Bioresource Technology 213 (2016) 270–275

Contents lists available at ScienceDirect

Bioresource Technology

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

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

Meera Christopher, Murali Anusree, Anil K. Mathew *, K. Madhavan Nampoothiri, Rajeev Kumar Sukumaran, Ashok Pandey

Centre for Biofuels and Biotechnology Division, CSIR-National Institute for Interdisciplinary Science and Technology, Industrial Estate P.O., Thiruvananthapuram 695019, India

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.

ARTICLE INFO

Article history:
Received 9 January 2016
Received in revised form 8 March 2016
Accepted 10 March 2016
Available online 12 March 2016

Keywords:
Biorefinery
Dilute acid pre-treatment
Detoxification
Adsorption
Lysine

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.

© 2016 Elsevier Ltd. All rights reserved.

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

* Corresponding author.
E-mail address: anilaykara@gmail.com (A.K. Mathew).

http://dx.doi.org/10.1016/j.biortech.2016.03.054
0960-8524/© 2016 Elsevier Ltd. All rights reserved.

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.

2.5.3. Statistical analysis

Statistical analysis was carried out using the tool GraphPad Prism 6.02 from GraphPad Software, Inc.

3. Results and discussion

3.1. Detoxification of APL using adsorbent or ion-exchange resins

Detoxification of APL was studied using adsorbent (ADS 400 & ADS 800) and ion-exchange (A-27MP & A-72MP) resins (Fig. 1). The adsorbent resins were found to be efficient in removing furfural from APL. Approximately 95% and 75% of furfural was removed when ADS 400 and ADS 800 was used for detoxification, respectively. These adsorbent resins were not effective in removing organic acids present in the APL. A maximum furfural removal of approximately 50% was observed when A-72MP was used for detoxification. The adsorbent resins had a higher capacity of removing the furans from the APL which might be correlated to their higher surface area. To determine the number of cycles for which the adsorbent resins could be reused, the resins were regenerated using 70% ethanol and water for desorbing the adsorbed inhibitors. These regenerated resins were further used for detoxifying the APL. It was observed that ADS-800 retained its adsorbing capacity, whereas the adsorbing capacity of ADS-400 was drastically reduced from 95% to 40% (Fig. 2) during second cycle of detoxification. A drastic reduction in adsorbing capacity of ADS-400 might have occurred due to the structural collapse or changes in surface properties during regeneration of the resins. Analysis of variance showed that there is no significant difference in the percentage removal of the inhibitors between 6 and 12 h of treatment. Further experiments showed that ADS-800 can be reused for up to 6 cycles (Table 3) with approximately 63% removal of furfural. According to a study by Tsuge et al., furfural concentrations above 21 mM can be inhibitory to bacteria. The concentration of furfural present in the APL used in this study was approximately 36 mM. To overcome the inhibitory effect of furfural, a detoxification efficiency above 50% is vital.

Ion exchange resins (A-27MP and A-72MP) were more selective for the removal of organic acids. The furfural and HMF adsorption by these resins were found to be much lower than adsorbent resins. A-27MP resin was found to be better in comparison with A-72MP for the removal of organic acids. Approximately 60% and 40% of formic and acetic acid was removed when A-27MP was used for APL detoxification, respectively (Fig. 1). Organic acids especially the acetic acid removal was found to be lower forA-72MP in comparison with A-27MP.

A comparison of efficiencies of detoxification of spruce hydrolysate using different methods is presented in Table 1. Although a number of physical, chemical and biological detoxification processes have been reported, most of them have significant drawbacks which affect the usability of the liquor for fermentation. For example, while vacuum evaporation reduces the levels of volatile components like furfural, it causes increase in the concentration of toxic non-volatile substances like lignin derivatives. Chemical methods like over-liming causes degradation of the sugars (Mussatto and Roberto, 2004). And while polymeric adsorbents like XADs can remove up to 70% of furfural, nearly 100% HMF and have high specificity for the inhibitors (Weil et al., 2002), their cost and reusability is a concern for industrial-scale applications.

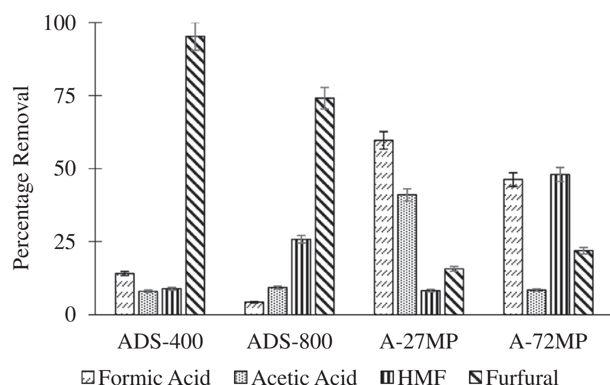

Fig. 1. Percentage removal of inhibitors using different resins.

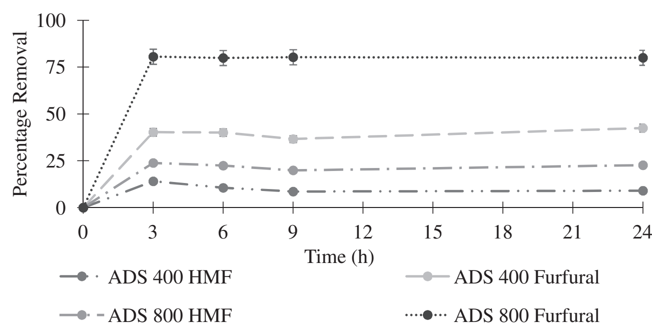

Fig. 2. Comparison of efficiencies of ADS-400 and ADS-800 during second cycle of use.

Table 1
Detoxification of spruce hydrolysate using different methods (Guo et al., 2013).

Detoxification method	Furfural (%)	HMF (%)	Acetic acid (%)	Formic acid (%)
Activated Charcoal	6	6	72	61
NaOH	92	92	98	94
$Ca(OH)_2$	65	55	100	111
NH_4OH	90	85	100	102
Anion exchanger pH10	78	74	78	67
Anion exchanger pH 5.5	94	91	80	72
Cation exchanger pH 10	88	85	90	90
Cation exchanger pH 5.5	96	94	93	91
Sodium sulfite	102	100	99	106

During the detoxification process, the resins could also adsorb the sugars present in APL. An ideal resin for large scale processes should have minimal sugar adsorption as the sugars are the primary requirement for fermentation to value added products. Sugar adsorption byADS 400 and ADS 800 was approximately 18% and 10% of the total sugar present in the APL (Fig. 3). A higher amount of glucose was adsorbed onto the resins as compared to xylose. The sugar adsorption by both ion exchange resin was found to be higher than that by the adsorbent resins. A-27MP adsorbed approximately 22% of total sugar present in the APL whereas A-72MP adsorbed approximately 32% of sugar present in the APL (Fig. 3).

3.1.1. Sequential use of ADS 800 and A-27MP

The sequential use of ADS 800 and A-27MP was investigatedas this might condition the APL much better by removing both the furan derivatives and organic acids present in the APL. The APL was first detoxified using ADS-800 followed by detoxification using A-27MP. However, it was observed that while about 77% of the furfural was removed, there was only a negligible amount of organic acid removal when A-27MP was used. Since the resins adsorb a lot of other components in addition to the inhibitors, some characteristic change might be occurring in the APL which renders it unsuitable for treatment by the ion-exchangers. When used in sequence, the resins also demonstrated the ability to remove the pigments

Table 2
Properties of adsorbent and ion-exchange resins.

Resin	Type	Matrix structure	Functional group	Particle size (mm)	Max temp (°F/°C)	pH range	Total exchange capacity (meq/ml)	Features
A-27 MP	Strong base	Polystyrene copolymer	Quaternary NH^4 Type I	0.3–1.2	175/80	0–14	1.2	Resistance to organic fouling
A-72 MP	Strong base	Polystyrene copolymer	Quaternary NH^4 Type I	0.3–1.2	175/80	0–14	1.0	Controlled pore size
ADS-400	Polyacrylic adsorbent	Polyacrylic copolymer	NIL	0.3–1.0	205/95	0–14	375	High organic removal capacity
ADS-800	Polystyrenic adsorbent	Polystyrenic copolymer	NIL	0.4–1.2	300/150	0–14	750	High organic removal capacity

Table 3
Reusability of ADS-800 after regeneration.

Cycle	Percentage removal of HMF	Percentage removal of furfural
3	59.76	85.15
4	57.12	77.66
5	57.15	75.47
6	24.99	63.58

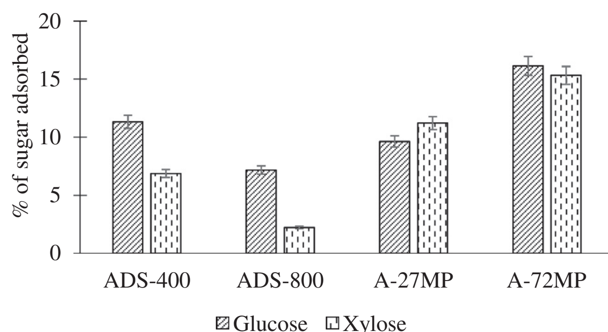

Fig. 3. Sugar adsorption by the resins.

present in APL. After treating with ADS 800, the APL turned from dark brown to yellowish indicating the furfural or other phenolic compounds present in the APL. When the APL treated with ADS 800 was passed through A-27MP, it became an almost clear solution. The sugar adsorption property of these resins remain unchanged during sequential use. Analysis of the sugar adsorption capacity of the resins showed that ADS 800 absorbed approximately 9% of the total sugars present in the APL, on the other hand, nearly 30% of the sugars was adsorbed by A-27MP.

It has already been reported that in the case of ethanol production by growth-arrested cells, typical inhibitors like organic acids or phenolic compoundsdid not substantially disturb *C. glutamicum* (Lange et al., 2012; Sakai et al., 2007). In principal overcoming inhibition can be achieved by different ways, e.g. by simple resistance to the inhibitory substances due to efflux pump or prevention of uptake by degradation of the relevant substances (Gorsich et al., 2006; Miller et al., 2010; Weingarten et al., 2010). Since the amount of acids present in the APL (37 mM acetic acid and 17 mM formic acid) is lesser than the reported tolerance limits of many xylose-fermenting microbes (Hasunuma et al., 2011; Sakai et al., 2007), and the sugar adsorption is too high to warrant further use, A-27MP was also not used in subsequent experiments.

3.1.2. Adsorption maxima of furfural and HMF onto ADS 800

To determine the maximum adsorption of inhibitors onto ADS 800, batch adsorption studies were carried out using pure furfural and HMF. The maximum amount of pure furfural and HMF that could be adsorbed on to ADS-800 was found to be in excess of 3840 mg/g and 4850 mg/g, respectively. However, the capacity for adsorbing these compounds from the pretreatment liquor was much lower (4.71 mg/g furfural and 0.1 mg/g HMF). This could be due to the other compounds like pigments, phenolics etc. which may be more preferentially adsorbed by the resin as compared to the inhibitors.

Three different solvent namely ethanol, acetone and methanol were evaluated for the desorption of inhibitors from ADS 800. All the three solvents that were assessed showed a similar capacity to desorb the inhibitors from the resin. But when the washed resins were reused for a second cycle of detoxification, the resins that were washed using 70% acetone did not adsorb as much as the resins washed with 70% ethanol or methanol (Data not shown). A possible explanation for the reduction might be the expansion of polystyrene in the presence of acetone (Marra and Hair, 1988).

3.2. L-lysine fermentation using wild and engineered strains of *C. glutamicum*

The wild-type *C. glutamicum* is a gram-positive soil bacterium that grows on a variety of sugars and organic acids and is the workhorse for the fermentative production of the amino acids L-glutamate (1.5×10^6 tons/year) and L-lysine (0.9×10^6 tons/year). Due to the growing world market and steadily decreasing market prices, great efforts have been made to develop more powerful and efficient production strains (Blombach and Seibold, 2010; Blombach et al., 2009). The natural substrate spectrum of *C. glutamicum* includes sugars like ribose or maltose, alcohols like ethanol or myo-inositol, organic acids like acetate, citrate, lactate, propionate and pyruvate, and amino acids like L-glutamate. Within the flexible feedstock concept, the substrate spectrum of *C. glutamicum* has been extended by metabolic engineering to allow access to starch, cellobiose, lactose, galactose and glycerol as well as succinate, fumarate and malate as carbon sources (Meiswinkel et al., 2013). *C. glutamicum* has proven a good choice for utilizing complex mixtures of carbon sources such as hemicellulosic hydrolysates because, unlike *Escherichia coli* and *S. cerevisiae*, *C. glutamicum* efficiently co-utilizes different carbon sources when present in blends (Arndt and Eikmanns, 2008; Blombach and Seibold, 2010; Wendisch, 2006). Consequently, besides proof-of-concept using pure chemicals, growth and production of amino acid using hemicellulosic hydrolysates obtained from rice straw have already been achieved (Gopinath et al., 2011).

In this study, a maximum biomass production of 1.65 g/l and 1.75 g/l was attained when the engineered *C. glutamicum* grown on the native and detoxified APL, respectively. In the detoxified APL, the wild strain of *C. glutamicum* also attained a similar biomass production as the construct. A lower amount of biomass (0.87 g/l) was produced when the wild strains of *C. glutamicum* were grown in non-detoxified (native) APL. The biomass production indicates the importance of detoxification of APL for the production of value added compounds. However, much higher

biomass productions have been obtained with *C. glutamicum* strains grown on inorganic media. These organisms have been known to reach growth rates of 4.5 g/l in 0.9% w/v glucose medium (Hadiati et al., 2014) and 8.25 g/l in 4% glucose medium (Seibold et al., 2006). A lower biomass production observed in the present study may might be due to the inhibitory effect of the aromatic inhibitors arising from phenols, as these might affect the key enzymes of metabolic pathways (Canilha et al., 2013; Ezeji et al., 2013; Klinke et al., 2004). 4-Hydroxybenzaldehyde, vanillin, and syringaldehyde are generated by the partial breakdown of lignin through the p-*hydroxyphenyl* residue, guaiacyl residue, and syringyl residue, respectively, and exert inhibitory effects on microbial growth (Sakai et al., 2007). *C. glutamicum* shows sensitivity to phenols similar to that of yeasts such as *S. cerevisiae* CBS1200, *Candida shehatae* ATCC22984, and *Pichia stipities* NRRL Y7124 and of bacteria such as *Zymomonas mobilis* ATCC 10988, *E. coli* ATCC 1175, and *E. coli* LY01 (Delgenes et al., 1996; Zaldivar et al., 1999). It is difficult to propose a general mechanism or cause of inhibition, because different molecules affect growth differently. The molecular structures of these inhibitors and the number of methyl groups that the inhibitor possesses affects the ability of each compound to penetrate the cell membrane.

The fermentability of detoxified APL was assessed by L-lysine production using wild and engineered strains of *C. glutamicum* and was compared with the L-lysine production from native APL. The growth and sugar utilization profiles (Fig. 4) demonstrate the ability of the mutant strain to co-utilize the pentose present in the APL. When grown on detoxified APL, even the wild strain was able to achieve a similar growth profile as the construct, indicating that the furan inhibitors significantly affect the growth of *C. glutamicum*. The detoxified APL was superior to APL in L-lysine production when engineered *C. glutamicum* was used fermentation. At 72 h of fermentation, the engineered *C. glutamicum* DM1729 (pEKEx3-$xylA_{Xc}$-$xylB_{Cg}$)(pVWEx1-*araBAD*) grown on detoxified pretreatment liquor was able to produce approximately 30 mM L-lysine whereas from the native APL,the L-lysine production was 21 mM. A significantly lower amount of L-lysine (9 mM) was produced from the wild strain of *C. glutamicum* grown on either native or detoxified APL (data not shown). The highest reported yields of L-lysine from strains improved for xylose utilization is 47.5 ± 2 mM at 144.7 ± 6.1 mg/l/h (Gopinath et al., 2011; Meiswinkel et al., 2013), using CGXII media supplemented with pure xylose.

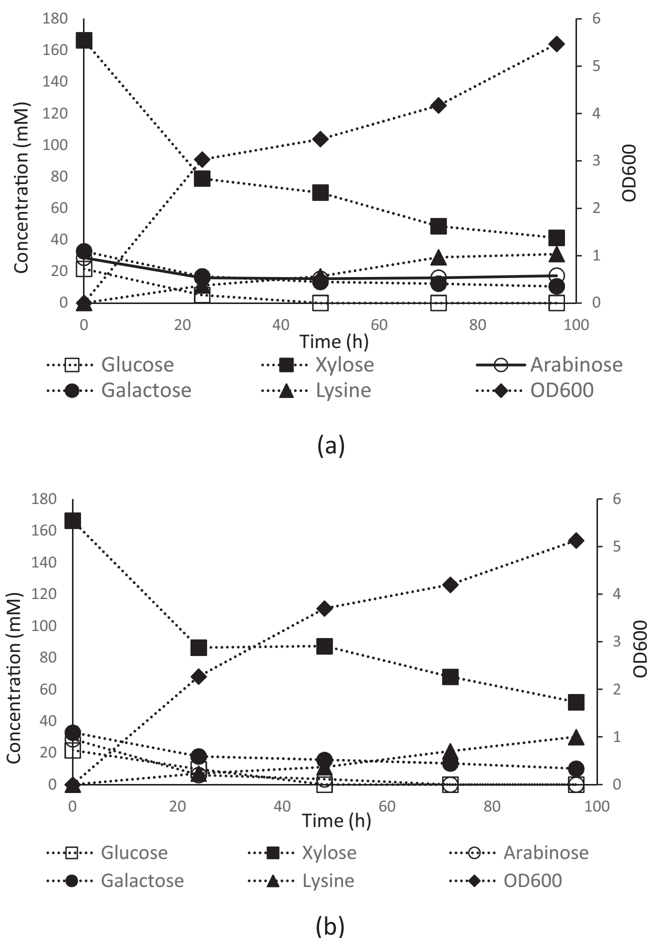

Fig. 4. Growth, L-lysine production and sugar utilization by engineered *C. glutamicum.* (a) detoxified APL as medium (b) non-detoxified APL as medium.

4. Conclusion

The production of value added compounds from waste streams such as APL is essential to improve the economics of lignocellulosic bioethanol production.Using biorefinery concept, this study focused on detoxification of the APL and further utilising it for L-lysine production. Of the four resins that were evaluated, ADS-800 was the most effective in adsorbing furfural and HMF from the liquor, and its lower affinity for sugars makes it suitable for processing the APL prior to fermentation. Finally, using the detoxified APL for fermentation enhanced the production of L-lysine by both wild and engineered strains of *C. glutamicum*.

Acknowledgements

The authors would like to thank the Technology, Information, Forecasting and Assessment Council (TIFAC), Department of Science and Technology, India for providing the financial support for this work and Thermax, India for providing the resins. The authors K.M.N. & M.A. thank the Wendisch Lab, University of Bielefeld, DST (Department of Science and Technology, India) and DBT (Department of Biotechnology, India) for the Indo-German collaborative projects.

References

Alvira, P., Tomás-Pejó, E., Ballesteros, M., Negro, M.J., 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. Bioresour. Technol. 101, 4851–4861. http://dx.doi.org/10.1016/j.biortech.2009.11.093.

Arndt, A., Eikmanns, B.J., 2008. Regulation of Carbon Metabolism in Corynebacterium glutamicum. Corynebacteria Genom. Mol. Biol., 340 http://dx.doi.org/10.2307/40079518.

Blombach, B., Hans, S., Bathe, B., Eikmanns, B.J., 2009. Acetohydroxyacid synthase, a novel target for improvement of L-lysine production by *Corynebacterium glutamicum*. Appl. Environ. Microbiol. 75, 419–427. http://dx.doi.org/10.1128/AEM.01844-08.

Blombach, B., Seibold, G.M., 2010. Carbohydrate metabolism in *Corynebacterium glutamicum* and applications for the metabolic engineering of L-lysine production strains. Appl. Microbiol. Biotechnol. 86, 1313–1322. http://dx.doi.org/10.1007/s00253-010-2537-z.

Canilha, L., Rodrigues, R.D.C.L.B., Antunes, F.A.F., Milessi, T.S.D.S., Felipe, M.D.G.A., Silva, S.S.Da., 2013. Bioconversion of hemicellulose from sugarcane biomass into sustainable products. INTECH 15–45, dx.doi.org/10.5772/53832.

Delgenes, J.P., Moletta, R., Navarro, J.M., 1996. Effects of lignocellulose degradation products on ethanol fermentations of glucose and xylose by *Saccharomyces cerevisiae*, *Zymomonas mobilis*, *Pichia stipitis*, and *Candida shehatae*. Enzyme Microb. Technol. 19, 220–225. http://dx.doi.org/10.1016/0141-0229(95)00237-5.

Eggeling, L., Reyes, O., 2005. Experiments. In: Eggeling, L., Bott, M. (Eds.), Handbook of Corynebacterium glutamicum. CRC Press, Boca Raton, pp. 3535–3566.

Ezeji, T., Qureshi, N., Ujor, V., 2013. Bioenergy Research: Advances and applications, in: Bioenergy Research: Advances and Applications. Newnes, p. 500.

Gopinath, V., Meiswinkel, T.M., Wendisch, V.F., Nampoothiri, K.M., 2011. Amino acid production from rice straw and wheat bran hydrolysates by recombinant pentose-utilizing *Corynebacterium glutamicum*. Appl. Microbiol. Biotechnol. 92, 985–996. http://dx.doi.org/10.1007/s00253-011-3478-x.

Gorsich, S.W., Dien, B.S., Nichols, N.N., Slininger, P.J., Liu, Z.L., Skory, C.D., 2006. Tolerance to furfural-induced stress is associated with pentose phosphate pathway genes ZWF1, GND1, RPE1, and TKL1 in *Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 71, 339–349. http://dx.doi.org/10.1007/s00253-005-0142-3.

Hadiati, A., Krahn, I., Lindner, S.N., Wendisch, V.F., 2014. Engineering of *Corynebacterium glutamicum* for growth and production of L-ornithine, L-lysine, and lycopene from hexuronic acids. Bioresour. Bioprocess. 1. http://dx.doi.org/10.1186/s40643-014-0025-5.

Hasunuma, T., Sanda, T., Yamada, R., Yoshimura, K., Ishii, J., Kondo, A., 2011. Metabolic pathway engineering based on metabolomics confers acetic and formic acid tolerance to a recombinant xylose-fermenting strain of *Saccharomyces cerevisiae*. Microb. Cell Fact. 10, 2. http://dx.doi.org/10.1186/1475-2859-10-2.

Klinke, H.B., Thomsen, A.B., Ahring, B.K., 2004. Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. Appl. Microbiol. Biotechnol. 66, 10–26. http://dx.doi.org/10.1007/s00253-004-1642-2.

Lange, J.P., Van Der Heide, E., Van Buijtenen, J., Price, R., 2012. Furfural-A promising platform for lignocellulosic biofuels. ChemSusChem 5, 150–166. http://dx.doi.org/10.1002/cssc.201100648.

Marra, J., Hair, M.L., 1988. Interaction between adsorbed polystyrene layers in acetone-heptane solvent mixtures. Effect of segment-surface adsorption affinity. Macromolecules 21, 2356–2362. http://dx.doi.org/10.1021/ma00186a010.

Meiswinkel, T.M., Gopinath, V., Lindner, S.N., Nampoothiri, K.M., Wendisch, V.F., 2013. Accelerated pentose utilization by *Corynebacterium glutamicum* for accelerated production of lysine, glutamate, ornithine and putrescine. Microb. Biotechnol. 6, 131–140. http://dx.doi.org/10.1111/1751-7915.12001.

Metzger, J.O., Hüttermann, A., 2009. Sustainable global energy supply based on lignocellulosic biomass from afforestation of degraded areas. Naturwissenschaften 96, 279–288. http://dx.doi.org/10.1007/s00114-008-0479-4.

Miller, E.N., Turner, P.C., Jarboe, L.R., Ingram, L.O., 2010. Genetic changes that increase 5-hydroxymethyl furfural resistance in ethanol-producing *Escherichia coli* LY180. Biotechnol. Lett. 32, 661–667. http://dx.doi.org/10.1007/s10529-010-0209-9.

Mussatto, S.I., Roberto, I.C., 2004. Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: a review. Bioresour. Technol. 93, 1–10.

Olah, G.A., Goeppert, A., Prakash, G.K.S., 2009. Beyond Oil and Gas: The Methanol Economy: Second Edition, Beyond Oil and Gas: The Methanol Economy: Second Edition. doi:10.1002/9783527627806.

Sakai, S., Tsuchida, Y., Okino, S., Ichihashi, O., Kawaguchi, H., Watanabe, T., Inui, M., Yukawa, H., Nakamoto, H., Okino, S., Ichihashi, O., Kawaguchi, H., Watanabe, T., Inui, M., Yukawa, H., 2007. Effect of lignocellulose-derived inhibitors on growth of and ethanol production by growth-arrested *Corynebacterium glutamicum* R. Appl. Environ. Microbiol. 73, 2349–2353. http://dx.doi.org/10.1128/AEM.02880-06.

Sandhya, S.V., Kiran, K., Kuttiraja, M., Preeti, V.E., Sindhu, R., Vani, S., Kumar, S.R., Pandey, A., Binod, P., 2013. Evaluation of polymeric adsorbent resins for efficient detoxification of liquor generated during acid pretreatment of lignocellulosic biomass. Indian J. Exp. Biol. 51, 1012–1017.

Schwartz, T.J., Lawoko, M., 2010. Removal of acid-soluble lignin from biomass extracts using Amberlite XAD-4 resin. BioResources 5, 2337–2347.

Seibold, G., Auchter, M., Berens, S., Kalinowski, J., Eikmanns, B.J., 2006. Utilization of soluble starch by a recombinant *Corynebacterium glutamicum* strain: growth and lysine production. J. Biotechnol. 124, 381–391. http://dx.doi.org/10.1016/j.jbiotec.2005.12.027.

Weil, J.R., Dien, B., Bothast, R., Hendrickson, R., Mosier, N.S., Ladisch, M.R., 2002. Removal of fermentation inhibitors formed during pretreatment of biomass by polymeric adsorbents. Ind. Eng. Chem. Res. 41, 6132–6138. http://dx.doi.org/10.1021/ie0201056.

Weingarten, R., Cho, J., Conner Jr., W.C., Huber, G.W., 2010. Kinetics of furfural production by dehydration of xylose in a biphasic reactor with microwave heating. Green Chem. 12, 1423. http://dx.doi.org/10.1039/c003459b.

Wendisch, V.F., de Graaf, A.A., Sahm, H., Eikmanns, B.J., 2000. Quantitative determination of metabolic fluxes during coutilization of two carbon sources: comparative analyses with Corynebacterium glutamicum during growth on acetate and/or glucose. J. Bacteriol. 182 (11), 3088–3096.

Wendisch, V.F., 2006. Genetic regulation of *Corynebacterium glutamicum* metabolism. J. Microbiol. Biotechnol. 16, 999–1009.

Yu, Q., Xu, C., Zhuang, X., Yuan, Z., He, M., Zhou, G., 2015. Xylo-oligosaccharides and ethanol production from liquid hot water hydrolysate of sugarcane bagasse. BioResources 10, 30–40.

Zaldivar, J., Martinez, A., Ingram, L.O., 1999. Effect of selected aldehydes on the growth and fermentation of ethanologenic *Escherichia coli*. Biotechnol. Bioeng. 65, 24–33 (doi:10.1002/(SICI)1097-0290(19991005)65:1<24::AID-BIT4>3.0.CO;2-2).