J. appl. Res in Plant Sci. Vol. 2(2), 121-131, 2021 eISSN 2708-3004; pISSN: 2708-2997
www.joarps.org https://doi.org/10.38211/joarps.2021.2.2.16

Effect of Pretreatment, Fermentation Medium and Solid Loading Rate on The Production of Bio- Ethanol from Fruit Waste Using *Saccharomyces cerevisiae*

Muhammad Shahzaib[1,*], Muhammad Usman[2], Muhammad Moaaz Ali[3], Shaista Gull[4], Talha Javed[5], Ahmed Fathy Yousef[3,6], Muhammad Waleed Shafique[7] and Ayesha Tahir[8]

[1]Department of Energy Systems Engineering, University of Agriculture, Faisalabad 38000, Punjab, Pakistan
[2]Institute of Soil and Environmental Science, University of Agriculture, Faisalabad 38000, Punjab, Pakistan
[3]College of Horticulture, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China
[4]Department of Horticulture, Bahauddin Zakariya University, Multan 66000, Punjab, Pakistan
[5]College of Agriculture, Fujian Agriculture and Forestry University, Fuzhou 350002, Fujian, China
[6]Department of Horticulture, College of Agriculture, University of Al-Azhar (branch Assiut), Assiut 71524, Egypt
[7]Department of Agronomy, University of New England, Armidale 2351, New South Wales, Australia
[8]Seed Physiology Lab, Department of Plant Breeding and Genetics, University of Agriculture, Faisalabad 38000, Punjab, Pakistan

[*]Correspondence: engr.shahzaib04899@yahoo.com

Article Received 18-01-2021, Revised 10-02-2021, Accepted 15-02-2021

Abstract

The rapid increase in world's population and growing industrialization are major sources of energy consumption, therefore energy demand is expanding continuously. The first-generation feedstock like maize, sugarcane, wheat, etc. can be used to produce bioethanol, but due to food and feed security issues, first-generation feedstock cannot be used to produce bioethanol. To overcome the feed and food security issue related to first-generation feedstock, waste fruit can be used to produce bioethanol. In this experiment, firstly the effect of pretreatment technique on glucose generation is observed. Simultaneous saccharification and fermentation (SSF) experiment carried out at a pH of 4.5 and temperature of 30°C for 48 h with fermentation helping nutrients using *Saccharomyces cerevisiae*. A nearly equal amount of glucose concentration is observed from samples treated with hot water, 1% H_2SO_4, 5% H_2SO_4, and without any pretreatment. SSF results also revealed that fermentation helping nutrients has no significant effect on the production of bioethanol at the same concentration. Second part of the experiment deals with the effect of solid loading rate, which is directly proportional to glucose concentration 10-20% (w/w) and time for fermentation (48-96 hours) on generation of bioethanol from fruit waste. Solid loading rate and reaction time for SSF had a significant effect on the production of bioethanol. Optimized 41.19 gL^{-1} bioethanol concentration was observed with solid load rate of 20% (w/w) and fermentation period of 58.8 h. High yield of bioethanol can be achieved using fruit waste at domestic scale with minimum operational requirements.

Key Words: Fruits Waste, Biofuel, Bioethanol, Renewable Energy, Bioenergy, Simultaneous saccharification and fermentation (SSF)

Introduction

In 20th century, energy consumption has been increased, which is about 13 times greater than growing population. Energy is considered a fundamental element for economic and social growth and has become an integral requirement of the modern world (Umar *et al*., 2020). However, a report indicates that 13% of the population still don't have access to modern electricity (WHO, 2020). A major

portion of fossil fuels is used by transportation, which is 60% of fossil fuel around the world, which leads to the harmful effect on the environment causing the depletion of ozone layer by the formation of greenhouse gases (GHGs) in the atmosphere (Carli 2018). The 82% of energy requirements fulfill by petroleum oil, natural gas (CH_4), and coal, about 20% of CO_2 emission is due to consumption of non-renewable energy sources (WEC, 2017). Human activity has a major impact on climate change. It is affected by various reasons like improper combustion of fossil fuels which leads to the emissions of various harmful gases like methane (CH_4), Nitrous oxide (N_2O_2), Carbon dioxide (CO_2), and Carbon monoxide (CO) (Poorter, 2004). Various pollutants are emitted by different energy-producing industries like Sulphur oxides (Sox), Carbon monoxide (CO), Methane (CH_4), Nitrogen oxides (NOx), and various organic compounds (Hashim *et al*., 2020). The use of fossil fuel is an integral part of the energy sector having many consequences including the emission of GHGs (Jursová *et al*., 2018). About 66.3 % (16000 Terra-watt) of electricity was generated from fossil fuels in 2015 and remaining part of electricity (8255 Terra-watt) produced using renewable and nuclear energy sources (IEA, 2020). Renewable fuels are environment friendly, nonpolluting, and economical for the users (Alaswad *et al.,* 2015). Due to the rapidly increasing demand for fossil fuels, scientists have been working to find out the alternatives of fossil fuels. Biofuels are getting more attention over conventional fuels due to their high availability, environment-friendly nature, and economic feasibility against fossil fuels which are much costly and hazardous to environment (Singh. *et al*., 2020). The emission of GHGs can be minimized by replacing blends of bioethanol with gasoline and biodiesel with conventional diesel. Blend of bioethanol with 95% of petroleum oil can reduce about 90% of CO_2 and 60-80% SO_2 (Makur and Birhanu, 2020). Monosaccharides e.g., sucrose, glucose are the building blocks for the production of bioethanol from juices of crops containing free sugar in the presence of microorganisms via fermentation (Ali *et al*., 2021a; Ali *et al*., 2021b). Feedstock to produce bioethanol is sugar crops and starch that are 60% and 40% respectively (Zabed *et al*., 2014). Corn starch and sugarcane produced about 30 billion gallons of bioethanol in 2019, which can be used as renewable biofuel (Colombini, 2020). The utilization of agricultural wastes or municipal solid waste will supply a limited amount of biofuel (Srivastava *et al*., 2014). Hence, there is a need to investigate the utilization of different wastes, such as, organic product waste, vegetable wastes, and fruit waste which are expended at immense scales and create a lot of disposal issues. Since organic products are rich in sugars, fruit waste can be a decent feedstock for bioethanol production. Therefore, the focus of this study was to find a new and cheaper way of bioethanol production from fruit waste which used less energy and other resources.

Materials and Methods

Collection of raw fruit waste: The fruit waste was collected from the local wholesale fruit market (Sadhaar bypass Fruit and Vegetable Market, Jhang Road, Faisalabad, Pakistan) and brought to Department of Energy Systems Engineering, University of Agriculture, Faisalabad 38000, Punjab, Pakistan (Figure 1A).

Physical pretreatment: For physical pretreatment of fruits, a mincer machine was used (Figure 1B), because other methods of physical pretreatment are costly and complicated to adopt. The mincer machine is readily available in the market and cheaper than other milling and cutting machines.

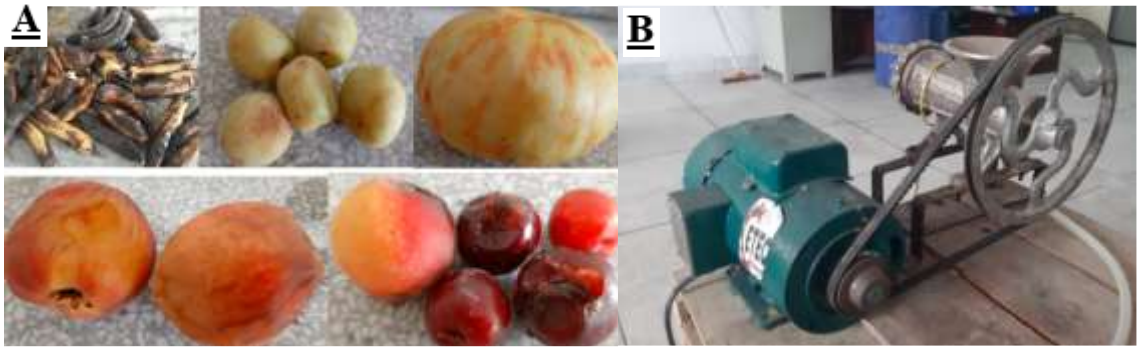

Figure 1. Fruit waste used to produce bio-ethanol (A), mincer machine used for physical pretreatment of fruits (B).

Acid pretreatment and hot water pretreatment: For acid (1% and 5% w/v) and hot water pretreatment a reactor of 250 ml was used with a time range (15-25 minute), fruit waste loading rate from 4 to 8 wt.%, and varying temperature of 75-145^{o}C (Table 1). After completion of the pretreatment reaction process solid and liquid parts were examined accordingly. The supernatant liquid was analyzed for estimation of total reducing sugars using 3, 5-dinitrosalicylic acid (DNS method).

Table 1. Values of fixed variables for the pretreatment process.

Working parameters	**Acid pretreatment**		**Hot water pretreatment**	
	Min.	**Max.**	**Min.**	**Max.**
Temperature (°C)	75	95	95	145
Fruit waste loading rate (wt.%)	4	8	4	8
Pretreatment Duration (minute)	15	25	15	25

Saccharomyces cerevisiae: Instant yeast (*Saccharomyces cerevisiae*) (92.89% dry weight) was purchased from a local departmental store and stored at 4^{o}C in a refrigerator. Inoculum from baker's yeast was prepared in distilled water with a concentration of 10 gL^{-1} at room temperature without any cultivation.

Fermentation media: In the fermentation reactor, 7.5 g of urea and 0.25 kg of sucrose was introduced as fermentation media for the fermentation of 1 kg fruit waste. The 50 g of *Saccharomyces cerevisiae* was used in distilled water to make a final volume of 5 L.

Proximate analysis of feedstock:

Moisture content

The gravimetric technique was used to find out the moisture content of the fruit waste. The 5g fruit waste was taken onto a Petri plate and kept in an oven at 105^{o}C for 30 to 60 minutes. After that, fruit biomass was cooled down for 20 minutes in a desiccator to get their moisture-free weight. With interval of an hour, Petri plate with the sample was weighed until a constant weight was obtained. At a constant weight, Petri plate was taken out from the desiccator. Then the final weight of sample was recorded and sample was examined using given relationship (Equation 1).

Moisture content (%) = [(A-B) / B] × 100……(Equation 1)

Where:

A = Wet sample weight (g)
B = Dry sample weight (g)

Ash Content: The ash content of sample was determined by dry oxidation method (oxidation at 550°C-600°C). One-gram sample of oven-dried (105°C) fruit waste was taken into the crucible and then burned for 6 hours into the furnace at 575°C. The given formula was used to examine the ash content (Equation 2).

Moisture content % = $[(W_i-W_f)/W_i] \times 100$…… (Equation 2)
Wi = Weight of sample before oxidation in the furnace (g)
Wf = Weight of sample after oxidation in the furnace (g)

Protein fraction: For the evaluation of protein, the technique described by Estefan *et al.* (2013) was implemented to find out the protein content. One-gram sample of known moisture was used for analysis. After performing different steps, the protein content was examined using Equations 3 and 4.

$$\%\ \text{N (Dry Basis)} = \frac{(V_{0.1N.H2SO4} - V_{Blank} - V_{0.1N.NaOH}) \times 0.0014 \times 100}{(W_s \times \frac{100 - \%X_s}{100})} \ldots\ldots\ldots\ldots\ldots\ldots$$ (Equation 3)

$$\%\ Protein = \%N \times 6.25 \ldots\ldots\ldots\ldots\ldots\ldots$$ (Equation 4)
Where:
N = Nitrogen present in the fruit waste sample (%)
Ws = Sample weight in grams
Xs = Sample moisture content (%)

Fat content: The 17 g fruit waste was added into two different flasks and weighed. Hexane (6 ml) and isopropanol (4 ml) were mixed and poured into the flasks for 5 minutes. The liquid portion was poured into empty beakers. Mixing and pouring of samples was carried out three times and then placed in oven for whole night drying at 105°C. To overcome the absorption of water, flasks were kept in desiccator, then cooled to ambient temperature and weighed.
The percentage of extracted fats was calculated according to Equation 5.

Moisture content (%) = $[(W_i-W_f)/W_i] \times 100$…… (Equation 5)
W_i = Initial weight(g)
W_f = Final weight(g)

Total carbohydrates: Total carbohydrates were determined by subtraction of fat, protein and ash content from total solids (Equation 6).

%Total Solids = % (Protein content +Fat content +Ash content+Total CHO's) ……(Equation 6)

Reducing sugars: Dinitro salicylic acid (DNS) technique was used to calculate the concentration of reducing sugars. The spectrophotometer was used to analyze the samples prepared by the DNS method. In the cold-water bath, temperature of samples was reduced and absorbance was recorded at 550 nm.

Fermentation: Basic sugars were started conversion into bioethanol and CO_2 when zymase from *Saccharomyces cerevisiae* acted in the fermentation process. A hydrometer was used to determine the specific gravity of the sample periodically during fermentation process. A constant value of specific gravity is the indication of the end of fermentation process.

Recovery of the product: The product of fermentation process was centrifuged. The upper layer of the centrifuged product was collected, and the volume of bioethanol was calculated by using hydrometer. The distillation process was carried out using a rotary evaporator. The distillation unit made of

three basic parts i.e., boiler, condenser, and distillate chamber.

Testing of product: Given test was conducted to check the quality of produced bioethanol. The presence of bioethanol was checked by the iodoform test. The 10 drops of bioethanol were added to a clean and dry test tube, 25 drops of iodine solution and sodium hydroxide (NaOH) solution were added to remove the color of the iodine and was mixed gently for a few minutes. A very pale-yellow precipitate of triiodo methane (previously known as iodoform) was formed which ensure the presence of ethanol. The pH of the bioethanol sample was measured using pH meter (Starter 3100, OHAUS). Density was measured using pycnometer, and calculated using Equation 7.

$$Density\ (g/ml^3) = \frac{\text{mass of sample}}{\text{volume of sample}} \quad \text{(Equation 7)}$$

Distilled irrigate was overflowing into the pycnometer, weighed, and recorded. The density of water and creation taster was used for the measurement of limited gravity. The aspect of gravity was calculated by using Equation 8.

$$Specific\ Gravity = \frac{\text{Density of ethanol}}{\text{Density of water}} \quad \text{(Equation 8)}$$

Results

The proximate analysis of feedstock: The growth and maintenance of yeast directly depend upon the available nutrients and compounds from the dried fruit waste. Fermentable sugars depend upon the existed content of total carbohydrate (CHO's) present in dried fruits. According to analytical tests, fruits were composed of approximately 60-70% water and 30-40% dry biomass. This 30-40% of dry biomass contains about 60% of carbohydrates, which show the potential of fruit waste as a perfect raw material for bioethanol production (Figure 2).

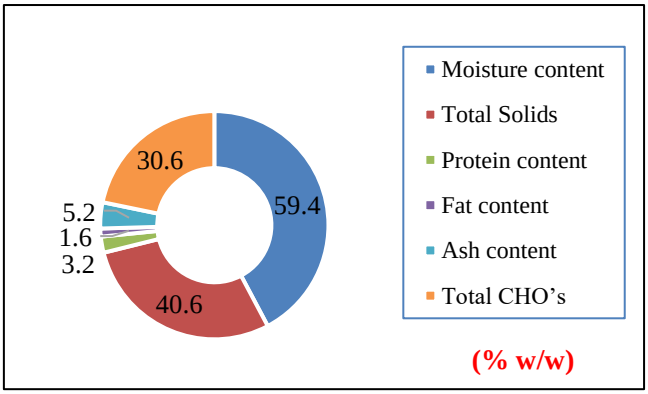

Figure 2. Biochemical composition of fruit waste used to produce bio-ethanol.

Effect of different pretreatment techniques on fruit waste: Raw fruit residues, rotten fruits, whole edible parts, and peels of fruits were used in this experiment, this fruit waste had a considerable amount of starchy and lingo-cellulosic materials (Figure 1). The yield of fermentation increased by using a suitable pretreatment technique (Table 2). The fermentation process with each pretreatment technique occurred under same circumstances and conditions. Thus, due to the different pretreatment techniques, the change in final glucose content was examined. Glucose content significantly affected by different pretreatments (Table 2). Sample treated with hot water and sample without any pretreatment had high glucose content than a sample with acid pretreatment. After 6-hours, the highest concentration of glucose was attained from un-pretreated samples was about 64.8 gL^{-1} and from the samples which were gone under boiled water treatment gave about 56.7 gL^{-1}. Therefore, we could directly undergo fermentation, as hot water pretreatment does

not have a significant impact on the yield. Similar results were obtained when hot water pretreatment was done which was significantly lower than the results achieved from mixture which does not undergo any pretreatment. From these results, it was found that fruit waste which was used in mixed form of carbohydrates fraction (without pretreatment) were more adequate in the production of glucose.

Table 2. Effect of pretreatment methods on glucose concentration of fruit waste.

Pretreatment conditions			Glucose concentration (gL^{-1})
Temperature °C	Time (Hour)	Pretreatment Method	
60	3	1% acid	48.0
60	3	4% acid	42.4
60	3	Hot water	56.7
WPT	WPT	WPT	64.8

WPT – without pretreatment.

Effect of fermentation medium on bioethanol production: For the batch production of the bioethanol, a mixture was subjected to fermentation at a temperature of 30°C for 2 days at a pH of 4.5. Table 3 showed the yield, final production of bioethanol, and initial glucose concentration. Results showed that yield and bioethanol production was the same for the fermentation of the glucose for both mediums regardless of the pretreatment method applied to the raw material. The yield and the bioethanol were attained as 0.36 gg^{-1} and 23.3 gL^{-1} for the mixture which was untreated sample and in which no fermentation medium was added. These results showed significantly higher results than the pretreated samples with hot water and fermentation medium was added in the samples. Productivity was about 0.49 $gL^{-1}hr^{-1}$. Thus, for the proper functioning of the *Saccharomyces cerevisiae* in bioethanol production, enough amount of nutrients was already present in the fruit waste.

Table 3. Effect of pretreatment and fermentation medium on bioethanol production by fruit waste.

Pretreatment	Glucose level before fermentation (gL^{-1})	Ethanol (gL^{-1})	Yield (g EtOH g^{-1} glucose)
Hot water+FW+WM	56.7	14.6	0.26
Hot water+ FW +NM	56.7	17.2	0.30
WPT+ FW +WM	64.8	17.4	0.27
WPT+ FW +NM	64.8	23.3	0.36

FW – fruit waste; WM – with fermentation helping nutrients; NM - without fermentation helping nutrients; WPT – without pretreatment.

Glucose-Ethanol trends and determination of fermentation time: Fermentative microbes (*Saccharomyces cerevisiae*) under anaerobic conditions performed alcoholic fermentation of organic material (fruit waste). Through, bioethanol was produced through fermentation of glucose (glycolysis). Temperature, inoculum, pH, and initial concentration of sugar were the factors involved in the good quality of bioethanol production. The study focused to find the time interval to ferment the fruit waste in some specific conditions like temperature (30°C), pH (4.5), and inoculum concentration ($1gL^{-1}$). Three different solid loading rates were introduced i.e., 10, 15, and 20% (w/w) with respect to different initial concentrations of sugar.

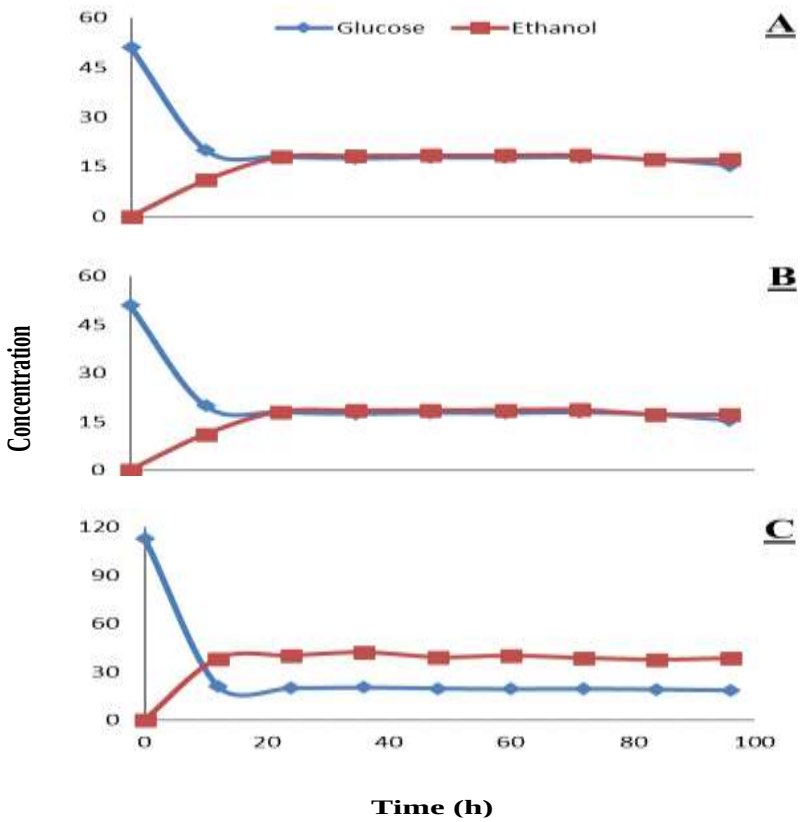

Figure 3. Glucose-ethanol trend with fruit waste fermentation time with 10% (A), 15% (B), and 20% (C) solid load.

Results given in Figure 3 showed that the conversion of 80% glucose (on an average). In all three levels of solid loading rate i.e., 10, 15, and 20% (w/w), production of bioethanol was increased with the increase in solid loading rate (glucose concentration). The 79% of glucose conversion was calculated when fermentation of fruit waste was carried out with a 10% (w/w) solid loading rate (51.3 gL^{-1} initial glucose concentration). After 72 hours of the fermentation process, the highest output of bioethanol was recorded as 0.37 $g.g^{-1}$. After 72 hours of the fermentation process, the highest output of bioethanol and desired conversion were examined. So, the productivity of 0.26 $gL^{-1}h^{-1}$ was observed (Figure 3A, Table 4). The trends in the fermentation of fruit waste at solid loading rate of 15% (w/w) is shown in Figure 3B. The initial glucose concentration and conversion of glucose were 74.67 gL^{-1} and 81%, respectively. After 72 hours of the fermentation process, the highest output of bioethanol was recorded as 0.38 $g.g^{-1}$. While the productivity was 0.39 $gL^{-1}h^{-1}$ bio-ethanol (Table 4). Similarly, after same period of fermentation, the highest bioethanol production (41.19 gL^{-1}) was observed with 85% glucose conversion rate with the highest productivity of 0.69 at solid loading rate of 20%. It had been seen a decline in fermentation yield when solid loading rate increased above 20%, which can be justified due to the increase in osmotic pressure. So, a maximum solid loading rate of 20% was set as the optimum operational parameter for said fermentation experiment. Also, in this case, maximum productivity of 0.69 EtOH $gL^{-1}h^{-1}$ was observed with a 20% solid loading rate. As can be seen from Table 4, with increasing solid loading rate, the initial glucose concentration was increased with high conversion of glucose. High concentrations and good consumption of glucose with increasing solid loading rate resulted in a higher yield of bioethanol and thus increased productivity

Table 4. Ethanol production, Glucose conversion, and yield as affected by solid loading rate.

Medium	Glucose Conversion	EtOH(max) gL^{-1}	Yield	Productivity
Waste 10% solid load	79	18.82	0.37	0.26
Waste 15% solid load	81	28.27	0.38	0.39
Waste 20% solid load	85	41.19	0.39	0.69

Discussion

About 0.72 Trillion tons/year biomass is produced through photosynthesis and we are using only 10-15% of that cellulosic raw material to meet current energy demand (Akia *et al*., 2014). Macroscopic, microscopic as well as sub-microscopic structure of the biomass alter after pretreatment. Pretreatment of fruit waste causes removal of hemicellulose and lignin from the lingo-cellulosic matrix of structure, pretreatment of fruit waste can decrease the crystals of the cellulose, enhance porosity and surface area of the biomass. Some fermentable sugars released by de-polymerizing the hemicellulose of the lignocelluloses in the process of retreatment of the lignocellulosic biomass. Till now, many methods of pretreatment had been suggested, these were simple or technically more complicated, they were categorized into three different classes, like physical pretreatment, biological pretreatment, and chemical pretreatment. These treatment techniques differ from one another in overall outcomes and mode of action of these pretreatment techniques different one another (Kamzon *et al*., 2016).

Table 5. Merits and Demerits of physical pretreatment.

Category	Type	Merit	Demerit
Physical pretreatment	Grinding	• Cracking of lignocellulosic material structure • Decrease crystallinity • maximize surface area	• Energy-intensive • Costly

Thermolysis (microwaves, gamma rays), Irradiation, and mechanical grinding all are forms of physical pretreatment. The basic purpose of physical pretreatment on the biomass to increase surface area, porosity, decrease the polymerization of cellulose, crystalline structure, hydrolysis of hemicelluloses, and partial de-polymerization of lignin content. They consume more energy, behave un-friendly for the environment, and not suitable for commercial process. Different techniques and methods are used to produce bioethanol on a commercial scale. All those methods and techniques are complex, difficult to follow, required sophisticated instruments and more trained unit operator for efficient conversion of biomass to biofuel, and also to get high yield of bioethanol. Previously discussed issues resolved using this study in which more convenient, reliable, cheaper, efficient, and user-friendly techniques are applied for efficient conversion of biomass and get a high yield of bioethanol. The major part of resources used during designing of the reactor, maintenance of optimal conditions for the desired product, and pretreatment of feedstock. So, these issues rectify using a domestically designed reactor with minimum requirements to get optimal conditions without costly pretreatment techniques. Results also showed that the applied process (only physical pretreatment, without stirring) is reliable and efficient because the experiment gives a high yield of bioethanol

.

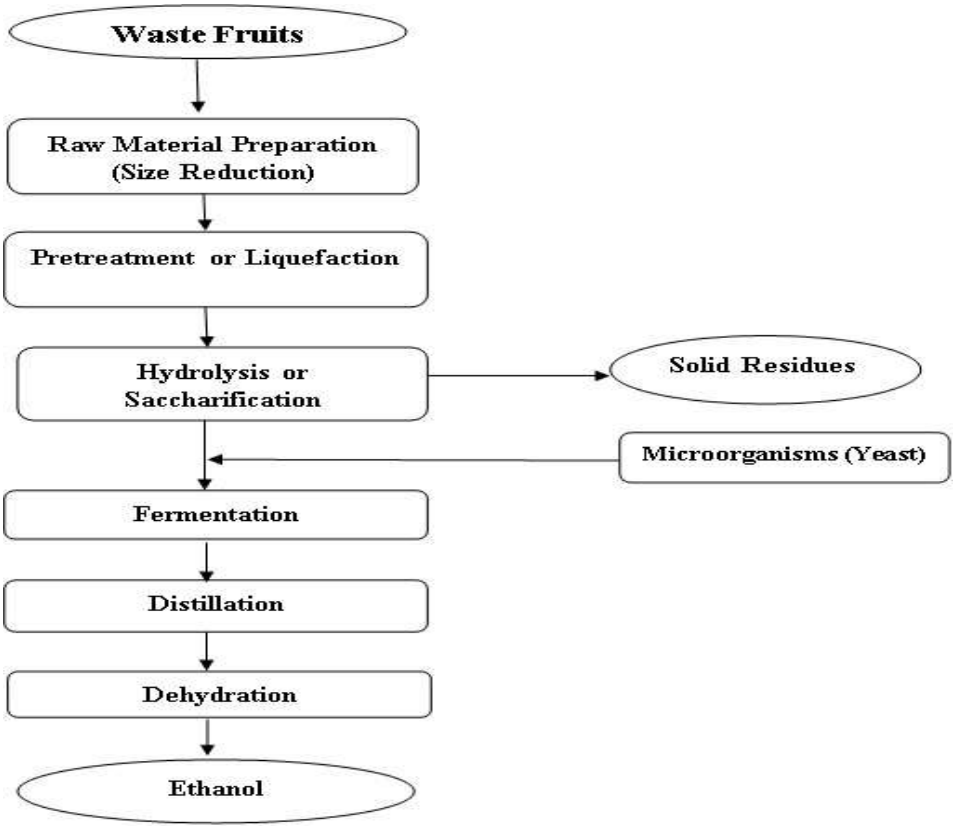

Figure 4. Process layout of bioethanol production using fruit waste.

The results of the proximate analysis are presented in Fgure 2. The results revealed that the moisture content, total solids, protein, fat, ash content and total CHO's in dry matter was 59.4%, 40.6%, 3.2%, 1.6%, 5.2% and 30.6%, respectively. The results of this study are in good arrangement with the previous research on the proximate analysis of date palm (Bouhlali *et al*., 2017). An analysis was performed on fruit waste from Egypt and found that these fruits contained crude fiber (5.20%), protein (3.00%), moisture (13.80%), fat (2.90%), and ash (2.13%) (El-Sohaimy and Hafez, 2010). The effect of temperature and hot water pretreatment on the production of ethanol is given in Table 2 and 3. It was observed that lower temperature resulted in a lower production rate of ethanol, which could be possibly due to less activity of the yeast cell, because during fermentation if the temperature is low the activity of yeast cells decreases and results in lower ethanol production (Zabed *et al*., 2017). Our results are also in similar to the findings of Fakruddin (2013) who stated that *S. cerevisiae* and *S. unisporous* worked best and produced the maximum ethanol. The efficiency of yeast to convert sugars into ethanol increased with increase in solid loading rate (Table 4). The results revealed that the increased solid loading rate produced maximum ethanol. The process of fermentation of sugars was active during the first 72 hr. After 72 hr, there was no consumption of sugars revealed by yeast. This may be due to the toxic effect of a longer fermentation period that is harmful to microbial growth (Zabed *et al*., 2014). However, the maximum yield of alcohol with the increase in sugar fermentation is justified from the findings of Bhatti *et al*. (2019) who stated that more sugar consumption increases the alcohol yield. Our results are also similar to the results of Ahmed *et al*. (2016) who reported the fermentation time of sugars from 36 to 72 hr.

Conclusion

This study gave satisfactory results with a high yield and productivity of 0.39 and 0.69, respectively. This new approach with minimum operational requirements can be used for bioethanol production most cost-

effectively. Due to high cost-effectiveness and environment-friendly aspects, any individual can develop this technology and can produce bioethanol on domestic as well as industrial level.

References

Ahmed, B., Mabrouk, K., Cherif, K. & Boudjemaa, B. (2016). Bioethanol production from date palm fruit waste fermentation using solar energy. African J. Biotechnol., **15**: 1621–1627

Akia, M., Yazdani, F., Motaee, E., Han, D. & Arandiyan, H. (2014). A review on conversion of biomass to biofuel by nanocatalysts. Biofuel Research Journal, **1**(1):16-25.

Alaswad, A., Dassisti, M., Prescott, T. & Olabi, A.G. (2015). Technologies and developments of third generation biofuel production. Renewable and Sustainable Energy Reviews, **51**:1446-1460

Ali, M.M., Anwar, R., Shafique, M.W., Yousef, A.F. & Chen, F. (2021a). Exogenous Application of Mg, Zn and B Influences Phyto-Nutritional Composition of Leaves and Fruits of Loquat (*Eriobotrya japonica* Lindl.). Agronomy, **11**(2): 224.

Ali, M.M., Anwar, R., Yousef, A.F., Li, B., Luvisi, A., Bellis, L. De, Aprile, A. & Chen, F. (2021b). Influence of Bagging on the Development and Quality of Fruits. Plants, **10**(2): 358.

Bhatti, Z.A., Rajput, M.-H. & Maitlo, G. (2019). Impact of Storage Time, Rain and Quality of Molasses in the Production of Bioethanol. Mehran Univ. Res. J. Eng. Technol., **38**: 1021–1032.

Bouhlali, E. dine T., Alem, C., Ennassir, J., Benlyas, M., Mbark, A.N. & Zegzouti, Y.F. (2017). Phytochemical compositions and antioxidant capacity of three date (*Phoenix dactylifera* L.) seeds varieties grown in the South East Morocco. J. Saudi Soc. Agric. Sci., **16**: 350–357.

Carli, R., Dotoli, M. & Pellegrino, R. (2018). A decision-making tool for energy efficiency optimization of street lighting. Computers & Operations Research, 96:223-235.

Colombini, K. (2020). RFA Urges Immediate EPA Action On 'Litany' Of Outstanding RFS Issues. Renewable Fuels Association. https://ethanolrfa.org/2020/08/rfa-urges-immediate-epa-action-on-litany-of-outstanding-rfs-issues/ [Accessed 22 September 2020].

El-Sohaimy, S.A. & Hafez, E.E. (2010). Biochemical and nutritional characterizations of date palm fruits (*Phoenix dactylifera* L.). J. Appl. Sci. Res., **6**: 1060–1067.

Estefan, G., Sommer, R. & Ryan, J. (2013). Methods of Soil , Plant , and Water Analysis : A manual for the West Asia and North. Int. Cent. Agric. Res. Dry Areas.

Fakruddin, M. (2013). Process Optimization of Bioethanol Production by Stress Tolerant Yeasts Isolated From Agro-Industrial Waste. Int. J. Renew. Sustain. Energy, **2**: 133.

Hashim, B.M., Sultan, M.A., Al Maliki, A. & Al-Ansari, N. (2020). Estimation of Greenhouse Gases Emitted from Energy Industry (Oil Refining and Electricity Generation) in Iraq Using IPCC Methodology. Atmosphere, **11**(6):662.

Hughes, S.R. & Jones, M.A. (2020). Fuel Ethanol Production from Lignocellulosic Materials Using Recombinant Yeasts. Green Energy to Sustainability: Strategies for Global Industries, 269-282..

IEA. 2020. Global Annual Average Change in Energy Production By Fuel, 1971-2018 –Charts – Data & Statistics - IEA. https://www.iea.org/data-and-statistics/charts/global-annual-average-change-in-energy-production-by-fuel-1971-2018 [Accessed 9 September 2020].

Jursová, S., Burchart-Korol, D., Pustějovská, P., Korol, J. & Blaut, A. (2018). Greenhouse gas emission assessment from electricity production in the Czech Republic. Environments, **5**(1):17

Kamzon, M.A., Abderafi, S. & Bounahmidi, T. (2016). Promising bioethanol processes for developing a biorefinery in the Moroccan sugar industry. International Journal of Hydrogen Energy, **41**(45):20880-20896.

Makur, M.M. & Birhanu, T. (2020). Review on the Production and Characterization of Glucose and Ethanol from Sugarcane Bagasse. International Research Journal of Science and Technology, **1**(2):169-176

Poorter, H. (2004). Physiological plant ecology. 4th edn. Ann. Bot. 93: 616–617.

Singh, D., Sharma, D., Soni, S.L., Sharma, S., Sharma, P.K. & Jhalani, A. (2020). A review on feedstocks, production processes, and yield for different generations of biodiesel. Fuel, **262**:116553.

Srivastava, N., Rawat, R., Sharma, R., Oberoi, H.S., Srivastava, M. & Singh, J. (2014). Effect of nickel–cobaltite nanoparticles on production and thermostability of cellulases from newly isolated thermotolerant Aspergillus fumigatus NS (Class: Eurotiomycetes). Applied biochemistry and biotechnology, **174**(3):1092-1103.

Umar, T., Egbu, C. & Ofori, G. (2020). Challenges towards Renewable Energy: An Exploratory Study from GCC Region. Energy, **173**(2): 1-34.

WEC. 2020. World Energy Issues Monitor | 2017: Exposing The New Energy Realities. https://www.worldenergy.org/publications/entry/world-energy-issues-monitor-2017-exposing-the-new-energy-realities [Accessed 30 September 2020].

WHO. 2020. Fuel For Life: Household Energy And Health. https://www.who.int/indoorair/publications/fuelforlife/en/ [Accessed 15 September 2020].

Zabed, H., Faruq, G., Sahu, J.N., Azirun, M.S., Hashim, R. & Nasrulhaq Boyce, A. (2014). Bioethanol production from fermentable sugar juice. The Scientific World Journal, 2014: 1-11.

Zabed, H., Sahu, J.N., Suely, A., Boyce, A.N. & Faruq, G. (2017). Bioethanol production from renewable sources: Current perspectives and technological progress. Renew. Sustain. Energy Rev., **71:** 475–501..