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Pentose rich acid pretreated liquor as co-substrate for 1,3-propanediol production

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ABSTRACT

Lignocellulosic biomass is considered to be a potential raw material for production of renewable fuels like bioethanol and biodiesel. Cellulose and hemicelluloses constitute major portion of the lignocellulosic biomass. Cellulose can be converted to glucose by hydrolysis and subsequently to ethanol by fermentation. The hemicellulosic portion mostly contains pentose sugars which cannot be utilized by many microorganisms for ethanol production. Acid pretreatment results in separation of a pentose-rich fraction which can be utilized for the production of various high value chemicals. The present study evaluates the utilization of pentose sugars as co-substrate, along with biodiesel industry-generated crude glycerol, for the production of 1,3-propanediol (1,3-PDO). Bioconversion of these low value byproducts into a high value chemical would be an economically advantageous strategy in terms of waste disposal for biorefineries. In this study, the production of 1,3-propanediol from the acid pretreated liquor obtained from rice straw was evaluated using *Klebsiella pneumonia*. Different carbon sources like pure hexose and pentose sugars, mixed pentose sugar containing acid pretreated liquor (APL) from rice straw and different concentrations of pentose sugars and acid pretreated liquor were evaluated. There is 65% increase in titers from 9.55 g/L to 15.75 g/L using APL as co-substrate. With addition of 0.5% (v/v) APL, 1,3-propanediol production reached 20.88 g/L with 0.69 g/g yield and 0.87 g/L/h productivity. The study comprehensively explains the behavior of *Klebsiella pneumoniae* strain utilizing pentose rich APL and crude glycerol which enroute to an integrated biorefinery approach.

1. Introduction

The sustainable production of chemicals and fuels from renewable sources has been drawing tremendous attention in recent years. The current energy crisis, shortage of raw material for production of value added chemicals and environmental concerns associated with the processes have lead to exploration of bio-based fuels and chemicals. The production of biodiesel has increased considerably in the past few years. In the transesterification reaction occurring during the biodiesel production, glycerol is released as byproduct. This crude glycerol contains impurities like salts, methanol and soap. Glycerol in pure form finds various applications as additives in food and cosmetic industries, but the refining of crude glycerol was not found to be economical [1]. Hence crude glycerol is considered as a waste product rather than a by-product of biodiesel industry. But this outlook can be changed if the crude glycerol could be utilized as raw material for the production of value added chemicals like 1,3-propanediol (1,3-PDO).

Glycerol is the only known natural substrate for 1,3-propanediol production by microbes. The glycerol is metabolized through dismutation process, via oxidative and reductive reactions. The oxidative branch leads to production of by-products like succinate, acetate, lactate, ethanol, CO_2, H_2, butanol or 2,3-butanediol, bioenergy and reducing equivalents for growth and 1,3-propanediol production. The reductive process is a simple two-step reaction:

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first, glycerol dehydratase enzyme coded by dha B gene reduces glycerol to 3-hydroxypropionaldehyde; the intermediate is then reduced to 1,3-propanediol by NADH-dependent 1,3-PDO dehydrogenase enzyme encoded by dha T gene. The dismutation process of glycerol is to regenerate reducing equivalents to maintain physiological equilibrium. The theoretical maximum yield of 1.3-PDOfrom glycerol is approximately 0.72 g $_{1,3\text{-PDO}}$/g $_{glycerol}$ [1,2]. As 1,3-PDO has high market demand due to its applications in production of polymers like polytrimethylene terephthalate (PTT), an economical process should be developed to increase the maximum conversion yield. If cheap raw materials are supplemented along with glycerol in a co-fermentation process, the yield of1,3-PDO might be improved, leading to a cost-effective process.

Lignocellulosic biomass, such as rice straw, is a renewableresource for production of biofuels. It contains cellulose, hemicellulose and lignin as primary cell wall components. In order to derive sugars from this biomass; various pretreatments need to be carried out. One among them is dilute acid pretreatment, wherein the pentose sugars get solubilized in the liquid fraction. The cellulose-rich solid residue can be enzymatically hydrolyzed to extract hexose sugars like glucose that could be subsequently fermented to ethanol. To improve the overall utility of the process, there should be effective utilization of pentose sugars for the production of ethanol or any other value added compounds [3].

In recent reports, efficient utilization of lignocellulosic hydrolysates as co-substrates in glycerol fermentation using *Clostridium diolis* [4], *Klebsiella pneumoniae* [5,6] and *Lactobacillus diolivorans* [7] have been demonstrated. But these studies were carried out using the oligomeric sugars obtained after enzymatic hydrolysis. In the present study, utilization of acid pretreated liquor (APL) as co-substrate for 1,3-PDO production using *Klebsiella pneumoniae* PD41was investigated. Comparisons of 1,3−PDO production from pure sugars as well as crude biomass hydrolysates were also carried out.

2. Materials and methods

2.1. Raw materials

Rice straw was obtained from the Kerala State Horticultural Products Development Corporation. The particle size was reduced to approximately 3 mm using knife mill. The milled biomass was stored in sealed bags under dry conditions.

2.2. Acid pretreatment of rice straw

The milled rice straw was pretreated at 10% (w/w) biomass loading and 1% (w/w) H_2SO_4 concentration at 121 °C for 1 h. After cooling, the mixture was neutralized to pH 6−7 using 10N NaOH. The acid pretreated liquid portion (APL) was separated from the pretreated slurry and was used for further studies [3]. The solid mass separated from APL was hydrolyzed enzymatically using enzymes, and the monomeric sugars obtained were fermented to ethanol, this part of the study is not discussed in the current report.

2.3. Microorganism and media composition

Klebsiella pneumonia was isolated and characterized in earlier works (Not published). The cultures were maintained on Luria-Bertani (LB) agar and glycerol stocks were maintained at −80 °C.

LB broth (containing 10 g/L casein enzymatic hydrolysate, 5 g/L Yeast extract, 10 g/L Sodium chloride) was used as seed culture medium to prepare the pre-inoculum. Production media components used were (composition in g/L) K_2HPO_4:5; KH_2PO_4: 3; $(NH_4)_2SO_4$: 2; $MgSO_4 \cdot 7H_2O$: 0.4; $CoCl_2 \cdot 6H_2O$: 0.004; yeast extract: 2; bacterial peptone: 2.5; beef extract: 1.5; glycerol: 30.

2.4. Shake flask experimental design

The first set of experiments were carried out in order to optimize the physical parameters considering the culturing of microorganism in micro-aerophilic conditions for 1,3-propaendiol production. The micro-aerophilic conditions were maintained by sparging sterile nitrogen at a flow rate of 0.2 vvm and incubation was carried out in air tight Schott-Duran bottles. Later, effect of various nitrogen sources like ammonium nitrate, sodium nitrate, potassium nitrate, yeast extract, peptone, beef extract, ammonium chloride, ammonium dihydrogen orthophosphate, casein hydrolysate, ammonium sulphate, tri-ammonium citrate and corn steep liquor on 1,3-PDO production were evaluated.

In order to increase the yield and titers of 1,3-PDO, the experiments were conducted by addition of co-substrates such as glucose or biomass derived sugars, which act as external carbon source for growth and generation of reducing equivalents. Hence glycerol will be available for the microorganism for 1,3-PDO production. Initially pure sugars and APL were supplemented as co-substrates to the production media respectively. Later the concentration of arabinose, xylose and APL was optimized. The pre-inoculum was prepared by transferring a loopful of culture into 250 ml Erlenmeyer flasks filled with 100 ml of sterile LB broth, and then incubated at 30 °C, 200 rpm for 12 h. Then 2% of pre-inoculum was transferred into sterile production media in screw cap Schott-Duran bottles and then incubated in a rotary shaker at 30 °C, 200 rpm for 24 h.

2.5. Pearson correlation coefficient

A Pearson correlation coefficient was calculated between the initial pH and final metabolites analyzed after fermentation. The correlation and significance calculation were made using Minitab 17 statistical software (Trial version).

2.6. Analytical methods

Cell growth analysis was carried out in LB broth and production media as mentioned above. Incubation was done at 30 °C in static conditions. Optical density of media noted at 600 nm in UV-Visible spectrophotometer (Schimadzu series UV 1601) at 1 h interval after inoculation.

The concentrations of the metabolites (1,3-PDO, lactic acid, acetic acid, succinate, and ethanol) and residual glycerol in the production media were measured using High Performance Liquid Chromatography (HPLC, Shimadzu, Japan) equipped with refractive index (RI) detector using Phenomenex Rezex-ROA Organic acid column (300 × 7.8 mm) with column temperature at 65 °C and 0.01N H_2SO_4 as the mobile phase at a flow rate of 0.6 ml/min. The samples were centrifuged at 8000 rpm for 5min, and then filtered using Nylon 6,6 membranes (pore size 0.22 μm) [8,9]. The sugars present in the acid pretreated liquor was analyzed using HPLC (Shimadzu, Prominence UFLC, Japan) with refractive index (RI) detector using Phenomenex Rezex RPM Monosaccharide column, with oven temperature maintained at 85 °C, and deionized water as mobile phase at a flow rate of 0.8 ml/min [3].

3. Results and discussion

3.1. Pretreatment of rice straw

Plant cell walls are composed of cellulosic microfibrils sheathed by hemicelluloses and lignin. Compared to cellulose, hemicellulose has branches with shorter lateral chains, thereby making them easier to hydrolyze [3]. Dilute acid pretreatment of rice straw solubilizes the hemicellulose fraction of the biomass, as is evident

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