Lactic acid production by *Lactobacillus brevis* mediated bioprocesses utilizing lignocellulosic agro-wastes

Jasneet Grewal and S.K. Khare
Enzyme and Microbial Biochemistry Laboratory
Department of Chemistry, Indian Institute of Technology Delhi, New Delhi-110016, India.
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Presenting author email: g.jasneet@gmail.com

Introduction

The production of renewable energy with green processes has received a great deal of attention due to depleting fossil reserves coupled with adverse environmental impact. Thus 40 million tons of non-edible lignocellulosic biomass generated annually is perceived as a promising feedstock for producing biobased platform chemicals and energy.

Lactic acid bacteria (LAB) are emerging as major bioresource for use in future biorefineries by converting plant-derived biomass into biofuels and many high value-added products (Mazzoli et al., 2014). Lactic acid, their main fermentation product, is an important C3-platform chemical with wide applications in food, cosmetic, tanning and pharmaceutical industries. It serves as the parent compound for production of biopolymer poly-lactic acid (PLA), biodegradable solvent ethyl lactate, acrylic acid, pentanol and many other important chemicals. The biodegradability and biocompatibility of PLA makes it indispensable for both biomedical and industrial sector (Jang et al., 2012). About 400,000t of lactic acid is produced per year by chemical synthesis or fermentation. However, fermentation method being more sustainable and environment friendly, is highly desirable. It is less energy intensive and operates under mild conditions at lower temperature. The raw materials constitute 68% of the total cost of lactic acid production. Hence, choice of appropriate fermentable feedstock and microorganism is critical for effective bioproduction.

Lignocellulosic wastes can serve as ideal feedstocks for lactic acid production due to their renewability, unlimited abundance, high polysaccharide content and non-competitiveness with food sources. However, pretreatment of lignocellulosic feedstocks, followed by enzymatic hydrolysis is necessary prior to sugar fermentation by LAB. The ionic liquids (IL), dubbed as green solvents, are very effective in pretreatment of various lignocellulosics making them amenable to enzymatic hydrolysis for efficient saccharification followed by fermentation (Khare et al., 2015).

The present study deals with lactic acid production by *Lactobacillus brevis* from lignocellulosic agro-waste feedstocks by solid-state fermentation (SSF) as well as simultaneous saccharification and co-fermentation (SSCF). The flow of bioprocess is shown in Fig. 1.

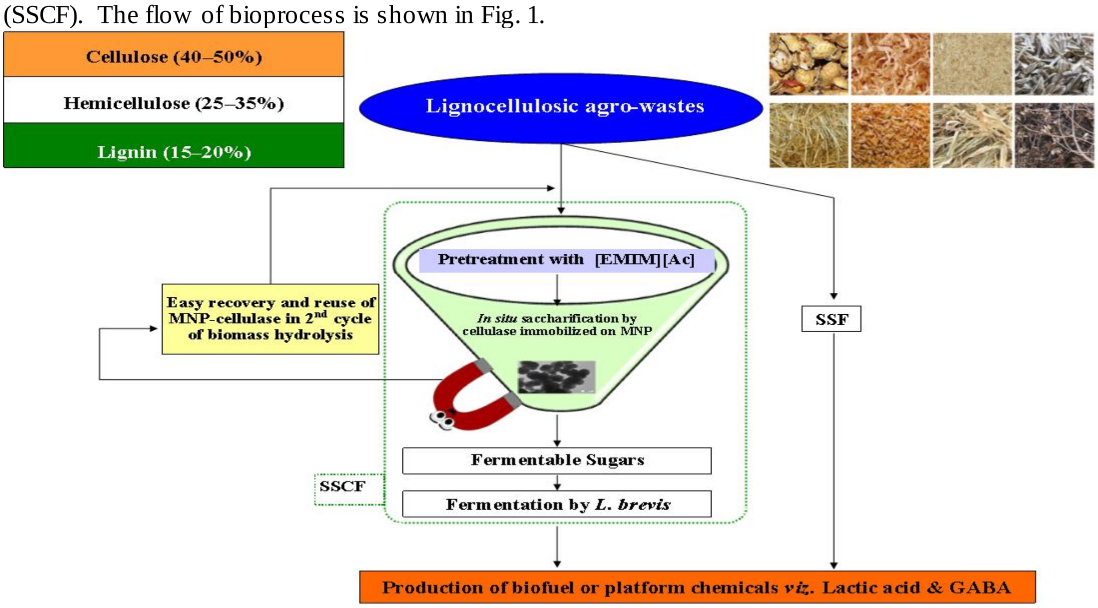

Fig.1- Lactic acid production by valorisation of agro-wastes

Material and methods

The bacterial strain *Lactobacillus brevis* (MTCC 4460) was procured from Microbial Type Culture Collection, IMTECH Chandigarh, India. Wheat straw and deoiled toxic cottonseed cake were used as agro-waste substrates for lactic acid production by *L. brevis* under SSF conditions (Grewal & Khare, 2017). The three lignocellulosic feedstocks i.e. deoiled cottonseed cake, wheat straw and sugarcane bagasse were pretreated by using 1-ethyl-3-methylimidazoliumacetate [EMIM][Ac] ionic liquid as described by Sadaf et al. (2016). For *in situ* saccharification of [EMIM][Ac] pretreated biomass, *Trichoderma reesei* cellulase was immobilized on amino-silane functionalized magnetic (iron oxide) nanoparticles (MNP). The overnight grown culture of *L. brevis* (A_{600}~ 1.011) was inoculated (5%, v/v) into this single pot system for fermentation of released sugars. The SSCF conditions i.e. pH 5 (0.05M, sodium acetate) and temperature (37°C) were suitable for operational activity of both *L. brevis* as well as immobilized cellulase. The reducing sugars and lactic acid were quantified on Agilent 1260 HPLC system equipped with Hi-Plex H column (7.7 × 300 mm) and refractive index detector (RID). The mobile phase was 5 mM H_2SO_4 at a flow rate of 0.5 ml/min.

Results and discussion

Solid state fermentation (SSF) is a cost effective tool for utilizing agro-waste feedstocks for growth of microbes resulting in bioproducts production. The presence of toxic gossypols in deoiled cottonseed cake restricts effective use of this abundant by-product. Grewal and Khare (2017) carried out bio-based economical production of γ-aminobutyric acid (GABA) from *L. brevis* by SSF using toxic deoiled cottonseed cake as substrate. GABA, a popular constituent of pharmaceuticals as well as nutraceuticals is an important C4 platform chemical. The microbially synthesized and purified GABA was used for synthesis of petro-derived 2-pyrrolidone, which is used as precursor for polymers especially nylon 4, polyvinylpyrrolidone (PVP). On similar lines, this study attempts lactic acid production by *L. brevis* fermentation of unutilized agro-wastes.

Wheat straw and deoiled cottonseed cake as a substrate in SSF simulates a natural habitat for microorganisms and are cheap substrates for lactic acid production. However, as *L. brevis* does not possess significant amylolytic or cellulolytic activity both agro-wastes were supplemented with glucose for lactic acid production. The overall lactic acid yield of 0.73 and 0.70g/g glucose was obtained with SSF of cottonseed cake and wheat straw respectively. Nonetheless, the SSF approach was a viable bioprocess as expensive synthetic media ingredients like yeast extract and other micronutrients were avoided coupled with valorization of waste biomass.

An IL stable and reusable cellulase was developed by immobilizing it onto MNP with immobilization efficiency of 85%. The immobilized cellulase being stable in residual [EMIM][Ac] allowed coupling of lignocellulosic pretreatment and saccharification in one pot with elimination of cumbersome and energy consuming washing process. The SSCF approach was used for lactic acid production by combining pretreatment, enzymatic saccharification and fermentation in a single one-pot system. The fermentable hydrolysate from three different agro-wastes i.e. deoiled cottonseed cake, wheat straw and sugarcane bagasse was prepared respectively by pretreating them with [EMIM][Ac] followed by enzymatic hydrolysis with immobilized cellulase. It gave high hydrolysis yields (≥85%) in first cycle and was consecutively used for next round of *in situ* saccharification after easy recovery by magnetic bar. The viability of *L. brevis* in presence of residual [EMIM][Ac] favoured the consolidated bioprocessing for lactic acid production. The SSCF process using lignocellulosic substrates viz. cottonseed cake, wheat straw and sugarcane bagasse resulted in lactic acid yield of 47%, 52% and 56% respectively. Similar lactic acid yields have been reported previously (Zhang & Vadlani, 2015; Mazzoli et al., 2014). The yields might be improved by co-inoculating a homofermentive LAB with *L. brevis*, which is a heterofermentive bacterium.

Conclusions

The lignocellulosic agro-wastes were optimally utilized for lactic acid production by *L. brevis*. Thus transformation of lignocellulosic biomass into valuable platform chemicals was achieved, providing an alternative feedstocks to petrochemical derived compounds. The green approach of pretreatment using ILs followed by subsequent hydrolysis, employing nanosupport immobilized cellulase for efficient one-pot SSCF process has not been explored previously for lactic acid production. The valorization of waste biomass, avoidance of synthetic media ingredients, easy recovery and reuse of immobilized cellulase for two consecutive SSCF cycles makes it a viable bioprocess for lactic acid production.

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