

## Can Microorganisms Produce Biobutanol after Agricultural Wastes Pretreatment?

Yusuf Owolabi

Biotechnology, University of Chester, Shrewsbury, United Kingdom

Article Info: Received: 18-09-2024 / Revised: 17-10-2024 / Accepted: 11-11-2024

Address for Correspondence: Yusuf Owolabi

Conflict of interest statement: No conflict of interest

### Abstract

This review investigates the possibility of biobutanol as a renewable energy source for addressing the environmental issues associated with traditional carbon-based fuels. As energy demand rises, carbon emissions from fossil fuels exacerbate climate change, emphasizing the importance of sustainable, low-emission alternatives. Bioenergy, which is produced from organic waste via biochemical processes, is a possible replacement. Biobutanol, in particular, has an advantage over bioethanol due to its cleaner combustion and applicability for a variety of applications; nevertheless, the toxicity of biobutanol to its producing bacteria reduces production efficiency. Gas stripping recovery systems are presented as solutions for increasing output.

Renewable energy is classified into four types based on its source: edible crops, non-edible biomass (such as lignocellulose), cellular materials, and carbon capture. Currently, first-type renewables dominate, but agricultural concerns are moving the focus to non-food sources. Second-generation renewables, particularly lignocellulose, offer considerable benefits by exploiting whole plant biomass with minimum land needs. Biobutanol, a second-type renewable, is recognized for its ability to satisfy energy demands while promoting environmentally good activities. This analysis emphasizes biobutanol's potential for bioenergy, notably from agricultural waste, and advocates for more research into production upgrades.

### Introduction

As energy consumption grows, carbon fuel supplies are depleted (Ni et al., 2012). Energy production increases carbon dioxide atmospheric concentrations, which affect the ecosystem since it is linked to climate change. Reliable methods of energy production are needed while also reducing carbon emissions (Bhatia et al., 2015). Perhaps, a biotechnological process may be a promising option. Since bioenergy is created via biochemical processes, it could be a suitable replacement for energy sources. Bioenergy may be made from various organic wastes (Palmer and Brigham, 2017). Biological

decomposition is involved in transforming waste to sugar which further promotes the action of microorganisms (Le et al., 2017). Climate change and energy security are the crucial reasons why butanol production draw global interest, and the bioenergy sector's systematic growth does have the capacity to help farming nations (Zhang & Jia, 2018). Butanol is a widely used product primarily manufactured from hydrocarbon biomasses, and it is used in diluents, butylamines, polymers, carboxylates, and other applications (Bhatia et al., 2017). Biobutanol may not be as well as bioethanol. On the other

hand, biobutanol offers several benefits above bioethanol, including being highly eco-friendly and cleaner combustible fuel (Zhang & Jia, 2018). Biobutanol is indeed poisonous to the bacteria that produce it, which lowers the efficiency of traditional biobutanol synthesis. To boost the efficiency of biobutanol production, an option would be to adopt the gas stripping recovery system (Ezeji et al., 2012). The review focus on the microbial synthesis of biobutanol from agricultural wastes after pre-treatments and its enhancement methods.

### Background

Renewables could be divided into distinct categories regarding their sources (Bhatia et al., 2017). Carbohydrates and polysaccharides are used to make first-type renewables. Renewables of the second-type are made from non-edible material; renewables of the third-type are made from cellular sources; and renewables of the fourth-type are made from Carbon dioxide capture (Aro, 2016). The majority of renewables generated now are first-type, and it is made solely from agricultural products in places like the USA, where maize is used (Bhatia et al., 2017). Recently, agricultural products and energy dilemmas have created numerous first-type bioenergy issues, prompting experts to consider additional renewable options (Qureshi et al., 2013). Biomass-based second-type renewables provide several benefits over first-type renewables. The highly abundant and unexplored active polymers here on the planet (lignocellulose) might be a potential energy source (Hou et al., 2017). Crop residues require minimal usable area to manufacture since the entire plant could be used as a biomass feedstock, while seeds are used in the first-type bioenergy (Zhang & Jia, 2018). However, the usage of lignocellulose as a biomass feedstock also has disadvantages, as it is primarily composed of sugars with minimal micronutrients. Crops of various sorts

could be cultivated simultaneously, requiring lesser fertilizer and reducing the cost of bioenergy sources. (Bhatia et al., 2017). Although third and fourth-type renewables are lucrative, their manufacturing is still in infancy; perhaps biobutanol (second-type) could be an ideal product for energy production.

### Agricultural Waste Pre-Treatments

The fundamental goal of pre-treatment would be to decompose the constituents of lignocellulosic biomass, which include cellulose and lignin for biobutanol production (Chen et al., 2017). The pre-treatment methods for crop residues include mechanical, chemicals, and microbiological approaches. These methods often change the biomolecular composition of lignocellulosic wastes and release carbohydrates (Brodeur et al., 2011). Hence, agricultural wastes need to be pretreated before being used for biobutanol production (Figure 1).

### Mechanical / Physical Approach

This approach involves the physical breakdown of agricultural wastes before biobutanol production (Figure 1). Since the physical method reduces the bulkiness of agricultural wastes while increasing the surface area, it can promote microbial fermentation for biobutanol production (Bhatia et al., 2017). Various techniques are employed in the physical method for biobutanol production. The volume of material may be reduced by chipping to 9–31 millimetres using heating and mass movement. Correspondingly grinding reduces agricultural wastes to 0.09–2.1 mm via shearing forces (Maurya et al., 2015). Although the mechanical method promotes waste reduction, it is not cost-effective and requires some funds to purchase the equipment. In a study comparing various milling processes, Kim and colleagues found that hydrolysis of lignocellulose with enzymes via planetary milling yielded more significant levels of lactose-constituents (Kim et al., 2013).

Furthermore, microwave irradiation seems to be a common technique for decomposing lignocellulosic materials. The method is simple, uses electromagnetic wavelength, and generates lesser inhibitory chemicals (Moodley & Kana, 2017). The process can transform large organic waste into gases, including hydrogen, methane, carbon monoxide,

or carbon dioxide (Huang et al., 2016). However, microwave irradiation poses more risk as it could heat the body tissues, and hence proper handling procedures are needed for safety. Also, sound waves for bioconversion of renewables have now been researched. This is one of the physical methods for pretreating organic wastes—ultrasonic vibrations breakdown lignocellulose components, giving room for enzymatic activity. Sound waves combined with chemical treatments result in cellulose fragmentation and enhanced enzymatic degradation (Gabhane et al., 2014). Another technique used is devolatilization. Devolatilization involves pre-heating organic wastes under anoxic conditions at elevated temperatures (600–900°C), which degrade the fibre components (Akhtar et al., 2015). The above evidence implies that physical methods can pretreat biomass prior to biobutanol production. Still, it has limitations in the cost and maintenance of mechanical devices that lead to other approaches like chemical usage.

### **Chemical Approach**

This approach involves the chemical breakdown of agricultural wastes before biobutanol production (Figure 1). Different chemicals have been known and utilised in the structural breakdown of organic materials such as lignocellulose to enhance their availability for enzymatic action (Bhatia et al., 2017). The chemicals used in pretreating organic wastes include acid, ions, alkali and ozone (Chandra Rajak & Banerjee, 2016). Vinegar components, HCl (Hydrochloric acid) and carboxylic acid have been employed to bioconvert

organic matter into carbohydrates (Maurya et al., 2015). The development of secondary features in addition to carbohydrates seems to be the major downside of chemical treatment. Lignocellulosic biomass was digested to form simple sugars following acid addition, which then deteriorated, producing other compounds (Jönsson & Martín, 2016). Such by-products can be harmful to bacteria and create a variety of oxidative stress, which destroy cells and limit the chemical approach (Allen et al., 2010). In commercial settings, diluted acid is preferably used, performed at an elevated temperature for a limited duration and vice-versa (Sindhu et al., 2011; Taherzadeh & Karimi, 2008). Another chemical substance used in pretreating complex organic waste is alkali (NaOH, Ca(OH)<sub>2</sub>, KOH, NH<sub>4</sub>OH) at room condition. The chemicals successfully decompose lignocellulose and increase enzymatic availability (Chang & Holtzapfel, 2000). Similarly, other chemicals such as ozone that involve the oxidation of organic matter are effective in waste treatment. Ozone targets heterocyclic groups, leaving lignocellulose unaffected. However, water activity and material type could limit ozone effectiveness (Maurya et al., 2015). Organic salts containing cations and anions components had been known to assist in pretreating complex wastes. For example, salt of chloride and oxide were found by various researchers to be involved in wastes treatment (Viell et al., 2016; Reddy, 2015). The side products formed in this method may be harmful and non-biodegradable, which could cause long-term problems; thus, there is a need for safe pretreatment methods, and microbial usage may be a better option.

### **Microbiological Approach**

This approach involves the microbial breakdown of agricultural wastes before biobutanol production. Microbiological approaches are often environmentally

sustainable compared to previously discussed techniques, and different microbes can be used in this method (Bhatia et al., 2017). Shirkavand and colleagues (2017) study confirmed the usage of fungi in the pretreatment of organic matters (radiata pine). However, fungi can digest lignocellulosic biomass, but they are less effective on only cellulosic materials (Sánchez, 2009). In addition, various researchers found that microbes (fungi) could promote the enzymatic breakdown of lignocellulosic wastes (Kumar et al., 2009; Maurya et al., 2015). Although many bacteria (*Bacillus*) were documented for bioconversion complex organic matter, microbes do not really promote enzymatic action on lignocellulosic materials (Masran et al., 2016). Hence, the combination of fungi and bacteria could be employed to improve the treatment of biomass. Enzymatic activity plays a vital role in degrading lignocellulosic materials (Plácido & Capareda, 2015; Masran et al., 2016). The microbial method takes longer and needs a wide surface area, making the technique not appealing for commercial use (Agbor et al., 2011). A combination of approaches while maintaining standard procedures would enhance the proper pretreatment of organic wastes regarding the evidence supplied for biobutanol production (Figure 1).

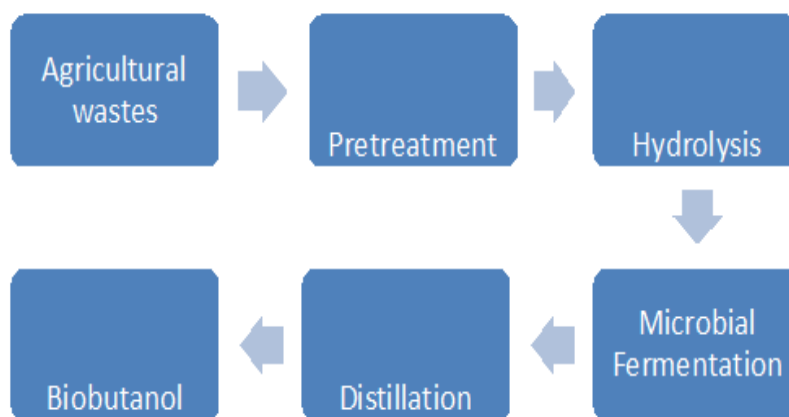
### **Synthesis of Biobutanol**

Biobutanol is a type of biofuel that may be utilised in place of petroleum. Biobutanol has a comparable energy value to petroleum and may be employed without any alteration in machines (Kumar et al., 2009). Biobutanol is insoluble in H<sub>2</sub>O (water), is simple to use, and maybe blended with petrol prior to usage (Dürre, 2007). However, biobutanol produces higher carbon emissions when burned, limiting its usage (Jin et al., 2011). Biobutanol synthesis has been documented using a variety of microorganisms and renewable sources (Figure 1). The study of

Komonkiat and colleagues showed about 14.5 g/L biobutanol synthesis using oil palm fluid as just a feedstock for bacteria culture (Komonkiat et al., 2013).

Biochemical modification could stimulate microorganisms that do not usually make biobutanol to produce it. In another study, biofuels were improved by genetically engineering bacteria with cellulosic genetic materials (Bokinsky et al., 2011). Grasses containing ions was used as a feedstock for engineered bacteria (*Escherichia coli*), which produced about 0.03 g/g biobutanol (Bokinsky et al., 2011). Similarly, researchers demonstrated biobutanol synthesis via a coculture of bacteria species under anoxic conditions. The technique yields about 15 g/L biobutanol using rotting plants as the biomass (Abd-Alla & Elsadek El-Enany, 2012). In other studies, microbes have been involved in the production of high biobutanol ranging from 80 to 100 g/L (Sun et al., 2009; Zhang et al., 2016).

Furthermore, iso-biobutanol is an energy-rich biofuel that is less flammable than bioethanol, with microorganisms involved in their production (Atsumi et al., 2008). Iso-biobutanol is miscible with petroleum because of its decreased oxygen level and is made by carbonylating polypropylene (Atsumi et al., 2008). Although microbes are involved in isobutanol biosynthesis, they can't independently produce it. Hence, biochemical modification could be employed in microorganisms to create iso-biobutanol. For example, Lin and colleagues used engineered thermophiles (bacteria) to produce iso-biobutanol (about 0.7 g/L) at a high condition (50°C) (Lin et al., 2014). Various fungi could stimulate enzymatic action that breaks down complex organic matter into a simpler form, then converted by modified bacteria to iso-biobutanol (about 2.0 g/L) (Minty et al., 2013). The above evidence implies that microorganisms can produce iso-biobutanol upon modifying the microbes.



**Figure 1: Flow chart for biobutanol production from agricultural wastes by microorganisms**

### Enhancement of Biobutanol Synthesis

Engineered bacteria may be created by modifying their genes, which would be a method to act against potential inhibitors of agricultural wastes in biobutanol production (Allen *et al.*, 2010). This makes the microorganisms more resistant to inhibitors while maintaining microbial fermentation for biobutanol production. Some compound (furfural) are potential inhibitor in biobutanol production and seems to be more harmful than others because it causes intracellular O<sub>2</sub> (Oxygen) formation, which damages essential cellular organelles (Bhatia *et al.*, 2016). For instance, Miller and colleagues confirmed that when this inhibitory compound is present, the level of Nicotinamide adenine dinucleotide phosphate becomes reduced in bacteria because of aldehyde reductase activity, which in turn harm microorganisms (Miller *et al.*, 2009). Removal of aldehyde reductase while adding amino acid (cysteine) could promote bacteria resistance to lignocellulosic biomass inhibitors (Miller *et al.*, 2009). Also, scientists have reported that overexpression of a specific biological catalyst (FuO) could result in a high amount of biobutanol synthesis (Zheng *et al.*, 2013; Seo *et al.*, 2016). Similarly, biochemical stimulation of yeast has been

demonstrated by researchers using an enzyme (G6PD) to ascertain their resistance to biomass inhibitor (Gorsich *et al.*, 2006). Glebes and colleagues study found that genetic engineering of bacteria could induce their resistance to potential lignocellulosic biomass inhibitor (Glebes *et al.*, 2014). This suggests that biobutanol synthesis could be enhanced by making bacteria resist biomass inhibitors.

Furthermore, after hydrolysis of complex organic substrates, harmful materials could pose a health risk to humans (Cavka & Jönsson, 2013). Therefore, scientists have utilised different techniques (microbial and chemicals) to destroy toxic materials after biomass hydrolysis (Guo *et al.*, 2013). Various studies have confirmed alkaline treatments such as calcium and sodium hydroxide to remove harmful compounds in biomass after hydrolysis (Alriksson *et al.*, 2011). In addition, some researchers used a liquid recovery technique to destroy toxic lignocellulosic biomass compounds (Persson *et al.*, 2002). Certain biological catalysts (peroxidase) have been reported to detoxify harmful materials from organic matter (Moreno *et al.*, 2015). Moreover, microbes have been found to detoxify inhibitory compounds of biomass to improve biobutanol production (Nichols *et al.*, 2008). This implies that chemicals and microbes play a critical role in promoting biobutanol production via detoxification of



harmful compounds after biomass hydrolysis.

Lastly, lignocellulosic material is really a readily accessible substrate that may be utilised to make biofuels. Several enzymes like glucanases are produced by microbes for the hydrolysis of lignocellulosic biomass into simple carbohydrates for biobutanol synthesis (Lynd *et al.*, 2002). For example, the research conducted by Lynd and colleagues (2002) proved the cellulolytic enzymes are capable of hydrolysing large organic substrates to a simpler unit, making them available for microbial utilization. Another study done by Biwas and colleagues (2014) confirmed the hydrolytic role of enzymes for lignocellulose utilization. However, many microorganisms are unable to digest and use lignocellulose independently; thus, metabolic modification may be a feasible option for improving microbial performance (Kurosawa, 2014). Some scientists have been able to engineer microbes to produce biobutanol. For instance, Morais and colleagues found that metabolically engineered fungi are efficient in lignocellulose utilization, yielding about 0.43 g/g of biobutanol (Hu *et al.*, 2016). Another research conducted by Lim and colleagues synthesized about 2.0 g/L of biofuel using metabolically engineered fungi (Kim, Baek, *et al.*, 2013). The above evidence indicated that microbes' metabolic engineering could improve the synthesis of biobutanol via efficient lignocellulose utilization.

### Conclusion

Renewables should safeguard the ecosystem and replenish the rising fuel consumption. There've been significant advancements in designing methods for producing first-type biofuels during the last 20 years. However, due to energy and agricultural product shortages and manufacturing costs, first-type renewables isn't a viable option. Third and fourth-type biofuel synthesis is at an early stage of development, and additional

investigation is required to create promising techniques. Because organic matter could be used as potential substrates, scientists have properly designed technologies for its utilization, second-type renewables like biobutanol might be a possible strategy. Perhaps, these procedures should be improved. The type of lignocellulosic biomass and microbes' inability to utilise it are critical impediments to producing biobutanol from crop residues. Biobutanol synthesis might be beneficial from the usage of microbiological consortiums. Therefore, microbes are essential in biobutanol production, which could be enhanced through effective substrates utilization, genetic and metabolic engineering.

### References

1. Abd-Alla, M. H., & Elsadek El-Enany, A.-W. (2012). Production of acetone-butanol-ethanol from spoilage date palm (*Phoenix dactylifera* L.) fruits by mixed culture of *Clostridium acetobutylicum* and *Bacillus subtilis*. *Biomass and Bioenergy*, 42, 172–178. <https://doi.org/10.1016/j.biombioe.2012.03.006>
2. Agbor, V. B., Cicek, N., Sparling, R., Berlin, A., & Levin, D. B. (2011). Biomass pretreatment: Fundamentals toward application. *Biotechnology Advances*, 29(6), 675–685. <https://doi.org/10.1016/j.biotechadv.2011.05.005>
3. Akhtar, N., Gupta, K., Goyal, D., & Goyal, A. (2015). Recent advances in pretreatment technologies for efficient hydrolysis of lignocellulosic biomass. *Environmental Progress & Sustainable Energy*, 35(2), 489–511. <https://doi.org/10.1002/ep.12257>
4. Allen, S. A., Clark, W., McCaffery, J. M., Cai, Z., Lanctot, A., Slininger, P. J., Liu, Z. L., & Gorsich, S. W. (2010). Furfural induces reactive oxygen species accumulation and cellular damage in *Saccharomyces cerevisiae*. *Biotechnology for Biofuels*, 3(1), 2. <https://doi.org/10.1186/1754-6834-3-2>

5. Alriksson, B., Cavka, A., & Jönsson, L. J. (2011). Improving the fermentability of enzymatic hydrolysates of lignocellulose through chemical in-situ detoxification with reducing agents. *Bioresource Technology*, 102(2), 1254–1263. <https://doi.org/10.1016/j.biortech.2010.08.037>
6. Aro, E.-M. (2015). From first generation biofuels to advanced solar biofuels. *Ambio*, 45(S1), 24–31. <https://doi.org/10.1007/s13280-015-0730-0>
7. Atsumi, S., Hanai, T., & Liao, J. C. (2008). Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. *Nature*, 451(7174), 86–89. <https://doi.org/10.1038/nature06450>
9. Bhatia, S. K., Kim, S.-H., Yoon, J.-J., & Yang, Y.-H. (2017). Current status and strategies for second generation biofuel production using microbial systems. *Energy Conversion and Management*, 148, 1142–1156. <https://doi.org/10.1016/j.enconman.2017.06.073>
10. Bhatia, S. K., Lee, B.-R., Sathiyarayanan, G., Song, H.-S., Kim, J., Jeon, J.-M., Kim, J.-H., Park, S.-H., Yu, J.-H., Park, K., & Yang, Y.-H. (2016). Medium engineering for enhanced production of undecylprodigiosin antibiotic in *Streptomyces coelicolor* using oil palm biomass hydrolysate as a carbon source. *Bioresource Technology*, 217, 141–149. <https://doi.org/10.1016/j.biortech.2016.02.055>
11. Bhatia, S. K., Yi, D.-H., Kim, Y.-H., Kim, H.-J., Seo, H.-M., Lee, J.-H., Kim, J.-H., Jeon, J.-M.,
12. Jang, K.-S., Kim, Y.-G., & Yang, Y.-H. (2015). Development of semi-synthetic microbial consortia of *Streptomyces coelicolor* for increased production of biodiesel (fatty acid methyl esters). *Fuel*, 159, 189–196. <https://doi.org/10.1016/j.fuel.2015.06.084>
13. Biswas, R., Persad, A., & Bisaria, V. S. (2014). Production of Cellulolytic Enzymes. *Bioprocessing of Renewable Resources to Commodity Bioproducts*, 105–132. <https://doi.org/10.1002/9781118845394.ch5>
14. Bokinsky, G., Peralta-Yahya, P. P., George, A., Holmes, B. M., Steen, E. J., Dietrich, J., Soon Lee, T., Tullman-Ercek, D., Voigt, C. A., Simmons, B. A., & Keasling, J. D. (2011).
15. Synthesis of three advanced biofuels from ionic liquid-pretreated switchgrass using engineered *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 108(50), 19949–19954. <https://doi.org/10.1073/pnas.1106958108>
16. Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K. B., & Ramakrishnan, S. (2011).
17. Chemical and Physicochemical Pretreatment of Lignocellulosic Biomass: A Review.
18. *Enzyme Research*, 2011, 1–17. <https://doi.org/10.4061/2011/787532>
19. Cavka, A., & Jönsson, L. J. (2013). Detoxification of lignocellulosic hydrolysates using sodium borohydride. *Bioresource Technology*, 136, 368–376. <https://doi.org/10.1016/j.biortech.2013.03.014>
20. Chandra Rajak, R., & Banerjee, R. (2016). Enzyme mediated biomass pretreatment and hydrolysis: a biotechnological venture towards bioethanol production. *RSC Advances*, 6(66), 61301–61311. <https://doi.org/10.1039/c6ra09541k>
21. Chang, V. S., & Holtzapfel, M. T. (2000). Fundamental Factors Affecting Biomass Enzymatic Reactivity. *Applied Biochemistry and Biotechnology*, 84-86(1-9), 5–38. <https://doi.org/10.1385/abab:84-86:1-9:5>
22. Chen, H., Liu, J., Chang, X., Chen, D., Xue, Y., Liu, P., Lin, H., & Han, S. (2017). A review on the pretreatment of lignocellulose for high-value

- chemicals. *Fuel Processing Technology*, 160,196–206. <https://doi.org/10.1016/j.fuproc.2016.12.007>
23. Dürre, P. (2007). Biobutanol: An attractive biofuel. *Biotechnology Journal*, 2(12), 1525–1534. <https://doi.org/10.1002/biot.200700168>
24. Ezeji, T. C., Qureshi, N., & Blaschek, H. P. (2012). Microbial production of a biofuel (acetone– butanol–ethanol) in a continuous bioreactor: impact of bleed and simultaneous product removal. *Bioprocess and Biosystems Engineering*, 36(1),109–116. <https://doi.org/10.1007/s00449-012-0766-5>
25. Gabhane, J., William, S. P., Vaidya, A. N., Anand, D., & Wate, S. (2014). Pretreatment of garden biomass by alkali-assisted ultrasonication: effects on enzymatic hydrolysis and ultrastructural changes. *Journal of Environmental Health Science and Engineering*,12(1). <https://doi.org/10.1186/2052-336x-12-76>
26. Glebes, T. Y., Sandoval, N. R., Reeder, P. J., Schilling, K. D., Zhang, M., & Gill, R. T. (2014). Genome-Wide Mapping of Furfural Tolerance Genes in *Escherichia coli*. *PLoS ONE*, 9(1), e87540. <https://doi.org/10.1371/journal.pone.0087540>
27. 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*. *Applied Microbiology and Biotechnology*, 71(3), 339–349. <https://doi.org/10.1007/s00253-005-0142-3>
28. Guo, X., Cavka, A., Jönsson, L. J., & Hong, F. (2013). Comparison of methods for detoxification of spruce hydrolysate for bacterial cellulose production. *Microbial Cell Factories*, 12(1). <https://doi.org/10.1186/1475-2859-12-93>
29. Hou, Q., Ju, M., Li, W., Liu, L., Chen, Y., & Yang, Q. (2017). Pretreatment of Lignocellulosic Biomass with Ionic Liquids and Ionic Liquid-Based Solvent Systems. *Molecules*, 22(3), 490. <https://doi.org/10.3390/molecules22030490>
30. Hu, M.-L., Zha, J., He, L.-W., Lv, Y.-J., Shen, M.-H., Zhong, C., Li, B.-Z., & Yuan, Y.-J. (2016). Enhanced Bioconversion of Cellobiose by Industrial *Saccharomyces cerevisiae* Used for Cellulose Utilization. *Frontiers in Microbiology*, 7. <https://doi.org/10.3389/fmicb.2016.00241>
31. Huang, L., Zhao, L., Zan, X., Song, Y., & Ratledge, C. (2016). Boosting fatty acid synthesis in *Rhodococcus opacus* PD630 by overexpression of autologous thioesterases. *Biotechnology Letters*, 38(6), 999–1008. <https://doi.org/10.1007/s10529-016-2072-9>
32. Jin, C., Yao, M., Liu, H., Lee, C. F., & Ji, J. (2011). Progress in the production and application of n-butanol as a biofuel. *Renewable and Sustainable Energy Reviews*, 15(8), 4080–4106. <https://doi.org/10.1016/j.rser.2011.06.001>
33. Jönsson, L. J., & Martín, C. (2016). Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. *Bioresource Technology*, 199, 103–112. <https://doi.org/10.1016/j.biortech.2015.10.009>
34. Kim, H. J., Chang, J. H., Jeong, B.-Y., & Lee, J. H. (2013). Comparison of Milling Modes as a Pretreatment Method for Cellulosic Biofuel Production. *Journal of Clean Energy Technologies*, 45–48. <https://doi.org/10.7763/jocet.2013.v1.11>
35. Kim, S., Baek, S.-H., Lee, K., & Hahn, J.-S. (2013). Cellulosic ethanol production using a yeast consortium displaying a minicellulosome and  $\beta$ -glucosidase. *Microbial Cell Factories*, 12(1). <https://doi.org/10.1186/1475-2859-12-14>



36. Komonkiat, I., & Cheirsilp, B. (2013). Felled oil palm trunk as a renewable source for biobutanol production by *Clostridium* spp. *Bioresource Technology*, 146, 200–207. <https://doi.org/10.1016/j.biortech.2013.07.067>
37. Kumar, P., Barrett, D. M., Delwiche, M. J., & Stroeve, P. (2009). Methods for Pretreatment of Lignocellulosic Biomass for Efficient Hydrolysis and Biofuel Production. *Industrial & Engineering Chemistry Research*, 48(8), 3713–3729. <https://doi.org/10.1021/ie801542g>
38. Kurosawa, K. (2014). Triacylglycerol Production from Corn Stover Using a Xylose-Fermenting *Rhodococcus opacus* Strain for Lignocellulosic Biofuels. *Journal of Microbial & Biochemical Technology*, 06(05). <https://doi.org/10.4172/1948-5948.1000153>
39. Le, R. K., Wells Jr., T., Das, P., Meng, X., Stoklosa, R. J., Bhalla, A., Hodge, D. B., Yuan, J. S., & Ragauskas, A. J. (2017). Conversion of corn stover alkaline pre-treatment waste streams into biodiesel via *Rhodococci*. *RSC Advances*, 7(7), 4108–4115. <https://doi.org/10.1039/c6ra28033a>
40. Lin, P. P., Rabe, K. S., Takasumi, J. L., Kadisch, M., Arnold, F. H., & Liao, J. C. (2014). Erratum to “Isobutanol production at elevated temperatures in thermophilic *Geobacillus thermoglucosidasius*” [Metab. Eng. 24C (2014) 1–8]. *Metabolic Engineering*, 24, 192. <https://doi.org/10.1016/j.ymben.2014.06.008>
41. Lynd, L. R., Weimer, P. J., van Zyl, W. H., & Pretorius, I. S. (2002). Microbial Cellulose Utilization: Fundamentals and Biotechnology. *Microbiology and Molecular Biology Reviews*, 66(4), 739–739. <https://doi.org/10.1128/mmbr.66.4.739.2002>
42. Masran, R., Zanirun, Z., Bahrin, E. K., Ibrahim, M. F., Lai Yee, P., & Abd-Aziz, S. (2016).
43. Harnessing the potential of ligninolytic enzymes for lignocellulosic biomass pretreatment. *Applied Microbiology and Biotechnology*, 100(12), 5231–5246. <https://doi.org/10.1007/s00253-016-7545-1>
44. Maurya, D. P., Singla, A., & Negi, S. (2015). An overview of key pretreatment processes for biological conversion of lignocellulosic biomass to bioethanol. *3 Biotech*, 5(5), 597–609. <https://doi.org/10.1007/s13205-015-0279-4>
45. Miller, E. N., Jarboe, L. R., Turner, P. C., Pharkya, P., Yomano, L. P., York, S. W., Nunn, D., Shanmugam, K. T., & Ingram, L. O. (2009). Furfural Inhibits Growth by Limiting Sulfur Assimilation in Ethanologenic *Escherichia coli* Strain LY180. *Applied and Environmental Microbiology*, 75(19), 6132–6141. <https://doi.org/10.1128/aem.01187-09>
46. Miller, E. N., Jarboe, L. R., Yomano, L. P., York, S. W., Shanmugam, K. T., & Ingram, L. O. (2009). Silencing of NADPH-Dependent Oxidoreductase Genes ( *yqhD* and *dkgA* ) in Furfural-Resistant Ethanologenic *Escherichia coli*. *Applied and Environmental Microbiology*, 75(13), 4315–4323. <https://doi.org/10.1128/aem.00567-09>
47. Minty, J. J., Singer, M. E., Scholz, S. A., Bae, C.-H., Ahn, J.-H., Foster, C. E., Liao, J. C., & Lin, X. N. (2013). Design and characterization of synthetic fungal-bacterial consortia for direct production of isobutanol from cellulosic biomass. *Proceedings of the National Academy of Sciences*, 110(36), 14592–14597. <https://doi.org/10.1073/pnas.1218447110>
48. Moodley, P., & Kana, E. B. G. (2017). Microwave-assisted inorganic salt pretreatment of sugarcane leaf waste: Effect on physiochemical structure and enzymatic saccharification. *Bioresource Technology*, 235, 35–42. <https://doi.org/10.1016/j.biortech.2017.03.031>

49. Moreno, A. D., Ibarra, D., Alvira, P., Tomás-Pejó, E., & Ballesteros, M. (2015). A review of biological delignification and detoxification methods for lignocellulosic bioethanol production. *Critical Reviews in Biotechnology*, 35(3), 342–354. <https://doi.org/10.3109/07388551.2013.878896>
50. Ni, Y., Xia, Z., Wang, Y., & Sun, Z. (2012). Continuous butanol fermentation from inexpensive sugar-based feedstocks by *Clostridium saccharobutylicum* DSM 13864. *Bioresource Technology*, 129, 680–685. <https://doi.org/10.1016/j.biortech.2012.11.142>
51. Nichols, N. N., Sharma, L. N., Mowery, R. A., Chambliss, C. K., van Walsum, G. P., Dien, B. S., & Iten, L. B. (2008). Fungal metabolism of fermentation inhibitors present in corn stover dilute acid hydrolysate. *Enzyme and Microbial Technology*, 42(7), 624–630. <https://doi.org/10.1016/j.enzmictec.2008.02.008>
52. Palmer, J. D., & Brigham, C. J. (2017). Feasibility of triacylglycerol production for biodiesel, utilizing *Rhodococcus opacus* as a biocatalyst and fishery waste as feedstock. *Renewable and Sustainable Energy Reviews*, 56, 922–928. <https://doi.org/10.1016/j.rser.2015.12.002>
53. Persson, P., Larsson, S., Jönsson, L. J., Nilvebrant, N.-O., Sivik, B., Munteanu, F., Thörneby, L., & Gorton, L. (2002). Supercritical fluid extraction of a lignocellulosic hydrolysate of spruce for detoxification and to facilitate analysis of inhibitors. *Biotechnology and Bioengineering*, 79(6), 694–700. <https://doi.org/10.1002/bit.10324>
54. Plácido, J., & Capareda, S. (2015). Ligninolytic enzymes: a biotechnological alternative for bioethanol production. *Bioresources and Bioprocessing*, 2(1). <https://doi.org/10.1186/s40643-015-0049-5>
55. Qureshi, N., Singh, V., Liu, S., Ezeji, T. C., Saha, B. C., & Cotta, M. A. (2013). Process integration for simultaneous saccharification, fermentation, and recovery (SSF): Production of butanol from corn stover using *Clostridium beijerinckii* P260. *Bioresource Technology*, 154, 222–228. <https://doi.org/10.1016/j.biortech.2013.11.080>
56. Reddy, P. (2015). A critical review of ionic liquids for the pretreatment of lignocellulosic biomass. *South African Journal of Science*, Volume 111 (Number 11/12). <https://doi.org/10.17159/sajs.2015/20150083>
57. Sánchez, C. (2009). Lignocellulosic residues: Biodegradation and bioconversion by fungi. *Biotechnology Advances*, 27(2), 185–194. <https://doi.org/10.1016/j.biortechadv.2008.11.001>
58. Seo, H.-M., Jeon, J.-M., Lee, J. H., Song, H.-S., Joo, H.-B., Park, S.-H., Choi, K.-Y., Kim, Y. H., Park, K., Ahn, J., Lee, H., & Yang, Y.-H. (2016). Combinatorial application of two aldehyde oxidoreductases on isobutanol production in the presence of furfural. *Journal of Industrial Microbiology and Biotechnology*, 43 (1), 37–44. <https://doi.org/10.1007/s10295-015-1718-2>
59. Shirkavand, E., Baroutian, S., Gapes, D. J., & Young, B. R. (2017). Pretreatment of radiata pine using two white rot fungal strains *Stereum hirsutum* and *Trametes versicolor*. *Energy Conversion and Management*, 142, 13–19. <https://doi.org/10.1016/j.enconman.2017.03.021>
60. Sindhu, R., Kuttiraja, M., Binod, P., Janu, K. U., Sukumaran, R. K., & Pandey, A. (2011). Dilute acid pretreatment and enzymatic saccharification of sugarcane tops for bioethanol production. *Bioresource Technology*, 102(23), 10915–10921. <https://doi.org/10.1016/j.biortech.2011.09.066>

61. Sun, L.-H., Wang, X.-D., Dai, J.-Y., & Xiu, Z.-L. (2009). Microbial production of 2,3-butanediol from Jerusalem artichoke tubers by *Klebsiella pneumoniae*. *Applied Microbiology and Biotechnology*, 82(5), 847–852. <https://doi.org/10.1007/s00253-008-1823-5>
62. Taherzadeh, M., & Karimi, K. (2008). Pretreatment of Lignocellulosic Wastes to Improve Ethanol and Biogas Production: A Review. *International Journal of Molecular Sciences*, 9(9), 1621–1651. <https://doi.org/10.3390/ijms9091621>
63. Viell, J., Inouye, H., Szekely, N. K., Frielinghaus, H., Marks, C., Wang, Y., Anders, N., Spiess, C., & Makowski, L. (2016). Multi-scale processes of beech wood disintegration and pretreatment with 1-ethyl-3-methylimidazolium acetate/water mixtures. *Biotechnology for Biofuels*, 9(1). <https://doi.org/10.1186/s13068-015-0422-9>
64. Zhang, C., Li, W., Wang, D., Guo, X., Ma, L., & Xiao, D. (2016). Production of 2,3-butanediol by *Enterobacter cloacae* from corn-cob-derived xylose. *TURKISH JOURNAL of BIOLOGY*, 40, 856–865. <https://doi.org/10.3906/biy-1506-66>
65. Zhang, J., & Jia, B. (2018). Enhanced butanol production using *Clostridium beijerinckii* SE-2 from the waste of corn processing. *Biomass and Bioenergy*, 115, 260–266. <https://doi.org/10.1016/j.biombioe.2018.05.012>
66. Zheng, H., Wang, X., Yomano, L. P., Geddes, R. D., Shanmugam, K. T., & Ingram, L. O. (2013). Improving *Escherichia coli* FucO for Furfural Tolerance by Saturation Mutagenesis of Individual Amino Acid Positions. *Applied and Environmental Microbiology*, 79(10), 3202–3208. <https://doi.org/10.1128/aem.00149-13>