Research Article

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Lactic acid production from cane molasses

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Abstract: Molasses, a by-product of the sugar manufacturing process, generally comprises approximately 50% (w/w) of total sugars, but it is currently used primarily [1] as an animal feed and as a raw material in alcohol production. Currently, the sugar production is more than 160 million tones worldwide. Its byproduct molasses contain heavy metals which have growth-inhibitory effect. The main sugar content in molasses is sucrose which often need to be hydrolyzed to glucose and fructose especially for utilization by Lactobacillus species. *Lactobacillus* species can convert sugar content to lactic acid with great efficiency, which is a valuable chemical. Lactic acid production from sugar molasses using batch fermentations with *Lactobacillus casei* and *Lactobacillus sp*. *MKT878* were investigated in this study. Results showed, that both examined *Lactobacillus* species could grow on molasses despite the heavy metals inhibitory effects. The conversion of sugar content to lactic acid was successful with yield between 55-80 g/g.

1 Introduction

Lactic acid is a chemical that can be used as a preservative, acidulant, and flavor in the food, textile, and pharmaceutical industries. Lactic acid can also be used as a raw material for production of lactate esters, propylene glycol, 2,3-pentanedione, propanoic acid, acrylic acid, acetaldehyde, and dilactide in chemical industries [2–4]. The current demand on lactic acid has increased considerably due to its use as a monomer in the production of polylactic acid (PLA), which is a promising biodegradable, biocompatible, and environmentally friendly alternative to plastics derived from petrochemicals. [5][6]. Lactic acid can be commercially manufactured either by chemical synthesis or by biotechnological fermentation [7]. Biotechnological fermentation offers a cleaner, more ecological (environmentally-friendly) alternative to current processes used in petrochemical industries, thus preventing environmental pollution and the depletion of petrochemical resources [1]. While chemically synthesized dl-lactic acid is racemic (i.e. optically inactive) produced from petrochemical resources (e.g. ethylene), the optically pure L(+)- or D(–)-lactic acid can be produced by biotechnological fermentation processes using renewable resources. However, this can only be accomplished if the correct microorganisms are chosen for lactic acid fermentation. The optical purity of lactic acid is crucial to the physical properties of PLA, and only L(+)-lactic acid can be polymerized to a high-purity crystalline PLA. For ideally commercialization, PLA based plastics should manufactured from a given ratio of pure L- and D-dilactides to earn usefull fibers and films [6].

The manufacturing cost of lactic acid would be significantly reduced if a waste product, such as molasses, would be used as a raw material in the production of lactic acid[8].

Molasses, a by-product from the sugar manufacturing process, generally comprises approximately 50% (w/w) of total sugars [9]. Molasses is dark brown in color, has a strong odor, and a very high Chemical Oxygen Demand (COD) as well as high Biochemical Oxygen Demand (BOD) with concentrations up to 60,000 mg/L and 25,000 mg/L, respectively [10]. It is currently used as raw material in alcohol and yeast production and also used as an animal feed [11][12]. The global sugar production is over 160 million tonnes [13].

Additional pretreatments of molasses with sulfuric acid, tricalcium phosphate, potassium ferrocyanide and EDTA may be necessary to enhance the fermentation efficiency, because molasses contains low concentrations of heavy metals (namely, iron, zinc, copper, manganese, alumina) and alkali earth ions (as magnesium, calcium,). The above heavy metals might inhibit cell growth, can affect the pH of the media, and inactivate the enzymes

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associated with product formation [14][15]. Therefore the further purification of molasses is recommended, but may increase the fermentation costs due to complex raw material pretreatment [16].

Added invertase or β-fructofuranosidase (EC 3.2.1.26) can catalyzes the hydrolysis of sucrose into glucose and fructose without formation of hydroymethyl-furfural (HMF) and other inhibitory compounds in case of thermal hydrolysis of molasses. The enzyme acts on non-reducing fructofuranoside terminal residues of β-fructofuranosides. This process is favoured before fermentation by such microorganism, which cannot utilize sucrose and are sensitive against HMF, like most of *Lactobacilli*. Invertase enzyme plays furthermore a key-role in the food industry, especially as a catalytic agent to obtain an artificial sweetener [17]. Therefore invertase is easily available.

In this article, we describe the appropriate molasses pre-treatment for biotechnological production of lactic acid applying and comparing two *Lactobacillus species*.

2 Materials and methods

2.1 Microorganism

Lactobacillus casei (NCAIM B.02467) and *Lactobacillus sp. MKT878* (NCAIM B.02375) were used in this study. These strains are efficient lactic acid producing bacteria, which can convert glucose into L(+)-lactic acid through the homolactic fermentation pathway.

2.2 Pretreatment of molasses

Thermal pre-treatment was done after acidifying to pH=2.5 with sulphuric acid due to autoclaving at 121°C for 20 minutes.

Enzymatic inversion was carried out by

Invertase (Merck, Art. 7686), catalyzing the hydrolysis of sucrose into fructose and glucose. The activity of the enzyme was 200 U/mg at pH 4.5 and 25°C. The used dosage was 100 mg/L

The enzymatic pretreatment started with the addition of enzyme and continued for 30 minutes at pH 4.5, followed by sterilization. Finally the pH was set to pH 6.5 by addition of 20 % NaOH.

2.3 Culture medium

The MRS medium for cell growth was composed of the following (per liter): 10 g peptone, 10 g meat extract, 5 g yeast extract, 20 g glucose, 1 g Tween-80, 2 g K_2HPO_4, 5 g sodium acetate ($NaCH_3COO$), 2 g ammonium citrate $(NH_4)_3C_6H_5O_7$, 0.2 g $MgSO_4$ x 7 H_2O and 0.05 $MnSO_4$ x H_2O.

The medium for inoculum preparation and for fermentation studies contained (per liter): 215 mL enzymatically hydrolyzed molasses, 20 g yeast extract and 83 g $CaCO_3$. The $CaCO_3$ was only added to the inoculated culture vessel.

2.4 Inoculum preparation

Stock cultures were inoculated into 125 mL of MRS growth medium in 250 mL Erlenmeyer flasks for pre-inoculum, and incubated at 37°C for 24 h in a shaking incubator at agitation speed of 200 rpm. Pre-inoculum cultures were transferred into 125 mL of fermentation medium in 250 mL Erlenmeyer flasks for inoculum preparation. These were incubated at 37°C for 3 days on a shaking incubator with an agitation speed of 200 rpm.

2.5 Fermentation conditions

A 1-L jacketed glass bioreactor (Biostat Q, BBRaun) equipped with magnetic stirrer and pH electrode containing 630 mL of fermentation medium was used for the fermentation experiments. The culture temperature was controlled at 37°C, and the agitation speed was set at 300 rpm without aeration. The pH was controlled with 20% NaOH solution at 6.5. The samples were removed at regular intervals for further analysis.

2.6 Analytical methods

Cell growth was monitored by measuring the optical density (OD) after a five-fold dilution of both blank and sample with distilled water at 600 nm in a 1 mL cuvette using a spectrophotometer (Pharmacia LKB Ultrospec Plus, 80-2092-26). Lactose and organic acids were quantified by using high performance liquid chromatography (Waters Breeze) with an organic acid analysis column (Aminex HPX- 87H, Bio-Rad) operated at 65°C eluted by 0.5 mM H_2SO_4 as mobile phase with a flow rate of 0.5 ml/min.

3 Results and discussion

Preliminary fermentation tests were done to find the optimal molasses pre-treatment. Lactic acid fermentations with neither used *Lactobacilli* could start on any of three tested molasses after thermal hydrolysis (pH=2.5). Therefore we tested some modified hydrolysis methods: 1) acidification with sulphuric acid combined with boiling, followed by sterilization in autoclave (pH=7); 2) enzymatic hydrolysis followed by sterilization in autoclave (pH=7); 3) enzymatic hydrolysis followed by microfiltration (pH=7). All fermentation could start, but while fermentations following the 3rd pretreatment method were successful in 48h (having yield over 90% and LA concentration over 100g/L), the other fermentations reached LA concentration of 100g/L only after 100h.

To summarize these results it can be observed, that low pH sterilization generate inhibitory compounds, while both enzymatic or low pH boiling can be used, if pH is set to neutral before sterilizing.

Laboratory fermentations were conducted in bench top fermenters after enzymatic hydrolysis method.

History plots of fermentations are shown on Fig 1 and Fig 2. *Lactobacillus casei* utilized glucose and fructose content in parallel at similar rate (due to spontaneous isomerization into each other), while *Lactobacillus sp. MKT878* utilized glucose bit faster than fructose.

There was a break in the fermentation of *Lactobacillus casei* (Fig. 1) at around 40 hours caused by incomplete molasses hydrolysis. This observation was reinforced with additional enzyme supplementation to both bioreactors which initiated the stagnant OD to increase again.

During the fermentation, all of the glucose and a substantial amount of the fructose was consumed. The average initial substrate (glucose+fructose) concentration 162 g/L, which decreased to 16 g/L at the 150th hour. The initial lactic acid concentration was 35 g/L, half part of which originated from the molasses and the rest originated from the inoculum. At the end of the fermentation, the lactic acid concentration was 118 g/L. Therefore, there was a 0.57 g/g yield from the consumed 146 g/L substrate. The lactic acid productivity for the fermentation with *Lactobacillus casei* was 0.59 g/L*h.

The fermentation with *Lactobacillus sp. MKT878* was slower than that of *Lactobacillus casei*. As shown in Fig. 2, glucose was consumed faster than fructose. The glucose content was nearly depleted at the end of the fermentation. Fructose was subsequently consumed but was not depleted at the 150th hour. The average initial substrate concentration was 150 g/L, which decreased to 59 g/L at the 150th hour.

The initial lactic acid concentration was 35 g/L, part of which originated from the molasses and the remaining lactic acid was added with the inoculum. The lactic acid concentration at the end of the fermentation was 101 g/L, with a final yield of0.73 g/g and productivity efficiency of 0.44 g/L*h.

As shown in Table 1, *Lactobacillus casei* consumed more substrate than *Lactobacillus sp. MKT878*. Therefore, *L. casei* produced more lactic acid but with lower yield. Interestingly, *Lactobacillus sp. MKT878* consumed

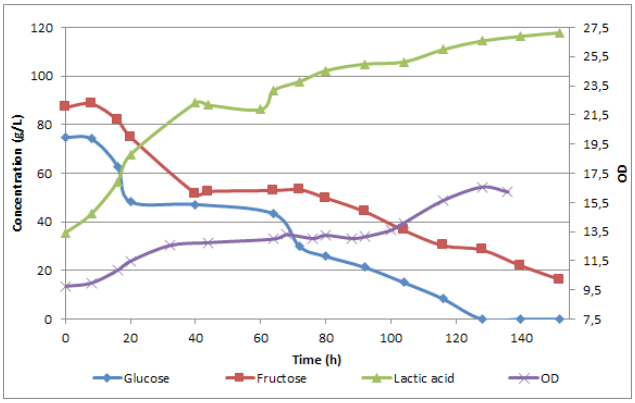

Figure 1. Cane molasses fermentation with *Lactobacillus casei*.

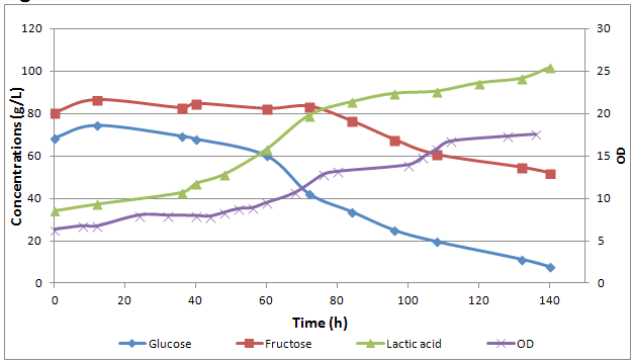

Figure 2. Cane molasses fermentation with *Lactobacillus sp.* MKT878.

Table 1 Cane molasses fermentation by *Lactobacillus species*

Microorganism	Consumed glucose	Consumed fructose	Consumed substrate	Produced lactic acid	Lactic acid yield	Productivity
L. casei	75 g/L	71 g/L	146 g/L	83 g/L	0.57 g/g	0.55 g/L*h
L. sp. MKT878	61 g/L	28 g/L	89 g/L	68 g/L	0.76 g/g	0.45 g/L*h

primarily the glucose content while *Lactobacillus casei* consumed glucose and fructose simultaneously.

The fermentation with *Lactobacillus sp. MKT878* was slow due to the long initial phase.

4 Conclusion

Cane molasses fermentations with *Lactobacillus species* were examined in this study. The two *Lactobacillus species* were capable of growing in cane molasses despite the growth-inibiting heavy metal content in the molasses. The amount of enzyme utilized or the length of time for pretreatment was insufficient as sucrose remained in the fermentation medium. The concentration of lactic acid produced after 150 hours was 68 g/L and 83 g/L with yields of 0.76 g/g and 0.57 g/g for *Lactobacillus sp. MKT878 and L. casei*, respectively. *Lactobacillus casei* utilized glucose and fructose in parallel, while *Lactobacillus sp. MKT878* utilized glucose faster than fructose. The reached productivities are far lower, than reported by other authors, but this could be happened because the lack of invertase for full hydrolysation of saccharose. Further improvements are required to improve the efficiency in the initial phase for the *Lactobacillus sp. MKT878* and to achieve a better yield for *Lactobacillus casei*.

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