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Detoxification of acidic biorefinery waste liquor for production of high value amino acid

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HIGHLIGHTS

- Detoxification of APL using adsorbent (ADS 800 & ADS 400), ion-exchange (A-27MP & A-72MP) resins.
- ADS 800 resin removed 85% furfural and 60% of HMF from APL.
- Detoxified APL was superior to APL L-lysine production.
- ADS 800 resin could reuse up to six cycles after regeneration.

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ABSTRACT

The current study evaluates the detoxification of acid pretreatment liquor (APL) using adsorbent (ADS 400 & ADS 800) or ion-exchange (A-27MP & A-72MP) resins and its potential for amino acid production. The APL is generated as a by-product from the pretreatment of lignocellulosic biomass and is rich monomeric sugars as well as sugar degradation products (fermentation inhibitors) such as furfural and hydroxymethyl furfural (HMF). Of the four resins compared, ADS 800 removed approximately 85% and 60% of furfural and HMF, respectively. ADS 800 could be reused for up to six cycles after regeneration without losing its adsorption properties. The study was further extended by assessing the fermentability of detoxified APL for L-lysine production using wild and mutant strains of *Corynebacterium glutamicum*. The detoxified APL was superior to APL for L-lysine production.

1. Introduction

The global energy demand is expected to rise by 33% between 2013 and 2040, with the major surge coming from non-OECD countries. Energy related CO_2 emissions are projected to be 16% higher by 2040 (IEA, 2015). Currently, fossil fuels, especially crude oil, are the predominant energy source, but this sector is vulnerable to price fluctuations and supply issues. Due to rising concerns regarding their limited availability and adverse impacts on the environment, it is now critical to develop feasible technologies for the production of alternate fuels such as bioethanol. Biomass as an energy source has many advantages because the use of biomass is essentially carbon neutral, avoids competition for the existing arable land and aids in stopping the increase of and even slowly reducing the CO_2 content of the atmosphere (Metzger and Hüttermann, 2009; Olah et al., 2009).Though a huge amount of lignocellulosic biomass is generated as surplus, the lack of an economically feasible platform for the efficient conversion of this biomass is a concern. Addressing this issue might be one step towards the commercialization of biomass processing.

Pretreatment process is essential for biomass-based biorefineries as the lignocellulosic biomass is not susceptible to enzymatic hydrolysis. Dilute-acid (DA) pretreatment is one of the strategies employed to pretreat the biomass in order to make it more amenable to subsequent hydrolysis (Alvira et al., 2010). To date, the industry is still in a nascent state due to higher operational costs associated with biomass processing. The liquor obtained after pretreatment (acid pretreatment liquor, or APL) is a hemicellulose rich by-product having approximately 15.2 mg/ml xylose, which can then potentially be fermented to a variety of products like sugar alcohols, organic acids, amino acids etc. The value addition from waste streams generated during the biomass processing might improve the overall economics of the process (Yu et al., 2015).

Unfortunately, the liquor obtained after pretreatment contains several inhibitors of microbial growth, which can be broadly

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sub-divided into furan derivatives, aliphatic acids and phenolic-compounds. Furan derivatives include furfural, formed from the degradation of pentoses, and hydroxymethylfurfural (HMF), that results from the degradation of hexoses. HMF can be further degraded to levulinicacid and formic acid. Acetic acid, the major aliphaticacid present, is released from the hemicellulosic acetyl-groups. During dilute acid pretreatment, a portion of ligninis also degraded to a wide range of aromatic compounds, the low molecular mass phenolics being the most toxic (Schwartz and Lawoko, 2010). However, the presence of sugar degradation products such as furfural, HMF, acetic acid and formic or levulinic acid can be inhibitory to the microorganisms as they prevent the utilization of sugars present in the APL. In order to be able to use the DA pre-treatment liquor for fermentation, it is essential to remove or to reduce the concentrations of these inhibitors via detoxification.

Detoxification can be carried out by different chemical and enzymatic methods like treatment with alkalis, sulphites, laccases etc., or by adsorbing these compounds on to carriers like activated carbon and other synthetic resins (Sandhya et al., 2013). In the present study, two adsorbent resins (ADS-400 and ADS-800) and two ion-exchange resins (A-27MP and A-72MP) were evaluated for their ability to remove inhibitors from the APL. The potential of different solvents to regenerate the resins and the reusability of these resins was also assessed. The study was further extended by comparing the growth and fermentability of *Corynebacterium glutamicum* (wild and engineered strains) in the native as well as detoxified APL.

2. Methods

2.1. Biomass

Sugarcane trash (excluding the stem and roots) was harvested from Erode, Tamil Nadu, India. 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. Dilute acid pretreatment

The milled sugarcane trash was pretreated at 25% (w/w) biomass loading and 4% (w/w) acid concentration at 121 °C for 1 h. After cooling, the mixture was neutralized to pH 6–7 using 10 N NaOH. The liquid portion (APL) was separated from the pretreated slurry and was used for detoxification studies.

2.3. Detoxification of APL

The Tulsion® resins were activated as recommended by the supplier before detoxification studies. ADS-400 and ADS-800 (Thermax, India) were washed with warm water for 30 min. The anion-exchange resins A-27MP and A-72MP (Thermax, India) were activated using 5% NaOH, after which they were washed with distilled water until the pH was lowered to 7. The properties of resins are presented in Table 2. Detoxification studies carried out in batch mode at room temperature. The resins were added to the APL in the ratio 1:15 (w/v). Samples were collected at different time intervals and analysed for inhibitor and monomeric sugar content. After each cycle of detoxification, the resins were regenerated by washing with 70% ethanol at 50 °C followed by hot water wash at 80 °C.

Regeneration of ADS 800 was carried out by using different solvents such as 70% ethanol, 70% methanol and 70% acetone. The reusability of the resins was evaluated by using them for repeated cycles of detoxification. The adsorption maxima of furfural and HMF on ADS-800 was determined by varying the inhibitor loading (10–120 mg) on 100 mg of the resin. For the adsorption maxima studies, the reaction volume was made up to 1.5 ml using distilled water and after 6 h of continuous mixing, the amount of unadsorbed inhibitors was determined.

2.4. Fermentation

The fermentability of APL and detoxified APL was studied by using *C. glutamicum* DM1729 and the engineered *C. glutamicum* DM1729 (pEKEx3-$xylA_{Xc}$-$xylB_{Cg}$)(pVWEx1-*araBAD*) having xylose and arabinose utilizinggenes and point mutations in $lysC^{P458S}$, hom^{V59A}, pyc^{T311I} (Gopinath et al., 2011). The strains used in the study were obtainedfroman Indo-German collaborative project with Prof. Volker F. Wendisch, University of Bielefeld, Germany.

2.4.1. Pre-inoculum and inoculum preparation

The two strains of *C. glutamicum*were maintained on Luria Bertani (LB) agar plates, and then cultivated overnight in LB broth containing 0.5% glucose. CGXII media (Eggeling and Reyes, 2005) was used to grow the L-lysine producers. One loopful eachof the two strains mentioned above was inoculated and incubated at 30 °C and 200 rpm for 18 h. The growth was followed by measuring the OD_{600} with UV 160A spectrophotometer (Shimadzu). The biomass concentration was calculated from OD_{600} values using an experimentally determined correlation factor of 0.25 g cell dry weight/l for OD_{600} = 1 (Wendisch et al., 2000).

2.4.2. L-lysine fermentation

APL obtained from the pre-treatment of sugarcane trash was concentrated using rotavapor (Buchi, India) to maintain the sugar concentration at 4%. The plasmid harbouring cultures (having Spectinomycin and Kanamycin selectable markers) were induced with 1 mM IPTG. Amino acid fermentation was carried out in the specified production media (CGXII) with the carbon source replaced by either the APL or detoxified APL. All the flasks were incubated at 30 °C and 200 rpm for 120 h. The samples were retrieved at intervals of 24 h for biomass, amino acid and sugar utilization analysis.

2.5. Analysis

2.5.1. Sugar and inhibitor analysis

The monomeric sugars present in the APL analysed by using HPLC (Shimadzu Prominence UFLC) fitted with Rezex® RPM Monosaccharide Pb^{+2} column (8% cross-linked) (column dimensions- 300 × 7.8 mm) (Phenomenex, India) and RI detector. The column temperature was maintained at 80 °C and the flow rate was 0.6 ml/min. MilliQ water (TKA-GenPure) was used as mobile phase. The concentration of inhibitors was determined using HPLC (Shimadzu Prominence UFLC) fitted with Rezex® ROA-Organic Acid H^+ (column dimensions – 300 × 7.8 mm) (Phenomenex, India) using 0.01 N H_2SO_4 as mobile phase. The flow rate was 0.6 ml/min and the column temperature was maintained at 50 °C. The inhibitor analysis was carried out by using photo diode array (PDA) detector. Organic acids weredetected at a wavelength of 210 nm and furan derivatives were detected at 254 nm.

2.5.2. Amino acid analysis

The quantitative determination of L-lysine in the supernatant was carried out by using Shimadzu HPLC system equipped with Agilent zorbax eclipse AAA column employing pre-column derivatization with o-Phthaldialdehyde. The reaction was carried out at a buffering pH of 10.2 which allows direct derivatization. The mobile phases were 40 mM Na_2HPO_4 and Acetonitrile:Methanol:Water (45:45:10, v/v/v), with gradient elution and run time of 32 min.

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