International Journal of ChemTech Research
CODEN (USA): IJCRGG, ISSN: 0974-4290, ISSN(Online):2455-9555
Vol.14 No.02, pp 290-298, 2021

Effect of different Pretreatment, Processing and Optimization methods on Bioethanol Production from a variety of Fruits and Fruit wastes

Shreya Iyer[1], Aabha Tengeri[2], Dikshita Ghawalkar[3], Satyajeet Deshmukh[4]

[1,2,3] Student,Chemical Engineering Department, DattaMeghe College Of Engineering, Airoli, Navi Mumbai, India
[4] Assistant Professor, Chemical Engineering Department, DattaMeghe College Of Engineering, Airoli, Navi Mumbai, India

Email id: shreyaiyer1999@gmail.com

Abstract : Due to rising energy demands and environmental concerns, there is an urgent need for the development of biofuels made from economical, renewable, and environmentally safe raw materials. Fruit biomass rich in sucrose, cellulose, and hemicellulose is cheap, readily available, and underutilised; it has the potential to be a substrate for bioethanol, one of the world's most widely produced liquid biofuels. Fruits being highly perishable, amounts to large quantities of waste as they are simply discarded due to spoilage during harvesting, transportation, storage and processing by fruit vendors and food processing industries, which can be industrially utilized to produce bioethanol. This bioethanol can be made more effective by using different pretreatment, processing methods and parameters that govern its efficacy.This paper explores those pre-treatment and processing methods used to improve bioethanol yield, as well as the parameters for optimising bioethanol production from a variety of fruits and fruit wastes.
Keywords : Bioethanol, Fruit wastes, Pretreatment, Fermentation.

Introduction

The global interest in biofuels has grown over the last decade as a result of the energy crisis and environmental concerns. Biofuel is one of the best alternatives because it is renewable and helps to mitigate greenhouse gas emissions caused by fossil fuels, which are the primary cause of global warming [12,19], and thus the food industry is one of the largest in the world, producing large amounts of fruit waste as fruits are highly perishable due to their nature and composition and spoilage occurs easily during harvesting, transportation to markets, storage and processing by industries, resulting in the generation of waste such as peel,

DOI= http://dx.doi.org/10.20902/IJCTR.2021.140204

seed, pomace, kernels, and so on [18]. These include sucrose-containing(like banana, apple, mango, pineapple, papaya, citrus) and lignocellulosicfeedstocks (peels,shells ,pomace) [4,18,7,19,21], bioethanol from them is produced in two ways; first, sugar is converted directly into ethanol through a fermentation process and second, cellulose and hemicellulose are run through a pre-treatment process followed by hydrolysis and fermentation.The first generation of ethanol production used corn as a substrate; later, corn was considered a feedstock, which resulted in the second generation of ethanol production, which used microorganisms and various wastes as substrates.Sucrose-containing feedstocks are appealing because they require a simple and straightforward process, whereas starch-bioethanol requires an additional step[15].

The production of bioethanol begins with extraction of sugars, which may be then taken straight to fermentation. However, in the case of lignocellulosic materials such as commonly used chestnut shells and empty palm oil bunches, pre-treatment and hydrolysis are required to change the structural and chemical composition of the lignocellulose as well as to improve cellulose enzymatic digestibility and enzymatic reactivity towards the remaining carbohydrates[7]. There are also various pre-treatment methods ,some of which are acid pre-treatment, alkaline pretreatment, steam explosion, ammonia fibre explosion ,liquid hot water; and hydrolysis and processing methods with variations in parameters such as the enzyme used, pH, temperature, specific gravity, enzyme concentration that affect the efficacy of bioethanol produced and contribute to optimising the bioethanol production process[15,12]. As a result, fruits and fruit wastes are indeed being considered as a potential substrate for bioethanol production because they are inexpensive, readily available, and currently underutilised, despite their high sucrose or cellulose and hemicellulose content, antimicrobial and antioxidant properties[1,12]. This paper reviews the different pre-treatment and processing methods used on several fruits and fruit wastes, with variations in parameters that affect the bioethanol production and its optimization.

Pre-Treatment Methods for Lignocellulosic Fruit Wastes

[1] Pretreatment of lignocellulosic biomass, containing cellulose and hemicellulose as well as lignin, is necessary to make the carbohydrates present in them available for enzymatic hydrolysis and fermentation. With reference to this, ShuvashishBehera et.al reported that one of the most important parameters (other than biomass crystallinity, degree of polymerization, exposed or accessible surface area, and lignin and hemicellulose contents) is the conditions used in the pre-treatment method.The efficacy of pre-treatment, and therefore the efficacy of enzymatic saccharification, was governed by a variety of pre-treatment methods and parameters. The main categories of pre-treatment include physical pre-treatment(to increase the accessible surface mainly by size reduction), physio-chemical pre-treatment (improve accessibility of the cellulose for hydrolytic enzymes), biological pre-treatment (produce enzymes to degrade lignin, hemicelluloses and polyphenols), electrical pre-treatment and last but not the least chemical pretreatment which was considered most promising. Many pre-treatment methods have demonstrated high sugar yields, i.e. >90% of the theoretical yield from lignocelluloses. However, it was concluded that defining the best pre-treatment method was not possible as it depends on numerous factors such as the type of lignocellulosic biomass, processing method and parameters, environmental impact, economic feasibility, and so on.

[2] TuVyThuy Nguyen et.al stated that each stage in bioethanol production plays a distinct role with pre-treatment being the most important. This stage essentially involves the breakdown of the cell wall, which contains cellulose, hemicellulose, and lignin with the tightest connection, in order to accelerate the further steps. The products used in this analysis are low-quality, degraded longan fruits and the process started with about 10 g of dried longans boiled in 50 mL of distilled water for 30 minutes at 90–95 ° C. These samples were then physically pretreated for 0, 15, and 30 minutes in an autoclave at 121 ° C and 15 psi. Following pre-treatment, 2% cellulase and 0.125% Tween-20 were applied to the samples, which were then held in the incubator for 1, 2, and 3 days at 30 °C and the total sugar and reducing sugar were calculated. To boost the experiments for sugar processing, the response surface methodology with a standard protocol of central composite design was chosen. To determine the reducing sugar during hydrolysis, the kinetic parameters are determined using reducing sugar productivity and reducing sugar yield coefficient equations. The best hydrolysis product with the highest sugar content was selected for ethanol fermentation.The maximum reducing sugar concentration of the longan fruit waste model was obtained after 15 minutes of pre-treatment and 24 hours of hydrolysis. Furthermore, the fermentation with the free cell of S. cerevisiae from 108 h produced the maximum ethanol concentration at 48 h. As a result, the current study's findings indicated that it is possible to achieve stable activity using dried low-

grade longan fruit wastes as the sole substrate for bioethanol processing in pilot- or large-scale biogas plants in the future.

[3] On similar grounds, AlemayehuGashaw et.al emphasized on the fact that biofuels have the potential to be used both domestically and internationally for energy security. Ethanol, one such biofuel, is generated both petro-chemically (by ethylene hydration) and biologically (by fermentation using microorganisms).Some factors that influence the ethanol production process include temperature, pH, substrate concentration, and yeast concentration. Bioethanol is made from fermentation of sugars derived from wood, such as sucrose, fructose, or lignocellulosic products. Hence, pre-treatment is required to improve the structure of cellulosic biomass by using enzymes that transform poly-carbohydrates into fermentable sugars so that cellulose forming microorganisms may access it. There are many strategies for increasing the digestibility of cellulose using pretreatment methods before it is subjected to enzyme or microbial conversion that include mechanical, physical, chemical, or biological pre-treatment, as well as a mixture of these methods. Mechanical size reduction and pyrolysis are part of the physical pre-treatment and steam explosion, a CO2 explosion, ammonia fibre explosion and ozonolysis are the part of physiochemical and chemical pre-treatment respectively.The alkaline pre-treatment method like oxidative delignification, wet oxidation and the organosolv process and enzymatic pre-treatment in biological pretreatment are also some of the well known ones. Enzymatic hydrolysis turned out to be the most promising alternative method for saccharification of complex polymers that supported the possibility of producing ethanol while maintaining optimal parameters.

[4] R. Arumugam M. and Manikandan stated that in the current energy shortage, the only way to satisfy the high demand for ethanol is to produce it from comparatively cheaper raw materials using effective fermentative microorganisms. Considering this, banana and mango fruit samples that were completely ripened were obtained. In addition, a mixed fruit pulp study was made by combining fruits in an equal ratio. The ash content, moisture content, total lipid content, and crude protein content were all calculated via chemical analysis. Dilute acid pre-treatment and liquid hot water preparation were the pre-treatment methods used. The hydrolysates obtained from the pretreated pulps and peels were saccharified enzymatically in two steps, DNS system was used to estimate the reducing sugar content of the enzymatic hydrolysates. This was followed by fermentation carried out with Saccharomyces cerevisiae in hydrolysates derived from pretreated and enzymatically hydrolyzed fruit pulps and peels. The fermentation studies revealed that mixed fruit pulps contributed to the highest ethanol efficiency of 0.747% and mango fruit peels had the lowest at 0.230%. The increased cell mass of yeast and the declining pattern in the sum of residual sugars in the fermentation medium also indicated a fast bioconversion of sugars to ethanol during the early stages in all of the fruit samples. Hence it was concluded that increasing the feedstock's available sugars at a fixed pre-treatment level can result in lower capital and operational costs for higher ethanol yield.

[5] Tye Ying Yinga, Lee KeatTeongb, Wan Nadiah Wan Abdullaha,LehCheuPeng conducted a study into the chemical composition of oil palm empty fruit bunch (EFB) and kenaf core fibres using various pretreatment methods used in the production of bioethanol. Water, acid and alkaline pre-treatments were carried out after the two non-wood fibres were collected and washed. This was followed by rinsing, spin drying and refrigeration of the pretreated fibres. Untreated and pre-treated EFB and kenaf core fibers were subjected to enzymatic hydrolysis using Celluclast 1.5 L in a shaking water bath at 100 rpm,50 °C for 48 h, followed by boiling, centrifugation and were stored frozen until sugar analysis. The chemical composition of the original and pretreated biomass was analyzed and the glucose content were determined.The enzymatic hydrolysis of the original EFB and kenaf core resulted in low levels of glucose i.e 2.6% and 0.4% glucose yield per weight of untreated fiber respectively. For both EFB and kenaf pretreated fibres, among the three pretreatment methods water pretreated fibre accounted for the highest glucose yield. On comparing the aqueous pretreatment of both the biomass, EFB gave a higher ethanol yield(34.9%) as compared to kenaf core fibres(19.3%).

[6] MaríaBoluda-Aguilar et.al, studied the effect of steam explosion pre-treatment on cell wall disruption, enzyme accessibility and efficiency of saccharification and fermentation processes of mandarin citrus peel wastes for bioethanol production. Steam explosion tests were performed in both dry and wet conditions and it was identified that the D-limonene content was higher in the dry conditions. As a result, wet conditions were used with steam at 160 C, and finally, this pretreated peel waste(with a final dry matter content of 14.70 percent, wet basis) and the condensed obtained were collected and stored separately where the limonene concentration, dry matter content, and sugars content were determined. These samples were then refrigerated until enzymatic

hydrolysis and posterior fermentation (HF process) or simultaneous saccharification and fermentation with Saccharomyces cerevisiae was performed. It was then observed that even with low enzymatic loading, the content of D-limonene in steam exploded wastes was reduced by a factor of ten (from 0.3–0.4 to 0.02–0.05 (% v/v)), and the sugar yield obtained was higher (ethanol production was 50–60 L/1000 kg raw wastes).

[7] Luisa Maurelli et.al, investigated various pre-treatment methods such as hot acid impregnation, steam explosion (acid-catalyzed or not), and aqueous ammonia soaking (AAS) on chestnut shell to achieve the highest conversion of cellulose and xylan into fermentable sugars. The pretreated chestnut shell was saccharified with two enzyme co-cultures prepared from commercial preparations, and the best pre-treatment and enzyme cocktail was determined based on the yield of fermentable sugars.Following preliminary experiments, AAS sugar production was attempted to increase the yield by varying the concentrations of ammonium hydroxide, enzymes, and chestnut shell. The best pre-treatment conditions were 10% ammonium hydroxide, 70°C, pH 5.0 for 72 hours with an enzyme cocktail containing chestnut shell at 5% solid loading. The residual activity in the soluble fractions after predetermined time intervals was used to assess enzyme adsorption. It was determined that AAS produced the best results, while alternative pre-treatments such as hot acid impregnation and steam explosion resulted in less cellulose degradation, and steam explosion also solubilized a significant amount of xylan. It was also stated that alternative assistive pre-treatment methods, such as microwave or ultrasound-assisted alkali pre-treatment, will be investigated in order to increase glucose yield even further.

[8] T.Keskin-Gundogdu conducted a study with the aim of producing and increasing bioethanol yield from fruit and vegetable waste by examining various pre-treatment methods such as microwave, ammonia, heat/acid, and heat pre-treatment for yeast fermentation as well as different fermentation processes i.e yeast and synthesis gas fermentation.Saccharomycescerevisiae was used in the study in three different forms including wet bakery yeast, beer yeast, dried bakery yeast. The yeast was grown along with pre-culture at 30 °C for 16 h, in a shaking incubator at 120 rpm and the wet bakery yeast was selected as the best yeast source. Fruits and vegetable wastes were collected,chopped into small pieces and the four pre-treatment methods were carried out simultaneously. For syngas fermentation, a nitrogen gas stream was passed through the system to a temperature of 200 degrees during pyrolysis treatment, the liquid product was obtained after passing it through an ice –water cooled traps and its amount was calculated and the pyrolysis gas was obtained. Bioethanol yield was the highest from microwave and acid/heat treatments, 0.471 and 0.463 g g–1 respectively. It was also mentioned that the acetate values in anaerobic mixed culture by syngas fermentation is high corresponding to an increase in ethanol production and the bioethanol yield was higher in yeast fermentation as compared to syngas fermentation with its limitations. In this study synthesis gas fermentation turned out to be more economical and environmentally safe due to lower amount of waste in the form of bio-oil.

[9] AnisKristiania et.al, conducted a study on the effect of using a combination of chemical and irradiation (physical) pre-treatment methods on Oil palm empty fruit bunches (OPEFB).The pretreatment process begins with the collection of oil palm's empty fruit bunches (EFB) and continues with the carrying out of combination of pretreatment methods.For chemical pre-treatment, 10% NaOH was used in a ratio of 5:1 to the EFB at a temperature of 150°C for 30 minutes at a pressure of 4-7 kg/cm2.The content of cellulose, hemicellulose and lignin in Lignocellulose biomass was determined by by using TAPPI T 13 m - 45 and ASTM 1104-56 method ,showing reduction in the lignin content and the cellulose and hemicellulose content increases indicating a rise in the amount of fermentable sugars. X-ray diffraction (XRD) method was employed in analysing the crystallinity of the raw material sample and the sample treated by chemical-irradiation pretreatment 200kGy gave the highest crystallinity index due to crosslinking and removal of hemicellulose and lignin and the crystallite size increased. Fourier Transform InfraRed spectroscopy was used to analyze chemical structure; the pretreated sample is flaky and brittle due to breakage of the intermolecular hydrogen bond.EDX for compositional analysis showed the increase in oxygen in the pretreated sample. Investigation of the above parameters demonstrated that a combination of chemical and irradiation methods improved the properties before the hydrolysis process in the production of bioethanol.

Bioethanol from Fruits : Processing & Optimization

[10] Mohammed Saifuddin et.al stated that fruit waste is abundant and can be repurposed for bioethanol production. As a result, it can help with waste management by reducing climate change and industrial waste.Mango peel and seed have a variety of qualities that make them potential bioethanol feedstocks, hence rotten mango was chosen, peeled and seeded. The fruit pulp and peel was carved into small cubes/pieces and

crushed. Saccharomyces cerevisiae was activated and inoculated into the mashed fruit for batch fermentation.The total soluble sugars and pH were determined. After a 120-hour incubation period, samples were taken out from the incubator and extracted via filter paper. The DNS method was used to evaluate the glucose content. Using a multi-element oil analyzer, various concentrations of yeast samples were analysed to determine the elemental value.The rate of alcoholic fermentation of mango waste was highly influenced by temperature.As a result, this massive volume of reducing sugars resulted in more generation of bioethanol. After five days of incubation, the glucose content, total soluble solid (TSS), and pH values were all reduced and the bioethanol processing from mango pulp with yeast concentration of 3 g/L at 30°C yielded the highest percentage of bioethanol.

[11]On similar grounds, Hari Shankar Vishwakarma* et.al stated that fruit waste is easily obtained and its residues after fermentation can be used as a fertiliser and hence conducted an experiment to determine the optimum conditions for maximising bioethanol yield obtained from a fruit waste batch. The procedure to determine bioethanol yield began with washing the fruits with 5% potassium permanganate and continues with crushing the fruits for fermentation. Yeast used is saccharomyces cerevisiae, which is added to the fruit waste mash collected in a beaker with urea, sucrose, and distilled water and brought up to 1000 ml after inoculation. The beaker was then placed in a 100 rpm incubator with a temperature of 32°C maintained for 36 hours, after which a sample was centrifuged and the alcohol content was determined using the specific gravity method. The distillate obtained after distillation is then subjected to an iodine test to determine the presence of ethanol. Various parameters affecting bioethanol production such as temperature, pH, and specific gravity, are measured and it was observed that rate of alcoholic fermentation was directly proportional to temperature and the performance of yeast was better in slightly acidic medium. It is then concluded the highest bioethanol yield is obtained at 32°C, pH 5.5, and specific gravity 0.865 for a fruit waste batch containing a variety of cheap fruits such as apples ,papaya, grapes, and bananas.

[12]Janani K et al stated that ethanol production and use has gone up significantly since it has been used as a fuel and a disinfectant, and thus ethanol was made from a variety of cellulosic fruits such as apple, papaya, grapes, banana, and other fruits and vegetables for the manufacture of ethanol in this paper.The process started with the mashing of fruits and was followed by yeast fermentation. The mash was then incubated for 3-5 days, during which the time, pH and specific gravity was determined. The mash was then subjected to distillation, after which the culture was placed in a reboiler, where it was boiled and vapours were extracted, concentrated, and liquid ethanol was produced. The effects of temperature, pH, and specific gravity were considered during this phase. As a result of this comparative analysis, the maximum yield of ethanol was obtained from grape wastes with a concentration of 6.21% at a pH of 5.4, temperature 30oC and specific gravity 0.860, which is appropriately similar to the constant value of ethanol.

[13] Ajay Kumar Singh et.al stated that banana peels are underutilised as a potential growth medium for a yeast strain and thus have the potential to produce significant bioethanol yield. At various temperatures and pH levels, co-cultures of a species of aspergillusniger and saccharomyces cerevisiae were used to perform simultaneous saccharification and fermentation. The process began with the isolation of microorganisms and the performance of a starch hydrolysis test to determine their ability to digest starch. The banana peels were then pretreated with 70% ethanol and fermented for 7 days, with the ethanol content being measured every 24 hours. The optimal pH and temperature for fermentation of banana peels was found and then fermentation was carried out at different yeast concentrations using the optimised pH and temperature. It is observed that amylolytic fungus Aspergillusniger and a non-amylolytic saccharomycescerevisiae gave a significant yield, and as the concentration of Saccharomyces cerevisiae increased, the time required to complete fermentation declined. Ethanol yield was measured using gas chromatography and the maximum was achieved in 2, 3, 5, and 7 days using a 12%, 9%, 6%, and 3% yeast inoculum, respectively. The results obtained supported the fact that the rate of fermentation was inversely proportional to the yeast concentration.

[14] RishabhChitranshi and Raj Kapoor conducted a study to investigate the efficiency and percentage of ethanol for high quality sanitizer production from different fruit wastes. The study used seasonal fruits such as apple, grape, and Indian blueberry with distinguished levels of fermentable soluble sugars having good antibacterial and antioxidant potency. Around 200 g of fruit waste was disinfected with 5% potassium permanganate solution, preceded by thorough washing, air-drying, crushing and collection in beakers. An activated yeast inoculum was made by combining 20g Saccharomyces Cerevisiae, 1.5 g urea, and 47 g sucrose

with 1000ml distilled water and pouring it into a 2L beaker. This method was used to obtain all three samples. The three flasks were then incubated for one week at 35 °C and 150 rotations per minute. Following fermentation, specific gravity was measured in order to determine the percentage of alcohol levels. To obtain the highest percentage of bioethanol in the final product, distillation was carried out for 4–6 hours followed by an iodine test to confirm the presence of ethanol. In this study, the highest amount of bioethanol was procured from Indian Blueberry at 33 °C, Ph 5.2 and specific gravity 0.875.

[15]With a different variety of fruits, SandeshBabu et al, carried out an experiment in which they used Saccharomyces cerevisiae to produce ethanol from a variety of fruit juices, including Sugarcane, Grapes,Watermelon, and Mosambi. Prior to inoculation, the pH was adjusted using hydrochloric acid at room temperature. The fermentation system was kept undisturbed for almost a week while experiments were conducted at various temperatures, lower sugar content, pH, and immobilised conditions.80 percent yield of pure ethanol was obtained and was further rectified to yield 99.2 percent pure ethanol. Dichromate reagent was used to determine the ethanol yield resulting in sugarcane (12.15%), grape (11.25%), followed by watermelon (10.10%) and lowest for Mosambi (6.23%). Fermentation increases with the rise in temperature therefore solutions were held at 25, 30, 35, and 40°C with a 20% initial sugar concentration to achieve the optimum temperature for ethanol fermentation. At 25°C in 48 hours, a low ethanol yield of about 6.8 percent was found. It was thus concluded that if the ethanol tolerance capability of yeast species is improved by mutation, a higher percentage of ethanol might be produced, which could then be utilised as a biofuel. This procedure was found to be environmentally beneficial and the leftover wastes from fermentation may be used as fertilizers in the soil.

[16] Even VenkatachalapathyGirish, Krishnappa Ravi Kumar and SirangalaThimmappaGirisha carried out a research which was intended to explore the practicability of using degrading fruits for the manufacture of bioethanol. In this cost effective process, raw materials used were pineapple, orange and lime. The decayed fruits were collected, washed with 5% potassium permanganate then with distilled water and extracted. The DNS method was followed before and after fermentation to determine the concentration of reducing sugars. Aerobic fermentation was carried out that involved keeping fruit extract with yeast (Saccharomyces cerevisiae) for 5 days in a fermenter covered with muslin cloth at around 30 C. In the anaerobic phase, fermentation lasted for around 9 days in an airtight container followed by simple distillation. The sugar content before and after fermentation was analysed by DNS method, the results showed that glucose level was more before fermentation with lime(21mg/mL), pineapple (20mg/mL) and orange (17 mg/mL) and extracted juices were diluted to 1:100 with distilled water. Hence when compared to after fermentation with lime(16mg/mL), orange (12.5mg/mL) and pineapple (11mg/mL). The author observed that the fermentation of fruit extract was allowed to take place for 9 days at 28 to 32 °C and concluded that production of alcohol was highest in pineapple (13%).This paper supported the use of decaying fruits for ethanol production, as it is a cheap and alternative fuel to meet the increasing energy demand.

[17] According to Naka TOUR et.al,the twenty-first century faces threats of dwindling fossil fuel supplies, dramatic increase in greenhouse gas emissions leading to global warming and a lack of ability to meet rising energy demands. Sustainable energy sources including biomass such as cashew apples, composed of glucose, fructose and sucrose, as well as minerals, vitamins, and some amino acids. In this paper, cashew apples were collected, extracted and pre-treatment was carried out. The strains were subcultured in liquid YEPD medium, consisting of 40 g/L glucose, 15 g/L yeast extract, and 10 g/L peptone. Agar slopes were used to stabilize the strain so as to inoculate the pre-cultures. Prior to use, these media were autoclaved to exterminate them. The pre cultures were then put in an orbital agitator, aerobic and anaerobic fermentations were carried out respectively. GPC and HPLC were used to calculate ethanol and sugars in the supernatants obtained after centrifugation of the sample, respectively. The amount of CO2 was calculated using the Guillou equation and the alcohol yield was estimated using gas chromatography. This paper supported the use of cashew apple as a raw material because of its molecular makeup for bioethanol processing.It was also revealed that the effect of temperature,which is characterised by the presence of oxygen reduces fermentation time and thereby increases efficiency.

[18] EzgiEvcan, Canan Tar studied the development of bioethanol from apple pomacehydrolysate using T.harzianum, A.sojae, and S.cerevisiaecocultures in order to develop a sustainable and low-cost alternative feedstock for fuel production and to illustrate a viable approach towards various environmental issues by reducing the accumulation of agro-industrial waste materials.First, hydrolysis was performed on apple pomace.

In fermentation tests, three strains, two fungi and one yeast i.eT.harzianum NRRL 31396 and A.sojae ATCC 20235 respectively were used. Aerobic as well anaerobic fermentation were carried out. HPLC was used to calculate the volume of bioethanol in the supernatant and a FCCD (face based central composite design) was created and tested with three variables: A.sojae inoculation rate, T.harzianum inoculation rate, and agitation speed varying between 100 and 300 rpm. A total of 20 experiments with 5 centre points for optimization experiments were carried out.As fungi may use both pentoses and hexoses, the inclusion of fungi in the fermentation flasks resulted in more effective sugar utilisation. As a result, this analysis was considered as a baseline for future research as the findings indicated that cocultures can be an efficient method of processing bioethanol due to their synergistic interactions.

[19] With a focus on hydrolysis, AlessiaTropea et.al found enzyme hydrolysis to be promising and tested three methods for producing bioethanol from pineapple waste.The methods included were direct fermentation (DF) with enzyme addition, separate hydrolysis and fermentation (SHF), and simultaneous saccharification and fermentation (SSF). The procedure started with the division of pineapple parts and progressed to yeast inoculation with the strain kept on 20 ml YM agar at 4 oC.S.cerevisiae was grown overnight at 30 oC on a rotary shaker at 200 rpm and the initial dry matter content was measured to conduct the tests. The pineapple waste was then blended and continuously mixed at 200 rpm for DF with dry matter content 9.2% , 500 rpm for SHF with dry matter content 8.5% , and 200 rpm for SSF with dry matter content 9%. The tests are performed at 30 degrees Celsius with the pH maintained at 4.5. CO2 evolution was also monitored until no more CO2 fluctuations were detected. The results of tests conducted in DF showed a rise of 3.4% in ethanol yield after enzyme addition, corresponding to a 86% of theoretical yield, SHF showed an increase of 3.7% and SSF showed an increase of 3.9% in ethanol yield, corresponding to 89 % and 96 % of theoretical yield, respectively, calculated on a dry matter basis. It was then concluded with a suggestion that the yield was significant but can be improved by the use of different nitrogen sources and an efficient pre-treatment method.

[20] Even, DelianaDahnuma et.al conducted a comparison study of two enzymatic hydrolysis methods, Separated Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF), to evaluate their effect on hydrolysis and enzyme concentration for producing ethanol. The process began with alkaline pre-treatment, in which the empty fruit bunch was mixed with a 10% NaOH solution in a batch reactor at 150°C for thirty minutes, which had a similar effect to Ammonia Fiber Explosion and was able to reduce lignin content by up to almost 50%. The pretreated empty fruit bunch samples were then subjected to a 72-hour Separated Hydrolysis and Fermentation (SHF) process, which included mixing in 0.05 M buffer citrate and fermentation. As a result, the total time for processes was 144 hours. All samples were placed in a shaking incubator at 150 rpm and were monitored every 24 hours.The data of glucose form HPLC was then measured as required to get the percentage of yield. The second process of Simultaneous Saccharification and Fermentation (SSF) began in the same manner as the first, with mixing in 0.05M buffer citrate and then adding each enzyme concentration as described in SHF, along with 1 percent (g/ml) dry yeast, Saccharomyces cerevisiae, at 32C in a shaking incubator (150rpm) for 72 hours. The highest yielding ethanol was 6.05 percent when 40 FPU of enzymes were used, and the optimum time reaction in SSF was 48 hours.The highest producing ethanol reached 6.05% using 40 FPU of enzymes and optimum time reaction in SSF was found to be 48 hours.Using 40 FPU of concentration enzyme, it could produce 4.74% of ethanol in 72 hour fermentation by SHF process and 6.05% of ethanol in 24 hour by SSF process. Finally it was concluded that SSF method was better than SHF because only within a day, ethanol had been formed.be used as fertilizers in the soil.

[21] Similarly, Seong Choi et.al, discovered a process to convert a combination of fruit wastes and citrus peel waste(CPW) fruit waste to ethanol without using pre-treatment, involving the development of an enzymatic hydrolysis process and a method to remove inhibitor terpenoid essential oil, D-limonene in CPW. The juices of Citrus (orange, mandarin, grapefruit and lime) fruits, apple, banana, and pear were extracted and collected. High performance liquid chromatography (HPLC) was used to measure the content of soluble sugar and hexane was used to homogenize CPW for 5 hours and a supernatant was obtained. Evaluation of the two in-house enzymes HEA and HEB produced from A. citrisporus and T. longibrachiatum was carried out, added to the fruit waste individually followed by enzymatic hydrolysis and the optimization of enzyme loading volume and the biomass hydrolysis time was recorded. An enzyme complex was developed from CPW as a carbon source to substitute for costly pre-treatment enzymes. D-limonene was retrieved via D-Limonene removal column (LRC), using hexane after fermentation, and the recovery rate was assessed using gas chromatography. 4 mL yeast peptone dextrose media (YPD) was used to activate Saccharomyces cerevisiae. The fermentation efficacy was analyzed

using an immobilized cell reactor (ICR), made from calcium alginate beads and cultured yeast. It was observed that Mandarin peel consisted of the most amount of fermentable sugars with a high conversion rate making it a very cost effective procedure to be employed on an industrial scale.

Conclusion :

Bioethanol is one of the most sustainable and low-cost biofuel . Out of the different pretreatment methods, chemical pretreatment, under optimal conditions, is the most prevalent and efficient for converting lignocellulosic fruit wastes to bioethanol. Under chemical pretreatment methods, aqueous ammonia soaking is found to be the most effective and even steam explosions reduce significant amounts of xylan and D-limonene. Also, alternative assistive pre-treatment methods such as microwave and heat/acid pretreatment are found to reduce lignin content Further, simultaneous saccharification and fermentation(SSF) and a combination of chemical and irradiation methods under hydrolysis, showed the best results. Various fruits were subjected to fermentation with saccharomyces cerevisiae, indian blueberry ,mango ,grapes and banana turned out to be the most suitable for production of bioethanol. It was also observed that with optimal yeast concentration, strict pH and temperature control we could optimize the overall process. As a result , the findings of this study showcases the effects of different pretreatment, processing and optimization methods for the production of bioethanol from a variety of fruits and fruit wastes.

References :

1. Shuvashish Behera, RichaArora, N. Nandhagopal, Sachin Kumar n,“Importance of chemical pretreatment for bioconversion of lignocellulosic biomass”, Renewable and Sustainable Energy Reviews, 2014
2. TuVyThuy Nguyen &Yuwalee Unpaprom & Numchok Manmai & Kanda Whangchai & Rameshprabu Ramaraj, “Impact and significance of pretreatment on the fermentable sugar production from low-grade longan fruit wastes for bioethanol production”, Springer-Verlag GmbH Germany, August 2020
3. AlemayehuGashaw, “Bioethanol Production from Fruit Wastes and Factors Affecting Its Fabrication”, International Journal of Chemical and Natural Sciences Vol. 2, No. 5 (2014): 132-140, 31 August 2014
4. R. ArumugamM.Manikandan, “Fermentation of Pretreated Hydrolyzates of Banana and Mango Fruit Wastes for Ethanol Production”, Society of Applied Sciences, 2011
5. Tye Ying Yinga, Lee KeatTeongb, Wan Nadiah Wan Abdullaha, LehCheuPeng,”The effect of various pretreatment methods on oil palm empty fruit bunch (EFB) and kenaf core fibers for sugar production”, 4th International Conference on Sustainable Future for Human Security,Sustain 2013
6. MaríaBoluda-Aguilar, Lidia García-Vidal, Fayiny del Pilar González-Castañeda, Antonio López-Gómez, “Mandarin peel wastes pretreatment with steam explosion for bioethanol production”, Bioresource Technology, Elsevier Ltd, 2009
7. Luisa Maurelli& Elena Ionata& Francesco La Cara & Alessandra Morana, “Chestnut Shell as Unexploited Source of Fermentable Sugars: Effect of Different Pretreatment Methods on Enzymatic Saccharification”, Applied Biochemical Biotechnology, 2013
8. T. Keskin-Gundogdu,”Comparison of Conventional and Novel Pre-treatment Methods for Bioethanol Production from Fruit and Vegetable Wastes”, Chem. Biochem. Eng. Q., 33 (4) 471–483 (2019),December 2019
9. AnisKristiania,*, NurdinEffendib, YosiAristiawana, FauzanAuliaa, YanniSudiyania,” Effect of combining chemical and irradiation pretreatment process to characteristic of oil palm’s empty fruit bunches as raw material for second generation bioethanol”, 2nd International Conference on Sustainable Energy Engineering and Application, ICSEEA 2014
10. Mohammed Saifuddin, Mohammad MoneruzzamanKhandaker, ABMS Hossain, MdSarwarJahan, NashriyahBinti Mat and AmruNasrulhaq Boyce “Bioethanol Production from Mango Waste (Mangiferaindica L. cv chokanan): Biomass as Renewable Energy”Australian Journal of Basic and Applied Sciences, June 2014
11. Hari Shankar Vishwakarma, Abhishek Kumar, Jyoti Singh, Shipra Dwivedi, Mahendra Kumar “Production of Ethanol from Fruit Wastes by using Saccharomyces cerevisiae”, International Journal of Renewable Energy Technology, December 2014
12. Janani K., Ketzi M., Megavathi S. , Dr.Vinothkumar D., Dr. Ramesh Babu N.G, “Comparative Studies

of Ethanol Production from Different Fruit Wastes Using Saccharomyces cerevisiae", International Journal of Innovative Research in Science, Journal Engineering and Technology, December 2013

13. Ajay Kumar Singh, Sanat Rath, Yashab Kumar, Harison Masih, Jyotsna K. Peter, Jane C. Benjamin, Pradeep Kumar Singh, Dipuraj, Pankaj Singh "Bio-Ethanol Production from Banana peel by Simultaneous Saccharification and Fermentation Process using cocultures Aspergillus niger and Saccharomyces cerevisiae",International Journal of Microbiology and Applied Sciences, 2014
14. Rishabh Chitranshi, Raj Kapoor," Utilization of over‑ripened fruit (waste fruit) for the eco‑friendly production of ethanol", Society for Plant Research 2021,August 2020
15. Sandesh Babu,K.M.Harinikumar, Ravi Kant Singh and AditiPandey,"Optimization of Bioethanol Production from Fruit Wastes using Isolated Microbial Strains", International Journal of Advanced Biotechnology and Research(IJBR),ISSN 0976-2612, Online ISSN 2278–599X,Vol5, Issue 4, 2014, p598-604,October 2014
16. Venkatachalapathy Girish, Krishnappa Ravi Kumar and SirangalaThimmappa Girisha," Estimation of sugar and bio ethanol from different decaying fruits extract", Pelagia Research Library Advances in Applied Science Research, 2014, 5(1):106-110 ISSN
17. Naka TOURE, KouaATOBLA , Mohamed CISSE and Soumaila DABONNE ", Bioethanol production from cashew (Anacardium occidentale L.) apple juice by batch fermentation using Saccharomyces cerevisiae", International Journal of Biological and Chemical Sciences, October 2019
18. EzgiEvcan, CananTari "Production of bioethanol from apple pomace by using cocultures: Conversion of agro-industrial waste to value added product", Elsevier Ltd May 2015
19. Alessia Trope, David Wilson1 , Loredana G. La Torre, Rosario B. Lo Curto , Peter Saugman , Peter Troy-Davies , GiacomoDugo& Keith W. Waldron "Bioethanol Production From Pineapple Wastes", Journal of Food Research, December 10 2013
20. Deliana Dahnuma, Sri Octavia Tasumb , Eka Triwahyunia , Muhammad Nurdinb , Haznan Abimanyua "Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch", 2nd International Conference on Sustainable Energy Engineering and Application, 2015
21. Seong Choi, Yoon Gyo Lee , Sarmir Kumar Khanal, Bok Jae Park, Hyeun-Jong Bae, "A low-energy, cost-effective approach to fruit and citrus peel waste processing for bioethanol production", Elsevier Ltd, November 2014

*****