PRODUCTION OF ETHANOL AND SECOND GENERATION BIOMASS BY USING YEAST WASTE FERMENTATION

Haider Ali [1], Mehmoona Saeed [1], M. Gulfraz [1], Aafia Islam[1] and Maryam Rasheed [2]

1. **University Institute of Biochemistry and Biotechnology**
2. **PMAS Arid Agriculture Rawalpindi**

ABSTRACT

Along with the technological unit processes of pretreatment, enzymatic hydrolysis, fermentation, distillation, and dehydration, the structural elements of the lignocellulosic biomass, such as cellulose, hemicellulose, and lignin, are provided. A study was conducted to assess various biomass potential for production of bio ethanol by using optimized fermentation conditions.

The pretreatment step's goal is to reduce the amount of inhibitors present and increase the amount of carbohydrate surface area accessible for enzymatic saccharification. Carbon dioxide emissions from engine fleets can be decreased by using biofuels. The typical method for producing bioethanol is by the microbial fermentation of sugars for example C6H12O6 into C2H5OH. Cereal grains, sugar cane, and sugar beets are examples of traditional feedstocks (for example, first-generation feedstock). However, 2^{ND} generation and 3^{RD}generation biomass have been researched due to worries about the sustainability of food. Numerous variables affect the amount of ethanol produced during fermentation.

Keywords; Biomass, Bio ethanol, Fermentation

INTRODUCTION

The issue over the buildup of greenhouse gases and their consequences on change in weather has grown along with the continued expansion of the world economy. Many nations are developing renewable energy in response, including the manufacturing of biofuels.Fuel which produced from biomass, such as waste materials, is referred to as a biofuel.(Xu *et al*., 2015)

To convert more complex organic compounds into simpler ones, fermentation is a naturally occurring process. Pretreatment procedures are necessary to get biomass ready for extraction and fermentation before alcoholic fermentation. Simple's sugars produces by enzymatic breakdown. In subsequent metabolic activities that can take place with oxygen or without oxygen, yeast transforms thesimple sugars into ethanol, CO2, and other byproducts. For instance, during glycolysis, two molecules of pyruvate are created from one molecule of glucose. The two pyruvic acid molecules are subsequently broken down into 2 ethanol and 2 carbon dioxide molecules.(Huang *et al*., 2015) Pyruvic acid changeinto acetaldehyde and release carbon dioxide when it is processed without oxygen. Acetaldehyde can then be converted to ethanol in a subsequent step by alcohol dehydrogenase.(Malakar *et al*., 2020)

Food crops, such as wheat, corn, potatoes, beets, and sugarcane, have been employed in generally fermentationof alcohol as feedstocks because they are main origin of the readily available starch and sugar needed for fermentation. But there has been growing worry about fuel generation from food crops as the world's population rises and the amount of arable land remains constrained**.**(Tse *et al*., 2021)

A huge variety of biomass raw materials may now be used to make ethanol, thanks to advances in ethanol production technology. A new generation of fermentation technology is frequently defined as one which enables the synthesis of bio-ethanol from an earlier unexplored biomass resource. Additionally, strategies for biomass generation are categorized according to elements affecting the fermentation environment. (Tse et al., 2021)Fermentation technology is categorized based on the amount of water and sugar present in the fermentation medium and whether batch or continuous methods are utilized. To increase ethanol production, additional methods can be used with the fermentation medium. The interrelated problems of global warming, dependency on fossil fuels, and food and energy security are foremost among the numerous difficulties that the modern world faces. Energy consumption rises as a result of population increase and expanding industrialization, yet traditional fossil fuels, especially petroleum, are limited resources that release greenhouse gases (GHG) when burned. In order to fulfil the world's future energy demands, sustainable and ecologically friendly energy sources are necessary (Chaudhary et al., 2017; Zhou et al., 2012)**.** Researchers, business partners, and governments are consequently very interested in biofuels, namely cellulosic bio-ethanol, butanol, and biodiesel. (Demain et al., 2005; Hill et al., 2006) Particularly, bio-ethanol is seen as a possible drop-in fuel that might serve as a substitute for gasoline in the transportation industry.

MATERIALS AND METHODS

Collection of Agricultural Substrates

A wide variety of samples, including wheat, rice, and cotton straws, in addition to corn stover wastes, were gathered from a variety of locations around the Punjab. After being dried and crushed, the samples were put through a sieve with a typical size of 40 mesh.

Analysis of Biomass Samples

The percentages of moisture, ash, dry matter, crude protein, crude fibre, and crude fat, in addition to the samples' respective dry weights, were determined for each and every sample (AOAC, 1990). The amounts of cellulose, hemicellulose, and lignin were determined with the use of a standardised approach that has been described by a number of writers, including Scharf and Tartar (2008). The pretreatment procedure was carried out using H2SO4 and NaOH (at concentrations of 1%, 1.5%, and 2%) at a range of temperatures, including 100, 110, and 120 degrees Celsius, and for a variety of timeframes (15, 30, and 45 minutes). During the experiment, the solid sample that was included in the reagent bottle contributed 10% (w/v). Following the completion of the pretreatment, the vacuum filtration assembly was used to filter the samples contained inside each bottle, and the contents of the bottles were then poured onto filter paper. Following the filtering process, the solid was removed by washing it with 300 ml of distilled water in order to bring the pH level back to normal. The filter paper was then dried at 105 degrees Celsius and weighed. After undergoing a pretreatment of 5% weight-to-volume, the biomass samples were hydrolyzed with cellulase and -glucosidases at a temperature of 50 °C and a rotational speed of 160 rpm for 72 hours in a water bath shaker containing 0.05 M buffer (sodium citrate) at 4.8 pH. Chloromphenicol (100 g/ml) and ampicilin (100 enzymes derived from T. Novozyme A/S, located in Bagsvaerd, Denmark, has provided reesei, cellobiase from Aspergillus niger and Novozyme 188. This enzyme has an activity of (30FPU g-1). In order to find out how much sugar was present, samples were taken out of the reagent bottle at regular intervals of 12 hours (Shields and Cathcart, 2010).

Acid protease is a protein-digesting enzyme that has its highest level of activity and stability in acidic environments (pH 2.0–5.0) and becomes inactive at pH values that are more than 6.0.

Following the completion of the enzymatic hydrolysis, either H2SO4 (l) or NaOH was added. The sample that had a greater quantity of sugar that had been released was chosen for the fermentation process, and the solid biomass was kept at a temperature of 4 degrees Celsius.

After adding the crude enzymes obtained from various bacterial species, the ratio of substrate to enzyme was adjusted to be 1:1, and the mixture was then heated to 50 degrees Celsius for a period of three days. In order to evaluate the specific enzymatic performance of each enzyme, we tested a distinct reaction mixture after adding each enzyme separately. Also, both enzymes were mixed together at a ratio of 1:1 in order to test the combined impact of the enzymes, and the sugar contents were analysed after the scarification process (Tokud and Watanabe, 2007).

At a temperature of four degrees Celsius, a strain of Saccharomyces cerevisiae was kept alive on an agar medium consisting of YPD (yeast extract 1% (w/v), peptone 2% (w/v), and glucose 2% (w/v)). According to Alfenore et al., yeast cells were cultured in a 5-mL tube of YPD medium that included 0.9% (w/v) of sodium chloride while being shaken at 100 revolutions per minute on a rotary shaker at a temperature of 30 degrees Celsius (2002).

RESULTS AND DISCUSSION

Results regarding this experiment are given in following sections

Utilizing C allowed for the successful completion of the fermentation experiment. thermocellum was grown in a medium containing glucose yeast extract for forty-eight hours, and then 10% of the resulting inoculum was added to a fermentation medium containing a solution that had been saccharified previously. This mixture was then stored at room temperature for three days. The fermentation experiment was carried out at a temperature of five hundred degrees Celsius with a

rotational speed of one hundred twenty revolutions per minute. Following the conclusion of the fermentation reaction, the obtained mixture comprised of methanol, butanol, ethanol, and acetone was subjected to a fractional distillation process, which was carried out in a fractional distillation apparatus on the basis of the boiling points of the respective solvents.

After treatment with enzymes cellulase and acid protease, it was shown that a greater quantity of sugar could be extracted from wheat straw. There was no mention made of the fact that the quantity of sugar released by any of the chemical treatments depended on the natural substrates that are utilised for the analysis. All of the agricultural and urban waste substrates that were used for the research had a sufficient quantity of sugars (Tables 2-3). Which is a useful predictor for the manufacture of ethanol on commercial scales, and findings very comparable to these have also been reported by (Zhao et al 2012)

When it was subjected to a treatment with a dilute acid concentration for a period of 30 minutes, during which time the temperature of the reaction was kept at 110 degrees Celsius, the solid portion of the samples produced a greater amount of glucose. During the pretreatment process, increasing the temperature to a moderate level while increasing the acid concentration was shown to have a vital function in increasing the amount of glucose present. A discovery that is comparable about the acid hydrolysis of orange peel at a low temperature was reported by Talo et al (2014).

The performance of celllases was actually enhanced (due to the absence of cellubioses), which resulted in higher sugar recovery after enzymatic hydrolysis (Yoon et al., 2007). The reason behind higher saccharification (80.54%) was achieved as there was no accumulation of sugar like cellobiose occurred although cellobiose was available in reaction mixture (Williams, 2009). The saccharification process of various biomass samples was carried out a number of times. The findings suggest that wheat straw is the most effective in releasing glucose, followed by rice straw, maize, cotton straws, and peel wastes (table 2-3). This sugar, after it has been liberated, may then be used further in fermentation research.

However, up to a specific time limit, the concentration of ethanol increased while the glucose concentration decreased as the time period progressed (Tables 1-2). Nevertheless, the glucose content was insufficient to keep the ethanol synthesis going after 72 hours. Comparing the cellulose and lignin contents of cogon grass to those of Peel wastes revealed that the former is a more favourable option for the generation of ethanol than the latter because to its higher cellulosic concentration.

Table 1. Pre treatment with 1 % H_2SO_4

H_2SO_4 (%)	Temp (C)	Time (min)	Wheat Straw	Rice Straw	Cotton stalk	Corn stover
1	105	10	11.9± 0.3	7.8 ± 0.5	6.7 ± 0.8	14.9 ± 0.4
		15	11.8± 0.4	9.8 ± 0.4	9.2 ± 0.5	15.2 ± 0.7
		20	11.5± 0.8	8.5 ± 1.5	9.8 ± 0.6	15.1 ± 0.7
	115	10	8.6± 0.5	11.5 ± 0.4	10.3 ± 0.5	13.8 ± 0.5
		15	7.9± 0.2	12.8 ± 0.3	11.7 ± 0.3	13.9 ± 0.4
		20	8.1± 0,7	9.5 ± 0.1	9.8 ± 0.1	10.8 ± 0.7

Chemical treatment of biomass samples for sugar . Mean ± ST

Table 2. Pre treatment with 1 % NaOH

H_2SO_4 (%)	Temp (C)	Time (min)	Wheat Straw	Rice Straw	Cotton stalk	Corn stover
1	105	10	10.9± 0.3	7.8 ± 0.5	6.7 ± 0.8	14.9 ± 0.4
		15	11.2± 0.4	9.8 ± 0.4	9.2 ± 0.5	13.2 ± 0.7
		20	11.5± 0.8	7.5 ± 1.5	8.8 ± 0.6	15.1 ± 0.7
	115	10	8.6± 0.5	11.5 ± 0.4	10.3 ± 0.5	11.8 ± 0.5
		15	6.9± 0.2	11.8 ± 0.3	11.7 ± 0.3	13.9 ± 0.4
		20	8.1± 0,7	9.5 ± 0.1	9.8 ± 0.1	10.8 ± 0.7

Chemical treatment of biomass samples for sugar . Mean ± ST

Spectrophotometric analysis and Comparison of sugar production in three agrowaste samples

After 72 hours of enzymatic hydrolysis, wheat straw consistently produced higher yields of glucose than any other material in all of the studies (Table 4). During the course of the experiment, it was discovered that elevating the concentration of H2SO4 from 0.5 to 1.5% resulted in an increase in the total quantity of sugar. A larger yield of glucose was also reported at a retention duration of 20 minutes rather than 10 and 15 minutes, which was the case in all of the studies. The optimal parameters for the acidic pretreatment of wheat straw were determined to be 120 degrees Celsius, a retention duration of 20 minutes, and 1.5% sulphuric acid. At this concentration, the amount of glucose present was at its highest point. During the alkaline pretreatment conditions, the glucose yield was improved by raising the temperature, and the highest yield was reported when the temperature was 120 degrees Celsius. During this time, using a NaOH concentration of 1.5% resulted in a significant yield of glucose being produced while maintaining the same temperature. Sugar yield was enhanced by prolonging the period of enzymatic hydrolysis from 0 to 48 hours, but sugar concentration was decreased when the time was raised further to 72 hours. The drop in glucose concentration was presumably related to the generation of inhibitors by greater acid concentrations. Rice straw has shown higher glucose

yield in acidic pretreatment conditions at 110 0C, acid concentration (1.5%) and retention time 10 minutes was used. The optimum condition used for rice straw analysis in the case of alkaline pretreatment was temperature (100 0C), sodium hydroxide concentration (0.5%), and retention time (20 min). Following enzymatic hydrolysis for seventy-two hours, a higher yield was achieved. During the acidic pretreatment process, a high yield of glucose was produced at a temperature of 1200 degrees Celsius and a concentration of 0.2 percent H2SO4.

Table 3. Products obtained after fermentation process

Substrate	**Total concentration (g/L)**	**Total theoretical yield of ethanol (g/L)**	**Actual yield of ethanol (g/L)**	**Fermentation Efficiency (%)**
Wheat straw	22.7	11.7	11.3	93.4
Rice straw	16.8	9.6	10.6	81.8
Corn stover	15.5	7.8	9.5	92.5
Cotton stalk	19.6	10.8	9.7	91.5

Ethanol production from biomass samples Mean ± standard deviation

When the bacteria are cultivated in the liquid substrate or submerged in the liquid, the process is referred to as submerged fermentation (Table 3).. As pulverized carbohydrate rich materials mixed with water and melt by heating and then breakdown by enzymes, this kind of fermentation is frequently utilized in the synthesis of 1stgeneration ethanol (Sadh et al. 2018). As a result, a liquid medium is created in which different nutrients and carbohydrates are suspended as particle solids or dissolved. Since submerged fermentation may swiftly produce a large output of bioactive metabolites, it is used in various bio-industrial processes, such as enzyme manufacturing. Unfortunately, there may be drawbacks to this process, such as the need for energy and water inputs, the need for high-volume of bioreactors.Fortunately, the extra thin stillage from the waste by-product wet distillers' grains can be removed using centrifugation. The thin stillage can then be dried to distillers' soluble with a minor amount of efficiency, and the

solids may then be dried to distillers' dried grain. Three products that are utilized as feed components result from these drying procedures are distillers' soluble, distillers' dried grains, and distillers' dried grain with soluble. Thin stillage can also be given to calves in neighboring feed lots as a water alternative, or it can be further processed fermentation by microbes to provide a high-quality protein feed. The transformation of low-value glycerol into the more valuable molecule 1,3-propanediol is one advantage of the latter method (Ratanapariyanuch et al. 2017; Tse et al. 2020).

Yeast is defined as a basidiomycetous or ascomycetous fungus that produces spores that are not enveloped in the fruiting body and reproduces by fission or budding (Kurtzman, 1996).

The majority of yeasts are capable of glycolyzing a variety of hexose carbohydrates into ethanol. However, due to its durability and tolerance, *Saccharomyces cerevisiae* is perhaps the most popular yeast strain for the process alcoholic fermentation. As a facultative anaerobe that can thrive in both aerobic and anaerobic environments while being supplied with glucose, *S. cerevisiae* has various improvements over other yeasts strains (Marcus Krantz, 2004) (P. A. M. Claassen, 1999). In anaerobiotic states, *S. cerevisiae* will start to yield acetaldehyde, which further reduces ethanol production (P. A. M. Claassen, 1999). The basis for the generation of bioethanol without resorting to the final oxidation products, CO2, is the capacity of yeasts to catabolize six-carbon compounds (Siti Hajar Mohd Azhar, 2017). Alcohol dehydrogenase (EC 1.1.1.1), which is reliant upon bioethanol, is generated by the diauxic shift and alcoholic fermentation digestion, which is finally regulated by the ADH1 site. ADH1 catalyzes the fermentation of glucose, which results in the formation of ethanol and the reduction of acetaldehyde. In a similar manner, the reverse reaction can also be catalyzed, though with less catalytic efficiency: the conversion of ethanol to acetaldehyde (Siti Hajar Mohd Azhar, 2017).

Yeast Stress

Various stressors, comprising of biological, chemical (toxicity from ethanol along with its byproducts, pH), and physical stressors (e.g., heat shock, osmotic tension) are applied to saccharomyces cerevisiae during inoculation and fermentation. These stress factors have the potential to reduce bioethanol production (Graeme M.Walker, 2020). Stress can cause a rise in mutations, contamination due to microbes, changed yeast flocculation, increase in the production of glycerol, reduced production of ethanol, and development of undesirable chemicals (such as taste and aromatic composites in fermented drinks) (Jonathan ACray, 2015) (Quinten Deparis, 2017).

The concentration of substrate ranging from 20 and 300 kg m-3 has an impact on the generation of ethanol. As seen in Figure 2, larger substrate concentrations can result in more production of bioethanol, however at temperatures of 30 C and higher starting glucose level over 80 kg m-3, a lengthier incubation period was needed. Additionally, whenever the pH value was not adjusted, larger initial levels of glucose, such as 300 kg m-3, may well have affected the productivity of ethanol conversion because the greater substrate and production concentration levels may have hindered the operation of bioethanol production (Yan Lin, 2012).

After incubation for 48 and 72 hours at 30 C, Figure 3 demonstrates, the distinct ethanol production levels and ethanol conversion efficiencies at a variety of starting glucose concentration. The information shown above shows that a greater starting glucose content may result in a lower conversion efficiency of ethanol. After 72 hours of incubation, the highest rates of glucose conversion were noted to be 48.0 percent, 59.9 percent, 28.3 percent, 13.7 percent, and 3.7 percent at glucose concentrations of 20, 40, 80, 160, and 300 kg/m-3, correspondingly.

When the pH value was not adjusted, addition of more substrate did not increase the particular ethanol manufacturing capacity.

Optimizing a number of factors can result in better bioethanol production efficiency. pH is a significant element that influences ethanol fermentation along with temperature and substrate concentration (Kaja Kasemets, 2007). In order to quantify the efficiency of the capacity to produce ethanol with variations in PH, variations in ethanol and VFAs were examined. In an anaerobic jar fermenter, this was tested at PHs 3.0, 4.0, 5.0, 5.5, and 6.0.

The findings of the batch test performed to look into how pH affects production of ethanol are shown in fig. 6. The incubation period for the highest ethanol concentration was lengthened when the pH was less than 4.0, although the highest concentration was not very low. The amount of ethanol generated reduced significantly as the PH level was greater than 5.0. As a result, the operating limit for the anaerobic ethanol generation process may be thought of as a pH range of 4.0-5.0.

With an ethanol energy conversion of 61.93 percent, pH 5.0 produced the greatest particular ethanol manufacture rate of all of the batch trials, which is 410 g kg-1h-1 of SS. At pH 4.0, the specific ethanol production rate was 310 g kg-1h-1 of SS, which is not materially inferior than the figure at pH 5.0. Thus, pH 4.0 may be thought as the functioning limit for the ethanol manufacture procedure while taking into account the chemical required for pH correction (Yan Lin, 2012).

A reduced fermentation period results in insufficient microbial development, which ultimately results in an ineffective production. As a consequence of rising concentration of ethanol in the

fermented broth, longer fermentation times have a harmful impact on microbial development, particularly when done in batch process.

While studying sweet sorghum, Nadir et al. (N. Nadir, 2009) found that ethanol concentrations peaked at 40.11 g/L after 64 hours of fermentation before falling to 37.24 g/L after 72 hours. Even though the ethanol production is lowest at lower temperatures, fermentation takes longer to finish. For instance, when fermentation was carried out at 15° C, only 44.0% of sugar was consumed in more than 240 hours, yielding the lowest amount of ethanol (A. M. Jones, 1994).

Bioethanol derived from sustainable feedstock is a profitable and environmentally benign substitute for non-renewable hydrocarbons as the world's demand for energy rises. However, lignocellulosic inedible biomass (second-generation bioethanol) and algal resources (third-generation bioethanol) are grow into more and more appealing feedstocks for bioethanol production as concerns about the world's food supply rise. The refractory lignocellulosic structure and algal cell wall must be disrupted by pretreatment conditions in order to make the fermentable glucose available in second- and third-generation feedstock. The feedstock, cultivar, and organism employed all have an impact on fermentation productivity and bioethanol production. To achieve the best rate and extent of fermentation, biotic (like microbial contamination) and abiotic (such nutritional, trace metal, and vitamin shortages) aspects must also be taken into consideration. Yeast fermentation techniques, such as fed-batch and continuous fermentation, can be used to solve some of these issues and assist reduce yeast stress. Additionally, supplementary additives and adaptive reactions can boost yeast organisms' resistance to stress (such as heat shock and ethanol shock) and enhance fermentation efficiency. In order to choose a feedstock option, commercial bioethanol manufacturers should consider the necessary pretreatment parameters, take into account the various fermentation technical designs,

and identify any potential fermentation-related difficulties. These actions improve fermentation efficiency and raise ethanol output when combined. To explore the viability and financial effects for the integration of these technologies in the upcoming manufacture of bioethanol, particularly in examining and encouraging the use of third-generation biofuels, techno economic considerations should also be assessed. The country ‘s energy demand is expected to increase three fold by 2050, but supply position is not inspiring. Due to similar situation renewable and sustainable energy resources are the best alternative of conventional fuels and energy sources

CONCLUSIONS

A sustainable and cost-effective route is the bioconversion of lignocellulosicbiomass into alcoholic fuels (butanol and ethanol). While efforts must be made to further create more efficient and cost-effective fermentation techniques and have a firm grasp on the foundations of the different pretreatment procedures. More efficient microbial strains are also needed to produce cost-effective detoxification methods. The manufacturing cost might be reduced and the company could become more competitive if it integrated and optimized the process of lowering energy consumption and raising yields of alcoholic fuels from raw materials.

REFERENCES

Achiras, Spyridon, and Gerrit Jan Willem Euverink. 2016. “Consolidated Briefing of Biochemical Ethanol Production from Lignocellulosic Biomass.” Electronic Journal of Biotechnology 23:44–53. doi: 10.1016/j.ejbt.2016.07.006.

Bai, F. W., W. A. Anderson, and M. Moo-Young. 2008. “Ethanol Fermentation Technologies from Sugar and Starch Feedstocks.” Biotechnology Advances 26(1):89–105. doi: 10.1016/j.biotechadv.2007.09.002.

Balat, Mustafa, Havva Balat, and Cahide Öz. 2008. “Progress in Bioethanol Processing.” Progress in Energy and Combustion Science 34(5):551–73. doi: 10.1016/j.pecs.2007.11.001.

Choudhary J, Surender S and Lata N (2017). “Bioprospecting Thermotolerant Ethanologenic Yeasts for Simultaneous Saccharification and Fermentation from Diverse Environments. J Bioscie Bioengineer 123(3):342–46. doi: 10.1016/j.jbiosc.2016.10.007.

Corbin, Kendall R., Caitlin S. Byrt, Stefan Bauer, Seth DeBolt, Don Chambers, Joseph A. M. Holtum, Ghazwan Karem, Marilyn Henderson, Jelle Lahnstein, Cherie T. Beahan, Antony Bacic, Geoffrey B. Fincher, Natalie S. Betts, and Rachel A. Burton. 2015. “Prospecting for Energy-Rich Renewable Raw Materials: Agave Leaf Case Study” edited by S. C. Davis. PLOS ONE 10(8):e0135382. doi: 10.1371/journal.pone.0135382.

Deesuth, Orawan, Pattana Laopaiboon, Preekamol Klanrit, and Lakkana Laopaiboon. 2015. “Improvement of Ethanol Production from Sweet Sorghum Juice under High Gravity and Very High Gravity Conditions: Effects of Nutrient Supplementation and Aeration.” Industrial Crops and Products 74:95–102. doi: 10.1016/j.indcrop.2015.04.068.

Demain, A L, Michael N and David W (2005). “Cellulase, Clostridia, and Ethanol. Microbiol Mol Biol Revi 69(1):124–54. doi: 10.1128/MMBR.69.1.124-154.2005.

FAO, ed. 2008. Biofuels: Prospects, Risks and Opportunities. Rome: FAO.

Hill J, Erik N, David T, Stephen P and Douglas T (2006). Environmental, Economic, and Energetic Costs and Benefits of Biodiesel and Ethanol Biofuels. Proceedings of the National Academy of Sciences 103(30):11206–10. doi: 10.1073/pnas.0604600103.

Huang H, Nasib Q, Ming-Hsu C, Wei L and Vijay S (2015). "Ethanol Production from Food Waste at High Solids Content with Vacuum Recovery Technology. J Agricul Food Chemi, 63(10):2760–66. doi: 10.1021/jf5054029.

Jambo, Siti Azmah, Rahmath Abdulla, Siti Hajar Mohd Azhar, Hartinie Marbawi, Jualang Azlan Gansau, and Pogaku Ravindra. 2016. "A Review on Third Generation Bioethanol Feedstock." Renewable and Sustainable Energy Reviews 65:756–69. doi: 10.1016/j.rser.2016.07.064.

Kang, Qian, Lise Appels, Tianwei Tan, and Raf Dewil. 2014. "Bioethanol from Lignocellulosic Biomass: Current Findings Determine Research Priorities." The Scientific World Journal 2014:1–13. doi: 10.1155/2014/298153.

Koller, Martin, Anna Salerno, Philipp Tuffner, Michael Koinigg, Herbert Böchzelt, Sigurd Schober, Simone Pieber, Hans Schnitzer, Martin Mittelbach, and Gerhart Braunegg. 2012. "Characteristics and Potential of Micro Algal Cultivation Strategies: A Review." Journal of Cleaner Production 37:377–88. doi: 10.1016/j.jclepro.2012.07.044.

Malakar S, Sanjib K P, and Jolvis P (2020). "Biotechnological Interventions in Beverage Production. (ed) Biotechnological Progress and Beverage Consumption. Elsevier ,pp:1-37

Mussatto, Solange I., Giuliano Dragone, Pedro M. R. Guimarães, João Paulo A. Silva, Lívia M. Carneiro, Inês C. Roberto, António Vicente, Lucília Domingues, and José A. Teixeira. 2010.

"Technological Trends, Global Market, and Challenges of Bio-Ethanol Production." Biotechnology Advances 28(6):817–30. doi: 10.1016/j.biotechadv.2010.07.001.

Puligundla, Pradeep, Daniela Smogrovicova, Vijaya Sarathi Reddy Obulam, and Sanghoon Ko. 2011. "Very High Gravity (VHG) Ethanolic Brewing and Fermentation: A Research Update." Journal of Industrial Microbiology & Biotechnology 38(9):1133–44. doi: 10.1007/s10295-011-0999-3.

Ramachandra, T. V., and Deepthi Hebbale. 2020. "Bioethanol from Macroalgae: Prospects and Challenges." Renewable and Sustainable Energy Reviews 117:109479. doi: 10.1016/j.rser.2019.109479.

Ratanapariyanuch, Kornsulee, Youn Young Shim, Shahram Emami, and Martin J. T. Reaney. 2017. "Production of Protein Concentrate and 1,3-Propanediol by Wheat-Based Thin Stillage Fermentation." Journal of Agricultural and Food Chemistry 65(19):3858–67. doi: 10.1021/acs.jafc.7b00772.

Rendleman, C. Matthew, Hosein Shapouri, C. Matthew Rendleman, and Hosein Shapouri. 2007. "New Technologies in Ethanol Production." doi: 10.22004/AG.ECON.308483.

Sadh, Pardeep Kumar, Surekha Duhan, and Joginder Singh Duhan. 2018. "Agro-Industrial Wastes and Their Utilization Using Solid State Fermentation: A Review." Bioresources and Bioprocessing 5(1):1. doi: 10.1186/s40643-017-0187-z.

Sadh, Pardeep, Suresh Kumar, Prince Chawla, and Joginder Duhan. 2018. "Fermentation: A Boon for Production of Bioactive Compounds by Processing of Food Industries Wastes (By-Products)." Molecules 23(10):2560. doi: 10.3390/molecules23102560.

Tse, Timothy J., Jianheng Shen, Youn Young Shim, and Martin J. T. Reaney. 2020. “Changes in Bacterial Populations and Their Metabolism over 90 Sequential Cultures on Wheat-Based Thin Stillage.” Journal of Agricultural and Food Chemistry 68(16):4717–29. doi: 10.1021/acs.jafc.9b07414.

Tse T J., Daniel J, Wiens M and Reaney J T (2021). Production of Bioethanol—A Review of Factors Affecting Ethanol Yield. Ferment, 7(4):268. doi: 10.3390/fermentation7040268.

Wang, Feng-Sheng, Chung-Chih Li, Yu-Sheng Lin, and Wen-Chien Lee. 2013. “Enhanced Ethanol Production by Continuous Fermentation in a Two-Tank System with Cell Recycling.” Process Biochemistry 48(9):1425–28. doi: 10.1016/j.procbio.2013.06.023.

Xu, Jiuping, Meihui Li, and Ting Ni (2015). “Feedstock for Bioethanol Production from a Technological Paradigm Perspective.” BioResourc 10(3):6285–6304. doi: 10.15376/biores.10.3.Xu.

Zhou H, Jing-sheng C, Benjamin L. W, Gerald R F and Gregory S (2012). “Xylose Isomerase Overexpression along with Engineering of the Pentose Phosphate Pathway and Evolutionary Engineering Enable Rapid Xylose Utilization and Ethanol Production by Saccharomyces Cerevisiae. Metabol Engineer, 14(6):611–22. doi: 10.1016/j.ymben.2012.07.011.

high gravity technology. Journal of Agricultural and Food, 42, 1242–1246.

Alawi Sulaiman, N. O. (2014). Enhancing the Halal Food Industry by Utilizing Food Wastes to Produce Value-added Bioproducts. Procedia - Social and Behavioral Sciences, 121, 35-43. doi:https://doi.org/10.1016/j.sbspro.2014.01.1106

Anshu Singh, A. K. (2012). Utilization of Vegetable Wastes for Bioenergy Generation. Agricultural Research, 1(3), 213-222.

Graeme M.Walker, T. O. (2020). Mitigating stress in industrial yeasts. Fungal Biology, 124(5), 387-397. doi:https://doi.org/10.1016/j.funbio.2019.10.010

Hossain Zabed, G. F. (2014). Bioethanol Production from Fermentable Sugar Juice. The Scientific World Journal, 2014, 1-11. doi:https://doi.org/10.1155/2014/957102

J. Itelima, F. O. (2013). Bio-Ethanol Production from Banana, Plantain and Pineapple Peels by Simultaneous Saccharification and Fermentation Process. International Journal of Environmental Science and Development, 4.

Jahid M, G. A. (2018). Production of bioethanol from fruit wastes (banana, papaya, pineapple and mango peels) under milder conditions. Journal of Bioprocessing & Biotechniques, 8(3), 1-11.

Jonathan ACray, A. P. (2015). Chaotropicity: a key factor in product tolerance of biofuel-producing microorganisms. Current Opinion in Biotechnology, 33, 228-259. doi:https://doi.org/10.1016/j.copbio.2015.02.010

Kaja Kasemets, I. N.-M. (2007). Growth characteristics of Saccharomyces cerevisiae S288C in changing environmental conditions: auxo-accelerostat study. Antonie Van Leeuwenhoek, 92(1), 109e28. doi:10.1007/s10482-007-9141-y

Khandaker MM, Q. K. (2018). Bio-ethanol production from fruit and vegetable waste by using saccharomyces cerevisiae. In Bioethanol Technologies (pp. 1703-1711).

Kurtzman, T. B. (1996). Nonconventional Yeasts in Biotechnology. (K. Wolf, Ed.) Berlin: Springer-Verlag Berlin Heidelberg. doi:10.1007/978-3-642-79856-6_1

Marcus Krantz, B. N. (2004). Anaerobicity Prepares Saccharomyces cerevisiae Cells for Faster Adaptation to Osmotic Shock. Eukaryot Cell, 6(3), 1381–1390. doi:10.1128/EC.3.6.1381-1390.2004

Mohammad Moneruzzaman Khandaker, U. A. (2020). Bio-Ethanol Production from Fruit and Vegetable Waste by Using Saccharomyces cerevisiae. In Bioethanol Technologies.

Muenduen Phisalaphong, N. S. (2006). Mathematical modeling to investigate temperature effect on kinetic parameters of ethanol fermentation. Biochemical Engineering Journal, 28, 36–43. doi:10.1016/J.BEJ.2005.08.039

N. Nadir, M. M. (2009). Comparison of sweet sorghum and cassava for ethanol production by using Saccharomyces cerevisiae. Journal of Applied Sciences, 9, 3068–3073. doi:10.3923/jas.2009.3068.3073

P. A. M. Claassen, J. B. (1999). Utilisation of biomass for the supply of energy carriers. Applied Microbiology and Biotechnology volume, 741–755.

Quinten Deparis, A. C.-M. (2017). Engineering tolerance to industrially relevant stress factors in yeast cell factories. FEMS Yeast Research, 17(4). doi:https://doi.org/10.1093/femsyr/fox036

Salman Khan Promon, W. K. (2018). Ethanol production using vegetable peels medium and the effective role of cellulolytic bacterial (Bacillus subtilis) pre-treatment. F1000Res. doi:10.12688/f1000research.13952.1

Siti Hajar Mohd Azhar, R. A. (2017). Yeasts in sustainable bioethanol production: A review. Biochemistry and Biophysics Reports, 10, 52-61. doi:https://doi.org/10.1016/j.bbrep.2017.03.003

T.A.McMeekin, J. D. (2002). Predictive microbiology: towards the interface and beyond. International Journal of Food Microbiology, 73(2-3), 395-407. doi:https://doi.org/10.1016/S0168-1605(01)00663-8

Yan Lin, W. Z. (2012). Factors affecting ethanol fermentation using Saccharomyces. Biomass and Bioenergy, 47, 395-401. doi:https://doi.org/10.1016/j.biombioe.2012.09.019