reduced by further enriching them with essential nutrients. The maximum lactic acid obtained was 60 g/l. The lactic acid concentration was 60 wt.%, depending on the amount of glucose used [85]. It is well known that sucrose is less favored by R. oryzae than glucose. Therefore, to see whether sucrose could be an alternative carbon-glucose source for lactic acid production, four different concentrations of sucrose, viz., 5, 30, 50 and 100 g/l were taken. The maximum amount of lactic acid was 21 g/l measured in 50 g/l sucrose. The use of sucrose at 100 g/l led to a significant reduction in lactic acid (Fig. 2). This might be due to the increased color of the little ones, possibly due to the amount of sucrose [3].

Currently, LAB are the main microorganisms used in LA production. However, there is a problem in its use. The low pH potential leads to the use of many decomposers including $CaCO_3$, resulting in the production of gypsum in the fermentation process. Compared to yeasts and bacteria, yeasts can tolerate low pH, reducing the need for neutralization and processing costs. The worst side effect of using wild yeast is reduced production of the main product, LA. However, synthetic hormones are the best solution to overcome these side effects [79]. Carob shells, a modern delicacy, have been shown to be a suitable carbohydrate source for lactic acid production. The supernatant, containing sugars extracted from carbohydrate capsules, can produce 58 g/l lactic acid, making it an alternative carbohydrate source.

Algae and cyanobacteria are classified as photosynthetic microorganisms and can grow character and produce a variety of chemicals (including organic matter (H_2), ethanol, lactic acid, AA, and FA) with a short harvest time of 1 to 10 days. Algal biomass, due to its high carbon and protein content and lack of lignin, may be presented as another candidate for low carbon LA production at reasonable costs. This study clearly shows that lactic acid production depends on the carbon source used in the fermentation process. As it is known, sugars defined as glucose and sucrose are the source of carbohydrates obtained from lactic acid fermentation, but it is also possible to use sub- or by-products such as carob pods, corn kernels and molasses [80]. Lactic acid in the form of other carbohydrate sources (1%) was investigated. Carbohydrate sources such as xylose, starch, lactose, maltose, sucrose, fructose and glucose were also investigated.

The most common substrate for fermentative LA production was the spread of waste from common yeasts used as animal feed. It contains proteins, salt and lactose. Whey can be hydrolyzed to glucose and galactose, purified and released from protein by ultrafiltration. Whey was added along with the yeast to remove the peptone from the milk powder or corn syrup. The most commonly used strength in the production of LA from whey was Lb. delbrueckii spp. Bulgaricus but in most studies Lb. Helveticus or Lb. [79] were used. Lactic acid was produced from food waste (FW) in a digester. Lactobacillus rhamnosus AW3, isolated from water-treated dates showed positive conversion of wastes to lactic acid. The combination of FW and MS to produce lactic acid resulted in a threefold increase in total production 4 ± 0.87 g/l. Using a batch fermenter with a good FW to MS ratio (2:0, 5%) reduced lactic acid production. Evolve from lactic acid, yeast, was a dietary ingredient with well pH (5.5) after 48 hours of fermentation. Lactic acid removal from L. rhamnosus AW3, lessened the soil pH and became greater soil phosphorus and therefore fertile ground. The uncovering was helpful for the biological conversion of FW into lactic acid and food waste for urban means [81]. Another commonly used substrate for LA production starch obtained from plants or waste. In order to be metabolized by LAB, it must be hydrolyzed to glucose and maltose. Legumes from a variety of sources, including wheat, maize, cassava, potato, rice, buckwheat, sorghum, and barley, have been used to produce LA. In some studies, starch was left untreated or soaked in water/gelatin and transformed with amylase producing organisms such as Lb. fermentum, Lb. amylovorus or Lb. amylophilus amylase can also be added to the hydrolysis of starch and LAB converts the glucose produced into LA. In one study, amylase was produced by Aspergillus awami and Lc. lactis produced LA from the obtained glucose. LAB used in the production of LA from starch contains: Lb. case, lb. plantarum, Lb. delbrueckii, Lc. lactis and Lb. helveticus.

3-6. Effect of nitrogen sources

Nitrogen source plays an important role in the production of lactic acid and its choice greatly influences the process. Different sources of nitrogen, such as urea, ammonium sulphate, malt sprout, and corn steep liquor (CSL), exhibit different levels of effectiveness. With slower bacterial growth rates, urea and ammonium sulphate generated LA yields of 26.68% and 19.14% respectively. Malt sprout and corn steep liquor produced significantly lower yields of 14.10% and 5.6

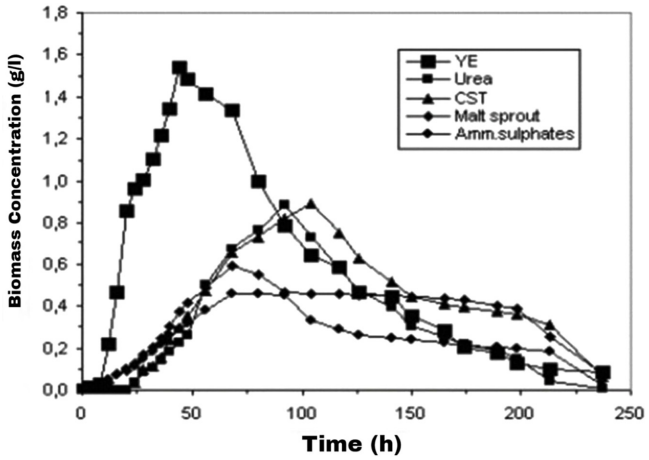

Fig. 6. A study on the growth of bacteria in pineapple waste fermentation with different nitrogen sources [88].

%. Although urea and ammonium sulphate were less expensive than YE, their support for a high yield or growth rate was diminished. For example, CSL obtained an 80% yield, which was lower than YE's yield but much cheaper, despite supplying a more cost-effective nitrogen source at a cost of about $90.78 per ton of D-lactic acid [86].

A recent study investigated the effect of nitrogen sources on LA production as shown in Fig. 6. The best nitrogen source was determined to be yeast extract, which gave an excess LA of 78.52%. Subsequently, urea, ammonium sulphates, maize steep liquor, and malt sprout yielded 26.68%, 19.14%, 14.10%, and 5.6%, respectively. The bacterial growth rate was fastest when yeast extract was used, with a lag phase of just 4 hours, opposite to 12 hours when ammonium sulphate, malt sprout, maize steep liquor and urea were used. In previous reports yeast extract has been characterized as the most effective nitrogen source for L. delbrueckii growth [87]. This was because yeast extract contains organic acids like pyruvic and glyceric acid, in addition to vitamins, amino acids, and peptides.

But on the other hand, yeast extract is a substrate that might impair the profitability of industrial scale LA synthesis due to its relatively high cost. Various other sources of nitrogen were investigated in an effort to overcome this limitation. In an effort to identify nitrogen source for LA biosynthesis, i.e., both inexpensive and abundant, the nitrogen concentration of nineteen different substrates both organic and inorganic was assessed. These substrates include food items, medium components, waste products, and leguminous plant seeds [89]. There were number of nitrogen sources which were investigated; the one with the greatest nitrogen source concentration were $CO(NH_2)_2$ (46.7%), NH_4NO_3 (35.0%), and peptone (8.8%). Yeast extract and peptone which have lower C:N ratio than NH_4NO_3 and $(NH_4)_2SO_4$, seem to produce more biomass, which may be explained by the fact that microbes have easy access to organic nitrogen sources [90]. YE offers excellent growth rates and yields, but its production has a huge environmental footprint and demands a lot of resources, therefore it's crucial to keep in mind when analyzing the environmental effect of nitrogen sources. Repurposing waste materials, agro- industrial byproducts like peanut meal hydrolysates, cottonseed meal and corn steep liquor (CSL) provide a more sustainable option. Nevertheless, these byproducts could include impurities that compromise lactic acid's optical purity. Cottonseed meal is a more cost-effective solution for preserving high choral purity since it has been shown to be devoid of mixed L-/D-lactic acid. Furthermore, both nitrogen sources based on ammonia and urea have different effects on the environment. Despite its widespread usage and low cost, urea may breakdown and generate strong greenhouse gases like nitrous oxide and ammonia. Climate change and air pollution are exacerbated by this. In addition, improper handling of urea can result in water contamination and soil acidification. Despite being a useful source of nitrogen, ammonia has a significant negative impact on the environment.

The cost of nitrogen is a critical factor. Depending on the concentration employed, using YE at optimum concentrations can lower the cost to $117-$500 per ton of lactic acid. On the other hand, although having a lesser yield using CSL lowers the cost to around $90.78 per ton, making it a more economical option. When utilized in larger concentrations, corn steep liquor and other agro-industrial wastes can be competitive with YE and offer considerable cost savings [91]. Effect of different nitrogen sources on lactic acid is shown in Table 4.

4. Conclusion

This study magnifies the critical role of several parameters impacting the production of lactic acid. Like the variation in optimal conditions such as pH, temperature, fermentation mode and substrate type highlight the need for a nuanced approach in optimizing production processes. Utilization of different LAB's and their effects has also been stated like, heterofermentative LABs, which employ the pentose phosphate route, are more versatile in substrate utilization but usually results in lower lactic acid yield, in contrast to homofermentative LABs.

Table 4. Effect of nitrogen source

Nitrogen source	L-Lactic acid ($g \cdot L^{-1}$)	Productivity ($g \cdot L^{-1} \cdot h^{-1}$)	Lactic acid yield ($g \cdot g^{-1}$)	Ref
Yeast extract and gluten	106	3.04	0.99	[92]
Yeast extract	88.5	0.92	0.896	[93]
Corn steep liquor	118	1.84	0.92	[88]
Soybean hydrolysate	114.6	2.61	1.02	[94]
Flax seeds	30.01	-	30	[89]
$(NH_4)_2SO_4$	33	-	33	[89]

The use of fungi like Rhizopus species offers promising avenues for direct conversion of starchy biomass, but their application requires addressing issues related to morphological control and fermentation aeration. Additionally, the review points out the huge potential of utilizing lignocellulosic biomass and agro-industrial waste as substrates, identifying an opportunity to shift towards more sustainable production Methods. However, challenges remain, particularly in balancing the different temperature and pH requirements for saccharification and fermentation and in improving the efficiency of lignocellulose pretreatment. Future research should focus on developing strategies for overcoming these challenges, such as enhancing microbial strain tolerance through genetic engineering, refining enzyme combinations, and improving pretreatment processes.

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