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Use of wheat straw biomass in production of L-lactic acid applying biocatalysis and combined lactic acid bacteria strains belonging to the genus *Lactobacillus*

Dalia Cizeikiene*, Grazina Juodeikiene, Jonas Damasius

Kaunas University of Technology, Department of Food Science and Technology, Radvilenu rd. 19, 50254 Kaunas, Lithuania

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ABSTRACT

The aim of the study was to investigate the usability of wheat straw in the production of L-lactic acid via fermentation applying by newly isolated lactic acid bacteria (LAB) strains belonging to the genus *Lactobacillus* and its combinations. Biotreatment of wheat straw was carried out through a three-step procedure consisting of: (i) physical pre-treatment, (ii) enzymatic hydrolysis and (iii) fermentation with LAB strains under laboratory conditions. Lactic acid production, residual sugar, cell biomass, pH medium and the influence of ions such as magnesium, calcium and sodium on efficiency of enzymatic hydrolysis of wheat straw were the main features examined. Moreover, optimal parameters for enzymatic hydrolysis were selected. Increased lactic acid production was observed, when mixed LAB cultures were used in comparison to individual ones. The results confirmed that tested wheat straw could be used for lactic acid and L-lactic acid production using selected enzymes and combined LAB strains.

1. Introduction

Lactic acid or 2-hydroxypropionic acid is a platform chemical, and its salts have a long history of commercial uses and wide applications (Wee et al., 2006). The interest in lactic acid is related to many aspects, among which is its relatively high added-value. In particular, the pharmaceutical and food industries have a preference for the L(+) lactic acid isomer, the only one that can be metabolized by the human body. However, the chemical industry requires one of the pure isomers or a mixture of both, according to the application (Martineza et al., 2013). This chemical has a market with great growth potential, can be alternatively produced by fermentation or chemical synthesis and can employ a large variety of different waste materials as substrates. The economics of lactic acid production by fermentation is dependent on many factors, of which the cost of the raw materials is very significant. It is very expensive when sugars, e.g., glucose, sucrose, starch and other etc., are used as the feedstock for lactic acid production (Abdel-Rahmana et al., 2011a). Therefore, lignocellulosic biomass is a promising feedstock for lactic acid production considering its great sustainability, availability, and low cost compared to refined sugars. According to the worldwide economic and environmental pollution issues the research interest in the recycling of lignocellulosic biomass into the various valuable chemicals such as lactic acid and other have been increased (Abdel-Rahman et al., 2011b; Anwar et al., 2014). Despite its advantages, commercial use of lignocellulose in lactic acid production is still under research. Cheap cellulosic materials such as agricultural waste, industrial solid waste are regarded as economically attractive feedstocks for lactic acid fermentation, which allow the utilization of agro-industrial waste as source of carbohydrate (Tang et al., 2011; Juodeikiene et al., 2016). Higher economical effect has been determined applying biotechnological conversion of wheat biomass to lactic acid vs chemical synthesis (Juodeikiene et al., 2015). The chemical route produces a racemic mixture of DL-lactic acid, while optically pure L(+)- or D(−)-lactic acid can be obtained by microbial fermentation. Since elevated levels of D-isomer are harmful to humans, L-(+)-lactic acid is the preferred isomer in food and pharmaceutical industries (Hofvendahl and Hahn-Hagerdal, 2000). Therefore, the search for microorganisms producing high content of L-lactic acid from lignocellulosic material is of outstanding importance. Many species have been used for lactic acid production. However lactic acid bacteria (LAB) belonging to the genus *Lactobacillus* are the most frequently used bacteria (Abdel-Rahmana et al., 2011a). LAB can be classified into 2 groups on the basis of the end product of their fermentation: homofermentative and heterofermentative. Homofermentative LABs virtually produce only lactic acid, whereas other products are generated by heterofermentative LABs along with lactic acid (Hofvendahl and Hahn-

* Corresponding author.
E-mail address: dalia.cizeikiene@ktu.lt (D. Cizeikiene).

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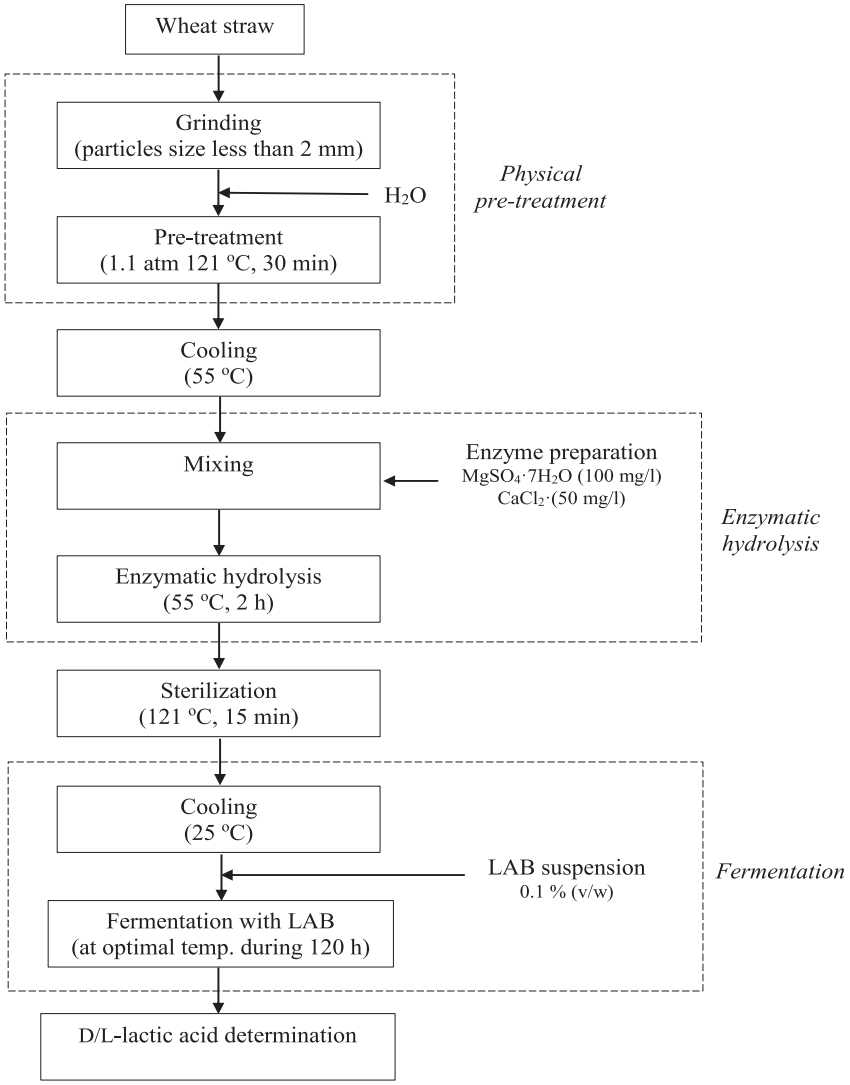

Fig. 1. A general flow chart of the "conventional" process for lactic acid production from wheat straw biomass.

Hagerdal, 2000). Recently synergistic effects of LABs have been reported regarding enhanced lactic acid production (KiBeom et al., 2005; Plessas et al., 2008). Lignocellulosic material requires pre-treatment applying physicochemical or enzymatic methods prior to lactic acid fermentation because the microorganisms cannot directly convert this material (Okano et al., 2010) into lactic acid. Therefore, the selection of specific enzymes and positive enzyme effectors for degree of hydrolysis increasing is necessary to obtain high content of bioproduct.

The aim of the study was to investigate the usability of wheat straw in the production of L-lactic acid via fermentation through a three-step procedure consisting of: (i) physical pre-treatment, (ii) enzymatic hydrolysis and (iii) fermentation with single LAB strains and their combinations under laboratory conditions.

2. Material and methods

2.1. Enzymes and microorganisms

Commercial enzyme preparation CeluStar XL containing xylanase, cellulase, β-glucanase and additional side activities such as pectinase, mannanase, xyloglucanase, laminarase, β-glucosidase, β-xylosidase, α-L-arabinofuranosidase, amylase and protease, a branded product of Dyadic International, Inc, was used.

Lactic acid bacteria (LAB) were previously isolated from wheat and rye sourdough and dairy environments. LABs were cultivated in a MRS broth (CM0359; Oxoid Ltd., Hampshire, UK) at optimal temperatures (for *Lactobacillus sanfranciscensis* MR29, *Lactobacillus frumenti* H10, *Lactobacillus rossiae* M2, *Lactobacillus crustorum* W19 and *Lactobacillus sanfranciscensis* MW15 at 25 °C; for *Lactobacillus rossiae* GL14 strain at 37 °C; for *Lactobacillus helveticus* DSM 20075, *Lactobacillus delbrueckii* subsp. *bulgaricus* MI, *Lactobacillus delbrueckii* subsp. *bulgaricus* DSM 20081 at 42 °C) for 24 h until further use.

After preliminary LAB screening for further experiments to evaluate synergetic activity of LABs three LAB mixtures ((i) *L. sanfranciscensis* MW15, L. *crustorum* W19 and *L. sanfranciscensis* MR29; (ii) L. *delbrueckii* subsp. *bulgaricus* DSM 20081 and L. *delbrueckii* subsp. *bulgaricus* MI; (iii) L. *crustorum* W19 and *L. sanfranciscensis* MR29) were prepared by mixing freshly grown single LAB strains. Equal quantity of mixed LAB strains was used for wheat straw fermentation at 25 °C in case of *mesophilic* strains and at 42 °C in case of thermophilic strains.

2.2. Plant material

The wheat was grown in experimental field located in Kaunas (Lithuania) in 2016. Wheat straw was dried to the level where it contained approx. 7% of water and was stored in dark environment at ambient temperature.