International Journal of Advance Research and Innovation
Vol. 7(1), Jan-Mar 2019, pp. 35-40
Doi: 10.51976/ijari.711906
www.gla.ac.in/journals/ijari

Article Info

Received: 17 Jan 2019 | Revised Submission: 30 Jan 2019 | Accepted: 05 Feb 2019 | Available Online: 25 Mar 2019

Preliminary Assessment for the Potential of Citrus Limetta Peel Waste for Bioethanol Production under Indian Conditions

Vinay Prabhakar and SK Singh***

ABSTRACT

Brazil is the top producer of citrus fruits in the world with 20 million tons annually while India stands 5th on the list with total production of 6,286,000 tonnes annually. Citrus limetta being the third most cultivated species of citrus after orange and lemon with 4,200,000 metric tonnes produce annually in the world. CLPW is pectin rich agro waste and can utilised for the production of bioethanol which can be used as a substitute for the conventional fuel as it is cleaner and cheaper fuel source. On overviewing different research work it can be concluded that fruit peel waste especially citrus fruit has a huge potential for bioethanol production rather discarded into the environment. The carbohydrates content of the solid waste can be determined by using Anthrone method. The fermentation is done in the bioreactor using the combination with the maximum reducing sugar yield and using S. cerevisiae as the microorganism. Dichromate method used for the analysis of ethanol production. The moisture content of waste was 80.59% of total weight. The average carbohydrates content of sample was found out to be 62.35% on wet weight whereas the ash content was 10.51% on dry weight basis. With increase in acid concentration there is decrease in reducing sugar yield and vice-versa. With increase in temperature there is increase in reducing sugar yield. It can also be concluded that reducing sugar yield was maximum at 150˚C and without acid. It can be also concluded that rather throwing the peel waste it can be used as a source of energy production. It can be also concluded that the method used for treatment of waste can be serve as a tool for waste management.

Keywords: *Bioethanol; waste management; reducing sugar; fermentation; moisture content.*

1.0 Introduction

Human society produced waste materials as it is one of an inevitable part of their life. In ancient times, the waste generation was less and land area available for disposal are more make it very easy to dispose the waste. But, with industrialisation and urbanisation the waste generated are more and it is difficult to rely on land for disposal.

There is a need of other treatment options for the wastes generated due to human activities. The per capita waste generation in India is around 0.37 kg/person/day[1].

The other treatment option available for waste treatment and disposal includes composting, incineration, land filling and production of different biofuels. The choice among them is based on different technical and economic factors.

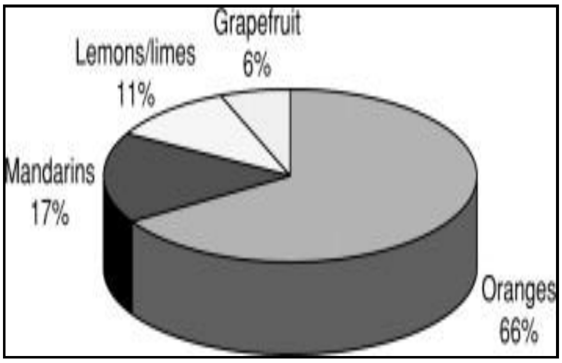
According to an estimate by Food and Agriculture Organisation (FAO) the citrus fruit production around the world is around 115.6 million tons in 2011. It is one of the most abundant crops in the world. Brazil is the top producer of citrus fruits in the world with 20 million tons annually while India stands 5th on the list with total production of 6,286,000 tonnes annually.

Citrus limetta being the third most cultivated species of citrus after orange and lemon with 4,200,000 metric tonnes produce annually in the world[2]. The distribution of citrus fruits types grown in the world is shown in figure 1. Around 57% of processed citrus limetta contributes to peel wastes[2]. Citrus limetta peel waste (CLPW) unlike other citrus peel wastes (CPW) may be used for cattle feed[3]. However, due to unavailability of cost efficient process for treatment of CLPW a large amount of it is

**Corresponding Author: Department of Environmental Engineering, Delhi Technological University, Delhi, India
(E-mail: prabhakarvinay01@gmail.com)
*Department of Environmental Engineering, Delhi Technological University, Delhi, India
(E-mail: sksinghdce@gmail.com;)*

lost[4]. CLPW is pectin rich agro waste and can utilised for the production of bioethanol which can be used as a substitute for the conventional fuel as it is cleaner and cheaper fuel source[5].

Fig 1: Average Production Percentages of Main Types of Citrus Fruit Grown in the World



Karimi and Karimi study on the ethanol production from kitchen and garden wastes and their residue left after for biogas production found that the addition of fermentation biomass to the pre-treated solidsignificantly improved the bio methane production yield.

They also found that after pre-treatment at 150 °C for 30 min and without detoxification, the maximum amounts of gasoline equivalent of 162.1 and 120.6 L were obtained with and without starch hydrolysis, respectively, where methane production yield was 157.4 mL/g VS and ethanol yields were 75.9% and 94.2%[6]. Kiran and Liu his study uses a fungal mash rich in hydrolytic enzymes produced from waste cake in-situ and applied it for the hydrolysis of mixed food wastes. Around 127 g/L glucose and 1.8 g/L free amino nitrogen act as a balanced nutrient stream produced from the enzymatic pre-treatment of food wastes using this fungal mash at 24 h. Also, using this solution as sole fermentation feedstock gives 58 g/L of ethanol corresponding to an ethanol yield of 0.5 g/g glucose was obtained within 32 h. It can be concluded from this study that the pre-treatment of mixed food wastes with the fungal mash produced is an effective option for food waste saccharification and bioethanol production[7].

Agro wastes like carrot peel, onion peel, potato peel and beetroot peel are put forward for saccharification process by *Penicillium sp.* for the hydrolysis followed by the fermentation using yeast *Saccharomyces cerevisiae* for the production of alcohol. They found that 14.52% ethanol was produced on 28th day from beetroot peel using dichromate method and 17.3% ethanol from same waste and time using Gas chromatography method for analysis[8].

Castro et. al. work on production of ethanol using three sweet sorghum cultivars. The fermentation by *Saccharomyces cerevisiae* and *Escherichia coli* followed pre-treatment of residual bagasse with dilute phosphoric acid steam explosion. They found that sugar concentration in the juice ranging from 140 to 170 g/L and almost complete conversion into ethanol by yeast. They also found that 27.5 g EtOH/L at enzyme concentrations of 11.5 FPU/gDW is produced. They also found that 10,600 L of ethanol per hectare was produced from the method they used[9].

The biofuels produced from agricultural waste such as ethanol and hydrogen is a renewable energy source. The maximum sugar production from pineapple peel is 34.03±1.30 g/L was obtained after 24 h of incubation time and maximum yield of ethanol using *S. cerevisiae* after 72h is 9.69g/L with no hydrogen production whereas using *E. aerogenes* ethanol and hydrogen production are 1.38g/L and 1,416mL/L after 72h and 12h cultivation respectively. But, when immobilized bacterial cell in matrix of loofah were used the biofuel production increased by 1.2-folds[10]. Ethanol fermented from renewable sources for fuel or fuel additives are known as bio-ethanol. Many food crops have been specifically grown for the production of bio-ethanol in Nigeria.

Itelima et. al. study on banana, plantain and pineapple peels for the production of bioethanol as these wastes are in abundance and do not interfere with food security were put forward for simultaneous saccharification and fermentation for 7days by co–

culture of *Aspergillus niger* and *Saccharomyces cerevisiae*.

They found that after 7 days of fermentation, pineapple peels had the highest biomass yield of 1.89 (OD), followed by banana peels 1.60 (OD), while plantain peels had the least 0.98 (OD). They also found that reducing sugar concentrations ranged between 0.27 – 0.94 mg/cm^3 for pineapple, 0.20 – 0.82 mg/cm^3 for banana and 0.16 – 0.45 mg/cm^3. They also found that optimal ethanol yields were 8.34% v/v, 7.45 % v/v and 3.98 % v/v for pineapple, banana and plantain peels respectively.

They also conclude from the findings that pineapple and banana peels ethanol yields were significantly higher ($P<0.05$) than plantain peel ethanol yield. It can also be concluded that fruit wastes are no longer discarded into environment rather converted into the useful products like bioethanol which can serve as an alternative energy[11].

Santi et.al. uses orange peel waste (OPW) for conversion into bioethanol by consecutive acid-catalysed steam-explosion (ACSE), enzymatic saccharification and fermentation with *Saccharomyces cerevisiae*. They found that OPW pre-treated at 180°C for 150s yield the highest glucose solubilisation degree of around 56% at the end of the saccharification step and the maximum ethanol yield coefficient around 0.495g/g and productivity of 4.85g/L/h at the end of the 3rd repeated fermentation batch in shaken-flasks[12].

Boluda and Lopez have applied steam explosion and enzymatic hydrolysis for pre-treatment of Citrus limon L. peel wastes to obtain bioethanol, galacturonic acid and other co-products, such as d-limonene and citrus pulp pellets.

They observed significant antimicrobial activity of lemon essential oils on *S. cerevisiae* at concentrations above 0.025%. They found that steam explosion pre-treatment reduces the residual content of essential oils below 0.025% and significantly decreases the hydrolytic enzyme requirements. They also found that ethanol production in excess of 60 L/1000 kg fresh lemon peel biomass can be produced[13].

Choi et. al. developed a novel approach for converting single-source CPW (i.e., orange, mandarin, grapefruit, lemon, or lime) or CPW in combination with other fruit waste (i.e., banana peel, apple pomace, and pear waste) to produce bioethanol. They used two in-house enzymes produced from *Avicel* and CPW and were tested with fruit waste at 12-15% (w/v) solid loading.

They observed rate of enzymatic conversion of fruit waste to fermentable sugars were approximately 90% for all feed-stocks after 48 h. They also designed a d-limonene removal column (LRC) for successfully removal of inhibitor from the fruit waste.

They also found that ethanol concentration is 14.4 to 29.5 g/L and yield is 90.2 to 93.1% when using LRC coupled with an immobilized cell reactor (ICR) which is 12-fold greater than products from ICR fermentation alone[14].

Citrus-processing industries generate every year huge amounts of wastes and citrus peel waste alone accounts for almost 50% of the wet fruit mass. Citrus waste is of immense economic value as it contains an abundance of various flavonoids, carotenoids, dietary fiber, sugars, polyphenols, essential oils, and ascorbic acid, as well as considerable amounts of some trace elements. Citrus waste also contains high levels of sugars suitable for fermentation for bioethanol production. However, compounds such as D-limonene must be removed for efficient bioethanol production[15].

On overviewing different research work it can be concluded that fruit peel waste especially citrus fruit has a huge potential for bioethanol production rather discarded into the environment. The main focus of this study was to produce bioethanol from citrus limetta peel waste.

The specific objective of the study were to optimize the factors mainly temperature and acid concentration for hydrolysis of CLPW to obtain the kinetics of bioethanol production and calculating the theoretical bioethanol potential of Delhi.

2.0 Methodology

The waste i.e. citrus limetta peel commonly known as mousambi was collected from the fruit juice vendor outside the campus gate. It is followed by shredding of waste which is the size reduction technique for solid waste. In our study for shredding mixer grinder was used and the sample was put in air tight container and stored at 4˚C for further analysis i.e. Characterisation of Waste and Acid Hydrolysis followed by Estimation of Reducing Sugars for knowing the potential of waste to produce bioethanol. Characterisation of waste includes estimation of moisture content, ash content and carbohydrates content using the standard methods. Carbohydrates content was estimated using spectrophotometer by anthrone method at 630 nm wavelength. Acid Hydrolysis is done by using different concentration of acid at different temperature (130˚C to 150˚C). The reducing sugar yield was analysed by DNS method using spectrophotometer at 540 nm wavelength with acid hydrolysed supernatant. Some statistical analysis like Principal Component Analysis (PCA) and Factor Analysis (FA) are applied for interpreting the results obtained from acid hydrolysis and reducing sugars yield. The maximum reducing sugars yield is further used for fermentation.

3.0 Results and Discussions

3.1 Characteristics of sample

The sample was analysed for moisture content, ash content and carbohydrates as described in the previous chapter. As per the analysis carried out, the moisture content was 80.59% of total weight. The average carbohydrates content of sample was found out to be 62.35% on wet weight whereas the ash content was 10.51% on dry weight basis. The composition of 62.35% total carbohydrate shows the enormous potential for ethanol conversion. The carbohydrate content is less than the carbohydrate content in a study by Khatiwada was 72.62%[16]. The only difference was that they used organic portion of kitchen waste and in our study instead of kitchen waste citrus limetta peel waste was used.

3.2 Acid hydrolysis and estimation of eeducing sugar in the sample

As mentioned in the methodology acid hydrolysis is done with different concentration of acid ranging from 0 to 10% at different temperature ranging from 130 to 150℃. It was found that the reducing sugar concentration at 0% acid concentration and 150℃ has maximum value i.e. 110.13 ppm. The combination of acid and temperature which has highest reducing sugar yield will be used for the fermentation. In our study, it can be recommended to use the sample with no acid at 150℃ hydrolysed sample for fermentation process. The variation of reducing sugar yield with acid and temperature is shown in the figure 2.

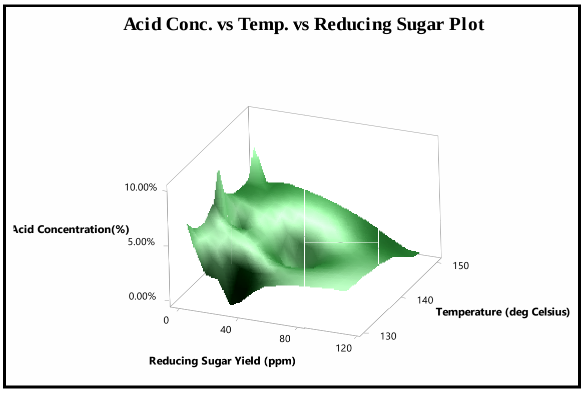
3.3 Effect of acid on reducing sugar yield

It can be seen from figure 2 that with increase in acid concentration there is decrease in reducing sugar yield and vice-versa.

3.4 Effect of temperature on reducing sugar yield

It can be seen from figure 2 that with increase in temperature there is increase in reducing sugar yield.

Fig 2: Variation of Reducing Sugar Yield with Acid Concentration and Temperature



3.5 Statistical analysis for acid hydrolysis and reducing sugar estimation

The statistical tools like principal component analysis (PCA) and factor analysis (FA) had been applied for the result obtained for acid hydrolysis followed by reducing sugar estimation. The figure 3 and 4 shows the PCA and FA of the result obtained respectively. It can be found from the PCA that the first two principal components have eigenvalues greater than 1 and these two components explain 89.8% of the variation in the data. Also, it can be seen that first component has large positive association with reducing sugar yield and second and third components have large negative association with temperature. There is negligible association of acid based on the PCA analysis. So, it is recommended to use distilled water for the hydrolysis of waste. It can be found from the FA that first factor has variance greater than 1 and the percentage of variability explained by factor 1, 2 and 3 are 56.5%, 33.3% and 10.2% respectively. Together, all the three factors explain 100% variation in the data.

Fig 3: Principal Component Analysis (PCA) of Results Obtained

Principal Component Analysis: Acid Concentration(%), ... r Yield (ppm)

Eigenanalysis of the Correlation Matrix

Eigenvalue	1.6938	1.0000	0.3062
Proportion	0.565	0.333	0.102
Cumulative	0.565	0.898	1.000

Eigenvectors

Variable	PC1	PC2	PC3
Acid Concentration(%)	-0.687	-0.238	0.687
Temperature (deg Celsius)	0.168	-0.971	-0.168
Reducing Sugar Yield (ppm)	0.707	0.000	0.707

Fig 4: Factor Analysis (FA) of Results Obtained

Factor Analysis: Acid Concentration(%), Temperature ... ar Yield (ppm)

Principal Component Factor Analysis of the Correlation Matrix

Unrotated Factor Loadings and Communalities

Variable	Factor1	Factor2	Factor3	Communality
Acid Concentration(%)	-0.894	-0.238	0.380	1.000
Temperature (deg Celsius)	0.219	-0.971	-0.093	1.000
Reducing Sugar Yield (ppm)	0.920	0.000	0.391	1.000
Variance	1.6938	1.0000	0.3062	3.0000
% Var	0.565	0.333	0.102	1.000

Factor Score Coefficients

Variable	Factor1	Factor2	Factor3
Acid Concentration(%)	-0.528	-0.238	1.241
Temperature (deg Celsius)	0.129	-0.971	-0.304
Reducing Sugar Yield (ppm)	0.545	0.000	1.278

4.0 Conclusions

It can be concluded that the citrus limetta peel waste have the potential for bioethanol production as it contain 62.35% of total carbohydrates. It can also be concluded that reducing sugar yield was maximum at 150˚C and without acid. It can be also concluded that maximum sugar yield of the waste with combination of acid and temperature will be used for fermentation using S. cerevisiae for the production of bioethanol.

It can also be concluded that keeping in mind about the sustainability the use of simple hydrolysis with distilled water rather than acid hydrolysis is used for preparing the final sample for the fermentation as the less consumption of acid is done.

It can be also concluded that rather throwing the peel waste it can be used as a source of energy production. It can be also concluded that the method used for treatment of waste can be serve as a tool for waste management.

References

[1] RK Annepu. Sustainable Solid Waste Management in India, 2012.

[2] E González-Molina, R Domínguez-Perles, DA Moreno, C García-Viguera. Natural bioactive compounds of Citrus limon for food and health, Journal of Pharmaceutical and Biomedical Analysis. 2010.

[3] F Talebnia, M Pourbafrani, M Lundin, MJ Taherzadeh, Optimization study of citrus wastes saccharification by dilute-acid hydrolysis, BioResources, 2008.

[4] M Boluda-Aguilar, L García-Vidal, FdP González-Castañeda, A López-Gómez. Mandarin peel wastes pretreatment with steam explosion for bioethanol production, Bioresour. Technolgy, 2010.

[5] E Molnár, N Nemestóthy, K Bélafi-Bakó.

Utilisation of bipolar electrodialysis for recovery of galacturonic acid, Desalination, 2010.

[6] S Karimi, K Karimi. Efficient ethanol production from kitchen and garden wastes and biogas from the residues,J. Clean. Prod., 2018.

[7] E Uçkun Kiran, Y Liu. Bioethanol production from mixed food waste by an effective enzymatic pretreatment, Fuel, 2015.

[8] I Mushimiyimana, P Tallapragada. Bioethanol production from agro wastes by acid hydrolysis and fermentation process, J. Sci. Ind. Res. (India)., 2016.

[9] E Castro et al. Potential for ethanol production from different sorghum cultivars, Ind. Crops Prod., 2017.

[10] A Choonut, M Saejong, K Sangkharak. The production of ethanol and hydrogen from pineapple peel by Saccharomyces cerevisiae and Enterobacter aerogenes, in Energy Procedia, 2014.

[11] J Itelima, F Onwuliri, E Onwuliri, I Onyimba, S Oforji. Bio-Ethanol Production from Banana, Plantain and Pineapple Peels by Simultaneous Saccharification and Fermentation Process, Int. J. Environ. Sci. Dev., 2013.

[12] G Santi et al.Orange peel pretreatment in a novel lab-scale direct steam-injection apparatus for ethanol production, Biomass and Bioenergy, 2014.

[13] M Boluda-Aguilar, A López-Gómez. Production of bioethanol by fermentation of lemon (Citrus limon L.) peel wastes pretreated with steam explosion, Ind. Crops Prod., 2013.

[14] IS Choi, YG Lee, SK Khanal, BJ Park, HJ Bae. A low-energy, cost-effective approach to fruit and citrus peel waste processing for bioethanol production, Appl. Energy, 2015.

[15] K Sharma, N Mahato, MH Cho, YR Lee. Converting citrus wastes into value-added products: Economic and environmently friendly approaches, Nutrition. 2017.

[16] NR Khatiwada. Production of Ethanol from municipal solid waste of India and Nepal, December, 2017.