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(Research Paper)

Optimization of Bio-ethanol Production via Solid-state Fermentation using *Saccharomyces cerevisiae* PTCC 1212 and Fruit and Vegetable Wastes

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Abstract

Introduction: Global municipal solid waste generation is growing progressively due to social urbanization. Fruit and vegetable waste are a significant part of solid waste. This waste can be recovered to produce biofuels. *Saccharomyces cerevisiae* is consumed as a first-choice microorganism for industrial processes of alcoholic fermentation.

Materials and Methods: The potential of ethanol production by *S. cerevisiae* PTCC 1212 on a laboratory scale was investigated using fruit and vegetable wastes over solid-state fermentation (SSF) using a colorimetric distilling system. First, we have estimated the ethanol production potential of solid waste substrates taken from the Shahrekord Center of Fruits and Vegetables. Next, and over-optimization experiments, fermentation parameters for ethanol production and yield including substrate type, pH, humidity, inoculum volume, and time of fermentation were evaluated using the Taguchi statistical method. Bioethanol assay was done by the distillation-colorimetric method and sugar consumption was determined using the DNS colorimetric method.

Results: Bio-ethanol production contents from solid waste substrates including fruit mix, carrot, potato, cattle food, eating vegetable, and beet molasses as an alcoholic fermentation additive were 2.8, 2.3, 0.98, 0.56, 0.07, and 3.5 (w/v), respectively. The optimized conditions for ethanol production were 4 days of fermentation, pH 6, 90% humidity, and 5% inoculum volume. The ethanol achievement in the final test based on optimized

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parameters was up to 3.3%. The optimized conditions for the best ethanol yield (ethanol per one gram of substrate) were, pH =5, humidity level as natural (without the addition of water) and substrate mixed fruits plus molasses.

Discussion and Conclusion: High sugar rate conversion showed appropriate efficiency in the fermentation process and fruit and vegetable wastes have an exciting potential for bio-ethanol production in solid-state fermentation. It could be considered for environmental challenges with garbage management. Moreover, adding molasses seems to be an appropriate supplement for boosting the production of ethanol.

Key words: *Saccharomyces cerevisiae*, Fruit and Vegetables Waste, Taguchi Optimization, Bioethanol

Introduction

Growing energy demand is one of the most significant challenges of the 21st century. In the 20th century, energy consumption has increased, which is about 13 times greater than the growing population. According to reports, global primary energy consumption increased more than 200% from 1978 to 2018, rising from 270.5 to 580 exajoules (EJ). Oil price increments during 2006 approached US$80 per barrel, causing a challenging global economic issue.

Besides these concerns, the energy supply must be concentrated on the reduction of greenhouse gas emissions. Global energy-related CO2 emissions were raised by 87% from 18.0 to 33.7 billion tons over the 1978-2018 period. China and the United States are the leading emitters of greenhouse gas emissions, together responsible for about 35% of global emissions (1-3). Various scientific research and policies toward this aim have been initiated. China has announced a target for carbon emission control. This plan aims to achieve a 40–45% reduction in CO2 levels by 2020 and 2030 compared to 2005 (4). The European Commission has targeted the substitution of up to 10% of fossil fuels with alternative fuels in the transport sector from 2020 (5). The US Energy Policy Act of 2005 aimed to mix 7.5 billion gallons of alternative fuels in 2012 (6).

At the beginning of the 1970s, the Organization of Petroleum Exporting Counties (OPEC) decreased total oil production, and oil prices increased, which persuaded oil consumers to seek alternatives. Ethanol is widely concerned about substituting fuels with renewable sources. Ethanol was formerly produced from carbohydrates and grains, which has human food concerns. Therefore, liquid biofuels from renewable resources, especially lignocellulosic materials, could have a constitutional role in this way. Ethanol is nowadays produced on a large scale in Brazil (from sugar cane), the USA, China (mainly from corn), and some European countries. It is expected to be one of the major renewable fuels in the transport sector within the next 20 years. The United States and Brazil are the leading global ethanol producers. They produce more than 80% of the global commercial alcohol (7, 8, 10, 11). Therefore, liquid biofuels from renewable resources, especially lignocellulosic materials, could have a constitutional role in this way (9).

World ethanol production grows annually. Global ethanol production was about 33.3 billion liters in 1998 (12). It is anticipated that it will reach up to 114 billion liters in 2022 (13). The future of bioethanol appears to be bright since renewable energy demand to decrease dependence on foreign oil is highly concerned. Many countries are also trying to reduce petroleum imports and improve their air quality (14).

The solid-state fermentation (SSF)

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technique is defined as the growth of microorganisms in moist solids without any free-flowing water. The moisture levels in this condition vary between 30 and 85%. SSF has many potential advantages, including a) fewer water requirements, b) fewer physical energy requirements, c) less capital investment, d) fewer operating costs, e) less liquid waste, and hence f) fewer pollution challenges. Besides these advantages, SSF has some limitations. There are a limited number of microorganisms capable of growing under these conditions. In addition, controlling parameters such as temperature, pH, humidity, and airflow is difficult (7, 15, 16). SSF provides an original approach for researchers and industries, especially in solid waste management areas, due to a few advantages of submerged fermentation (16, 17).

The global municipal solid waste (MSW) generation is growing progressively due to social urbanization. In 2010, approximately 1.3 billion metric tons of MSW were generated worldwide, and it is estimated to reach 2.2 billion metric tons by 2025. Fruits and vegetable waste (FVW) are a significant part of solid waste due to their high global generation in agriculture and marketing processes (18). About 40% of organic waste is composed of fruit and vegetable waste (19). The FVW generation during cultivation and transport increases the marketing cost (18). Based on the Food and Agriculture Organization (FAO) stats, the per capita fruit consumption in Iran was around 120 kg in 2017 (Figure S1) (20), which will be around 9.5 million tons of fruit consumption. In 2015, up to 35% of agriculture and fruit production went to waste in Iran. It causes a loss of up to 15 billion m^3 of drinking water and 15 billion dollars annually (21). Food waste contains carbohydrates up to 65% of its total solids and, therefore, is a suitable substrate for producing ethanol (22).

FVW can be recovered by various microorganisms such as microalgae, yeast, bacteria, and fungi (19). *Saccharomyces cerevisiae* generally is consumed as a first-choice microorganism for industrial processes of alcoholic fermentation (23). The most important reasons include the capability of production and tolerance of 20 % v/v ethanol, regarded as a Generally Recognized as Safe (GRAS) organism and high biosafety, the ability to fast growth in anaerobic conditions, excessive amounts of biological and biochemical information, and the potential for the use of baker yeast biomass as cattle food, which led to its being an economic factor (24, 25).

Any plant material containing significant sugar content could be used as a raw substrate source for bio-ethanol production. (26) There are various reports of fruit and agricultural crops' application for bioethanol production. Apple pomaces (27), grape and sugar beet pomaces (28), sweet sorghum stalk (29), wheat straw (30), mahula (Madhuca latifolia L.) flowers (31), wheat bran particles (32), sugarcane bagasse (33), sweet potato (34), *Ulva fasciata* (a common green alga) (35), citrus fruit waste (36), watermelon, pineapple, carrot, papaya, banana, and cucumber peels (37), cassava (38), and potato cassava pulp (39) have been previously reported as a substrate for ethanol production under SSF conditions.

Fruits and vegetable wastes contain several types of polymer sugars, including pectins (1.50–13.40%), cellulose (7.20–43.60%), hemicelluloses (4.26–33.50%), and lignins (15.30–69.40%).

Also, vegetable wastes are renewable sources of carbohydrates, amino acids, lipids, and phosphates. Vegetable wastes are also renewable sources of carbohydrates, amino acids, lipids, and phosphates (40, 41). The sugar content of this type of waste is around 5% (42). They

contain cellulose and hemicellulose polymers, which can be converted into monomer sugar through the hydrolysis processes (43). All of these lignocellulose-containing biomasses could be used for bioethanol production (44).

In the present study, we first investigated ethanol production from various waste substrates. Next, we designed solid-state and submerge fermentation tests for bio-ethanol production using waste fruit, vegetables, and molasses. The influence of various process parameters on ethanol yield, including pH, substrate type, and inoculum volume, was studied. The outcome could be used to stimulate urban and agricultural waste refineries.

Materials and Methods

Microorganism and Culture Method: *S. cerevisiae* strain was purchased from Persian Type Culture Collection (PTCC 1212). The yeast strain was cultured in YPDA medium (yeast extract, 10; peptone, 10; dextrose, 20; agar 15,g/l Merck, Germany) using the strike plate method. An isolated colony was used for producing inoculum in the YPD broth medium. The inoculum preparation was carried out under the following conditions: temperature, 30 C; agitation speed, 120 rpm; and OD=1. Cell growth was measured by optical density at 600 nm (gene quant pro, Germany). All the media and substrates were autoclaved at 121 °C for 15 min.

PH Determination: One gram of each sample was transferred to 9 ml of distilled water and mixed by vortex (45). Then, pH values were registered using a conventional pH meter (JENWAY, UK). All sample pH was settled to desired pH levels using 1 N NaOH and HCl solutions.

Sugar Content Determination: One ml of the above solution was used for sugar content determination (monosaccharide sugars, glucose, and fructose mainly) using the DNS colorimetric method (46). By comparing it to the standard curve (Figure S1), the amount of sugar was calculated. Ten ml of PBS buffer per gram of sample was added to each Erlenmeyer and mixed for 5 minutes to determine secondary sugar (residual sugar after fermentation). One ml of this solution was used for sugar determination, using the DNS colorimetric method as mentioned above. Standard samples, including a serial dilution of pure glucose (MERCK, Germany) 0.5 up to 2.5 % W/V (gr/100 %) sugars, were used as standard curve samples. Beet molasses sugar content was determined after acidic hydrolysis, a general DNS test was carried out, and the amount of each sugar type was determined. Molasses brix was determined using the ABBE refractometer (ABBE, Belgium) (47).

Humidity Determination: Constant amounts of samples were transferred to a 75 °C oven for three days for humidity content (w/w) measurement. Water (considering humidity change by inoculum volume humidity) was added to each sample to reach humidity levels based on Taguchi tests (Table 1).

Ethanol Determination: Ethanol determination was carried out using a colorimetric distilling system employing sulfochrome as the ethanol determinant factor. During distillation, separated ethanol transfers to the sulfochrome solution, changing the color of the solution. The concentrations of ethanol were calculated by comparing them to the ethanol standard curve (48).

The standard equation used for ethanol determination is mentioned below (48).

$$y = 0.0402\,x + 0.00718$$

Statistical Analysis: Fermentation parameters, including inoculum volume, pH, humidity, temperature, and substrate type, were regarded as 1-4 values. Three levels for each parameter were considered.

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The test was designed and analyzed using specific Taguchi software (Table 2).

Table 1- Details of each Sample Based on Taguchi Designed Test

Test No.	Substrate Type	pH	INC Volume (ml)	Water Addition (ml)	Final humidity (%)
1	MIX F	5	2.5	0	Natural (86.6)
2	MIX +VEG	6	2.5	10.62	86
3	MIXF+MOL	7	2.5	36.3	90
4	MIXF+MOL	6	7.5	6.9	86
5	MIX F	7	7.5	12.2	90
6	MIXF+VEG	5	7.5	0	Natural (86.6)
7	MIXF+VEG	7	10	5	86
8	MIXF+MOL	5	10	0	Natural (86.6)

Table 2- Taguchi Statistical Method Details

Level 3	Level 2	Level 1	parameter
7	6	5	pH
35	30	25	Temperature (č)
90 %	86 %	Natural (no added water)	Humidity (w/v)
10 %	7.5 %	5 %	Inoculum volume (ratio to sample volume v/w)
Mix F +MOLASSES 10 grams of each fruit (40 grams)+ 30 grams of molasses with brx≈30	Mix F + VEG 10 grams of each fruit (40 grams)+ 10 grams of each vegetable* (30 grams)	Mix F 10 grams of each fruit (40 grams)	Substrate type (50 grams of each mix used)

Fermentation Condition Design: Primary tests were carried out on 3, 4, and 5 days. Fifty grams of each substrate (fruit mix, potato, carrot, molasses (brix=20), eating vegetables, cattle food (concentrated residue from the molasses processing industry), and YPD media (as control media) were transferred to 250 ml flasks and inoculated with 2 ml of yeast suspension with OD 1.0. No pretreatment was applied to evaluate direct sugar fermentable potential. Table 3 shows the details of each substrate type. Fruits and vegetables were collected from the Shahrekord Center of Fruits and Vegetables (Shahrekord, Chaharmahal va Bakhtiyari Province, Iran) and transferred to the lab using sterile glass containers. The fresh beet molasses was also collected from the Charmahal Sugar Factory in Shahrekord, Iran.

Table 3- Substrates for Media Preparation

Sample	Ingredients
Fruit mix	40 grams of mixed fruit
Carrot	40 grams of mixed carrots
Potato	40 grams of mixed potato
Molasses	40 grams of molasses brix=20
Cattle food	40 grams of mixed cattle food+100cc distilled water
Eating vegetables	40 grams of each mixed eating vegetables
Control media	YPD broth (1:1:1.5)

All Taguchi tests were carried out at 120 rpm and 30 °C in 250 ml Erlenmeyer flasks over 4 days of incubation (best fermentation time according to primary test results). Each substrate was mixed using a mixer, and 50 grams of each substrate was autoclaved for 15 minutes. All tests were carried out in triplicates. The average of each parameter data was used for statistical analysis. We have divided substrates into three types, including fruits mix (seven types of fruits were selected, based on their availability and abundance in the season, including orange, apple, lemon, sweet lemon, banana, tangerine, kiwi, and grape), fruit mix plus vegetables (carrot and potato), and eating vegetables consisting of a combination of a set of fresh herbs and raw vegetables (including Basil (49), parsley, and radishes). Fruit mix plus molasses were also used in order to simulate the urban waste ingredients in Iran based on the Iranian Ministry of Agriculture Statistics (50).

Below is more of each defined level for each parameter:

In Taguchi fermentation tests, variable

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parameters were inoculum volume, pH, substrate type, and humidity. The temperature was set at 30 °C based on the optimized temperature mentioned previously (51-53). According to mathematical calculations, volumes of water were added to each sample for changing humidity to candidate humidity rates in Taguchi tests. Table 1 shows the details of each parameter type.

Finally, the Taguchi optimized condition was evaluated, and the best ethanol production was verified in the final fermentation test.

Results and Discussion

Primary Tests: The primary test was done to determine the best fermentation time. The results are shown in Figure 1. According to our data, the best fermentation time was four days of incubation ($p<0.05$). The reduction of ethanol On the 5th day may refer to stressful conditions during the final steps of anaerobic fermentation, such as ethanol and acetaldehyde accumulation, poor nutrients, ROS accumulation, and glucose decrease (54, 55). The ethanol contents of the fruit mix, carrot, potato, molasses, eating vegetables, and control samples were 2.8, 2.3, 0.98, 3.5, 0.56, and 1.77 % w/v, respectively. The cattle food substrate, a concentrated, redundant, and low humidity substrate that could be used in SSF fermentation due to its redundancy and low cost, showed a low potential for ethanol production and was hence eliminated from candidate substrates. Subsequent fermentation trials were carried out based on these primary tests.

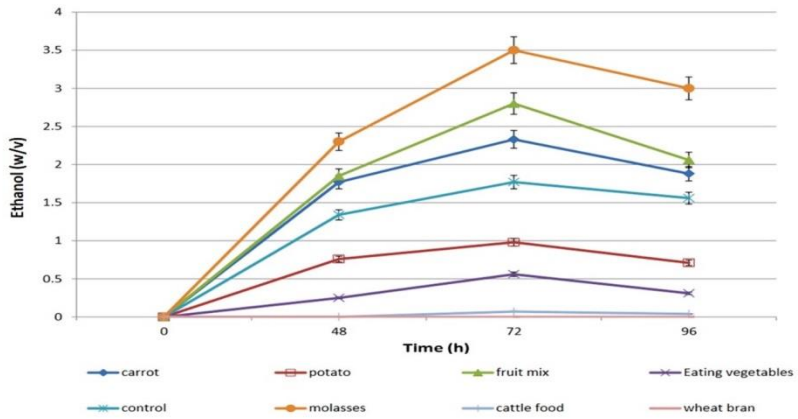

Fig. 1- The impact of fermentation time on ethanol production from each substrate, including fruit mix, carrot, potato, molasses, cattle food, and eating vegetables mix (ethanol Production from each substrate type for 96 hours was measured)

Taguchi Results: Achieved ethanol amounts were registered. Tables 1 and 4 show a summary of all the collected results ($p<0.05$). Within the Taguchi tests, produced ethanol reached an acceptable and racial amount as we expected. As seen in Table 4, sugar consumption and conversion to ethanol meet an acceptable rate. Final ethanol yields were 1.81, 0.56, 3.05, 3.05, 0.81, 0.56, 0.81, and 1.81% w/v for number 1 to number 8 samples, respectively. These substrates are defined so that they simulate urban fruit and vegetable waste content in Iran based on annually consumed fruits and vegetables. Molasses, as an abundant and relatively cheap sugar source, is regarded as a supplement additive to waste in order to improve sugar content and hence ethanol production.

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Table 4- The Amount of Carbohydrates Consumption and Bioethanol Yield in Taguchi Tests

Test No.	Cn1 (g sugar /100g substrate)	Cn2 (g sugar /100g substrate)	Sugar Consumption (%)	Yield (g Ethanol/1Glucose)
1	5.9	1.37	76	0.397
2	5.3	1.7	68	0.155
3	5.14 9.07(Sucrose)	2.95 7.18(Sucrose)	42 20	0.510
4	5.14 9.07(sucrose)	1.19 8(Sucrose)	76 11	0.500
5	5.9	1.7	71	0.192
6	5.3	0.28	94	0.121
7	5.3	0.98	81	0.155
8	5.14 9.07(Sucrose)	1.86 8.1(Sucrose)	63 10.7	0.346

All three types of substrates, including mixed fruits, mixed fruit plus molasses, and mixed fruits and vegetables, showed a reliable potential as waste sugar-containing sources for bio-ethanol production. Samples 3 and 4 showed higher ethanol production (molasses-containing samples) compared to sample 8, due to the impact of the humidity factor. Variation of ethanol production in vegetable-containing samples (samples 2, 6, and 7) showed the weak impact of inoculum volume for this substrate type, while the humidity of these samples was equal to the LSF (Liquid State Fermentation) situation. The difference in ethanol production of Samples 1 and 5, which are mixed fruit substrates, is due to the humidity differences. Availability and redundancy of sugars may be the rolling factor, and hence substrate type was considered the most effective factor by Taguchi software (Figure 3).

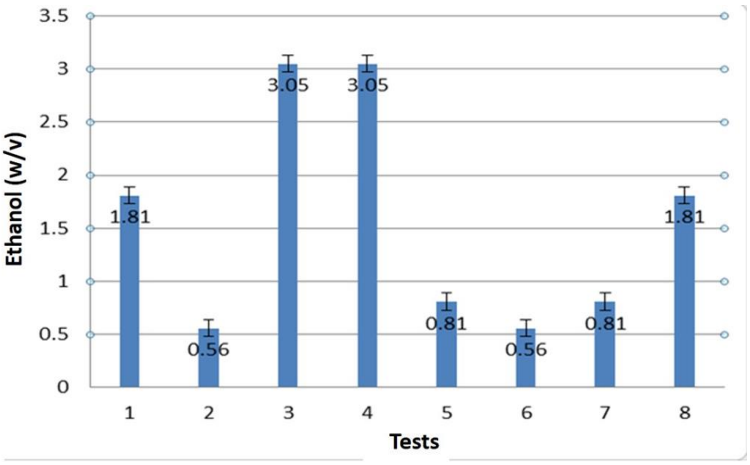

Fig. 2- Final ethanol production content from Taguchi recommended tests based on distinct parameters. The ethanol production of each sample was achieved in triplicate and their averages were reported (samples 1-8 are Taguchi experiments which are explained in Table 1 in detail).

Optimized conditions for ethanol production include inoculum volume level 1(5%, sample volume v/w ratio), pH level 2 (pH = 6), substrate type level 3 (mixed fruits plus molasses), and humidity level 3 (90%).

Also, the optimized results of ethanol yield were calculated by Taguchi software. Table 4 shows the yield of each test. As shown in Figure 5, optimized conditions for the best ethanol yield are pH level 1(5), humidity level 1 (natural), and substrate level 3 (mixed fruits + molasses). Inoculum volume levels did not show any effect on ethanol yield. The significance order of each

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parameter on ethanol yield is shown in Figure 6.

According to our data, substrate type has the most affecting factor rolling the ethanol production and yield (Figures 3 and 6). The substrate type impact orders are shown in Figures 4 and 5. Substrate level 3 (optimized level) may simply have many available amounts of fermentable sugar content, which leads to better ethanol production, as Salvadó Z et al. mention consistently (56). Also, the liquid form of molasses can be considered an affecting factor, increasing sugar availability compared to solid-form substrates. Vegetables usually have low fermentable sugars for *S. cerevisiae* (57). It should be mentioned that, as we expected, the vegetable addition would decrease ethanol production and have a negative impact on alcohol production. It may occur due to the vegetable ingredients (eating vegetables), especially the low fermentable sugar content, or the presence of fermentative inhibitor substances (58).

Humidity was considered the second effecting factor the ethanol production and yield ethanol production and yield by Taguchi software (Figures 3 and 6). The humidity level impact order is shown in Figures 4 and 5. Changing humidity to near 90 percent shifts the fermentation to an LSF type. A comparison between SSF (natural humidity) and LSF (=90 % humidity) shows better ethanol production over the LSF condition. It may be due to the better shaking of media ingredients and hence better substrate availability for cells, better mass, gas, and oxygen transfer. It was consistent with the work of Yu et al., which announced increased ethanol production during humidity increment (7). It is unclear why over humidity level 2, both ethanol production and yield are in the minimum state. Natural humidity (without the addition of water) showed the best ethanol yield (Figure 5). It is an enthusiastic outcome from our Taguchi tests. Water consumption is one of the challenging factors in ethanol production (59). It would be applied to produce ethanol in solid form without the need for additional water.

PH was mentioned as the third affecting factor for ethanol production and yield (Figures 3 and 6). The significant orders of pH are shown in Figures 4 and 5. PH = 5 (level 1) showed the highest ethanol yield. The optimized pH level for ethanol production is level 1 (pH = 6, Figure 4). It is inconsistent with Tesfaw and Assefa who reported a reduction in ethanol production over a pH range beyond pH = 6 (52), and Wong and Sanggari who reported a reduction beyond pH = 5 (60), as well as Md. Fakruddin et al., who reported the best ethanol production at pH = 6 and a reduction at pH = 5.4 and pH = 6.5 (53). Kim et al. also mentioned that pH = 6.8 was an optimized value for ethanol production from kitchen waste (61). At a lightly low pH, cell membrane permeability to some essential nutrients is ruled by the H+ concentration in the fermentation media (62). High pH fermentation was reduced by high pH. The cell cycle was held at pH 9.0, probably due to an arrest as a result of modifications required by the cells to fight the changes under these situations (63).

Inoculum volume was considered as the last effecting factor by Taguchi for both ethanol production and yield (Figures 3 and 6). Inoculum volume levels did not show any effect on ethanol yield. Lower inoculum volume showed a better impact on ethanol production, which was consistent with Tesfaw and Assefa (52). Inoculum volume level one is suggested as the optimized volume level. It may be due to the sugar consumption correlation between initial phase growth and ethanol production anaerobic phase. It was previously reported that inoculum size does not have a notable influence on final ethanol concentration but significantly influences sugar consumption rate and ethanol productivity. However, it was announced that ethanol production was enhanced with the rise in the primary cell

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numbers from 1×10^4 to 1×10^7 cells/mL and no vital variation in ethanol production was found between 107 and 10^8 cells/mL. High cell density within a particular scale also decreases fermentation time due to the fast growth of cells in the fermentation media that rapidly absorb fed sugar during producing ethanol. Breisha stated that by raising inoculum density from 3.0% to 6.0%, a drop-in fermentation time from 72 h to 48 h was observed (64).

Based on our data, humidity and substrate type seem to be major rolling factors (Figures 3 and 4). The waste fruits seem to be an adequate and cheap source of ethanol production, which can be mixed with molasses as available and relatively inexpensive sugar sources. Beyond the environmental benefits, they have economic advantages. They can exceptionally be considered as suitable waste processing potential by metropolis managers especially in waste management problems.

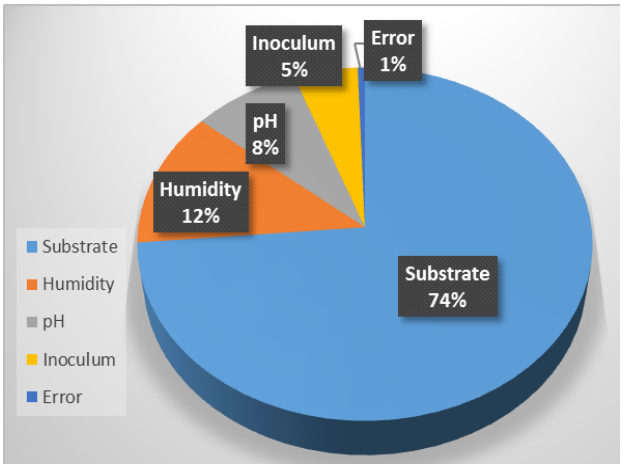

Fig. 3- The impact of Taguchi factors on ethanol production. The influence of Taguchi factors, including pH, humidity, substrate type, and inoculum volume on bio-ethanol production was assessed. The figure illustrates the significance of each parameter in percent.

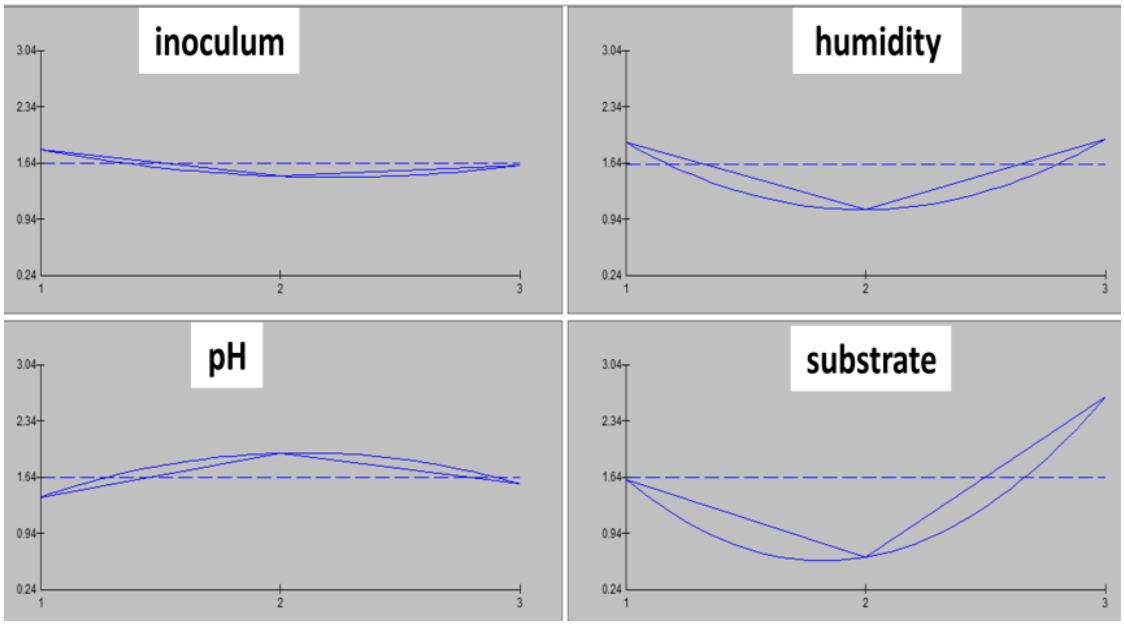

Fig. 4- The impact of Taguchi parameters on Ethanol production by parameter levels. The level of each parameter (levels 1-3) is explained in Table 2.

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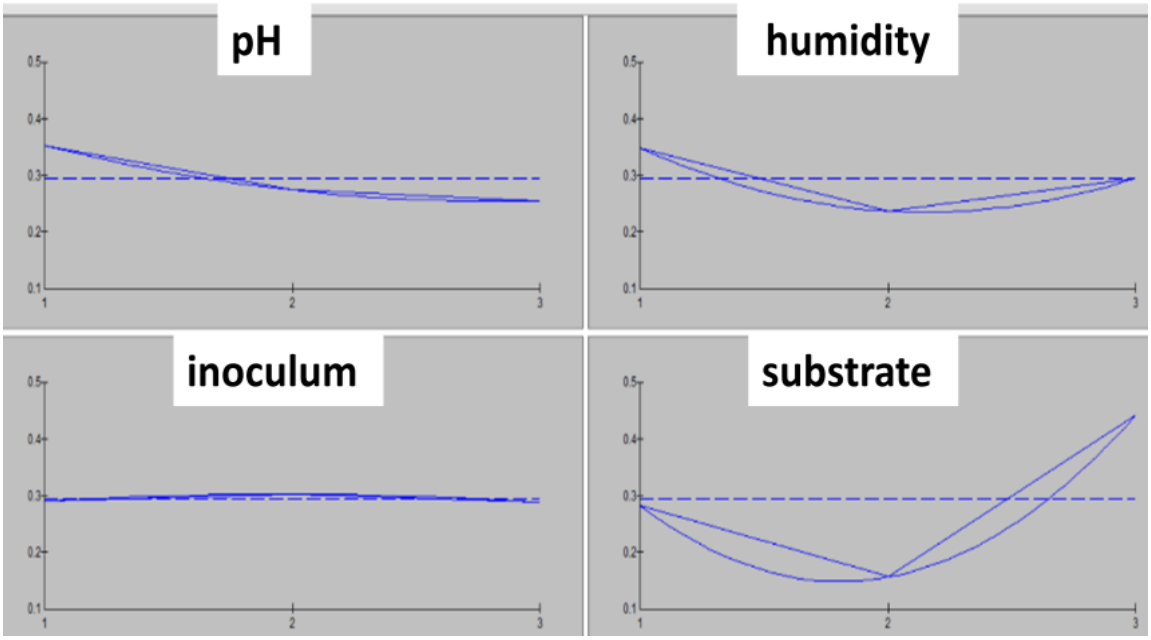

Fig. 5- The impact of Taguchi parameters on Ethanol yield (ethanol/gram substrate) by parameter levels. The level of each parameter (levels 1-3) is explained in Table 2.

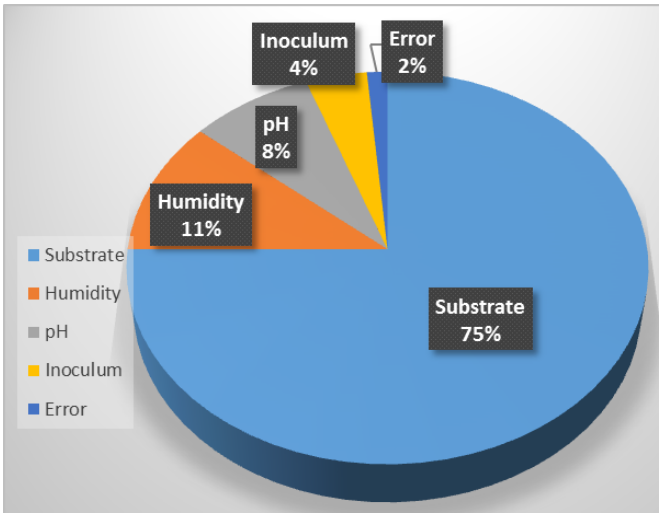

Fig. 6- The impact of Taguchi Factors on ethanol yield (ethanol/gram substrate). The influence of Taguchi factors, including pH, humidity, substrate type, and inoculum volume on bio-ethanol production was assessed. The figure illustrates the significance of each parameter in percent.

The final test was performed based on the optimized parameters predicted by the Taguchi software result. During this final test, ethanol achievement was up to 3.3 %. This amount of archived ethanol is similar to pure molasses ethanol production (over primary tests), which shows the potential of this waste as a co-substrate for ethanol production. Further analysis is needed for industrial evaluation of this supplemental fermentation method to approve this method over industrial ethanol production. Due to economic considerations, the low rate of fermentation (prolonged time) is a challenging issue in industrial bioethanol production (65). Hence, it should be considered for further experiments.

Sugar Content Determination: Figure s2

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shows the standard curve of the DNS sugar determination method. Sugar consumption, primary, and final concentrations are summarized in Table 4. Monosaccharide sugar consumption was 76, 68, 42, 76, 71, 94, 81, and 63 % for each sample. Sucrose consumption was 20, 11, and 10.7 % for samples 3, 4, and 8. Glucose and fructose consumption show an excellent rate, which indicates appropriate fermentation activity. As shown, the addition of molasses (samples 3 and 8) causes a decrease in the level of monosaccharide sugar consumption, which is the natural response of molasses sugar redundancy. Low amounts of monosaccharide consumption by *S. cerevisiae* may be due to the high concentration of sucrose. Sample 4, besides molasses content, showed high monosaccharide consumption, but the rate of sucrose consumption decreased. Also, it should be mentioned that high sugar concentration may cause a stressful condition for the yeast due to high osmotic pressure and hence reduce fermentation activity (66). The reduction of monosaccharide consumption decreased to 40 % in sample 3, which means nearly half of the fruit substrate is not converted during fermentation. There is a balance between monosaccharide and sucrose (disaccharide) consumption. The sucrose-converting enzymes of baker yeast mostly retain their maximum activity at pH 4–7 (67, 68). So, it would be predictable that water availability is the most significant factor in invertase activity in our experiment.

Further experiments are needed for sugar consumption optimization. Also, various reports mentioned the challenges of the ethanol process techno-economy. It is regarded as a critical and profitable profile concerning the process (69, 70). Therefore, fine evaluations and precious analysis are needed to optimize supplemented molasses to build an economical and optimized balance between molasses addition and fruit waste sugar consumption.

Conclusion

The potential of using waste fruits and vegetables for SSF ethanol production was approved. In contrast to fruit mix, carrot, potato, eating vegetables, and molasses substrates, the cattle food showed inappropriate potential for ethanol production. The waste fruits are an adequate source of ethanol production. Beyond the environmental benefits, it has economic advantages and could be considered by metropolis managers when dealing with waste management problems. Optimized conditions for ethanol production from kitchen waste as a potential source for producing ethanol by *S. cerevisiae* include inoculum volume = 5 %, pH = 6, humidity = 90%, and a mix of fruits plus molasses as substrate. The best condition was evaluated when the ethanol achievement was up to 3.3%. Also, optimized factors for the best ethanol yield were pH = 5 and substrate level 3 (mixed fruits + molasses). The inoculum volume factor did not show any significant effect on ethanol yield. Also, natural humidity was calculated as the optimized humidity condition. It provides a great option to produce ethanol in solid-state conditions without the need for water consumption. Based on the results of the study, the sugar rate conversion and ethanol production using different substrates showed an appropriate efficiency in the fermentation process. Beyond the environmental benefits of bioethanol production from kitchen wastes, it could also be considered a suitable waste processing tool. Also, molasses is an ideal supplement for increasing ethanol production from these substrate types.

Conflict of Interest

The authors declare there are no conflicts of interest regarding this manuscript's publication.

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