

COLLEGE OF NATURAL AND COMPUTATIONAL SCIENCE

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REVIEW ON BIOETHANOL PRODUCTION FROM FRUIT WASTES USING *SACCHAROMYCES CEREVISIAE* FERMENTATION

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LIST OF ABBREVIATIONS

C-5……....Carbon five

CBP……..Consolidated bioprocessing

CO_2.....Carbon Dioxide

CWs……..Citrus Wastes

H_2SO_4.....Sulfuric acid

S. cerevisiae……...*Saccharomyces cerevisiae*

SSF……Simultaneous Saccharification and Fermentation

TSS…….Total Soluble Solvent

SUMMARY

Bioethanol production from lignocellulosic biomass has been demonstrated as alternative to conventional fuel, as it is considered to be renewable and clean energy. Fruit wastes are available in plenty as wastes world over. Fruit wastes are rich in sugars and carbohydrates which can be recovered and utilized for the production of bioethanol. This review paper aims to provide an overview of works done on bioethanol production from fruit wastes there by giving incites to the reader that bioethanol can be used as substitute for fossil fuel that could diminish the combined ill effect of Air, soil, and water pollution and global warming. The literature indicates that the best results have been obtained with acid and steam pretreatment of fruit waste while enzymatic hydrolysis using cellulase and xylanase enzymes resulted in giving good yields of fermentable sugar. S. cerevisiae has been obtained as the best microorganism in the production of ethanol due to its wide tolerance of P^H *making it less susceptible to infection. Recombinant S. cerevisiae was also obtained as the best bioethanol producer compared to non recombinant one. Production of Bioethanol can be maximized by controlling fermentation factor such as temperature, pH, and time. Different integrated methods for hydrolysis and fermentation have been proposed but Simultaneous saccharification and fermentation process has been the preferred step for the production of bioethanol. Fruit waste including rambutan, mango, banana, date palm, pineapple, orange and apple pomace can efficiently be used for ethanol production with the help of Saccharomyces cerevisiae. Bioreactors equipped with new agitation systems like a special segmented helical stirrer, the double helical ribbon impeller and S-shaped impellers were constructed to achieve better yield of ethanol. Blending bioethanol with gasoline or other additives under different ratios take advantage of bioethanol high octane number and low octane number. Ethanol production from fruit waste can be used as green alternative for fuel production. This green production is important for environmental sustainability.*

Key words: Bioethanol, *Saccharomyces cerevisiae*, Fruit wastes, Fermentation, Saccharification

1. INTRODUCTION

A bio-fuel is a generated fuel through biomass fermentation rather than the one produced from geological process of oil and fossil. As a result, of biomass fermentation it can be technically utilized directly as fuel.

The term bio-fuel and biomass are interchangeably used. Biomass with complex or free sugar that can later form soluble sugar is used for the production of bioethanol. The feedstock is divided mostly into three major groups; starchy crops, sugar crops, and by-products of sugar refineries) and lignocellulosic biomass, they differ respectively from the sugar solutions in them (Zabed et al., 2016). Production of bioethanol from conventional feedstock like starch-rich feedstocks (corn, potato) and sugarcane has been previously reported as the first-generation process. Nevertheless, they have economic and social barriers (mabee and saddler, 2005). Bioethanol second-generation process is gaining momentum. Lignocellulosic biomass (corn stover, fruit wastes, bagasse, straws, stalks, and switchgrass) are used for the second-generation process. One of the significant alternative processes of bioethanol production is easy adaptability of this biofuel to prevailing engines with better octane rating (Grad, 2006). Any plant material with significant amounts of sugar is utilized as a source of raw materials in bioethanol production. Sugarcane, pineapple, and potato are one of the major plants that resulted in a high yield of bioethanol (Khandaker *et al*., 2018.)

Fruit wastes are renewable sources of carbohydrates for microbial conversion to chemicals and fuels (Geddes *et al*., 2011).This substrate can be converted into liquid fuel. This in turn improves the CO_2 balance, and since it is a waste resource, it does not interfere with human food chain (Soccol *et al*., 2010). Fruit waste are composed of three main components: hemicellulose (20 % to 40 % dry wt.), cellulose (30 % to 50 % dry wt.), and lignin (10 % to 20 % dry wt.) (Sebayang *et al*., 2016). Hemicellulose is polymer of pentose sugars (D-xylose an L -arabinose) and hexose (D-glucose, D-mannose, and D-galactose) (Kuhad, 2011). Xylose can be fermented in to bioethanol by yeasts with the ability to ferment pentose (Pereira *et al*., 2008). Cellulose is a polymer of hexose sugar which is insoluble in water, and allows the hydrolysis process to break down the polysaccharide to free sugar molecules by increasing water content and converted to

bioethanol by yeast (Hamelinck, 2005). Lignin is among the component of fruit waste and the obstacles to fermentation process, which makes it unaffected by chemical and biological degradation, in turn affects the quality of bioethanol production (Taherzadeh & Karimi, 2008).

Conversion of ligninocellulosic material to bioethanol needs fermentative microorganism such as *S. cervisiae*. *S. cerevisiae* is a unicellular fungus, possessing a nuclear genomic DNA of 12068 Kb organized in 16 chromosomes. The genome of *S. cerevisiae* has been completely sequenced by Goffeau *et al*., (1996) and was found to contain approximately 6000 genes, of which, 5570 are predicted to be protein-encoding genes (Wood *et al*., 2001). *S. cerevisiae* is the most valuable species for a variety of industrial applications. The important characteristics of *S. cerevisiae* for some industrial applications are its remarkable resistance/tolerance to high sugar concentrations and production of a number of aromatic and volatile compounds. *S. cerevisiae* produces and accumulates ethanol which is toxic, or static, for most other microbial species able to compete with it for the sugar compounds and thus eliminate competition. After *S. cerevisiae* has cleared the particular ecological niche from most of its competitors, it then proceeds in the consumption of the produced ethanol, thus promoting its own growth (Hagman *et al*., 2013).

Nowadays the population is extremely increasing throughout the world. It is well known that environment becomes more polluted than the past few years. As the demand of energy is increasing and is being utilized at a rapid rate, thus creating a concern over the depletion of fossil resources. As a result of the depletion in oil, global warming and the effects of greenhouse gases making the earth on the condition of alarming (Saiffudin *et al*., 2014). Despite seeing the world are completely dependent on the limited sources of fossil-based petroleum that can later not withstand to meet future demands. The depletion of fossil fuel resulted in the continual price rising and the pressure for independence of oil and environments concerns lead to strong bioethanol markets for biofuel (Arapoglou *et al*., 2010). The rapid increment of CO_2 level in the environment resulted in the global warming emanating from the burning of fuel from petroleum based resources (Hossain and Fazliny, 2020).

The world populations are concern about climatic change and the consequent need to decreasing of greenhouse emissions of gasses leading to the encouragement of the usage of bioethanol as an alternative or replacement (Zacchi and Galbe, 2002). Another challenge is as a result of waste dumping arises in an open place resulting in malignant effects to the natural inhabitants at the surrounding environments of the dumpsite.

The globe needs urgently to device another option of sources of energy as a result of the rapid world energy supply exhaustion (Khan, 2011). The production of energy in the form of a solution by utilization of the waste is affordable, cheap and efficient in reduction of municipality wastes. Recently, an enormous number of renewable sources of energy are rapidly growing through technologies utilizing and producing renewable energy sources including solid biomass, liquid fuels and biogases (Chin, 2013). Fruit waste is one of the daily wastes discarded across the cities and towns and contributes a significant amount on the environmental pollution and hampers the health of the surrounding community. However, Fruit wastes are a rich source of carbohydrates/ sugars and the conversion of these wastes in to useful product have many advantages, amongst which generation of clean environment, keeping the health of community and obtaining important product from such wastes are the major ones (Gaddafi *et al*., 2016). Bioethanol is better replacement of petroleum-based fuels and has emerged as the best alternative. Fruit waste is widely available and cheap which is best alternative feedstock for ethanol production and overcomes problems related to energy security, promotes rural development through job creation, promotes environmental conservation and decreases greenhouse gas emission and reduce cost of fuel from fossil. Utilization of such waste does not only help as a source of bio-ethanol, but also protects our environment from pollution. This review is important for fruit waste management and recycling of its biomass to industrially useful substrate.

Therefore, this paper aims to review previous works done on bioethanol production from fruit wastes thereby giving incites to the reader that bioethanol can be used as substitute for fossil fuel that could diminish the combined ill effect on air, soil, and water and also on global warming.

2. LITERATURE REVIEW

2.1. Bioethanol production from fruit wastes using *Saccharomyces cerevisiae*

2.1.1. Principles and Steps used for Bioethanol Production

For production of ethanol from fruit waste, four major steps are commonly used: pretreatment, hydrolysis, fermentation, and distillation of the product mixture to separate ethanol (braide *et al*., 2018). Pretreatment determines the yield of that would be obtained after fermentation (Gaddafi *et al*., 2016). The aim of pretreatment is to reduce the compactness, strength, and crystalline nature of cellulose aiding in hydrolyzing the lignocellulosic biomass to simple sugar units. Alkaline treatment was done at three different concentrations of Sodium hydroxide 1%, 2%, and 4% (Zhu *et al*., 2016). Several researchers performed acid pretreatment. For example, Yasir *et al*., (2018) pretreated banana peels of dry powder with H2SO4 (0.5%), 121^0c for 60 min and obtained a better yield of cellulose. Many other researchers performed pretreatment using steam. For example, Jahid *et al*., (2018) and Madhumala *et al*., (2020) soaked fruit peels in water (200 ml) for 30-180 min and then autoclaved in steam at 120°C for 15 min. The pretreated wastes were filtered and the filtrate obtained was analyzed for the total reducing sugars and pentose sugars. The authors reported that Simple water soaking and steaming were found to be an inexpensive way to recover free sugars from fruit wastes. The acid and steam pretreatment was performed to improve the further fermentation process. Most of the cellulosic components can be converted to reducing sugar after acid pretreatment followed by autoclaving which thereby improve hydrolysis efficiency (Hsu *et al*., 2011). Microorganisms can also be used to treat lignocelluloses and enhance enzymatic hydrolysis. The applied microorganisms usually degrade lignin and hemicellulose but very little part of cellulose. Taniguchi *et al*., (2005) evaluated biological pretreatment of rice straw using four white-rot fungi (*Phanerochaete chrysosporium*, *Trametes versicolor, Ceriporiopsis subvermispora*, and *Pleurotus ostreatus*) on the basis of quantitative and structural changes in the components of the pretreated rice straw as well as susceptibility to enzymatic hydrolysis. This is also done for fruit waste. For instance, Srilatha *et al*., (1995) performed orange peel hydrolysis by fungal strain *Sporotrichum, Fusarium, Aspergillus*, and *Penicillum* and enhanced the availability of feed constituents.

Low energy requirement, no chemical requirement, and mild environmental conditions are the main advantages of biological pretreatment. However, the treatment rate is very low in most biological pretreatment processes (Sun and Cheng, 2002).

Researchers perform hydrolysis to produce simple sugar for example Gaddafi *et al.,* (2016) observed the use of different concentration of H_2SO_4 for the hydrolysis of cellulosic and hemicellulosic contents of the fruit waste. However, the acidic hydrolysis also results in the formation of fermentation inhibitors which are known to affect the ethanol production performance of fermenting microorganisms (Chandel *et al.,* 2006b). Enzymatic hydrolysis using cellulase and xylanase enzymes resulted in giving good yields of total reducing sugars and pentose sugars respectively (Jahid *et al*., 2018). Arumugam and Manikandan, (2011) reported that hydrolysate from enzyme shows fermentation efficiency of 70.3% compared to 27.1% of hydrolysate from acid hydrolysis after 48 hr of incubation. The authors have reported that production of bioethanol was far better from enzyme hydrolyzates than that from acid hydrolyzates with *S. cerevisiae* (Sun and cheng, 2002). However, there is a need to develop a convenient process of enzymatic hydrolysis of fruit wastes by firstly recovering the easily recoverable sugars by milder water-steam treatment (Jahid *et al*., 2018). The limiting factor in the enzymatic conversion of fruit waste to bioethanol is the cost of cellulase enzymes for use in the hydrolysis of pretreated biomass. Therefore, to achieve cost effective biomass conversion much information is available on the preparation of cellulase enzymes using different substrates and a variety of cellulolytic microorganisms for application in lignocellulosic bioethanol production have been reported such as *Phanerochaete chrysosporium* (Johansson *et al*., 1996), *Tricoderma reesei, Aspergillus niger* (Panday *et al*., 2009)). The optimum conditions for cellulase have been reported as temperature of 40 to 50^0C and pH 4 to 5 and assay conditions for xylanase have also been reported to be 50^0C temperature and pH 4 to 5 (Ferreira *et al*., 2009).

In addition to the five- and six-carbon sugars produced during hydrolysis of ligninocellulosic biomass several inhibitors of ethanol fermentation are also generated. For example, furans (furfural and hydroxylmethylfurfural), carboxylic acids (acetic acids, levulinic acids, and formic acids), and phenolic compounds (syringaldehyde, 4-hydroxybenzaldehyde, catechol, vanillin, 4-hydroxybenzoic acid, dihydroconiferyl alcohol, coniferyl aldehyde, and syringic acid) are the

most common inhibitors for ethanol production by *S. cerevisiae* from lignocellulosic hydrolysates (Ludwig *et al*., 2013). In addition to the three inhibitor categories, glycolaldehyde was reported as another inhibitory compound in lignocellulosic hydrolysates (Jayakody *et al.*, 2011). These inhibitors pose hindrances such as inhibition of cell growth and sugar consumption during *S. cerevisiae* cultivation for ethanol production (Kim *et al.*, 2013). Different approaches have been used to solve the inhibitory effects of these chemicals in the production process. For example, treatment with reducing agents (Alariksson *et al*., 2011), over liming (Martinez *et al*., 2000), detoxification with laccase (Chandel *et al*., 2006b), biological detoxification (Lopez *et al.*, 2004), and charcoal adsorption (Chandel *et al*., 2006b) were performed.

S. cerevisiae is the most popular yeast in the production of ethanol due to its wide tolerance of pH making it less susceptible to infection (Hossain *et al*., 2017). Fermentation of hydrolysate was carried out using *S. cerevisiae* as 100ml from the Inoculum was added to 0.5g of *S. cerevisiae*. The flask was covered with aluminum foil to prevent air entrance and growth was made anaerobically (Gebregergs *et al*., 2016). The problem for the use of *S. cerevisiae* is it cannot utilize the main C-5 sugar xylose of the hydrolysate (Walker, 2010). Therefore, genetically modified *S. cerevisiae* and non *Saccharomyces* yeasts are used for fermenting pentose sugars (Sinumvayo *et al*., 2015). *S. cerevisiae* ATCC 26603 has been developed to improve bioethanol production (Moniruzzaman, 1995). It is illustrated that bioethanol yield difference between these two *S. cerevisiae* yeast types, usual *S. cerevisiae* showed a bioethanol yield of 0.41 g/l without nutrients and 0.49 g/l with nutrients, which is much lower than the recombinant *S. cerevisiae* bioethanol yield of 0.91 g/L (Hossain & Jalil, 2015b) but recombinant yeast has not been established in industrial usage for bioethanol production because of its very high instrumental cost. The optimum pH range of *S. cerevisiae* used in fermentation for ethanol production is 4.0-6.0 (khandaker *et al*. 2020). Ethanol production is maximized by controlling fermentation temperature (Periyasamy, 2009). According to Periyasamy, (2009), the sample maintained at an optimum pH (4) was fermented to different temperatures like 25^0c, 30^0c, 35^0c, and 40^0c. Bio-ethanol production was increased with the increase in temperature and reaches maximum value at 35^0c. In contradiction to previous work done by Priyasamy, (2009), Azhara *et al*, (2017) and Lin *et al*., (2012) determined the optimum temperature for ethanol yield at 30°C.

Hadeel *et al*, (2011) reported that the maximum ethanol production from rambutan fruit biomass using yeast *S. cerevisiae* was at temperature of 30ºC. This is also investigated by other substrates. For example, high yield of ethanol was obtained from molasses at the fermentation temperature of 30°C (Arslan *et al*., 2021). Ethanol productivity from fruit hydrolysate can also optimized by controlling fermentation time. The decrease in ethanol with increase in fermentation time can be attributed to the accumulation of inhibitors and toxic metabolic by-products during fermentation which influence yeast growth leading to decrease in yeast cell biomass (Gawande and Patil, 2017). Reduction of hydrolysate sugar occur as incubation time increases and this may lead to decrease in bioethanol because availability of limited sugars causes progression of yeast cells into the stationary phase (Braide *et al*., 2018). Sharma *et al*., (2007) reported decrease in bioethanol concentration after 48 hours using citrus and banana wastes. Arumugam and Manikandan, (2011) also reported decrease in ethanol content after 48 hours of incubation from banana and mango wastes. Similar result was obtained by Gutierrez *et al*., (2015) from ripe mango peel. At the end of fermentation TSS content of fruit and wastes decreased because sugar converted to ethanol after fermentation. The highest decrease on TSS content of pineapple was observed (Khandaker *et al*., 2018).

Through a fermentor, the fruits waste can be fermented into a liquid wash which contains approximately 15% to 20% ethanol by weight (Abashar and Elnashaie, 2010). To produce usable ethanol, the excess water contents from the liquid wash must be removed through distillation process. To achieve high purification, several distillations were done. This was because all materials have intermolecular interactions with each other, and two materials will co-distill during distillation (Onuki, 2005).

2.1.2. Production of Bioethanol from Fruit Wastes through the use of S. *cerevisiae*

Several researchers have reported on the use of fruit wastes for the production of bioethanol (Manikandan *et al.*, 2008; Shilpa *et al*., 2013; Alemayehu, 2014). Mishra *et al*., (2012) investigated the production of bioethanol from fruit peels of orange, sweet lime, and pineapple. They obtained maximum sugar from Pineapple. Arumugam and Manikandan, (2011) evaluated the chemical composition of banana and mango fruit waste for bioethanol production. In their study dilute acid pretreatment was followed by enzymatic hydrolysis and maximum sugar was produced from the mixed fruit pulps, followed by the banana fruit pulp, and then followed by banana peels. In other experiment Rambutan fruit waste produced high amount of bioethanol when compared to Mango, Banana, and Pineapple as it produces bioethanol of (v/v) 7.5 % at pH 5 and 30^0c (Hossain *et al*., 2015). Apple pomace was tested for bioethanol production by Margaret *et al*., (2016) and overall, ethanol yields were 13.4 %. This result was by far better than that of the previous experiment done by Hossain *et al*., (2015).

Production of ethanol from citrus wastes was investigated by Mohammad Pourbafrani *et al.*, (2010) and they found one ton of CWs with 20% dry weight resulted in 39.64 liter ethanol, which means about 15.6 % yield which was even better than that of Apple pomace.

Another researcher Maina *et al.*, (2017) obtained highest yield of ethanol from mango waste as 19.98% this was clearly incomparable with the result that Hossain *et al*., (2015) have got (less than 7.5%, Rambutan fruit waste). The result of the above researcher revealed that the fruit including rambutan, mango, banana, and pineapple can efficiently be used for ethanol production with the help of *S. cerevisiae* in the process of fermentation.

Date palm is a good source of various nutrients, and these nutrients support microbial growth used in the fermentation process for ethanol production (Abd- Alla & El- Enany, 2012). Date palm fermentation resulted in maximum ethanol yield at (30°C), pH (4.5), fermentation time (36– 72 hr), sugar concentration (38 g/100 g) and inoculum size (25%) (Azhara *et al*., 2017).

Rishabh and Kapoor, (2021) obtained ethanol concentration of 4.52%, 5.26% and 6.5% from apple, grapes, and Indian blueberry respectively. This study also observed that the polysaccharides present in the fruit were degraded during fermentation. Similarly, Singh *et al*., (2014) obtained maximum ethanol yield from banana peels was 6.540 %. They conducted Simultaneous fermentation of starch to ethanol efficiently by using cocultures of fungus *Aspergillus Niger* and *S. cerevisiae*.

Demiray *et al*, (2019) reported ethanol production as pomegranate peels were used as substrate for enzymatic hydrolysis from different concentrations of cellulase. The resulted ethanol yield was 13 g/L.

Comparative studies of bioethanol production from different fruit wastes such as water lemon, banana, pineapple, papaya, and sweet lime using *S. cerevisiae* was done by Suryaprabha *et al*., (2017) at different temperature (25^0c, 30^0c, 35^0c, 27^0c, and 29^0c) respectively and Pine apple fruit waste shows the higher yield of ethanol (6.5%) than the other fruit wastes. This result is comparable with the previous work done by Rishabh and Kapoor, (2021).

2.2. Current methodologies and advances in bio-ethanol production

2.2.1. Advances in fermentation method

The hexose and pentose sugar formed are subsequently converted to bioethanol by a variety of microorganisms via the process of fermentation (Kang *et al*., 2014). In this scenario, different integrated methods for hydrolysis and fermentation have been proposed.

Simultaneous saccharification and fermentation is an approach, where the saccharification of biomass is combined with simultaneous fermentation of the released sugars in a single reactor. Therefore, as soon as the sugars are formed from biomass, they are rapidly converted into ethanol thus minimizing the accumulation of inhibitory sugars in the medium. Oberoi *et al.*, (2011) optimized the bioethanol production from banana peels using enzyme hydrolysis and Simultaneous saccharification and fermentation by *S. cerevisiae*. Another worker Boluda-Aguilar *et al*., (2010) produced bioethanol from mandarin peel waste and obtained 6.8 g ethanol per 100 g biomass using simultaneous saccharification and fermentation and *S. cerevisiae*.

The process can be easily operated and the presence of ethanol in the broth makes the medium less vulnerable to contamination (Ferreira *et al*., 2010). Simultaneous saccharification and fermentation process has been the preferred step for the production of biofuel because the operations of both hydrolysis and fermentation are conducted in the same reactor vessel thus reducing costs (Brodeur *et al*., 2011). This utilizes the sugars by fermenting organisms that could reduce the extent of feedback inhibition of enzymes and chances of contamination are also minimal due to the presence of ethanol in Simultaneous saccharification and fermentation (Stenberg *et al.*, 2000). However, the major disadvantage is that both saccharification and fermentation are carried out under suboptimal conditions (Galbe & Zacchi, 2002).

In simultaneous saccharification and co-fermentation process, glucose and xylose are co-fermented in the same reactor. Strains of *S. cerevisiae* and *Zymomonas mobilis* are genetically engineered to co-ferment both glucose and xylose (Hahn-Hägerdal *et al*., 2007). In this method, glucose inhibition is reduced; however, ethanol yield from both glucose and xylose is considerably lower than that formed from glucose by *S. cerevisiae*) (Koppram *et al*., 2013).

Another method of process integration is the Consolidated BioProcessing, in which one single microorganism is used for hydrolysis and fermentation steps. This potentially reduces the capital costs and increases process efficiency. However, obtaining microorganisms which can both produce enzymes for hydrolysis of biomass and then ferment released sugars is difficult (Lynd *et al*., 2005).

2.2.2. Bioethanol production using genetically modified organisms

Majority of CBP organisms are generated by either improving biofuel yield of naturally existing cellulose-degrading microorganisms or by engineering cellulolytic microbes to be ethanologenic or ethanologenic microbes to be cellulolytic (Parisutham *et al*., 2014). Different species of bacteria like *Clostridium thermocellum* (Maki *et al*., 2013), *Escherichia coli* (Shin *et al*., 2014), *Zymomonas mobilis* (Feng *et al*., 2014) and yeasts including *Pichia stipitis* (Puseenam *et al*., 2015) and *S. cerevisiae* (Yamada *et al*., 2011) have been studied for their widespread use in bioethanol production.

Genetic modifications in microorganisms may sometimes lead to loss in activity and robustness of enzymes. In this case, a microbial consortium rather than a single microbe could be used to perform consolidated bioprocessing, utilizing the substrate more efficiently thereby increasing product yield. Brethauer *et al*., (2014) proposed a consortium in a biofilm membrane reactor comprising *Trichoderma reesei*, for enzyme secretion; *S. cerevisiae*, to ferment hexose sugars; and *Scheffersomyces stipitis*, to utilize pentose sugars, and convert lignocellulosic biomass to ethanol. The control of consortium and difficulty to find identical fermentation conditions for all concerned strains are the major challenges associated with this approach. However, the application of diverse strains will not only aid in the utilization of a broader range of substrates but also produce more enzymes and efficiently utilize pentose and hexose sugars. The fermentation of enzymatic and acid hydrolysates were done and the resulted ethanol was 17.7 and 5.16g/L with *S. cerevisiae* and *Pichia stipitis* after 16 and 24h, respectively (Kuhad *et al*., 2010). In another study, Karagöz *et al*., (2012) obtained 14.07 g/100 g raw straw using *S. cerevisiae* and *Pichia stipitis.*

2.2.3. Bioreactor design for fermentation

Gupta *et al*, (2012) exploited the saccharification and ethanol production of pretreated mesquite wood in batch experiment. Enzymatic hydrolysis of pretreated lignocellulosic biomass was performed in a 3.0-L stirred tank reactor equipped with rushton impeller. Fermentation was performed at 30°C a constant speed of 200 rpm, and an aeration of 0.4 vvm. The yield of ethanol obtained was $3.16gL^{-1}$.

Isci *et al*, (2009) performed SSF process of ammonia soaked switchgrass using a 50-L pilot-scale bioreactor. They conducted SSF with steam-jacketed fermenter equipped with three Rushton-type impellers at 35 °C and 130 rpm for 72 hr after aseptical addition of yeast inoculum at the end of the process they obtained an ethanol yield of 73 %. Brethauer *et al*, (2014) performed SSF of acid-pretreated corn stover in batch system. ß-glucosidase was employed for the saccharification step, while the *S. cerevisiae* was used for the fermentation at 38 °C. They observed an ethanol yield of 70 %. The result obtained by Isci *et al*, (2009) was better when compared with the previous work done by Brethauer *et al*, (2014).

Zhang *et al*, (2010) designed a reactor with a new agitation system namely double helical impeller to perform the SSF of corn stover. Accellerase 1000 and *S. cerevisiae* were used for the saccharification and fermentation steps respectively. The yield of Ethanol obtained was 51.0 g L−1.This indicates how the new agitation system improved the yield of ethanol.

Bioreactors equipped with new agitation systems like a special segmented helical stirrer, the double helical ribbon impeller, and S-shaped impellers were constructed to achieve an efficient fiber homogenization, reducing the required energy in conditions of high substrate loading (Du *et al*. 2014).

2.2.4. Immobilization of yeast

Immobilization of yeast cells has been considered as potential alternative for enhancing ethanol productivity, because immobilizing yeasts reduce risk of contamination ,make the separation of cell mass from the bulk liquid easy, retain stability of cell activities, minimize production costs ,enable biocatalyst recycling, reduce fermentation time, and protect the cells from inhibitors (Razmovski and Curovi, 2014). Immobilized yeast cells were found to be superior to the free yeast cells since immobilized cells are more tolerant to ethanol and lower substrate inhibition (Karagoz and Ozkan, 2014) Different researchers concluded that immobilized S. cerevisiae produced more ethanol compared to free cells (Behera *et al*., 2011; Razmovski and Curovi, 2014)

2.3. Bioethanol as alternative fuel

The classification of bioethanol fuel from different lignocellulosic materials and processes are currently being developed to meet sustainability and fuel quality standards, as well as the need for roads, aviation, and electricity. Pretreatment, hydrolysis and fermentation have significant effects on the internal combustion engines (Abdulfatah *et al*., 2019). Blending bioethanol with gasoline or other additives under different ratios take advantage of bioethanol high octane number and low octane number, which increases thermal efficiency and compression ratios of the engine compared to gasoline alone (Zhao *et al*., 2011; Yusuf and Inambao, 2018). Bioethanol provides additional oxygen in combustion when blended with gasoline its burns relatively more completely.

Therefore, emitting lower Carbon monoxide and hydrocarbon compared to pure gasoline (Zhao *et al*., 2011). They also reported that lower boiling point of bioethanol helps to obtain better combustion efficiency. However, it has lower energy density than gasoline.

2.4. Techno-economic evaluation of bioethanol production

Technologies that are being developed for commercial pretreatment and value addition of fruit waste face technical and economical impediments. Therefore, cost effective technical innovations that allow cost-effective conversion of biomass into fuels and chemicals are mandatory. These technologies include low cost pretreatment, highly effective enzymes and efficient and robust fermentative microorganisms (Cardona and Sánchez, 2007; Vertès *et al*., 2008). The high cost of enzymes presents a significant barrier to commercialization of bioethanol production from fruit waste. In recent years, significant effort has been made to reduce the cost by focusing on improving the efficiency of known enzymes, identification of new enzymes and minimization of enzyme production costs (Merino and Cherry, 2007). Combined effect of higher hydrolysis and fermentation efficiency, lower capital investments, and using genetically engineered *S. cervisiae* that can convert sugar to ethanol can greatly improve ethanol production efficiency and reduce the cost of the production (Sun and Cheng, 2002). Yield improvements in all major steps of fruit processing would enable lower capital requirements, thus improving the economics and lowering investment risk (Bohlmann, 2006).

3. CONCLUSIONS

It has been proven that conversion of fruit waste wastes to bioethanol has paved way for underutilized resources and encouraged environmental sustainability, hence ethanol-from this waste holds great potential due to the widespread availability, abundance, and relatively low cost of biomass resource. Increase on world energy demand and the progressive depletion of oil reserves can be balanced by this alternative energy source.

Fruit waste based bioethanol requires a multi-step complex conversion technology due to its rigid structure, comprised of pretreatment, hydrolysis, and fermentation. Optimization of the pretreatment strategy aimed at reducing compactness, strength, and crystalline nature of cellulose and optimization of enzyme mixtures for efficient conversion of pretreated biomass together with improving fermentation efficiency. Acid pretreatment followed by autoclaving can afford the recovery of cellulose and hemicellulose from fruit wastes and enzymatic hydrolysis route is obtained as a best method for the recovery of fermentable sugar.

S. cerevisiae is undeniably the best studied and one of the most widely used yeast in ethanol production process. It has been obtained as the most efficient yeast for hexose sugar fermentation.

Inhibitory compound which may cause inefficient fermentation are circumvented using a variety of optimization techniques. Overliming, adsorption with activated charcoal, detoxification with laccase, biological detoxification and treatment with reducing agents potentially reduce the inhibitors.

Fruit waste serve as potential feedstock for bioethanol due to high sugar content and cost effective substrate. According to the review, mango peel and CWs hold better potential of yielding bioethanol through enzymatic hydrolysis followed by fermentation using *S. cerevisiae*. Effective implementation of all fuel production process would ultimately pave the way towards the establishment of sustainable environment and cost effective fuel development.

4. RECOMMENDATIONS

Studies show that using lignocellulosic materials such as agricultural residues, municipal waste and other low-cost biomass can significantly reduce the cost of raw materials for ethanol production and provide green environment as well but performing the following is important.

- ✓ Reducing the cost of ethanol production by minimizing the cost of enzymes and the use of genetically engineered raw materials with higher carbohydrate content combined with the improvement of conversion technology.

- ✓ Maintaining stable performance of the genetically engineered *S. cerevisae* in commercial scale fermentation operations, developing more efficient pretreatment technologies for lignocellulosic biomass, and integrating the optimal components in to cost effective production system.

- ✓ Acquiring best mechanism for reducing inhibitor that may enhance cell growth and sugar consumption rate.

- ✓ Using Microbial consortium rather than *S. cerevisae* alone utilize the substrate more efficiently thereby increasing product yield.

- ✓ Application of genetically modified microorganism for fermentation and hydrolysis process.

- ✓ Using Immobilized cells which is superior to the free cells in ethanol yield

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