Bioresource Technology 147 (2013) 299–306

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Impact of varying lignocellulosic sugars on continuous solvent production in an immobilized column reactor

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HIGHLIGHTS

- Study suggests the preference of biomass feedstocks appropriate for ABE-fermentation.
- Effect of varying lignocellulosic sugar concentrations in continuous column reactor.
- Multi linear regression (MLR) modeling successfully used for continuous production of solvents.
- Scale up of column reactor is successfully demonstrated.

ARTICLE INFO

Article history:
Received 28 June 2013
Received in revised form 6 August 2013
Accepted 8 August 2013
Available online 15 August 2013

Keywords:
ABE fermentation
Butanol
MLR modeling
Clostridium acetobutylicum

ABSTRACT

The effect of varying glucose, mannose and xylose concentrations on continuous solvent production at various dilution rates was studied by multiple linear regression (MLR) modeling using an immobilized column reactor. The factors affecting the solvent production were dilution rate and concentrations of glucose and mannose. MLR-models also showed a preference of glucose as well as its inhibitory effect on xylose consumption. The fermentation process was studied at bigger scale with a volume factor of 17 with an added recirculation loop in the system. The up-scaled reactor produced 12.5 g/l of acetone–butanol–ethanol (ABE) solvents at a dilution rate of 0.23 h^{-1}, as compared to 13.4 g/l with a smaller column reactor. The xylose utilization was significantly higher in the modified reactor (73%) as compared to the small scale (43%).

1. Introduction

The depletion of fossil fuels, increasing cost of fuels, adverse effect on climate and geopolitical tensions have intensified the research for renewable fuels. The reduction of greenhouse gas emissions is necessary to control the hazardous climate change (Ranjan and Moholkar, 2012). There are different biofuel options such as biodiesel, bioethanol, biobutanol and higher alcohols which are sought as replacements for fuels produced from fossils. Bioethanol is also an option with shortcomings such as corrosiveness, which limits its transportation with pipelines. It can also separate from the gasoline in the presence of water due to its solubility characteristics, if used as a blend (Jin et al., 2011). *n*-Butanol and isobutanol are regarded as potential substitutes for gasoline. These compounds have an energy density (27 MJ/l) close to that of gasoline (32 MJ/l) and lower hygroscopicity compared to ethanol. They provide advantages such as high energy content, possibility to mix with gasoline and diesel in any ratio, easy transport via pipelines, possibility to use in current engines and reduced risk of groundwater contamination due to lower water solubility (Ranjan and Moholkar, 2012; Lan and Liao, 2013).

The choice of a feedstock is extremely important in commercialization of the acetone–butanol–ethanol (ABE) fermentation. The feedstock cost is the most significant cost factor of the total production costs (Gapes, 2000). The investment on a sugarcane biorefinery with butanol production showed to be more attractive than the conventional 50:50 (ethanol:sugar) annexed plant when butanol is produced by an improved microorganism and traded as a chemical (Mariano et al., 2013). The feedstocks utilized in ABE-fermentation are divided into three groups such as starch containing biomass, biomass containing cellulose and hemicellulose and fruits and vegetables containing monosaccharides including fructose, glucose and xylose (Ranjan and Moholkar, 2012). Other alternative resources such as macroalgae biomass, waste proteins, syngas, and CO_2, have also been explored as renewable raw material to produce these compounds (Lan and Liao, 2013).

Lignocellulose is a prime example of a multi-carbon-source-feedstock. It contains significant fractions of glucose, xylose and mannose polymers which can be hydrolyzed and utilized as monosaccharides in fermentation. In addition to these three components, lignocellulose also contains small amounts of other sugar

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http://dx.doi.org/10.1016/j.biortech.2013.08.058

polymers such as galactan and arabinan. The sugar composition of lignocellulose varies among different feedstocks. Generally softwood contains relatively more mannose and hardwood contains more xylose (Rakkolainen et al., 2010).

The use of various lignocellulosic hydrolysates for ABE fermentation has been reported in the literature. There are very few reports on the use of these in continuous reactors. Survase et al. (2011) used SO_2–ethanol water (SEW) spent liquor from the spruce chips as a feed. They reported the maximum productivity of 4.86 g/l/h. The sugar composition of the feed used was (in g/l) glucose, 29.7, mannose 13.0, arabinose 1.3, galactose 2.4, and xylose 6.2. Qureshi et al. (2010a,b) reported use of various agricultural residues such as barley straw, corn stover and switchgrass hydrolysates for butanol production. The sugar composition for all the hydrolysates was varying. Recently, Moradi et al. (2013) suggested the alkaline and phosphoric acid pretreatments as promising pretreatment processes for efficient production of ABE from rice straw. They reported production of more than 44 g butanol and 17 g acetone was produced from each kg of rice straw using both of the pretreatments followed by enzymatic hydrolysis and fermentation. The effect of concentrations of glucose and xylose was studied by Chen et al. (2013) in an immobilized continuous column reactor using *Clostridium acetobutylicum* CGMCC 5234. They reported that, in mixed-sugar fermentation (30 g/l glucose plus 30 g/l xylose), the immobilized cultures produced 11.1 g/l butanol with a yield of 0.19 g/g, 28.3% higher than with the suspended cells (8.65 g/l). The glucose was completely utilized in both cases whereas, 3.46 and 13.1 g/l of xylose remained unutilized for immobilized and suspended cells, respectively. Thus the main reason for the lower yield with suspended cells is explained by the low utilization of the xylose while the yield from consumed sugars is close to that with immobilized cells (0.18 vs. 0.19 g/g).

There are reports on modeling of various parameters in ABE fermentation process including stoichiometric, kinetic and black box models. Recently, Mayank et al. (2012) reviewed mathematical models of ABE fermentation. Qureshi et al. (1988) developed a model for a continuous immobilized reactor system to assess the kinetics of cell growth and the differentiation of cells in the reactor. They found that the amount of solvent producing cells in the reactor was fairly constant during the operation although the inactive cell mass increased. This accounts for the relatively low specific productivity observed in the immobilized ABE-systems.

The purpose of this study is to investigate the continuous production of ABE in an immobilized plug flow reactor using *C. acetobutylicum* DSM 792 when media containing various mixtures of pure glucose, mannose and xylose were fed to the process at various dilution rates. Optimal design of experiments was used as a framework for regression modeling. The results could indicate the differences in the usage of various lignocellulosic feedstocks, depending upon their sugar composition and raw materials.

2. Methods

2.1. Materials

Glucose and xylose was purchased from VWR International, Finland. Yeast extract and tryptone were purchased from Lab M Ltd. UK. Mannose was purchased from Danisco, Finland. *p*-Amino benzoic acid, $MnSO_4$, $MgSO_4$, $FeSO_4$, were obtained from Fluka, Switzerland. L-cysteine hydrochloride and biotin were purchased from Sigma Aldrich, USA. Ammonium acetate, K_2HPO_4 and KH_2PO_4 were obtained from Merck, Germany. NaOH, HCl and H_2SO_4 were obtained from J.T. Baker, Holland. All the chemicals were analytical grade.

The wood pulp fibers were obtained from the Department of Forest Products Technology, Aalto University School of Chemical Technology, Espoo, Finland. The pulp fibers were prepared by using SEW fractionation method (Sklavounos et al., 2011).

2.2. Culture maintenance, inoculum and production medium

C. acetobutylicum DSM 792 was obtained from DSMZ, Germany (German Collection of Microorganisms and Cell Cultures). The spore suspension prepared as stock with 7% corn starch was activated by a heat shock at 80 °C for 10 min. The activated spore culture (2.5 ml) was inoculated in 100 ml sterile reinforced clostridia medium (RCM) in 125 ml air tight, anaerobic glass bottles and it was grown for 20 h at 37 °C and used as an inoculum for 1 l RCM medium. This was grown for 20 h at 37 °C and used for immobilization of column reactor. The RCM contained (in g/l) meat extract 10, peptone 5, yeast extract 3, D(+) glucose 30, starch 1, sodium chloride 5, sodium acetate 3 and L-cysteine hydrochloride 0.5 (final pH 6.8 ± 0.2).

The medium reported by Survase et al. (2011) was used as the production medium which contained (in g/l), magnesium sulphate 0.2, sodium chloride 0.01, manganese sulphate 0.01, iron sulphate 0.01, potassium dihydrogen phosphate 0.5, di-potassium hydrogen phosphate 0.5, ammonium acetate 2.2, biotin 0.01, thiamin 0.1 and *p*-aminobenzoic acid 0.1. The production medium was modified according to the sugar mixture composition for each experiment. The total sugar concentration was varied between 30 and 70 g/l. Individual sugars were varied as glucose (20–40 g/l), mannose (3–11 g/l) and xylose (7–19 g/l). The medium was adjusted to pH 6.5. After preparation, the medium was purged with nitrogen and autoclaved at 105 kPa (121 °C) for 20 min and cooled.

2.3. Preparation of the immobilization column

The immobilized column reactor was prepared using wood pulp as an immobilization material. The column reactor was prepared using the method reported by Survase et al. (2011, 2013). A piece of a polyethylene mesh (18 cm × 20 cm) was cut out to serve as a support structure for the wood pulp. The wet pulp fibers (40 g) was distributed evenly on the mesh and rolled as tightly as possible so that the support mesh formed the outer layer of the roll. The roll was inserted to a Pharmacia XK 26 purification column (Pharmacia, Sweden). The height of the pulp roll was 18 cm and the diameter 2.5 cm. The thickness of an individual pulp layer was about 0.3 cm. The whole immobilization matrix was sterilized with 70% ethanol for 24 h and used for the immobilization of cells. The immobilization was performed using an inoculum grown in RCM medium for 20 h, by recirculating through the column for 22 h. Production media with varying sugar contents were continuously fed to the immobilized cell reactor at different dilution rates. After changing the dilution rate, the culture was allowed to stabilize (approx. three column volumes) indicated by a stable solvent and acid production and substrate consumption. The samples were taken from the top of the column and centrifuged at 15,000 rpm for 5 min and supernatants were used for the substrate and product analysis. The column temperature was maintained at 37 °C by continuously circulating water through the jacket.

2.4. Design of experiments

Four different parameters including dilution rate and the concentrations of the three main lignocellulosic carbohydrates, namely glucose, mannose and xylose were chosen as factors to evaluate the effect on ABE solvent production and sugar component utilization in immobilized continuous column reactor. Full factorial design with four factors and three levels was chosen for