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FERMENTATION SUBSTRATES FOR THE PRODUCTION OF LACTIC ACID - A REVIEW

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ABSTRACT

Pure sugars or edible crops have been a traditional substrate for lactic acid production that is advantageous in obtaining a pure lactic acid product. This review looks at the various materials which have been considered as attractive alternative substrates and renewable resources. These include by-products of agricultural industries, food industries (food waste), and natural unutilized biomasses such as starchy biomass, lingo-cellulosic biomass, whey, yogurt, glycerol.

Keywords: Lactic acid, Lingo-cellulosic, Substrate, Industries, yogurt, glycerol

INTRODUCTION

Lactic Acid LA or three carbon acid is an organic acid which is used on very large scale in food, polymer, chemical, cosmetic, pharmaceutical, and biomedical industries. A rapid increment in the demand of LA has gained momentum due to its very wide industrial applicability both in food as well as in non-food industries. It is utilized in the food industry including beverage industry (as food preservative, fermentation agent, flavour enhancer, and decontaminant), antioxidant, prebiotic activity. The efficiency of lactic acid fermentation processes mainly depends on the lactic acid producer, fermentation substrate, and operational modes.

Raw material cost is one of the major factors in the economic production of lactic acid. Pure sugars or edible crops have been a traditional substrate for lactic acid production that is advantageous in obtaining a pure lactic acid product and lowering costs of pre-treatment and recovery

(Zakhodyayeva *et al.,* 2010; Zhou *et al.,* 2011). Since substrate cost cannot be reduced by process scale-up, extensive studies are currently underway to search for novel substrates for lactic acid production. Various materials have been considered as attractive alternative substrates and renewable resources, including by-products of agricultural industries, food industries, and natural unutilized biomasses such as starchy biomass, lingo-cellulosic biomass, whey, yogurt, glycerol, and algal biomass.

Food Waste

Food waste can include any compounds from the food production process to the wastes formed by the final consumer. Food waste contain a high amount of carbohydrate which causing it to be suitable as a substrate for lactic acid fermentation. Numerous studies stated food waste are suitable for lactic acid production such as kitchen residues/refuse and municipal solid wastes (Lopez-Gomez *et al.,* 2019), model kitchen refuse medium contain water, vegetables, meat/fish and cereals (Tashiro*et al.,* 2013), mixes of cooked rice, vegetables, meat, and bean curd (Zhang *et al.,* 2008; Kwan *et al.,* 2015) rice, noodles, meat, and vegetables (Kwan *et al.,* 2015; Pleissner*et al.,* 2013), vegetables such as carrot peel, cabbage, and potato peel, fruit such as banana peel, apple peel, and orange peel, baked fish, rice, and used tea leaves (Kitpreechavanich*et al.,* 2016; Tashiro*et al.,* 2016) rice, noodles, meat and vegetables, and unsold bakery products including cakes, breads and pastries (Neu*et al.,* 2016) rice, vegetables, and meat (Tang *et al.,* 2017a) coffee mucilage (Neu*et al.,* 2016) and coffee pulp (Pleissner*et al.,* 2016).

CARBOHYDRATES

Starchy biomass and sugar plant wastes (malt, molasses, and sugar beet juice)

Lactic acid can be produced from sugar plant wastes (molasses and sugar beet juice), starchy, and lignocellulosic biomasses. Disaccharides (lactose and sucrose) and monosaccharides hexoses (glucose, fructose, and galactose) and pentoses (xylose and arabinose) sugars can be fermented by LAB via EMP and/or the Pentose PK Pathway (Li *et al.,* 2010). Molasses are waste products containing a large amount of sucrose and other essential nutrients, which can derive from sugar cane and sugar beet from sugar manufacturing plants.

Several microorganisms can use molasses as a substrate including *Lb. delbrueckii* subsp. delbrueckii mutant Uc-3, *Lb. delbrueckii* NCIM 2025, *Lb. delbrueckii* NCIMB 8130, *Lb. delbrueckii* C.E.C.T. 286, *Lb. delbrueckii* IFO 3202, *Lb. delbrueckii*, *Lb. plantarum* (Coelho *et al.,* 2011), *Sporolactobacilluscellulosolvens* (Abdel-Rahman *et al.,* 2013), *Rhizopusarrhizus, Lb. casei* M-15 (Chaisu*et al.,* 2014), *Bacillus sp*. XZL9 and *E. faecalis*. Shukla *et al.* (2004) also reported that recombinant E. coli strain could produce D-lactic acid from molasses. Raw sugar beet juice with a Brix of at least 60 was used for LA production by lactic acid-producing microorganisms including bacteria (lactobacilli and moderately thermophilic bacilli due to fermentation at relatively high temperature such as *B. coagulans, B. thermoamylovorans,* and *B. smithii*, yeasts and fungi, such as, *Rhizopus* and *Aspergillus* (Visser*et al.,* 2012). Malt and date juice are another source for LA

production by *Lb. casei* subsp. rhamnosus in batch and fed-batch cultures with a maximum LA production level of 89.2 g/L already achieved (Nancib*et al.,* 2009; Oliveira et al., 2018).

There is a great interest to introduce cellulosic and starchy materials as substrates for LA production due to their abundance, low price and for being derived from renewable sources (Vink*et al.,* 2007). Amylolytic lactic acid bacteria (ALAB) such as *Lb. plantarum*, *Lb. fermentum* and *Lb. manihotivorans, Lb. amylophilus* and *Lb. amylovorus* can ferment starchy biomass into LA due to their α-amylases activity. Some ALAB were isolated from various amylaceous compounds, which include maize and maize-based fermented products, potato, cassava, and cassava-based fermented products (Abdel-Rahman *et al.,* 2013; Abdel-Rahman *et al.,* 2011), rice and rice-based fermented products, sweet sorghum, wheat, rye, oat, barley, and other starchy substrates (Komesu *et al.,* 2017).

Lignocellulosic biomass

There are abundant and cheap lignocellulosic materials worldwide, that include agricultural residues (corn stover, bagasse, and rice husk), forestry residues (sawdust), portions of municipal solid wastes (waste paper and brewer spent grains), herbs, switch-grass and shrubs (switch grass and water hyacinth), woody plants (poplar trees), Stems, straws, leaves, stalks, shells, husks, and peels from cereals like wheat, rice, barley, corn, sorghum and various industrial wastes (Idrees*et al.,* 2013). Cellulosic materials are composed mainly by cellulose, xylan, arabinan, galactan, and lignin (Abdel-Rahman *et al.,* 2013; Abdel-Rahman *et al.,* 2011). The addition of pectinases and cellulases in the fermentation medium can enhance LA production. However, fermentation of lignocellulosic hydrolysates is prevented by the inhibitory effect of some compounds including acetic acid, furfural, and 5-hydroxymethylfurfural, which are formed during pre-treatment of lignocellulose. To reduce this inhibition, studies were performed through physical and chemical detoxification of the hydrolysate, being this mentioned as the challenges that must be overcome for their efficient utilization.

For LA production, several cellulosic materials can be used as substrate, such as: pure cellulose (Ou *et al.,* 2011), lignocellulosic pentoses including xylose and arabinose, corncob, wastepaper (Abdel-Rahman *et al.,* 2013; Abdel-Rahman *et al.,* 2011), and wood. Yadav*et al.* (2020) indicated that *P. pentosaceus* SKL-7, *Lb. plantarum* SKL-19, *Lb. fallax* SKL-15, *Lb. plantarum* SKL-22, and *Lb. paracasei* SKL21 grew well in presence of 1-Ethyl-3-methylimidazolium-acetate, 1- Butyl-3-methylimidazolium methane-sulfonate and 1-Butyl-3-methylimidazolium chloride. The *L. plantarum* SKL-22 demonstrated relatively high tolerance with greatest specific growth rates in presence of 0.5% and 1% 1-Butyl-3-methylimidazolium methane-sulfonate and 1-Butyl-3-methylimidazolium chloride. L. plantarum SKL-22 formed reasonably good content of lactic acid 34.26 g/l, so promising strain for production of lactic acid from lignocellulosic biomass. Agricultural residues are another potential source of substrates for LA

production. This category includes: alfalfa fiber, wheat bran and straw, defatted rice bran (Jukonyte *et al.,* 2018), food wastes, corn stover and cob (Wang *et al.,* 2010b), barley bran husks, sugarcane and cassava bagasse (Laopaiboon *et al.,* 2010; Oliveira *et al.,* 2016), trimming vine shoots, wine-trimming wastes, apple pomace, banana wastes, mango peel (Jawad *et al.,* 2013), mussel processing wastes (Abdel-Rahman *et al.,* 2013; Abdel-Rahman *et al.,* 2011), cellulosic bio sludge, kitchen refuses and wastes, fish meal wastes, cardboard waste. (Yanez *et al.,* 2005) and sugarcane bagasse waste. Wastewater of paper sludges is another source that does not require pretreatment and have a high content of polysaccharide degradation products and short cellulose fibers.

LACTIC ACID PRODUCTION FROM DAIRY PLANT WASTES

Cheese Whey

Dairy industries all over the world generate ample amounts of whey from milk processing for various manufactured products. Whey is a by-product in wastewater discharged through the cheese production process, and its disposal is currently a major pollution problem for the dairy industry. Whey is a potent and suitable raw material for lactic acid production because it consists of lactose, protein, fats, water-soluble vitamins, mineral salts, and other essential nutrients for microbial growth (Panesar *et al.,* 2007).

Nevertheless, LAB requires supplementation with sources of amino acids and vitamins to the medium because they do not have sufficient proteolytic enzyme activities to utilize milk proteins in whey (Miller *et al.,* 2011). In addition, proteinized whey containing mainly lactose has been extensively studied for lactic acid production (Kim *et al.,* 2006; Li *et al.,* 2007; Schepers *et al.,* 2002, 2006). Theoretically, 4 mol of lactic acid should be produced from 1 mol of lactose through a homo-fermentative pathway after the cleavage of lactose to 1 mol of glucose and 1 mol of galactose. (Juodeikiene *et al.,* 2016)**.**

In addition, the produced lactic acid has an inhibitory effect in whey fermentation that can be alleviated to a certain extent by conducting fermentation in a continuous dialysis process in a hollow fibre fermentor or in an electro dialysis system with a mono-polar or dipolar membrane (Bazinet, 2004). Continuous fermentation of whey has resulted in high productivity and does not require high-volume fermenter. (Ghasemi *et al.,* 2017)

Yogurt

The market for yogurt has also grown rapidly over the past few years. Consequently, rejects of damaged or expired yogurt create a huge amount of waste products. Yogurt is usually sweetened with additional sugars, such as sucrose and glucose, which would result in higher lactic acid production than cheese whey containing fewer sugars. *Lb. casei* ATCC 393 achieved a bioconversion of total sugars of around 44% to 25.9 g/L of lactic acid at a yield of 0.9 g/g and productivity of 0.76 g/L/h in a pH-controlled batch fermentation process using yogurt whey (Alonso *et al.,* 2010); however, mixed sugar utilization of lactose, glucose,

and sucrose is a major problem that has to bead dressed for effective utilization of yogurt whey**.**

Dairy plant wastes have been used for LA production by Lactobacilli for a long history. Lactobacilli are fastidious microbes requiring complex macro and micronutrients. Due to the poor nutritional status of dairy plant wastes such as whey, which is the waste product produced during the cheese production and contains salts, lactose and small amounts of proteins, enrichment of whey with nitrogen sources such as yeast extract, peptone, soy flour or corn steep liquor significantly improved LA production (Abdel-Rahman *et al*., 2013).

LACTIC ACID PRODUCTION FROM GLYCEROL

The biodiesel industry is one of the most important technological biomass-based platforms because it has been considered as an environmentally friendly fuel. Its production reached more than11.1 million tons in 2008 with an annual growing production rate of close to 8%–10% (Posada *et al.,* 2012a). Different kinds of edible and inedible vegetable oils and algae oils have been widely used for biodiesel production worldwide. Consequently, over production of raw glycerol as a by-product is a continuous challenge, leading to by product wastes obtained at a weight ratio of 1:10 (glycerol: biodiesel) (Posada *et al.,* 2012b). Therefore, effective glycerol utilization as a cheap raw material would solve both economic and environmental drawbacks. Several microorganisms were reported to convert glycerol to lactic acid, including *Klebsiella* pneumonia, *Clostridium* pasteurianum, and *Lactobacillus* Brevis (Biebl, 2001; Cheng *et al.,* 2006). Kishimoto (2008) developed a method for producing lactic acid from glycerol using *Achromobacterdenitrificans* NBRC 12669.

However, only 3.9 g/L lactic acid was produced from 9.4 g/L consumed glycerol. A common characteristic of glycerol metabolism by wild type *E. coli under* either anaerobic or micro-aerobic conditions is the hetero-fermentation behaviour with the production of ethanol as the primary product and the negligible amount of lactic acid production (Dharmadi *et al.,* 2006; Durnin *et al.,* 2009; Gonzalez *et al.,* 2008; Murarka *et al.,* 2008). Hong *et al.* (2009) isolated *E. coli* AC-521, which produced 56.8 g/L and 85.8 g/L lactic acid with few by products in batch and fed-batch fermentations, respectively. Recently, lactic acid production from glycerol using engineered *E. coli* has been engineered a homo-fermentative route for D-lactate production by over expressing pathways involved in the conversion of glycerol to D-lactic acid and by disrupting the pathways leading to the synthesis of competing by-products. Using that engineered strain, 32 g/L of D-lactic acid was produced from 40 g/L glycerol in batch fermentation with a minimal medium.

CONCLUSION

Traditional or Fermentation substrate for lactic acid production show tremendous advantage in obtaining a pure lactic acid product and lowering costs of pre-treatment and recovery. Utilization of most substrates like glycerol, food waste, carbohydrate and so on, as a cheap raw material would solve

both economic and environmental drawbacks of different industries and reduce the issues of pollution both in our homes and the outside environment.

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