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Pichia stipitis: A Hospitable Host for Bioethanol Production

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ABSTRACT: Humans discovered the fire when they needed to cook, wheels when they needed to travel and fuel when they needed to escalate all this process. This evolution of humanity increased pressure on nature, which has enough to satisfy our need but not our greed. Humans bathed in a new shower of change with every coming generation. Fuel is one of the essential parts of this change, and hence, a cloud of exhaustion keeps on covering its reserves, and the day is not too far when this cloud will bring the ultimate shower of change which will exhaust all the fuel reserves. Hence, the need to shift to an unconventional source of fuel production has risen. This paper mainly focuses on providing a solution to this continued exploitation of conventional fuel production sources by suggesting a viable alternative method. One such alternative can be biofuel, which is considered one of the most promising alternative fuel production sources. However, it too has its challenges like a proper selection of feedstock, transportation of biomass, pretreatment, conversion to usable fuel, all of this while not competing over food crops. Hence, lignocellulosic biomasses are the most potential source for the production of biofuels. The best among them is bioethanol which has a current global market share of US $ 58 Billion. The main component of the lignocellulosic feedstock is cellulose. Its yield is 11.1% more than glucose, but it is also comparatively challenging to synthesize cellulose and convert it into bioethanol. *P. stipitis*, coming from the family of 'respiro-fermentative' yeasts, is used as the best xylose fermenter among all other microorganisms used in bioethanol production. Fermenting lignocellulosic feedstock using P. stipitis can reach up to 80% yield. *Scheffersomyces stipitis* or *Pichia stipitis* gives the best yield, approaching 91% when under optimal microaerophilic conditions.

Keywords: Biofuel, Bioethanol, *P. stipitis,*Lignocellulosic biomass

INTRODUCTION

The continuous increase in the world's energy requirement and awareness about the environment has led to a rise in the focus on alternatives to fossil fuels for energy requirements. The first such renewable alternative that comes to mind is biofuel. Among so many different kinds of biofuels, bioethanol is the most promising one. It is also the most used biofuel globally, with an ever-so-increasing annual market considering the current yearly market value being US $58 Billion. The biomass obtained from agricultural residues, municipal waste, wood etc., suffice for the need for a sustainable and renewable feedstock to produce biofuel, namely – bioethanol (Pundir *et al*., 2019; Ismael *et al*., 2020). Using cellulose as a bioethanol feedstock is more advantageous than using sugars or glucose.

$$C_6H_{12}O_6 \rightarrow 2C_2H_5OH + 2CO_2 \;\; (b = 0.511)$$
(glucose)

$$n(C_6H_{10}O_5) + nH_2O \rightarrow 2nC_2H_5OH + 2nCO_2 \;\; (b = 0.568)$$
(cellulose)

It is evident from the equations that cellulose has an ethanol yield of 11.1% more than glucose (Ghosh and Ghose, 2003). Bioethanol can be obtained from feedstocks sourced from glucose, starch and even lignocellulosic materials.Since lignocellulosic matter is just a by-product of food and fodder production, it does not put up the food vs fuel fight. Considering good and efficient conversion techniques are applied to the process, lignocellulosic materials can serve as the feedstock for a significant amount of bioethanol fuel. The essential elements of the lignocellulose based biomass are cellulose, hemicellulose and lignin. After cellulose, hemicellulose is the most abundantly found carbohydrate in nature. Thus, if efficiently converted to ethanol, it is readily available and can be the alternative to fossil fuels as the future fuel.

Yeasts and bacteria are those microorganisms that are very useful in converting hemicellulose to sugar. One such microbe is *Pichia stipitis* (*aka Scheffersomyces stipitis*), being the best xylose-fermenting organism.

This capability of *P. stipitis* has already been scrutinized extensively in laboratories across the globe by its ability to utilize pentose in addition to hexose giving high yields of ethanol.Its solid and robust ability to convert biomass into ethanol has become an essential part of the biofuel and energy industry (Surya and Kumar, 2018). This yeast shows its fermentation capabilities better in oxygen deficit environments. It also shares an endosymbiotic relationship with passalid beetles. The efficiency of conversion of xylose to ethanol is very quickly affected by the concentration of oxygen in the environment.In the symbiotic relationship with passalid beetles, *P. stipitis* uses its oxygen deficit digestive tract and the hindgut region to convert xylose. It has also been observed that *P. stipitis* can ferment glucose obtained from lumber products. These microbes are very robust and can survive even in landfills, contributing a significant amount of glucose for ethanol production by microbes. *P. stipitis*, if provided optimal conditions, can give yields as high as 84.7% to 90.7% on every one unit of the substrate.

PRETREATMENT METHODS

Pretreatment of a substrate essentially reduces the substrate's size, degrades the fibres, swells the biomass, makes it softer, decreases crystallinity, solubilizes hemicellulose removes lignin. Overall, it affects any or all of these to increase the effective surface area available for the enzymatic hydrolysis. Hemicellulose can be hydrolyzed to soluble sugars by pretreating it with dilute sulphuric acid at high temperatures (Agbogbo and Wenger, 2006).

This is an integral part of the production process of bioethanol from lignocellulosic biomass as it prepares the cellulose for hydrolysis to obtain bioethanol. However, it is tough to convert lignocellulose to bioethanol to make the cellulose easily accessible. The biomass feedstock is pretreated beforehand with the four fundamental pretreatment types – physical, chemical, physicochemical, and biological. Pretreatment helps improve the rate of hydrolysis.

In simple terms, physical pretreatment is the process of breaking the feedstock into smaller and smaller particles to increase the total surface area or volume available for hydrolysis. This makes the upcoming production steps easier (Abdu Yusuf and Inambao, 2019). Methods like enzymatic degradation, ionic and acidic hydrolysis saccharify cellulosic materials to produce fermentable sugars. A direct result of pretreatment is improved crystallinity along with a reduction in particle size.

Chemical pretreatment is the most promising method. It increases the biodegradability of cellulose which is done by the removal of lignin. It decreases the crystallinity and polymerization of the cellulosic component, decreases the amount of lignin, does not emit toxic residues, does not need chemical additives, operates at ambient temperature and pressure. Chemical pretreatment can use chemicals like oxidizing agents, hemicellulose and cellulose obtained from lignocellulosic wastes. Organic chemicals are also employed – oxalic acid, acetylsalicylic acid, salicylic acid.

Physio-chemical pretreatment is cost-effective, transforms lignin and makes hemicellulose soluble. In this two-step process, we get high yields of hemicellulose and glucose. It combines physical and chemical pretreatments in one. All the essential processes of the physical and chemical pretreatment dissolve hemicellulose, alters the lignin structure, and makes cellulose easily accessible by hydrolytic enzymes. Few pretreatment methods include liquid hot water,steam explosion, wet oxidation, ammonia fibre/freeze explosion, organosolv, among others.

Biological pretreatment is comparably cheaper than the physical, chemical and physicochemical pretreatment methods. The equipment does not suffer any corrosion; the inhibitors' cost is also reduced, with low energy consumption. This method counts on the fungi' action and ability, yeasts that can produce enzymes capable of degrading lignin, hemicellulose and phenols, etc., along with other such components of bioethanol feedstock. In the recent past, biological pretreatment methods have gained so much interest from scientists since it has many potential and visible advantages over the other methods such as specificity in reaction type and substrate (Behera *et al.,* 2014).

Goals of pretreatment :

- It makes the solid biomass highly digestible, which improves the sugar and glucose production figures from enzyme hydrolysis.
- Reduces and limits the inhibitor formation for the further steps of the production process.
- Pretreatment also helps make it easier to recover lignin for conversion into valuable products.
- Pretreatment also plays an essential role in NOT degrading sugars like pentose and also those obtained from hemicellulose.
- Furthermore,it makes the system more efficient and cost-effective by operating in moderate-size reactors while minimizing heat and power requirements (Brodeur *et al.,* 2011).

II. FERMENTATION

The conversion of carbohydrates to acids or alcohol is termed fermentation. Bioethanol, the renewable transportation fuel, is prepared under anaerobic conditions by converting corn sugar,i.e. glucose, toethanol with the help of yeast. Out of the so many possible feedstocks fermented by microbes, the most abundantly found feedstock is the lignocellulosic biomass, comprising cellulose, hemicellulose lignin. A cross-linked network of polysaccharides like cellulose and hemicellulose, bound tightly to lignin, forms a lignocellulose matrix. The lignocellulosic materials promise to be an alternative to fossil fuel resources due to their high abundance in nature. Because of the heavy rise in CO_2 rate by using existing fossil fuels leading to global warming. A polymer linked by glucosidic bonds at beta-1,4 and formed by glucose unitsis known as cellulose (Agbogbo *et al.,* 2006). The process by which the biomass of lignocellulose is converted to obtain bioethanol isbroken down into five basic operations:—reducing the size to expand the uniformity and the surface area. Using fermentation to produce ethanol from sugars that are monomeric and, finally, the process of distillation/separation to recover ethanol (Bellido *et al.,* 2011). After reducing the xylose to xylitol, oxidization of xylitol is done inside the cell in which proton symport is used to transport the xylose, marks the beginning of converting xylose to ethanol. In this process, xylose reduction and the oxidization of xylitol aremade using XR and XDH, respectively. The conversion of this xylulose into the compounds of C6 and C3 after phosphorylation and channelling into Penrose phosphate shunt occurs. After that, glycolysis is done using these compounds. In xylose to ethanol by yeasts (like *P. stipitis*), oxygen plays a vital role in the entire process.

Also, a stage is reached when ethanol is produced in large quantities, and cell production reduces oxygenation (Skoog and Hahn-Hägerdal, 1990). *Pichia stipitis* is among the most rarely occurring yeasts. It is obtained from woods that are decaying and larvae of insects that are native to the wood. This yeast has such an ecological niche, making it capable of utilizing the present sugars in the wood very smoothly. It can break cellobiose also as it can easily break it into cellulose monomer by using one of its enzymes, beta-glucosidase.*Pichia stipitis* is among the rarest yeasts capable of fermenting xylose from lignocellulosic materials for bioethanol production. Its capability under anaerobic conditions to ferment cellobiose, galactose, along xylose and glucose is unmatchable (Agbogboand Coward-Kelly, 2008). Among the known microbes, its native ability to ferment xylose is the highest (Ishizaki and Hasumi, 2014).

Additionally, *P. stipitis* has less ethanol resistance than *S. cerevisiae*and controlling the dissolve. Oxygen at low levels is of great importance for high ethanol yield using *P. stipitis*. Even though this yeast's proper growth and development require a temperature of around 30°C, it can quickly be brought into use for SSF set-ups. The cofactor imbalance in the enzyme used to metabolize xylose can be balanced using the non-cytochrome electron transport chain which they possess. Nitrogen significantly affects ethanol production by *P. stipitis*. Only under microaerophilic condition renders the most efficient ethanol production. On the other hand, there is zero ethanol production under aerobic environments,with an excess of sugars. Different hydrolysates have been used to evaluate the productivity measures with yields ranging from 0.31-0.48g ethanol per gram sugar consumed (Weber *et al.,* 2010).

Table 1: Ethanol production from the lignocellulosic substrate by microorganisms.

S. No.	Substrate	Microorganism	Ethanol Production (g/L)	Reference
1	Rice straw	Sesc engineered *Aspergillus niger with engineered Saccharomyces cerevisiae*	31.9	Yang *et al.,* 2018
2	Pomegranate peel	*Saccharomyces cerevisiae, Pichia stipitis*	5.58	Demiray *et al.,* 2018
3	Banana peels hydrosylate	*Zymomonasmobilis* CCT 4494, *Pachysolen tannophilus* CCT 1891	11.32	Ferreira *et al.,* 2018
4	Rice husk	*Escherihia coli* KO11	2.7	Tabata *et al.,* 2017
5	Sugarcane bagasse	*Pichia stipitis* BCC15191	0.29±0.02	Buaban *et al.,* 2010
6	Sugarcane bagasse	Recombinant *Escherichia coli* KO11	31.5	Takahashi *et al.,* 2000
7	Wheat straw	*Pichia stipitis* NRRL Y-7124	0.35	Nigam, 2001
8	Wheat straw	*Pichia stipitis*A	0.41	Nigam, 2001
9	Rice straw	*Candida shsehatae* NCL-3501	0.45	Abbi *et al.,* 1996
10	Rice straw	*Saccharomyces cerevisiae* ATCC 26603	4	Moniruzzaman, 1995
11	Rice straw	*Pichia stipitis NRRL Y-7124*	6	Moniruzzaman, 1995

COMMERCIAL APPLICATIONS AND FUTURE ASPECTS

Among the so many xylose-fermenting yeasts, the most promising one is *Pichia stipitis* owing to its ability to ferment xylose with high ethanol yield and very low to none xylitol accumulation. It shares a close relationship with the other yeasts,making endosymbiontof passalid beetles that inhibit and decompose white rotten hardwood (Abdu Yusuf and Inambao, 2019; Behera *et al.,* 2014; Agbogbo and Wenger, 2006; Ghosh and Ghose, 2003). This particular yeast is the best xylose-fermenting microorganism compared to any known microorganism. In addition to xylose, this can consume other sugars found in wood as well. Theoretically, the fermented lignocellulosic sugars by *P. stipitis* can approach 80% yield. Because of its natural ability to ferment hydrolysates along with all the genetic tools available, it has become a major choice by scientists and producers to convert lignocellulose into fuels or other chemicals (Ruchala *et al.,* 2020). A yeast group called 'respiro-fermentative' yeast tells us that excess oxygen can lead to lower yields (Agbogho and Wenger, 2007). Under controlled conditions of oxygen, the *P. stipitis*is very well capable of consuming xylose to produce up to 57g/L of bioethanol with a meagre yield of 0.36 g/g xylose at standard room temperature 30˚C. In essence, this yeast, *Pichia stipitis,* has its native strong capacity to ferment glucose, mannose and galactose along with the other important xylan and xylan rich oligomeric sugars. This built-in strength of the *P. stipitis* to effectively ferment and metabolize sugar is one of the most important yeast features. Pretreating the biomass obtained from agricultural waste with mild acids helps prevent side products that degrade sugars. Those by-products can significantly inhibit the fermentation process. They may also release up to 15% to 55% of all the soluble oligomeric sugars. Thus, we can understand that recovery and underutilization of hemicellulose biomass have lesser cost and higher yield when compared to glucose.Naturally, *Pstipitis*has an affinity towards glucose when it comes to ethanol production. Provided the working conditions are the same, glucose is consumed at a rate higher than xylose. Thus, to increase its usefulness in the commercial and industrial sectors, increasing the rate of xylose consumption by *P. stipitis* can go a long way (Selim *et al.,* 2020)· Although *P. stipitis* is not the most used yeast, its specific traits are of utmost importance. Many scientists use *P. stipitis* to genetically modify *Saccharomyces cerevisiae* to improve its xylan, xylose and cellulose metabolism.There are a few other bioconversion related traits of *P. stipitis* that are important: (1) ability to modify low molecular weight moieties, (2) ability to reduce acyclic enones to the corresponding alcohols, (3) ability to form various esters and smell/aroma components, and (4) engineered in such a way that it gives high yields while producing xylitol and lactic acid(Jeffries and Van Vleet, 2009).

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Conflict of Interest. The authors declare no conflict of interest

REFERENCES

Wu, Z., Hou, Y., Dai, Z., Hu, C.A.A., and Wu, G. (2019). Metabolism, nutrition, and redox signaling of hydroxyproline. *Antioxidants & Redox Signaling*, **30**: 674-682.

Abbi, M., Kuhad, R.C., and Singh, A. (1996). Fermentation of xylose and rice straw hydrolysate to ethanol by *Candida shehatae* NCL-3501. *Journal of industrial microbiology*, **17**: 20-23.

Abdu Yusuf, A., and L Inambao, F. (2019). Bioethanol production techniques from lignocellulosic biomass as alternative fuel: a review. *International Journal of Mechanical Engineering and Technology*, **10**: 34-71.

Agbogbo, F.K., and Coward-Kelly, G. (2008). Cellulosic ethanol production using the naturally occurring xylose-fermenting yeast, *Pichia stipitis*. *Biotechnology letters*, **30**: 1515-1524.

Agbogbo, F.K. and Wenger, K.S. (2006). Effect of pretreatment chemicals on xylose fermentation by *Pichia stipitis*. *Biotechnology letters*, **28**: 2065-2069.

Agbogbo, F.K., and Wenger, K.S. (2007). Production of ethanol from corn stover hemicellulose hydrolyzate using *Pichia stipitis*. *Journal of Industrial Microbiology and Biotechnology*, **34**: 723-727.

Agbogbo, F.K., Coward-Kelly, G., Torry-Smith, M., and Wenger, K.S. (2006). Fermentation of glucose/xylose mixtures using *Pichia stipitis*. *Process Biochemistry*, **41**: 2333-2336.

Behera, S., Arora, R., Nandhagopal, N., and Kumar, S. (2014). Importance of chemical pretreatment for bioconversion of lignocellulosic biomass. *Renewable and Sustainable Energy Reviews*, **36**: 91-106.

Bellido, C., Bolado, S., Coca, M., Lucas, S., González-Benito, G., and García-Cubero, M.T. (2011). Effect of inhibitors formed during wheat straw pretreatment on ethanol fermentation by *Pichia stipitis*. *Bioresource Technology*, **102**: 10868-10874.

Brodeur, G., Yau, E., Badal, K., Collier, J., Ramachandran, K. B., and Ramakrishnan, S. (2011). Chemical and physicochemical pretreatment of lignocellulosic biomass: a review. *Enzyme Research*, 2011.

Buaban, B., Inoue, H., Yano, S., Tanapongpipat, S., Ruanglek, V., Champreda, V., Pichyangkura, R., Rengpipat, S. and Eurwilaichitr, L. (2010). Bioethanol production from ball milled bagasse using an on-site produced fungal enzyme cocktail and xylose-fermenting *Pichia stipitis*. *Journal of Bioscience and Bioengineering*, **110**: 18-25.

Demiray, E., Karatay, S.E., and Dönmez, G. (2018). Evaluation of pomegranate peel in ethanol production by *Saccharomyces cerevisiae* and *Pichia stipitis*. *Energy*, **159**: 988-994.
Ferreira, J., Santos, V.A.Q., and Cruz, C.H.G. (2018). Ethanol production by co-culture of *Zymomonas mobilis* and *Pachysolen tannophilus* using banana peels hydrolysate as substrate. *Acta Scientiarum. Technology*, **40**: e35169-e35169.
Ghosh, P., and Ghose, T.K. (2003). Bioethanol in India: recent past and emerging future. Biotechnology in India II, 1-27.
Ishizaki, Haruki and Hasumi, Keiji (2014). Chapter 10- Ethanol Production from Biomass. (EdsTojo S, Hirasawa T) Research Approaches to Sustainable Biomass Systems. Boston: Academic Press. p. 243–258.
Ismael, M.H., Farhan, S.N., Jasem, Y.I. and Mahmood, W.A. (2020). Ethanol Productionfrom Modified Wheat Straw using *Saccharomyces cerevisiae*. *International Journal on Emerging Technologies*, **11**: 845–848.
Jeffries, T.W., and Van Vleet, J.R.H. (2009). *Pichia stipitis* genomics, transcriptomics, and gene clusters. *FEMS Yeast Research*, **9**: 793-807.
Moniruzzaman, M. (1995). Alcohol fermentation of enzymatic hydrolysate of exploded rice straw by *Pichia stipitis*. *World Journal of Microbiology and Biotechnology*, **11**: 646-648.
Nigam, J.N. (2001). Ethanol production from wheat straw hemicellulose hydrolysate by *Pichia stipitis*. *Journal of Biotechnology*, **87**: 17-27.
Pundir, A., Thakur, M.S., Mehta, V., Kumar, B. and Radha. (2019). Studies on Use of Waste Plastic: A Review. *International Journal of Theoretical and Applied Sciences*, **11**: 48-51.
Ruchala, J., Kurylenko, O.O., Dmytruk, K.V., and Sibirny, A. A. (2020). Construction of advanced producers of first-and second-generation ethanol in *Saccharomyces cerevisiae* and selected species of non-conventional yeasts (*Scheffersomyces stipitis*, Ogataea polymorpha). *Journal of Industrial Microbiology and Biotechnology*, **47**: 109-132.
Selim, K.A., Easa, S.M., and El-Diwany, A.I. (2020). The xylose metabolizing yeast *Spathaspora passalidarum* is a promising genetic treasure for improving bioethanol production. *Fermentation*, **6**: 33.
Skoog, K., and Hahn-Hägerdal, B. (1990). Effect of oxygenation on xylose fermentation by *Pichia stipitis*. *Applied and Environmental Microbiology*, **56**: 3389-3394.
Surya, K., and Kumar, D. (2018). Hydrothermal and Enzymatic Pretreatment of Apple Pomace for Bioethanol Production by Solid-state Fermentation. *Biological Forum – An International Journal*, **10**: 114-120.
Tabata, T., Yoshiba, Y., Takashina, T., Hieda, K., and Shimizu, N. (2017). Bioethanol production from steam-exploded rice husk by recombinant *Escherichia coli* KO11. *World Journal of Microbiology and Biotechnology*, **33**: 47.
Takahashi, C.M., de Carvalho Lima, K.G., Takahashi, D.F., and Alterthum, F. (2000). Fermentation of sugar cane bagasse hemicellulosic hydrolysate and sugar mixtures to ethanol by recombinant *Escherichia coli* KO11. *World Journal of Microbiology and Biotechnology*, **16**: 829-834.
Weber, C., Farwick, A., Benisch, F., Brat, D., Dietz, H., Subtil, T., and Boles, E. (2010). Trends and challenges in the microbial production of lignocellulosic bioalcohol fuels. *Applied Microbiology and Biotechnology*, **87**: 1303-1315.
Yang, P., Zhang, H., Cao, L., Zheng, Z., Mu, D., Jiang, S., and Cheng, J. (2018). Combining sestc engineered A. niger with sestc engineered *S. cerevisiae* to produce rice straw ethanol via step-by-step and in situ saccharification and fermentation. *3 Biotech*, **8**: 1-9.

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