Archives of Ecotoxicology, Vol. 3, No. 2, pp. 27-31, 2021

Archives of Ecotoxicology

Journal homepage: https://office.scicell.org/index.php/AE

OPEN ACCESS

Potential in Bioethanol Production from Various Agro Wastes Fermenting by Microorganisms using Carrot Peel, Onion Peel, Potato Peel and Sugar Beet Peel as Substrates

Isaie Mushimiyimana[1]*, Celestin Sirimu[2], Fidel Niyitanga[1], Leoncie Niyonsaba[2]

[1]Department of Food Science and Technology, College of Agriculture, Animal Science and Veterinary Medicine, University of Rwanda (CAVM /UR).
[2]Department of Rural Development and Agricultural Economics College of Agriculture, Animal Science and Veterinary Medicine, University of Rwanda (CAVM /UR).

Article info
Received 9 July 2020
Revised 8 March 2021
Accepted 8 March 2021
Published online 18 August 2021

Regular article

Keywords:
Agro wastes, Bioethanol, *Cladosporium cladosporioides*, *Saccharomyces cerevisiae*, Gas Chromatography

Abstract

Large amount of agro wastes is produced in Rwanda each year. The global annual potential bioethanol production from the major vegetables wastes such as carrot peel, onion peel, potato peel and sugar beet peel were estimated. Those wastes processing was successfully used as raw materials for the production of bioethanol, employing by cellulase produced from various filamentous fungi including *Cladosporium cladosporioides* was used for hydrolysis and the fermentation of the hydrolyzed samples was done using *Saccharomyces cerevisiae*. The fermented product was purified by primary distillation process at 79°C and the fraction was collected. The ethanol is then determined by specific dichromate method and Gas Chromatography. Instantaneous saccharification and fermentation process yielded maximum ethanol in the substrate of carrot peel was 16.9% at 21st day and further confirmed by Gas chromatography and the yield of ethanol obtained was 15.8%.

1. Introduction

During oil crisis in 1970s a rising crude oil price, political instability and unstable oil market in countries producing oil and climatic changes, biomass has high potential to replace the supply of energy **(Nagashima *et al.,* 1984).** The amount of wastes produced by society each day is increasing in line with increasing populations worldwide and Rwanda is no exception. Agro wastes are an important part of total solid wastes produced nationally; they have potential to become an environmental pollution or more logically, to be utilized for the production of energy and other products.

Production of bioethanol from lignocelluloses materials such as agro wastes can substitute fossil oil production. Today, raw materials producing bioethanol by fermentation are classified as sugars, starches and cellulosic materials because fermentation is cheaper and easily than other fermentation (Bailey, 1986). The potential of bioethanol production from agro wastes of four crops which are carrot, onion, potato and sugar beet have been investigated. Currently, agro wastes are burnt by the rural farmers as cookers in households. Production of bioethanol from agro wastes have been attempted with enzymes from different sources for hydrolysis of biomasses and with different organisms for fermentation **(Öhgren *et al.,* 2006; Eken-Saracoglu and Arslan 2000).**

The demand for bioethanol is expected to increase dramatically until 2020 where there is an increase in the world population with expected 9 billion in the year 2050 increasing the need for food and energy **(Galal *et al.,* 2014)**. *S. cerevesiae,* also known as brewer's yeast, is the most commonly used fermentation microbe because of the baking and beer brewing industries **(Michalka, 2007; Roehr, 2001)**. Many of the sugar crops that would be suitable for industrial fermentation include sugar cane, sugar beet, fruits, sweet potato, sweet sorghum, Jerusalem artichokes and agro wastes **(Atiyeh and Duvnjak, 2002; Pramanik, 2005).**

The objective of this study was producing bioethanol from carrot peel, onion peel, potato peel and sugar beet peel for submerged fermentation and management system to maximize economic benefits at the same time protection of the environment.

2. Material and methods

2.1 Raw materials

Carrot peels, onion peels, potato peels and sugar beet peels, were collected from the local restaurant in volcanic region at early morning. They were clean to make free from sand, stone and dust by washing it twice in water. They were sun dried then each raw material was grinded and sieved into a 1mm. Those agro wastes are favorable for bioethanol production due to their availability and cheapest throughout the year.

2.2 Microorganisms producing ethanol

*Corresponding author: muzayasi@gmail.com
https://doi.org/10.36547/ae.2021.3.2.27-31

Several bacteria, yeasts, and fungi have been used for bioethanol production. *S.cerevisiae*, is the most yeast, which can produce ethanol of the fermentation broth. The yeast *S. cerevisiae* can produce bioethanol up to 18% of the fermentation broth, **Pretorius (2000).**

Figure 1. Raw materials: agro wastes

2.3 Sources of microorganism

The isolated fungi were done from the rhizosphere of strawberry fields of College of Agriculture, Animal Science and Veterinary Medicine (Busogo) identified by serial dilution and wet mount technique **(Aneja, 2005).**

2.4 Culture medium chemical

The fermentation used was 0.2% yeast extract, 0.2% $(NH_4)\ NO_3$, 0.1% $MgSO_4{\cdot}7H_2O$, 0.2% KH_2PO_4 **(El- Gendy *et al.,* 2013)** and 5 g powdered of each substrate has been added.

2.5 Enzyme molecular weights

Poly-Acrylamide Gel Electrophoresis (PAGE) of the partial purified cellulase enzyme was performed according to **(Uk, 1970).** After electrophoresis, the gel was immersed in fixing solution. Staining of the band was done with coomassie brilliant blue, R-250 (CBB) for 2 h and later de-stained. The molecular weight of the cellulase was estimated using standard protein molecular weight marker consisting of Bovine Serum Albumin.

2.6 Protein estimation

The protein from partially purified samples of carrot peel, onion peel, potato peel and sugar beet peel were estimated **(Bradford,1976)** method. Optical density of the reaction mixture was observed at 660 nm against a blank prepared with 0.1 mL buffer.

2.7 Fermentation

Culture filtrate was further inoculated with *S. cerevisiae* and allowed for fermentation for 14^{th}, 21^{st} and 28^{th} days. After fermentation, it was filtered and ethanol content was determined **(Caputi, 1968).** As part of this study, we have reported a process for producing ethanol from agro wastes pre-hydrolysed by alkali followed by saccharification carried by co-cultivation of *C. cladosporioides* and fermentation of the released sugars to ethanol, using *S. cerevisiae* for ethanol production.

2.8 Distillation process

Distillation was carried in rotary vacuum flask at 80°C (boiling point of ethanol) and fraction is collected **(Kumnuanta *et al.,*1983)** as shown on Fig 2.

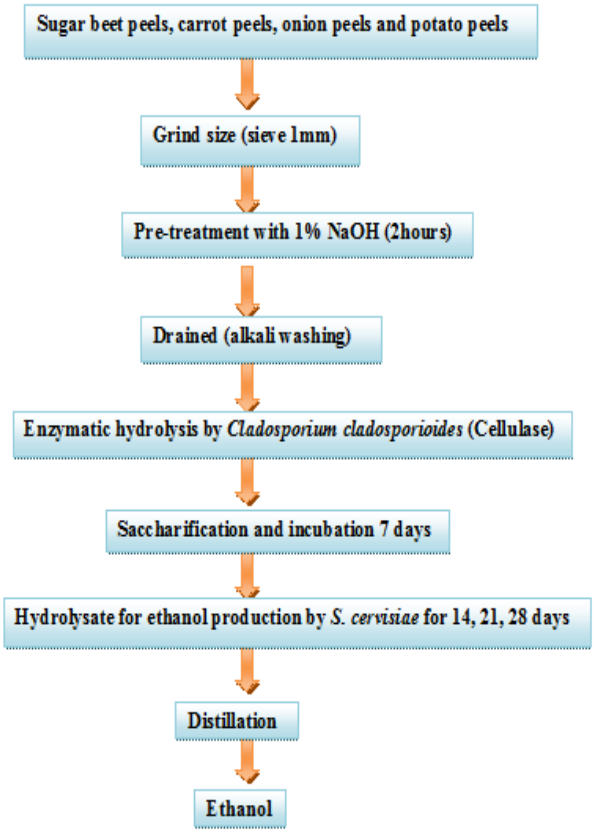

Figure 2. Bioethanol production from onion peel, sugar beet peel, carrot peel and potato peel.

2.9 Bioethanol estimation by potassium dichromate method

Standard ethanol was prepared from concentrations of 2% to 10% with blank. 2.5 ml of freshly prepared potassium dichromate solution (1g of potassium dichromate in 100 ml of pre-chilled $6H_2SO_4$) was mixed with 15ml of distillates and standards (2%, 4%, 6%, 8% and 10%) taken in separate test tubes and were incubated at 60ºC for 30 minutes (for color appearance) **Caputi, (1968).** Tubes were allowed to cool to room temperature and absorbance was estimated at 600nm **(William, 1950).**

2.10 Determination of quantity of ethanol produced

The distillate collected was measured using a measuring cylinder and expressed as quantity of ethanol produced in g/l by multiplying the volume of the distillate by the density of ethanol ($0.8033 g/cm^3$) **(Humphrey *et al.,* 2007).**

2.11 Confirmative analysis of ethanol by Gas Chromatography (GC)

The confirmation of ethanol qualitatively and quantitatively was done by gas chromatography method (Shimadzu Tokyo Japan). Gas Chromatography settings and characteristic features were selected to enable ethanol separation from the injected supernatant. 0.5 ml supernatant was dispensed into l ml capped sample and mixed with 5 ml of 1% internal standard solution. After mixing, 0.1 µL of the sample was directly injected into the Gas Chromatography **(Wang *et al.,* 2003).**

2.12 Statistical analysis

MS Excel version 2007 was employed for all statistical analysis. Data was recorded in triplicates and represented as a mean value.

3. Results and discussion

Currently bioethanol is produced from alcoholic fermentation of molasses or simple sugar, which are produced from crops generating starch or sugar. While technologies to produce ethanol from simple carbohydrates are well established, the technologies to produce bioethanol from agro wastes are still under development. It is possible that agro waste products may be economically converted to bioethanol. We used agro wastes peel as a source of lignocellulosic substrate for ethanol production (Figure1 and 2).

3.1 Enzyme molecular weights

The protein present in various agro wastes substrates showed several bands ranged from 30 to 130 kDa. The crude protein extract of carrot peel which contains maximum yield concentration of bioethanol confirmed its homogeneity and protein was resolved on 5% stacking and 12% running gel. The molecular weight of the protein bands was 30 kDa and 130 kDa for carrot peel (Figure 3).

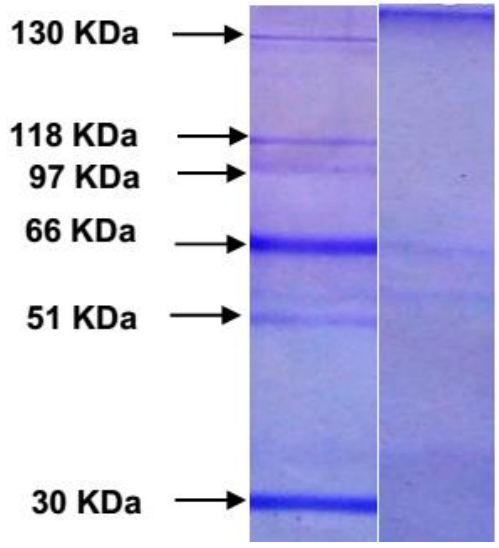

Figure 3. Molecular weight and cellulase activity from *Cladosporium cladosporioides*

Our results are close to the findings of **(Bai *et al.,*2013)** reported that the molecular weight of cellulase produced by different fungal species may vary from 12 kDa to 126 kDa. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) is the most commonly used method for judging the apparent molecular weight of enzymes **(Ramani *et al.,* 2012).** Cellulase produced by *Trichoderma viride* was purified to homogeneity using DEAE-sepharose column and the molecular weight was estimated at 87 kDa by SDS-PAGE, **Yasmin *et al.,* (2013).** *Penicillium pinophilum* MS 20 produced a monomeric cellulase with molecular weight of 42 kDa, which appeared as a single band on SDS-PAGE gel **(Pol et al., 2012).** The cellulase produced by *Aspergillus niger* revealed a molecular weight of 60 kDa on SDS-PAGE gel **(Baraldo *et al.,* 2014).**

3.2 Protein estimation

The protein content with *C. cladosporioides* was observed in carrot peel 643.48 µg/ml, onion peel 1336.5µg/ml, potato peel 1318.76µg/ml and sugar beet peel 1101.12µg/ml (Table1). **Ado (2008)** reported the mycelial protein production by *Aspergillus niger* using banana peel. The protein content obtained by *Cladosporium sp* with lignocellulosic biomass was about 0.224 (mg/g) and mycelial protein of about 60.6±1.12 (mg/g) reported **(Mohan *et al.,* 2013).**

Table 1. Substrates protein content (µg/ml) with Cladosporium *cladosporioides*

Carrot peel protein content (µg/ml)	Onion peel protein content (µg/ml)	Potato peel protein content (µg/ml)	Sugar beet peel protein content (µg/ml)
643.48 µg/ml	1336.5 µg/ml	1318.76 µg/ml	1101.12 µg/ml

Mango peels ranged from 1.2258–13.8715 mg/ml in which *Aspergillus tamarii* produced the maximum protein concentration released on day 12 of cultivation. Watermelon peels, it ranged from1.8926–5.2474 mg/ml in which *Aspergillus terreus* gave the maximum biosynthesis potential on day 3 of fermentation. The yield of extracellular protein on the rampage on medium containing banana peels ranged from 0.9247-4.0108 mg/ml in which *Mucor piriformis* had the maximum biosynthesis potential on day 3 of submerged cultivation. Furthermore, on medium with plantain peels, it ranged from 1.1725-8.3441 mg/ml in which *Aspergillus sclerotio niger* had the maximum biosynthesis potential on day 3 of cultivation. Aspergillus *sp* take over *Fusarium sp* and *Mucor sp*. in polygalacturonase (PG) production.

3.3 Bioethanol obtained by dichromate method

Lignocellulosic materials and various agro wastes with different methods have been employed for bioethanol production. The maximum level of bioethanol varied from day-to-day fermentation. During the fermentation period, the ethanol yield of substrates was found to increase gradually from the 14th, 21st to 28th day (Figure 4). The maximum concentration of ethanol was achieved on 28th day of fermentation and started to level off. From the results obtained on bioethanol production potential of various lignocellulosic wastes varied and can be concluded that carrot peel was a very promising raw material for bioethanol production with *C. cladosporioides*. **Mishra *et al.,* (2012)** founded increase in quantity of ethanol produced in sub-merged state fermentation as compared to the produced by solid state fermentation and founded optimal incubation period 72 hours for bioethanol production by orange peel using *S. cerevisiae.* **Senthilkumar and Gunasekaran (2005)** reported that some gram-positive bacteria *Clostridium cellulolyticum, Lactobacillus casei* have been engineered for bioethanol production. **Dien *et al.,* (2003)** worked on Gram-negative bacteria Escherichia *coli, Klebsiella oxytoca,* and Zymomonas *mobilis. E. coli* and *K. oxytoca* are naturally able to use a wide spectrum of sugars, and work has concentrated on engineering these strains to produce ethanol selectively.

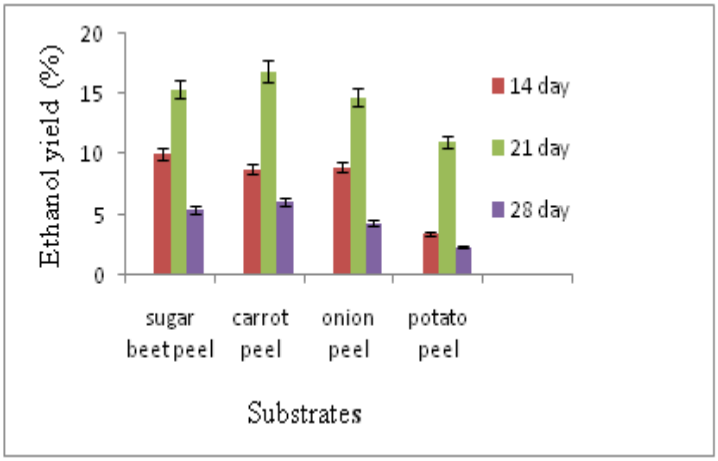

Figure 4. Ethanol obtained by dichromate method bioethanol yield (%) with agro wastes peel after 14th, 21stand 28th days of incubation of *Cladosporium cladosporioides*

3.4 Gas Chromatography and Bioethanol concentration

The Purity level for the ethanol through Gas Chromatography for the sample carrot peel was found to be to be 15.85%, the Retention Time [min] was 1.06, and Area [mV.s] was 2400, Height [mV] 168.364 and Area [%] 93.4 (Figure 5).

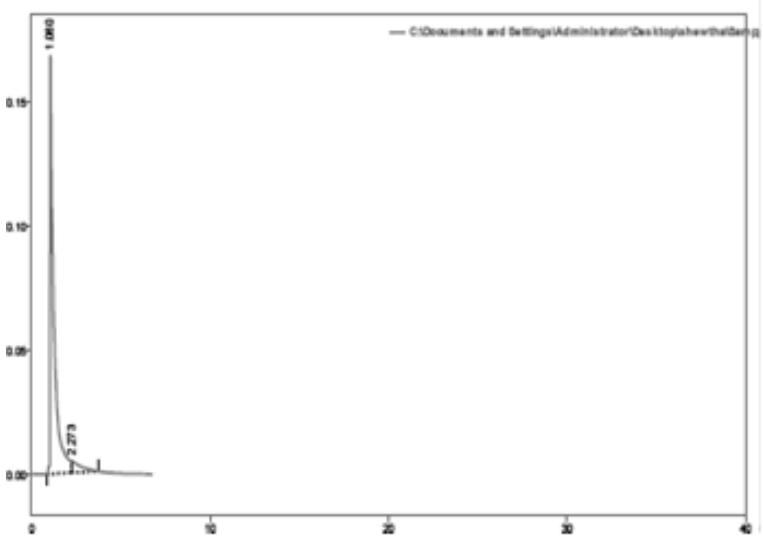

Figure 5. Gas Chromatogram of *Cladosporium cladosporioides* with carrot peel at 28 days

Isaie M and Padmavathi T (2016) reported that agro wastes such as carrot peel, onion peel, potato peel and sugar beet peel are products subjected to saccharification process by Penicillium *sp.* for the hydrolysis, this process was followed by the fermentation using yeast *S. cerevisiae* for the production of alcohol which was fermented at 14, 21, 28 days to produce alcohol. High yield of ethanol was obtained from sugar beet peel 14.52% on 28th day and further confirmed by Gas chromatography and the yield of ethanol obtained on 28th day was 17.3%. **Muchtaridi *et al.*, (2012)** determined alcohol contents of fermented black tape ketan based on different fermentation time with three different methods. Methods used are specific gravity, refractive index and GC-MS. Alcohol concentration obtained by using specific gravity method at 3, 10, 17, 24, and 31 days of fermentation, respectively, are 3.17% v / v; 3.02% v / v; 3.63% v / v; 3 , 12% v / v; and 4.47% v / v, using the method of refractive index is 3.90% v / v; 3.69% v / v; 4.31% v / v; 3.80% v / v and 5.04% v / v, and using GC-MS method was 4.30 % v/v; 4.23 % v/v; 5.01 % v/v; 4.75 % v/v; and 5.34 % v/v. The variation of fermentation time obviously did not influence the produce of alcohol contents statistically.

Table 2. Bioethanol concentration by *Cladosporium cladosporioides* (g/l)

Substrates + *Cladosporium cladosporioides*	**Bioethanol yield (g/l) at 14th day**	**Bioethanol yield (g/l) at 21st day**	**Bioethanol yield (g/l) at 28th day**
Sugar beet peel	79.136	121.506	42.606
Carrot peel	69.353	133.341	47.734
Onion peel	7.101	115.983	34.400
Potato peel	27.615	87.026	18.620

From the results obtained on bioethanol production potential of various lignocellulosic wastes varied and can be concluded that carrot peel was a very promising raw material for bioethanol production with *C. cladosporioides.* The maximum bioethanol concentration obtained in carrot peel at 21st day by *C. cladosporioides* was 133.341 g/l (Table 2). **Oyeleke *et al.*, (2009)** reported that the maximum volume of ethanol (27.10 g/l) produced from guinea corn husk and millet husk (18.24 g/l) at the 120th hours with *Zymomonas mobilis.* **Agulejika *et al.*, (2005)** reported maximum ethanol yield at 120th hour from fresh fruit (64.01 g/l) and waste fruits (21.14 g/l) using *Zymomonas mobilis*. **Micheal and Rosaline (2000)** reported that the highest ethanol yield from fresh fruit was due to higher presence of fructose and glucose in fresh fruits. **Ismail *et al.*, (2012)** has reported yields of bioethanol 0.475 g/g to 0.51 g/g of the Wheat Straw and corn cobs, and hulls acid hydrolysate respectively. Using green algae, **(Trivedi *et al.*, 2013; Ge *et al.*, 2011), (Wu *et al.*, 2014)** obtained an ethanol yield of 0.45 g/g from *U. fasciata,* 0.44 g/g from Laminaria *japonica* and of 0.47 g/g from hydrolysate Gracilaria *sp.*

4. Conclusion

The present study examined the influences of fermentation period on ethanol production ability of *S. cerevisiae* using the carrot peel, onion peel, potato peel and sugar beet peel as substrates. The results of this study indicate incubation time for fermentation using *S. cerevisiae* which may enhance ethanol yield and minimize the cost of production could be obtained from agro wastes as substrates. Bioethanol production by *S. cerevisiae* may be used as successful alternative of *S. cerevisiae* in bioethanol production.

Acknowledgments

Grateful acknowledgements are made to University of Rwanda, College of Agriculture, Animal Science and Veterinary Medicine, (CAVM / UR) for the infrastructural support.

Declaration of interest

The authors report no conflicts of interest.

References

1. Ado, S. A., Yabaya, A. (2008). Mycelial protein production by Aspergillus niger using banana peels. *Science World Journal*, *3*(4).
2. Agulejika, E.O., Olabode, F.I., Babatunde, K.A. (2005). Ethanol production from waste fruits. *Int J Food Agric* (2) (1and 2), 190-194.
3. Aneja, K.R. (2005). Experiments in Microbiology Plant Pathology and Biotechnology. New Age International Publishers New Delhi, 269 –274.
4. Atiyeh, H., Duvnjak, Z. (2002). Production of fructose and ethanol from sugar beet molasses using Saccharomyces cerevisiae ATCC

36858. *Biotechnology progress*, *18*(2), 234-239. https://doi.org/10.1021/bp010164z

5. Bai, H., Wang, H., Junde Sun, M. I., Han, M., Huang, Y., Han, X., Yang, Q. (2013). Production, purification and characterization of novel beta glucosidase from newly isolated Penicillium simplicissimum H-11 in submerged fermentation. *EXCLI journal*, *12*, 528-540.
6. Baraldo Junior, A., Borges, D. G., Tardioli, P. W., Farinas, C. S. (2014). Characterization of β-glucosidase produced by Aspergillus niger under solid-state fermentation and partially purified using MANAE-Agarose. *Biotechnology research international*, *2014*. https://doi.org/10.1155/2014/317092
7. Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical biochemistry*, *72*(1-2), 248-254. https://doi.org/10.1016/0003-2697(76)90527-3
8. Caputi, A., Ueda, M., Brown, T. (1968). Spectrophotometric determination of ethanol in wine. *American Journal of Enology and Viticulture*, *19*(3), 160-165.
9. Dien, B. S., Cotta, M. A., Jeffries, T. W. (2003). Bacteria engineered for fuel ethanol production: current status. *Applied microbiology and biotechnology*, *63*(3), 258-266. https://doi.org/10.1007/s00253-003-1444-y
10. Eken-Saraçoğlu, N., Arslan, Y. (2000). Comparison of different pretreatments in ethanol fermentation using corn cob hemicellulosic hydrolysate with Pichia stipitis and Candida shehatae. *Biotechnology Letters*, *22*(10), 855-858. https://doi.org/10.1023/A:1005663313597
11. El-Gendy, N. S., Madian, H. R., Amr, S. S. A. (2013). Design and optimization of a process for sugarcane molasses fermentation by Saccharomyces cerevisiae using response surface methodology. *International Journal of Microbiology*, *2013*. https://doi.org/10.1155/2013/815631
12. Ge, L., Wang, P., Mou, H. (2011). Study on saccharification techniques of seaweed wastes for the transformation of ethanol. *Renewable energy*, *36*(1), 84-89. https://doi.org/10.1016/j.renene.2010.06.001
13. Isaie, M., Padmavathi,T. (2016).Bioethanol production from agro wastes by acid hydrolysis fermentation process and agro wastes. *J Sc Ind Res.* 75,38-388.
14. Ismail, W. A., Braim, R. R., Ketuly, K. A., Awang, Bujang, D.S.S., Arifin, Z. (2012). Production ofbiocellulosic bioethanol from wheat straw. *Acta Polytech.* 52,28-34.
15. Michalka, J. (2007). *Optimization of sugar consumption in the fermentation of Temulose for ethanol production* (Doctoral dissertation). Available electronically from http://hdl.handle.net/1969.1/5683
16. Mishra, J., Kumar, D., Samanta, S., & Vishwakarma, M. K. (2012). A comparative study of ethanol production from various agro residues by using Saccharomyces cerevisiae and Candida albicans. *Journal of Yeast and fungal research*, *3*(2), 12-17.
17. Mohan, P. R., Pradeep, S., & Reddy, O. V. S. (2012). A comparative study on simultaneous saccharification and fermentation of agricultural wastes to bio-ethanol using two Saccharomyces strains. *Chiang Mai Journal of Sciences*, *39*, 1-14.
18. Muchtaridi, M., Musfiroh, I., Hambali, N. N., Indrayati, W. (2012). Determination of alcohol contents of fermentated black tape ketan based on different fermentation time using specific gravity, refractive index and GC-MS methods. *Journal of Microbiology, Biotechnology and Food Sciences*, *2012*, 933-946.
19. Nagashima, M.M., Azuma, S., Noguchi, K., Inuzuka, and H., Samejima.(1984).Continuous ethanol fermentation using immobilized yeast cells. *Biotechnol Bioeng*. 26,992-997.
20. Oyeleke, S. B., Jibrin, N. M. (2009). Production of bioethanol from guinea cornhusk and millet husk. *African Journal of Microbiology Research*, *3*(4), 147-152.
21. Öhgren, K., Bengtsson, O., Gorwa-Grauslund, M. F., Galbe, M., Hahn-Hägerdal, B., & Zacchi, G. (2006). Simultaneous saccharification and co-fermentation of glucose and xylose in steam-pretreated corn stover at high fiber content with Saccharomyces cerevisiae TMB3400. *Journal of biotechnology*, *126*(4), 488-498. https://doi.org/10.1016/j.jbiotec.2006.05.001
22. Pramanik, K. (2003). Parametric studies on batch alcohol fermentation using Saccharomyces yeast extracted from toddy. *.J Chin Inst Chem Eng*. 34,487-492.
23. Pretorius, I. S. (2000). Tailoring wine yeast for the new millennium: novel approaches to the ancient art of winemaking. *Yeast*, *16*(8), 675-729.
24. Pol, D., Laxman, R. S., & Rao, M. (2012). Purification and biochemical characterization of endoglucanase from Penicillium pinophilum MS 20. *Ind J Biochem Bio*. 49:189-194.
25. Ramani, G., Meera, B., Vanitha, C., Rao, M., & Gunasekaran, P. (2012). Production, purification, and characterization of a β-glucosidase of Penicillium funiculosum NCL1. *Applied biochemistry and biotechnology*, *167*(5), 959-972. https://doi.org/10.1007/s12010-012-9645-4
26. Roehr, M., Kosaric, N., Vardar-Sukan, F., Pieper, H. J., & Senn, T. (2001). The biotechnology of ethanol. Classical and future applications. Weinheim, Germany, Wiley-Vch Publishing 62-65.
27. Senthilkumar, V., Gunasekaran, P. (2005). Bioethanol production from cellulosic substrates: Engineered bacteria and process integration challenges. *J Sc Ind Res.* 64, 845-853.
28. Trivedi, N., Gupta, V., Reddy, C. R. K., Jha, B. (2013). Enzymatic hydrolysis and production of bioethanol from common macrophytic green alga Ulva fasciata Delile. *Bioresource technology*, *150*, 106-112. https://doi.org/10.1016/j.biortech.2013.09.103
29. Uk, L. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, *227*(259), 680-685.
30. Wang, M. L., Choong, Y. M., Su, N. W., Lee, M. H. (2003). A rapid method for determination of ethanol in alcoholic beverages using capillary gas chromatography. *Journal of Food and Drug Analysis*, *11*(2).
31. Williams, M. B., & Reese, H. D. (1950). Colorimetric determination of ethyl alcohol. *Analytical chemistry*, *22*(12), 1556-1561.
32. Wu, F. C., Wu, J. Y., Liao, Y. J., Wang, M. Y., Shih, L. (2014). Sequential acid and enzymatic hydrolysis in situ and bioethanol production from Gracilaria biomass. *Bioresource Technology*, *156*, 123-131. https://doi.org/10.1016/j.biortech.2014.01.024
33. Yasmin, S., Mottoo, R.,Nehvi, F.(2013).Isolation, characterization and molecular weight determination of cellulose from Trichoderma viride, *Afr J Biotechnol*.12,4503-4511.