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Determination of Brew Fermentation Rate Using *Tithonia diversifolia* Catalyst

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The consumption of alcoholic drinks have highly risen recently to a situation whereby there is a deficit in the stores, this is due to the higher demand compared to supply. Due to the high prices of most of the industrialized brews, consumers have opted for locally brewed drinks. Although locally manufactured brews are not recognized and certified by law, most are of good quality and with low cost of production. The use of *Tithonia diversifolia* can be employed to aid in improvement of the rate of production of local and industrialized brews. The main aim of this project was to improve the rate of fermentation of alcoholic beverage using both *Tithonia diversifolia* leaves extracts and iron II nanoparticles derived from it. It was observed that the plant catalyst reduced the time taken to produce alcohol. Alcohol fermentation rate in presence of yeast and with a tithonia extract as catalyst was measured, Rates of alcohol production was measured by UV VIS at intervals of one hour and deduced from a calibration curve. From the data, the alcohol content was higher in the sample catalyzed by the complexed extract and the one containing extracts as the catalyst as compared to the one without a catalyst. The percentage ethanol was able to be detected by finding absorbances (beer lambert law $A = e\,l\,c$.).

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Keywords: Tithonia diversifolia; brew fermentation; iron II nanoparticles.

1. INTRODUCTION

Fermentation of alcohol is a process whereby sugars are converted to ethanol, cellular energy and carbon dioxide and yeast is normally incorporated to initiate the process of conversion. Control of the fermentation process is prerequisite to production of high quality brews, it requires low cost, fast and non-destructive technique [1]. As observed, the rate of consumption of alcoholic drinks increase daily despite government effort to put them away [2]. This is due to the changes in life style among youths that is believed to be the fashion of the century [3]. In many developing countries at the village art, old techniques are still used for alcohol fermentation, this is seen not to be recognized at the industrial level [3,4] and they neglect the old age techniques that were used by the traditional Africans in brewing alcohol for faster fermentation as well as the improvement of the quality [5]. In research, special emphasis on fermentation period ought to be considered to ferment alcoholic beverage that serve as refreshment to the people [6,7].

Alcoholic products sold in bars, supermarkets and restaurants are expensive to low and middle class citizens. Due to increase in the cost of living, many people find it affordable to consume local brews which are cheap and available to quench their alcoholic thirst. This has led to increment in the production of local brews in Kenya. The local brews normally take very short time to ferment especially when a catalyst is used alongside a suitable strain of yeast [8]. Although many people due to monetary greed produce harmful brews that end up killing or paralyzing parts of the body, there are those brews that are of good quality due to the use of natural non harmful raw materials [9]. By using *Tithonia diversifolia* in the fermentation of local brews, can not only bring about quality but also increases consumption of the local naturally manufactured drinks. The beverage takes a short fermentation period and this helps in production of large quantity and with the best quality [10,11]. The beverage will be sold at a fair price and distributed widely in the market. Locally manufactured beverage is one of the most appreciated local drinks among the people from all over the nation, the government should therefore, take into consideration the acceptance of the drinks after checking for the standardization[12]. Also, the application of the catalyst in large scale would be of importance to reduce the processing time.

Alcohol production requires optimization of the factors contributing to the process, yeast, saccharomyces cerevisiae was found to be the best culture since it has high tolerance to ethanol production unlike the other species [13,14,2]. The culture can ferment both in aerobic and under anaerobic condition. The feedstock ranged from corn to sugarcane juices depending on the availability and the quantity of alcohol required [15]. Sugarcane could produce small amount of alcohol but with good quality since it has high sucrose level [16,17].

Consumption of alcohol traditionally was attributed to improvement of the digestive system, bringing happiness and adding flavor to meals, hence consumption before meals [5]. Alcohol consumption were consumed in large quantities in functions. During weddings, burials and circumcision gatherings among others, brews were consumed in large quantities. All genders and ages could take part in the celebration and alcohol drinking to enhance happiness, this led to production of more brews so as to satisfy everyone [5]. The desire to produce large quantities of brews and with good quality over a short time led to introduction of catalysts in the tradition community.

2. MATERIALS AND METHODS

2.1 Sample Collection and Extraction

The leaves and twigs of *Tithonia diversifolia* plant were procured from JKUAT botanical gardens, Kenya. Samples were washed and sliced to small pieces to ease grinding then dried at room temperature for one week [8]. It was then ground and extraction done using n-hexane, ethylacetate and methanol. The solvents were removed and samples concentrated by rotary evaporator.

2.2 Synthesis of Iron Ii Nanoparticles

Synthesis of iron II nanoparticles was done by adding 0.01M ferrous chloride to *Tithonia diversifolia* extract in the ratio of 2:5. The mixture was heated in a magnetic hot stirrer at 50-60^{0}c for four hours to allow reaction to take place. The colour changed from pale green to brown indicating oxidation of the metal ions. pH was kept constant at 8 by addition of ammonia since

it's the major influence to the reaction. The solution was centrifuged and the pellets collected and washed with distilled water.

2.3 Fermentation Process

5 g of yeast powder was dissolved in 100ml water to be used in initiation of fermentation.

Sugarcane juice was extracted using extractor and filtered to remove particles. The juice was diluted using warm distilled water in the ratio of 1:5 ml then 100 ml each introduced to six conical flasks for fermentation. Of the six flasks, three test samples were prepared as follows; into two flasks, 10ml of the yeast solution was added and left to stand, 10 ml yeast and 2 ml of 4% plant extract were added each to two flasks and to the remaining two flasks, 10ml yeast and 2ml of 4% iron II nanoparticles were added. The solutions were left to stand for an hour under room temperature. The alcohol content was measured at an interval of one hour using ultra-violet visible region (UV VIS).

2.4 Ethanol Standards Preparation

In the experiment, ethanol standards were prepared as follows;

10 ml of 3% ethanol were obtained and serial dilutions done to obtain five more 10ml solutions with concentrations from 0.0125%,0.025%, 0.05%,0.1% and 0.2% the process was done using test tubes and 10 ml volumetric flasks, distilled water was used to dilute the samples and all labelled according to their concentrations.

2.5 Uv Vis Data Collection

UV VIS spectroscopy was done to determine the wavelength at which ethyl alcohol absorbs light. Ethanol was diluted in the ratio of 1:10 and ran to obtain spectrum. Plain sugarcane was used to auto zero when running the samples under test, while distilled water used as the blank when ethanol spectrum was being drawn.

3. RESULTS AND DISCUSSION

Spectra were obtained to indicate the wavelength at which ethyl alcohol absorbs light. Spectrum for yeast and samples were also drawn. The outcome was as shown below. The figure shows spectra for ethanol and the samples under test, the samples were run after an hour to allow alcohol formation. Peaks were formed between 230-280nm wavelength by both ethanol and brews indicating the formation of ethanol in the reaction.

Fig. 2 shows spectra after two hours. From the peaks, the absorbance raised due to continuous production of alcohol. This indicates that the concentration of alcohol increases with time as per Beer Lamberts law. Yeast spectrum was far much different from the rest. Alcohol

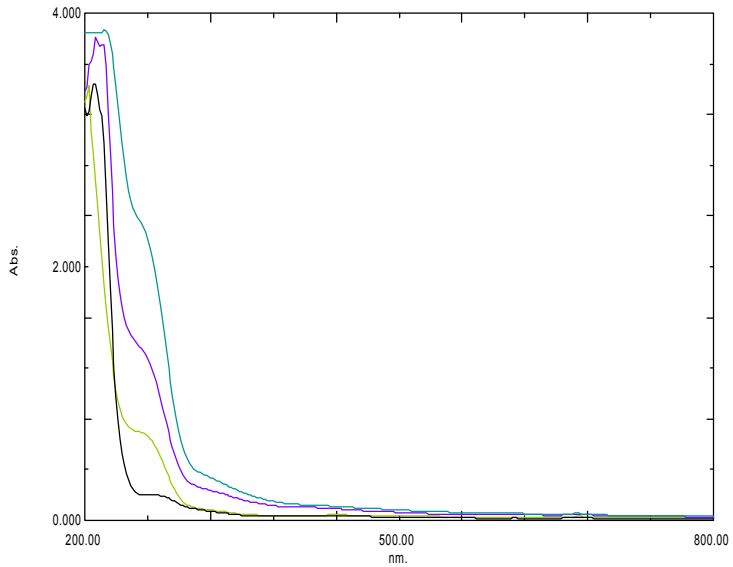

Fig. 1. Ethanol production after an hour

Key; laboratory ethanol Complex catalyzed reaction Crude extracts catalyzed reaction Uncatalyzed brew

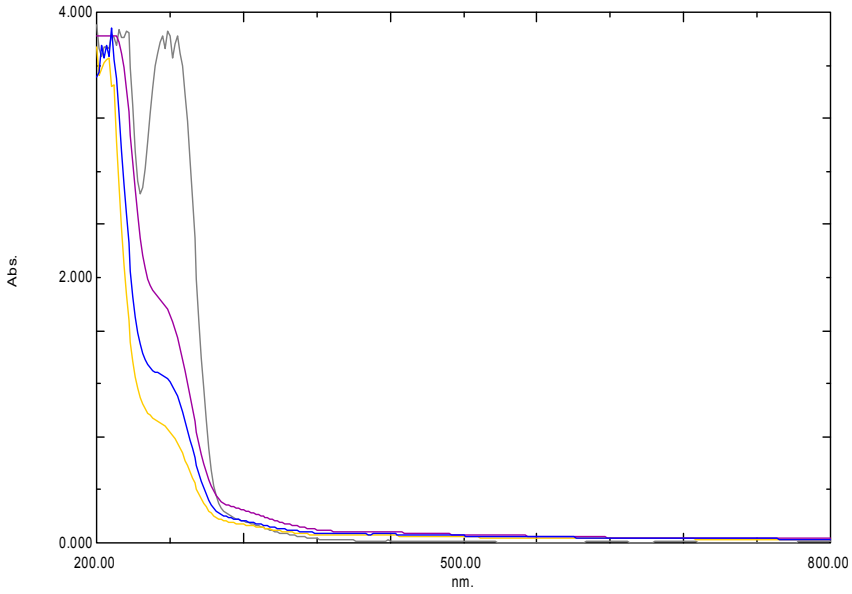

Fig. 2. Spectra after two hours

Key: yeast peak iron II catalyzed reaction Crude extracts catalyzed reaction Uncatalyzed brew

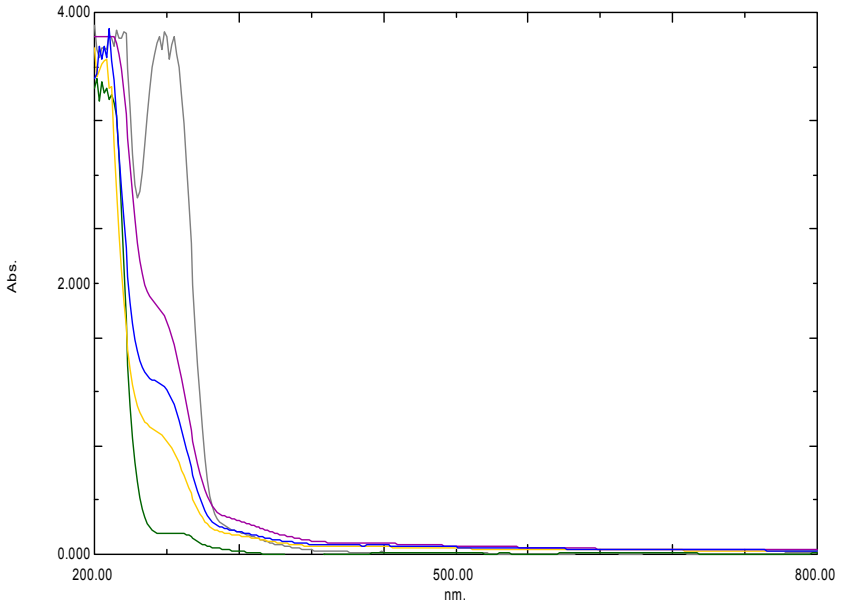

Fig. 3. Spectra for both yeast, ethanol and the brews after two hours

Key: yeast peak Iron II catalyzed reaction Crude extracts catalyzed reaction Uncatalyzed brew

concentration was higher in the reaction catalyst by nanoparticles compared to the other reactions; this indicated that the complexed sample had higher effect on the rate of production than the crude extracts and the uncatalyzed reaction. Crude extract showed effect on catalysis since the absorbance was higher than that of uncatalyzed reaction.

Fig. 3 shows the spectra for both yeast, ethanol and the brews after two hours. Yeast showed three sharp peaks between 220-250nm.

Standard Table Report

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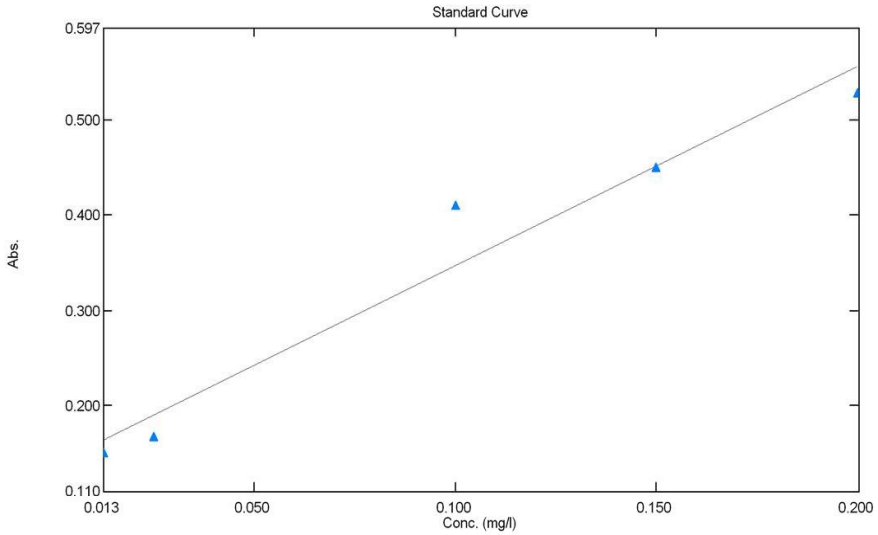

Standard Table

	Sample ID	Type	Ex	Conc	WL800.0_200.0	Wgt.Factor	Comments
1	sample 1	Std-Repeat		0.013	0.151	1.000	
2	sample 1-2	Std-Repeat		0.013	0.151	1.000	
3	sample 1-Avg	Average		0.013	0.151	1.000	Avg of preceding 2 Samples
4	sample 2	Std-Repeat		0.025	0.167	1.000	
5	sample 2-2	Std-Repeat		0.025	0.167	1.000	
6	sample 2-Avg	Average		0.025	0.167	1.000	Avg of preceding 2 Samples
7	sample 3	Std-Repeat		0.100	0.411	1.000	
8	sample 3-2	Std-Repeat		0.100	0.411	1.000	
9	sample 3-Avg	Average		0.100	0.411	1.000	Avg of preceding 2 Samples
10	sample 4	Std-Repeat		0.200	0.530	1.000	
11	sample 4-2	Std-Repeat		0.200	0.528	1.000	
12	sample 4-Avg	Average		0.200	0.529	1.000	Avg of preceding 2 Samples
13	sample 5	Std-Repeat		0.150	0.451	1.000	
14	sample 5-2	Std-Repeat		0.150	0.451	1.000	
15	sample 5-Avg	Average		0.150	0.451	1.000	Avg of preceding 2 Samples
16							

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Fig. 4. standard calibration curve for ethanol

3.1 Rate of Production

Calibration line was obtained using the ethanol concentrations made as indicated earlier. The concentrations ranged from 0.0125 to 0.2% and was taken in duplicates. Results were as shown.

The readings were done twice and average taken. From the curve above, concentrations of alcohol were deduced from the brews at intervals of an hour for four hours, the outcomes were as shown in Fig. 5.

From the standard curve, the concentrations of alcohol was derived and curve drawn as shown.

Alcohol content increased with time until when the maximum production of alcohol was reached.

The curve begins to flatten. The pH was taken and it was near neutral, and the decline was attributed to accumulation of ethanol that caused dormancy of the culture.

Rate of production

At 0hrs there was no alcohol production. After an hour the results were as shown below

Brew concentration with no catalyst

0.761% alcohol after one hour

$$\text{Rate of production} = \frac{\text{change in concentration}}{\text{Time}}$$

$$\frac{0.761}{1\text{hr}} = 0.761\%$$

Brew concentration with crude extract as a catalyst

$$\frac{0.782}{1hr} = 0.782$$

Brew concentration with nanoparticles as a catalyst

$$\frac{1.089}{1hr} = 1.089$$

Sample Table Report

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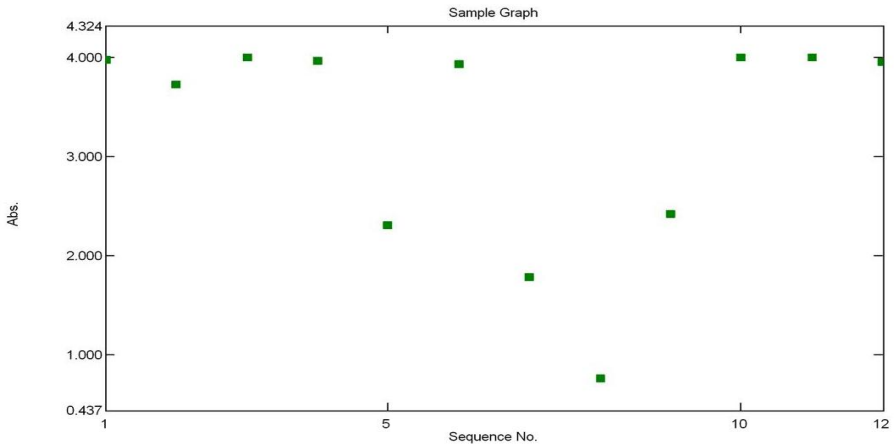

Sample Table

	Sample ID	Type	Ex	Conc	WL800.0_200.0	Comments
1	s+y+t	Unknown		1.834	3.984	3hrs
2	s+y 2	Unknown		1.716	3.736	3hrs
3	s+y+c	Unknown		1.842	4.000	3hrs
4	s+y+c1	Unknown		1.828	3.972	2hrs
5	s+y1	Unknown		1.031	2.301	2hrs
6	s+y+t1	Unknown		1.813	3.939	2hrs
7	s+y+t2	Unknown		0.782	1.777	1hrs
8	s+y2	Unknown		0.297	0.761	1hrs
9	s+y+c2	Unknown		1.089	2.421	1hrs
10	s+y+c3	Unknown		1.842	4.000	4hrs
11	s+y+t3	Unknown		1.842	4.000	4hrs
12	s+y3	Unknown		1.821	3.957	4hrs
13						

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Fig. 5. Samples catalyzed ethanol rate

Key: s+y sugarcane juice + yeast, s+y+t sugarcane juice + yeast + tithonia extract, s+t+c sugarcane juice + yeast + complexed extract.

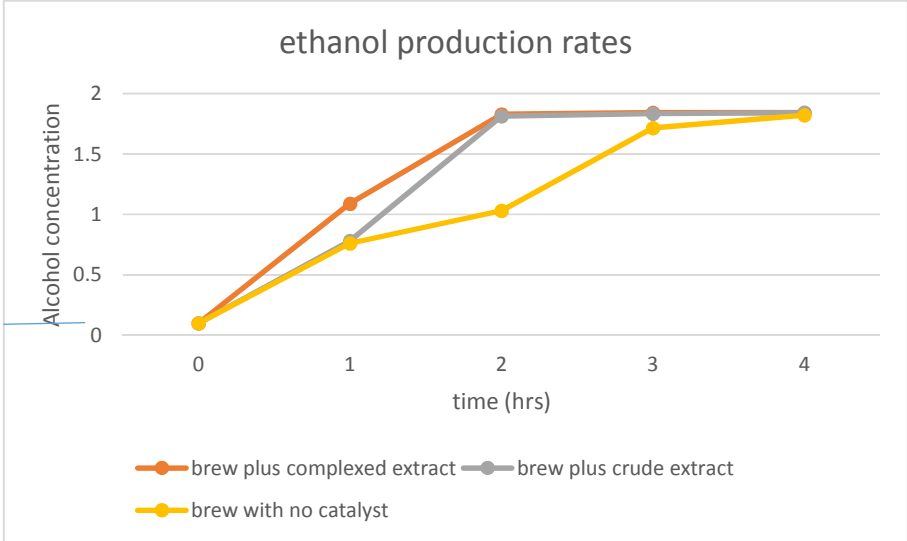

Fig. 6. Curves for ethanol rates

From the graphs above, the rate of ethanol production increased with time. The rate at which brew catalyzed by nanoparticles produced ethanol was highest compared to the others. The crude extract catalyzed reaction, showed significantly higher concentration of alcohol than the uncatalyzed reaction. This indicates that the extract and its complex induces catalytic effect on the fermentation rate. Temperature that is a crucial factor in fermentation rate was maintained at room temperature. The reactants temperature was kept constant so as not to stress the yeast. pH also was maintained since the sugarcane pH was at 3.4 and it is known that yeast works best at pH ranging from 3.0-5.0. It was also noted that the initial sugarcane concentration can highly affect the functioning of the microbes, this is so because when the concentration is too high, the uptake increases hence increasing the steady rate of fermentation. To solve this, the sugarcane juice was diluted as indicated earlier.

The reaction rate continued up to the maximum point whereby the concentration was saturated and there was no free molecules in the solution for further reaction. This lead to the curve flattening as seen from the graph.

4. CONCLUSION

Normally in the local production and at the industrial level, the rate of brew fermentation takes up to 3 days to get ready to drink, this indicates consumption of much time and therefore a need for a catalyst. The use of *tithonia diversifolia* extract and the nanoparticles derived from it, could be of greater benefit since it improves time taken to brew and moreso, a good remedy for ailments, the catalyst can be recommended for use in fermentation processes. The plant is readily available and has no side effect, it is also of a pharmaceutical importance.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Stanbury PF. Whitaker A, Hall SJ. Principles of fermentation technology; 2017.
2. Lozada CM. Metabolic analysis of Saccharomyces cerevisiae during alcoholic fermentation; 2014.
3. Hockings K, Dunbar, RIM. Alcohol and humans: A long and social affair; 2020.
4. Reungsang A, Sittijunda S, Sreela-or C. Methane production from acidic effluent discharged after the hydrogen fermentation of sugarcane juice using batch fermentation and UASB reactor. Renewable Energy. 2016;86:1224-1231.
5. Rasmussen SC. The quest for aqua vitae: The history and chemistry of alcohol from antiquity to the Middle Ages; 2014.
6. Monteiro B, Ferraz P, Barroca M, Da CSH, Collins T, Lucas C, Springer Link (Online service). Conditions promoting effective

very high gravity sugarcane juice fermentation. (Biotechnology for biofuels.) ; 2018.
7. Dombek KM. The declining rate of ethanol production during batch fermentation by Saccharomyces cerevisiae; 1987.
8. Grumezescu AM, Holban AM. Biotechnological progress and beverage consumption. 2020;19.
9. Kanchi S, Ahmed S. Green metal nanoparticles: Synthesis, characterization and their applications; 2018.
10. Kuila A, Sharma V. Principles and applications of fermentation technology; 2018.
11. Jin CK. Fermentation kinetics and process development for enhanced rate of production of ethanol from carbohydrates; 1981.
12. Grumezescu AM, Holban AM. Food processing for increased quality and consumption. 2018.
13. Reid JE. Factors affecting the rate of fermentation of apple juice; 1957.
14. White C, Zainasheff J. Yeast: The Practical Guide to Beer Fermentation. Lanham: Brewers Association; 2010.
15. Williams AT, Harbertson JF, Washington State University, Washington State University. Managing pH and acid composition to assess microbial ecology of wine fermentation; 2019.
16. O'Hara IM, Mundree SG, Robins Karen, Speight Robert. Chemicals manufacture from fermentation of sugarcane products. John Wiley and Sons; 2016.
17. O'Hara IM, Mundree SG. Sugarcane-based biofuels and bioproducts; 2016.

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