Proceedings of the
International Conference on Mechanical Engineering and Renewable Energy 2011
(ICMERE2011) 22- 24 December 2011, Chittagong, Bangladesh

ICMERE2011-PI-123

DETOXIFICATION OF LIGNOCELLULOSIC HYDROLYSATE TO INCREASE FERMENTABILITY FOR BIOFUELS PRODUCTION

Niloy Chandra Sarkar[1], Abu Yousuf[1]*, Mozammel Hoque[2], Abul Kalam Azad[2]

[1]Department of Chemical Engineering & Polymer Science, Shahjalal University of Science & Technology Sylhet-3114, Bangladesh
[2] Department of Genetic Engineering & Biotechnology, Shahjalal University of Science & Technology Sylhet-3114, Bangladesh

***Corresponding Author:** E-mail: ayousuf-cep@sust.edu

Abstract- *Lignocellulose is the most abundant biomaterial in nature but use is still limited due to their complex structure and high processing cost. The exhaustion of fossil resources and global warming, the attention has been redirected on biofuels preparation from lignocellulosic biomass as an alternative to fossil fuels. During the hydrolysis of lignocellulosic materials some non-carbohydrate compounds such as acetic acid, furfural, 5-hydroxymethylfurfural (HMF), and water soluble lignin generated with fermentable monosaccharides. These byproducts have various adverse effects on microbial cell growth, metabolism, sugar utilization and lipid accumulation. To remediate these problems, different detoxification methods have been developed such as overliming, vacuum evaporation, adsorption with active charcoal, ion-exchange resins or inhibitor degrading microorganisms etc. This paper portrays the technique of maximizing the recovery of fermentable sugars and minimizing costs associated with energy and chemical inputs of inhibitor removal.*

Keywords: lignocellulosic biomass, fermentable sugar, detoxification, biofuels

1. INTRODUCTION

Lignocellulosic biomass is considered as a renewable source of bioenergy like ethanol, methane, biodiesel and also hydrogen [1]. During hydrolysis of lignocellulosic materials a wide range of compounds which are inhibitory to microorganisms are formed or released. This is a major challenge in biological conversion of lignocellulosic biomass to remove antimicrobial agents from hydrolysate. Undesirably, during the hydrolysis process some non-carbohydrate compounds such as acetic acid, furfural, 5-hydroxymethylfurfural (HMF), and water soluble lignin generated with fermentable monosaccharides. Mainly, furfural and HMF originated from the decomposition of pentoses and hexoses, acetic acid from the acetyl group in hemicellulose and phenolic compounds including syringaldehyde, p-hydroxybenzaldehyde, vanillin, etc derived from lignin [2, 3]. These compounds limit efficient utilization of the hydrolysates for ethanol, methane, biodiesel and hydrogen production by fermentation. Detoxification is necessary in order to reach maximum productivity in the fermentation process. If the inhibitors are identified and the mechanisms of inhibition elucidated, fermentation can be improved by developing specific detoxification methods, choosing an adapted microorganism, or optimizing the fermentation strategy. Various detoxification methods have been studied such as extraction with organic solvents, overliming, evaporation, steam stripping, sulfite treatment, ion-exchange, enzyme treatment, zeolite treatment and activated carbon treatment.

The objective of this review is to find out which detoxification method will be successful and attractive to apply. This will be done by giving an overview of the specific lignocellulosic biomass for biodiesel, methane and ethanol production process.

2. STRUCTURE OF LIGNOCELLULOSIC MATERIALS

Lignocellulosic material consists of mainly three different types of polymers, namely cellulose, hemicellulose and lignin, which are associated with each other [4]. The major composition of mostly usable lignocellulosic biomass is presented in the Table 1.

2.1. Cellulose

Cellulose is a linear polymer of β(1-4) linked glucose units [4]. The individual chains vary in length from several hundred to more than 10,000 glucose monomers. It is highly crystalline and insoluble in water and most

organic solvents because of the extensive hydrogen bonding between chains. The insolubility and crystalline nature of cellulose make it highly recalcitrant to degradation

2.2 Hemicellulose

Hemicellulose is a complex carbohydrate structure that consists of different polymers like pentoses (i.e., xylose and arabinose), hexoses (like mannose, glucose and galactose), and sugars. Hemicellulose has a lower molecular weight than cellulose, and branches with short lateral chains that consist of different sugars, which are easy hydrolyzable polymers [4]. The solubility of the different hemicellulose compounds is in descending order: mannose, xylose, glucose, arabinose, and galactose. The solubility increases with increasing temperature.

Table 1: The composition of mostly useable lignocellulosic biomass

Sources	Composition	References
Spruce wood	Cellulose -41.9% Lignin-27.1% Xylan-6.1%	[5]
Pine wood	Cellulose-37.7% Lignin-27.5% Xylan-4.6%	[5]
Birch wood	Cellulose-38.2% Lignin-22.8% Xylan-18.5%	[6]
Poplar wood	Cellulose-49.9% Lignin-18.1% Xylan-17.4%	[6]
Corn stover	Cellulose-36.4% Lignin-16.6% Xylan-18.0%	[6]
Wheat straw	Cellulose-38.2% Lignin-23.4% Xylan-21.2%	[6]
Switchgrass	Cellulose-31.0% Lignin-17.6% Xylan-20.4%	[6]

2.3 Lignin

After cellulose, it is the most abundant organic material on Earth, making up one-fourth to one-third of the dry weight of wood, where it is concentrated in the cell walls. It is an amorphous heteropolymer consisting of three different phenylpropane units (p-coumaryl, coniferyl and sinapyl alcohol) that are held together by different kind of linkages. The main purpose of lignin is to give the plant structural support, impermeability, and resistance against microbial attack and oxidative stress. The amorphous heteropolymer is also non-water soluble and optically inactive; all this makes the degradation of lignin very tough [4].

3. Preparation techniques of lignocellulosic hydrolysates

Lignocellulosic biomass in its natural form is a tough feedstock for hydrolysis due to the crystallinity of cellulose and due to the compact packing of cellulose, hemicelluloses and lignin in the plant material. The basic objective of pretreatment is to make this complex polymer accessible to the action of cellulases which is achieved by removal of either hemicelluloses or lignin from the matrix or breaking up of the compact packing of these polymers [7,8]. A wide range of thermal, mechanical and chemical pre-treatment methods and combinations thereof have been reported for achieving these goals.

3.1 Acid hydrolysis

Pretreatment of lignocelluloses with acids at ambient temperature are done to enhance the anaerobic digestibility. The pretreatment can be done with dilute or strong acids. Concentrated acid processes are often reported to give higher sugar yield and consequently higher ethanol yield, compared to dilute-acid processes. Furthermore it can be operated at low temperature (e.g. 40 ºC), however severe problems with corrosion of hydrolysis equipment render high investment cost, and also the recovery of the acid are expensive and difficult [9]. Compared to a concentrated acid process a dilute acid process will consume much less acid, however high temperature required often lead to corrosion problems and sugar degradation, resulting in lower sugar yield and inhibition of the fermentation, but this problem can be solved by a two stage process, in which the hemicelluloses is mainly hydrolyzed in the initial step at temperature 150-190 ºC and the remaining cellulose subsequently hydrolyzed at more severe conditions at 90-230 ºC [10].

3.2 Basic hydrolysis

Alkali pretreatment refers to the application of alkaline solutions such as NaOH, $Ca(OH)_2$ (lime) or ammonia to remove lignin and a part of the hemicelluloses, and efficiently increase the accessibility of enzyme to the cellulose. The alkali pretreatment can result in a sharp increase in saccharification, with manifold yields. Pretreatment can be performed at low temperatures but with a relatively long time and high concentration of the base. In the case of alkali pre-treatment, lignin component is dissolved in alkali and removed in liquid fraction while the hemicelluloses and cellulose fractions are recovered together in the solid fraction [11]. Important aspect of alkaline pretreatment is the change of the cellulose structure to a form that is denser and thermodynamically more stable than the native cellulose [12].

3.4 Enzymatic hydrolysis

Enzymatic hydrolysis of the cellulosic component of

pretreated biomass is the key step in lignocellulosic biomass to ethanol technology. Three major types of cellulose enzymes are involved in the hydrolysis of native cellulose namely cellobiohydrolase (CBH), endo-b-1,4-glucanase (EG) and b-glucosidase [13]. Reduction in the costs of enzymes used in lignocellulosic hydrolysis is a key issue in commercial cellulosic ethanol production. The high cost of enzymatic hydrolysis is due to the poor activity of cellulase. Reducing the costs of enzymes used in the process is crucial to favorable cellulosic ethanol process economics and commercialization [14]. Given that loadings have been extensively optimized, improvements in enzyme performance or reduction in enzyme production costs will be required [4]. Main challenges of enzymatic hydrolysis are high enzyme costs; poor activity/long incubation times; optimized enzyme mixtures for specific feedstocks/ processes.

4. INHIBITION ACTIVITIES AND FERMENTABILITY

Lignocellulosic hydrolysates, however, contain substances that inhibit microbial fermentation to desirable products [15]. Based on their origin the inhibitors are usually divided in three major groups: weak acids, furan derivatives, and phenolic compounds. These compounds limit efficient utilization of the hydrolysates for ethanol and triglyceride production by fermentation. If the inhibitors are identified and the mechanisms of inhibition elucidated, fermentation can be improved by developing specific detoxification methods, choosing an adapted microorganism, or optimizing the fermentation strategy [16].

5. DETOXIFICATION METHODS

5.1 Over liming

In the case of chemical detoxification, alkali treatment such as overliming is often employed [17, 18]. The detoxification mechanism of overliming involves precipitation of the inhibitory compounds and increased instability of some inhibitory compounds at high pH [16]. The most significant effect of overliming was a sharp decrease in the concentration of furfural and hydroxymethylfurfural, whereas the concentration of acetic acid remained unchanged and the decrease in the total phenolic compounds was less than 30%. On the other hand, decrease in sugar concentration during overliming was a serious problem at pH 12, especially at the higher temperature, where up to 70% sugars were degraded.

Horvath [19] has obtained a 120% increase in ethanol production after overliming up to pH 11 at 30°C. Minimal amounts of weak organic (formic acid, acetic acid) acids were removed; however, up to 65% of the furans and 22% of the phenolic compounds contained in the water soluble fraction were removed. Similar effects of the overliming on the hydrolysates were confirmed by [20, 21]. On the other hand, it was also reported that the detoxification with lime was a costly method and may contribute to total ethanol production costs up to 22% [22].

5.2 Adsorption

Among the detoxification processes, adsorption treatment either stand-alone or in combination with chemicals such as alkali and ion exchange resins is effective for the removal of furfural, 5-hydroxymethyl furfural (HMF), and phenolic compounds in various hydrolyzates [2,23,24]. Though various adsorbents were studied like ion exchange resins [25, 26] wood charcoals [27], activated charcoal [28,29] but Villarreal and his coauthors (2006) investigated that ion exchange resins were more efficient than activated charcoal to remove all four major groups of inhibitory compounds without sugar loss. The ion exchange detoxification drastically enhanced the fermentability of the hydrolysate.

5.3 Bioabatement

Biological inhibitor abatement is a potential method to remove inhibitory compounds from lignocellulose hydrolysates that could be incorporated into a scheme for fermentation of ethanol from cellulose. In the case of environmental pollutants, microbes have been used for bioremediation of toxic chemicals. A fungus, *Coniochaeta ligniaria* NRRL30616, was identified as having desirable metabolic capabilities and inhibitor tolerance. Strain NRRL30616 was selected in particular for its ability to tolerate and metabolize the furan aldehydes furfural and HMF, and an aromatic acid, ferulic acid, as sole sources of carbon and energy. *C. ligniaria* also can grow on many other compounds commonly found in hydrolysates, including aromatic acids and aldehydes [30]. Nichols [31] characterized *C. ligniaria* NRRL30616 for its ability to metabolize and remove furans and organic acids and aldehydes from corn stover dilute acid hydrolysate and found very promising results. Other removal techniques are describe in the Table 2.

Table 2 : Inhibitors and their removal technique

Substrate	Inhibitors	Detoxification Method	References
Rice straw hydrolysate	Acetic acid, furfural, HMF, water soluble lignin	Overliming, oncentration, and adsorption	[25]
Wood hydrolysate	4-hydroxybenzoic acid, 4-hydroxy-3-methoxybenzaldehyde (vanillin) and 4-hydroxy-3-methoxybenzoic (vanillic) acid,	Biodetoxification , Laccase and lignin peroxidase from *Trametes versicolor*	[15]
Corn stover hydrolysate	Furfural, HMF, furoic acid	Biodetoxification, *Coniochaeta ligniaria* NRRL30616	[31]
Spruce chips (*Picea abies*)	furfural, 5-hydroxymethylfurfural, vanillin, vanillic acid, *p*-hydroxybenzoic acid and coniferylaldehyde	Adsorption, wood charcoals	[27]
Wood hydrolysate	formic acid, acetic acid, hydroxymethylfurfural (HMF) and furfural	Adsorption, activated carbon	[29]
Corn stover ,Rice straw and cotton stalk, Wheat straw and rape straw	acetic acid, formic acid, levulinic acid, furfural and HMF	Biodetoxification, *Amorphotheca resinae* ZN1	[32]

5.4 Vacuum evaporation

The evaporation under vacuum might effect the chemical composition of the hydrolysates in terms of sugar or inhibitor concentration. No sugar decomposition occurred during the evaporation, while more than 96% of furfural and in lesser extant formic and acetic acid disappeared from the hydrolysates. The evaporation resulted in decreased concentrations of some inhibitors. Evaporation under vacuum resulted in high-sugar and fermentable hydrolysates with no sign of carbohydrate degradation. These results may be interesting industrially, since higher sugar concentrations in the hydrolysates lead to less energy consumption in the distillation and downstream processes, while fermentation can be carried out successfully with no prior detoxification. The successful evaporation of hydrolysates under vacuum can be incorporated into a process design utilizing multi-effect evaporators, in which the hydrolysates can be evaporated with low consumption of energy. Dehkhoda [33] found that, the evaporation under vacuum did not decompose the sugars, but were able to remove the volatile inhibitors partially or completely. Therefore vacuum evaporation can be applied for concentrating hydrolysates in an industrial scale.

5.5 Enzymatic

Previously described detoxification methods resulted in many negative outcomes, including massive freshwater usage and wastewater generation, loss of the fine lignocellulose particles and fermentative sugars and incomplete removal of inhibitors [34]. Enzymatic or biodetoxification is An alternate option for removing toxins without causing these problems and this method relies on microorganisms to degrade the toxins as part of their normal metabolism by secreting peroxidase or laccase enzymes into the hydrolysates [30, 35, 36, 37]. Biodetoxification has many advantages, such as no loss of cellulose solids, greatly decreased use of water, and thus high concentrations of solids for fermentation.

Jonsson [15] achieved an increased rate of glucose consumption and ethanol production by enzymatic treatment of wood hydrolysates. Both laccase and lignin peroxidase had a positive effect, but, under the conditions used, laccase was more efficient. The laccase treated sample and the sample treated with both laccase and peroxidase displayed similar rates of glucose consumption and ethanol production, both of which were increased three times. The sample detoxified with only lignin peroxidase showed double the rate of glucose consumption and ethanol production.

6. CONCLUSION

The formation of inhibitors depends on the lignocellulosic sources. To increase the fermentability of lignocellulosic hydrolysate various steps can be considered. Firstly, the formation of inhibitors can be minimized through optimization of the pretreatment and hydrolysis conditions. Secondly, characterization of the hydrolysates depending on the lignocellulosic sources, thirdly understanding of the inhibitory mechanisms of individual compounds and their interaction effects and fourthly, specific detoxification methods can be developed for efficient removal of inhibitors prior to fermentation of strongly inhibiting hydrolysates.
However, the most promising way would be to develop the adapting power of the microorganism to the toxic compounds in the hydrolysate by genetically modification or like something.

7. REFERENCES

[1] A.T.W.M. Hendriks, and G. Zeeman, Pretreatments to enhance the digestibility of lignocellulosic biomass, *Bioresource Technology,* vol. 100, pp. 10-18, 2009.

[2] G. B. M. Carvalho, S. I. Mussatto, E. J. Candido, and J. B. Almeida e Silva. Comparison of different procedures for the detoxification of eucalyptus hemicellulosic hydrolyzate for use in

fermentative processes, *J Chem Technol Biotechnol*, vol. 81, pp. 152-7, 2006.

[3] C.Z.X. Hu, J. Zhao, S. Wu, and Z. K. Zhao, Effects of biomass hydrolysis by-products on oleaginous yeast Rhodosporidium toruloides, *Bioresource Technology,* vol. 100, I. 20, pp. 4843-4847, 2009.

[4] D. Fengel, and G. Wegener, 1984. Wood: Chemistry, Ultrastructure, Reactions. De Gruyter, Berlin. *Bioresource Technology,* vol. 101, pp. 4826-4833, 2010.

[5] M. Hayn, W. Steiner, R. Clinger, H. Steinmüller, M. Sinner and H. Esterbauer, Basic research and pilot studies on the enzymatic conversion of lignocellulosic. In: Saddler JN (Ed.). Bioconversion of forest and agricultural plant residues. *CAB International, Wallinford, UK*. pp. 33-72, 1993.

[6] A. Wiselogel, S. Tyson, and D. Johnson, Taylor & Framcis, PA Bristol, Biomass feedstock resources and composition. In: Wyman CE (Ed.). *Handbook of bioethanol: Production and utilization* , pp. 105-118, 1996.

[7] B. Venkatesh, S. Leonardo da Costa, P.S.C. Shishir, M. Derek, N.S. Lekh, C.K. Chambliss, and E.D. Bruce, Enzymatic digestibility and pretreatment degradation products of AFEX-treated hardwoods Populus (Populus nigra). *Biotechnol,* Prog. 25, pp. 365–375, 2009.

[8] R. Kumar, and C.E. Wyman, Effects of cellulase and xylanase enzymes on the deconstruction of solids from pretreatment of poplar by leading technologies. *Biotechnol,* Prog. 25, pp. 302–314. 2009.

[9] J. L. Jones, and K. T. Semrau. Wood hydrolysis for ethanol production – previous experience and the economics of selected process. *Biomass* 5: pp. 109-135, 1984.

[10] W. L. Faith. Development of the scholler process in the United States industrial and engineering chemistry, 37,pp. 9-11, 1954.

[11] R. K. Sukumaran , V.J.S. R. Sindhu, Parameshwaran Binod, K.V.S.K.U. Janu, K.P.Rjasree, and A. Pandey, Lignocellulosic ethanol in India: Prospects, challenges and feedstock availability, *Bioresource Technology,* vol. 101 pp. 4826–4833, 2010.

[12] R. C. Pettersen, The chemical composition of wood (chapter 2). In: Rowell, R.M. (Ed.), The chemistry of solid wood, Advances in Chemistry Series, *American Chemical Society*, vol. 207 , pp. 984, 1984.

[13] W.A.M. Schulein, and J.N. Abelson, (Eds.), Cellulases of Trichoderma reesei . In: Wood, Methods in Enzymology, *Academic Press, New York.,* vol. 160, pp. 234– 242, 1988.

[14] G. Stephanopoulos, Challenges in engineering microbes for biofuels production. Science 315: 801– 804; 2007.

[15] L. J. Jonsson, E. Palmqvist, N. O. Nilvebrant, and B. Hahn-HaÈgerdal, Detoxification of wood hydrolysates with laccase and peroxidase from the white-rot fungus Trametes versicolor, *Appl Microbiol Biotechnol*, vol. 49, pp. 691–697, 1998.

[16] E. Palmqvist , B. H. Hagerdal, Fermentation of lignocellulosic hydrolysates. II: inhibitors and mechanisms of inhibition, *Bioresource Technology*, vol. 74, pp. 25±33, 2000.

[17] R. Leonard, and G. Hajny, Fermentation of Wood Sugars to Ethyl Alcohol, *Industrial & Engineering Chemistry*, vol. 37, I. 4, pp. 390-395, 1945.

[18] C. Van Zyl, B. Prior, and J. Du Preez, Production of ethanol from sugar cane bagasse hemicellulose hydrolyzate byPichia stipitis. Applied Biochemistry and Biotechnology, vol. 17, I. 1, pp. 357-369, 1988.

[19] I. Horvath, A. Sjode, B. Alriksson, L. Jonsson, and N. Nilvebrant, Critical conditions forimproved fermentability during overliming of acid hydrolysates from spruce, *Applied Biochemistry and Biotechnology*, vol. 124, I. 1. pp. 1031-1044, 2005.

[20] N. Eken-Saraçoglu, and Y. Arslan, Comparison of different pretreatments in ethanol fermentation using corn cob hemicellulosic hydrolysate with *Pichia stipitis* and *Candida shehatae*. *Biotechnol Lett*,vol. 22, pp. 855–858.

[21] A. Martinez, M. E. Rodriguez, M. L. Wells, S. W. York, J. F. Preston, and L. O. Ingram, Detoxification of dilute acid hydrolysates of lignocellulose with lime. *Biotechnol Prog*, vol. 17, pp. 287–93, 2001.

[22] M. Von Silver, G. Zacchi, L. Olsson, and B. Hahn-Hägerdal, Cost analysis of ethanol production from willow-using recombinant Escherichia *coli*. *Biotechnol Prog* vol. 10, pp. 555–60, 1994.

[23] D. B. Hodge, C. Andersson, K. A. Berglund, and U. Rova, Detoxification requirements for bioconversion of softwood dilute acid hydrolyzates to succinic acid. *Enzyme Microb Technol*, vol. 44(5), pp. 309-316.

[24] S. I. Mussatto, and I. C. Roberto, Hydrolysate detoxification with activated charcoal for xylitol production by Candida guilliermondii. Biotechnol Lett , vol. 23, pp. 1681-4, 2001.

[25] C. Huang, M. Zong, Wu Hong, and L. Qiu-ping, Microbial oil production from rice straw hydrolysate by Trichosporon fermentans, *Bioresource Technology*, vol. 100, PP. 4535–4538, 2009.

[26] M. L. M Villarreal, A. M. R. Prata, M. G. A. Felipe, Almeida, and J. B. E. Silva, Detoxification procedures of eucalyptus hemicellulose hydrolysate for xylitol production by Candida guilliermondii , *Enzyme and Microbial Technology,*Vol. 40, Issue 1, pp. 17-24, 2006.

[27] H. Miyafuji, H. Danner, M. Neureiter, C. Thomasser, J. Bvochora, O. Szolar, and R. Braun, Detoxification of wood hydrolysates with wood charcoal for increasing the fermentability of

hydrolysates, Enzyme and Microbial Technology, vol. 32, pp. 396–400, 2003.
[28] L. Canilha, J. B. de Almeida e Silva and A. Irene Nápoles Solenzal, Ucalyptus hydrolysate detoxification with activated charcoal adsorption or ion-exchange resins for xylitol production, *Process Biochemistry*, Vol. 39, I. 12, pp. 1909-1912, 2004.
[29] J. Myoung Lee, A. R. Venditti, H. Jameel, and R. W. Kenealy, Detoxification of woody hydrolyzates with activated carbon for bioconversion to ethanol by the thermophilic anaerobic bacterium Thermoanaerobacterium saccharolyticum, biomass and bioenergy, vol. 35, pp. 626-636, 2011.
[30] N. N. Nichols, B. S. Dien, G. M. Guisado, and M. J. Lopez, Bioabatement to remove inhibitors from biomass-derived sugar hydrolysates, *Appl Biochem Biotechno*l, vol. 121, pp. 379-390, 2005.
[31] N. N. Nichols, L. N. Sharma, R. A. Mowery, C. K. Chambliss, G. P. Walsum, B. S. Dien, and L. B. Iten, Fungal metabolism of fermentation inhibitors present in corn stover dilute acid hydrolysate, Enzyme and Microbial Technology, vol. 42 ,pp. 624–630, 2008.
[32] J. Zhang, Z. Zhu, X. Wang, N. Wang, W. Wang, and J. Bao, Biodetoxification of toxins generated from lignocellulose pretreatment using a newly isolated fungus, Amorphotheca resinae ZN1, and the consequent ethanol fermentation, *Biotechnology for Biofuels*, 3:26, 2010
[33] A. Dehkhoda, Concentrating lignocellulosic hydrolysate by evaporation and its fermentation by repeated fedbatch using flocculating Saccharomyces cerevisiae, MSc Thesis, University College of Borås, Sweden, 2008.
[34] H. W. Dong, and J. Bao, Biofuel via biodetoxification, *Nat Chem Biol*, vol. 6,pp. 316-318, 2010.
[35] J. Yu, and H. Stahl, Microbial utilization and biopolyester synthesis of bagasse hydrolysates, *Bioresour Technol*, vol. 99, pp. 8042-8048, 2008.
[36] M. J. Lopez, N. N. Nichols, B. S. Dien, J. Moreno, and R. J. Bothast, Isolation of microorganisms for biological detoxification of lignocellulosic hydrolysates, *Appl Microbiol Biotechnol,* vol. 64, pp. 125-131, 2004.
[37] N. Wierckx, F. Koopman, L. Bandounas, J. H. De Winde, and H. J. Ruijssenaars, Isolation and characterization of Cupriavidus basilensis HMF14 for biological removal of inhibitors from lignocellulosic hydrolysates, *Microb Biotechnol*, vol. 3, pp. 336-343, 2010.