Journal Homepage: -www.journalijar.com
INTERNATIONAL JOURNAL OF ADVANCED RESEARCH (IJAR)
Article DOI:10.21474/IJAR01/5356
DOI URL: http://dx.doi.org/10.21474/IJAR01/5356

RESEARCH ARTICLE

BIO-ETHANOL PRODUCTION USING NON-EDIBLE TUBER (Amorphophallusflavovirens): AN EFFORT TOWARDS ENSURING FOOD SECURITY.

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Manuscript Info

Manuscript History

Received: 07 July 2017
Final Accepted: 09 August 2017
Published: September 2017

Abstract

Studies on manufacture of bio-ethanol have been carried out on different feed stocks including non-edible tubers. In this study, Amorphophallusflavovirens, a species of non edible tubers was used for the trial. Starch extracted from amorphophallusflavovirens was hydrolyzed with varying concentrations of 5% yeast (*Saccharomyenslerevisae*) at 27^0C for 72 hours. 0.5ml, 1.0ml, 2.0ml, 4.0ml, 8.0ml and 10.0ml of the yeast, produced 3.3%, 3.3%, 3.5%, 3.8%, 4.2% and 4.6% of alcohol respectively. The percentage alcohol yield using 10ml of baker's yeast was highest. The ethanol produced from Amorphophallusflavovirens was found to be chemically identical with alcohol produced from UMUCASS45 cassava variety from National root crops research institute Umudike. it can also be used as fuel ethanol in blends. The percentage ethanol produced was found to be directly proportional to the yeast added.

Introduction:-

The production of ethanol is surrounded by a lot of controversial issues. This is not very surprising because there are a variety of feedstock from which it can be produced and a number of the production processes including different uses of this commodity (Cedric *et al*. 2002).

There is confusion also with regards to the term ethanol. The term is usually used as a synonym for alcoholic beverages. This is misleading, although ethanol may be used as a raw material for the production of spirit (Sharma, 2006). The feedstock and processes by which ethanol can be produced are diverse. Synthetic alcohol can be distilled from crude oil or gas and coal (Evans, 2011).

Agricultural alcohol may be distilled from cereal crops, molasses, fruits, cellulose, sugarcane and many other sources. Both synthetic and agricultural alcohols are chemically identical (Evans, 2010). Nonetheless, synthetic feedstocks play a minor role on a global scale. In early 21st century, less than 4% of overall out put was accounted for by synthetic feedstock. More than 95% came from agricultural crops, giving the strong interest in fuel ethanol production Worldwide (Evans, 2010).

The annual bio-ethanol production worldwide was around 83.1 billion litres in 2012. USA are the largest producers of bio-ethanol with production reaching approximately 50 billion litres (primarily from maize and secondarily from

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wheat) and brazil with approximately 35 billion litres from sugarcane in 2012). Together, USA and Brazil produce 87% of the World's ethanol. This share is expected to increase in the future (Lintch, 2012).

Another distinction which is of important in the field of ethanol is one between anhydrous and hydrous alcohol (Fisher, 2007). The former is free of water and at least 99% pure. The ethanol may be used in fuel blend while the later (i.e. hydrous alcohol) contains some water and usually has purity of 96%.

Ethanol is produced by fermentation from raw materials that contain starch or sugar such as sugarcane, corn, cassava, potatoes, wheat, sorghum, *Amorphorphallusflavoviriens* which is our area of concentration in this research, rice sunflower etc. *Amorphophallusflavovirens*is a garden plant which usually grows on a fallow land. It has corn through which new plant grows.

Objective of the Research:-
Ethanol production by fermentation from plants containing starch and sugar were done using edible plants. This pose a very big threat to human nutrients since crops meant for human consumption are now channeled to the production of ethanol for energy usages.

Efforts are being made by scientists on how to use non-edible plants for the production of ethanol in order to avoid famine in the society.

The main objective of this work is to produce ethanol from a non-edible plant (*Amorphophallusflavovirens*) and compare it with ethanol produced from UMUCASS45 cassava variety (from National rootcrops research institute Umudike southeast Nigeria).

Materials and Methods:-

Sample collection:-
The samples were collected from Agbani in Nkanu local Government Area of Enugu State, Southeast Nigeria.

Processing starch from the sample plant:-
The sample plant tubers were collected in large quantity. The tuber's back were peeled off with small knife, exposing the whitish body, which was then washed with clean water and grinded into a slurry using a manual grinding machine. The slurry gave a net weight of 4053.73g. The slurry was sieved with a lot of water using a sieve cloth. The chaff which has been made starch free was discarded and the white starch solution was collected in a bowl.

The water was carefully decanted and the starch collected in an empty clean salt bag. The salt bag as squashed to remove excess water. The starch was collected in a tray for drying in an electric oven. 256g of dry mass of the starch was then collected as raw material for the next step of the research.

Preparation of sugar from starch (*Gelatinization* process*):-*
100g of the dry starch sample was measured into a conical flask. Then 600ml of distilled water was added to give a ratio of 1:6. The dry starch water mixture was stirred thoroughly to give a homogenous mixture. The mixture was heated and the temperature was maintained at 70^0C – 80^0C. the mixture was stirred gently for the first 5 minutes until a gel was formed. The formation of gel computes the gelatinization process.

***Saccharification* process:-**
After gelatinization, the sample starch slurry was cooled to 30^0C and 4g of diastase (enzyme) was added and stirred thoroughly. The temperature was maintained between 40^0C – 60^0C, which is the temperature for the activity of diastase, for 2hrs with constant stirring.

Meanwhile, calcium was added in form of calcium hydroxide. The calcium ion stabilizes the enzyme. The temperature was increased to 105^0C for 5 minutes and later cooled down to 90^0C for 2 hours to further hydrolyze the starch with constant stirring. The starch was converted into mactose or mact sugar and this completes the saccharification process.

$$(C_6H_{10}O_5)_n + nH_{20} \rightarrow nC_{12}H_{22}O_{11}$$
Starch mactose

Sample Analysis:-

Iodine test for starch (Confirmatory test):-

5g of the sample was collected, grinded and put into a test tube. The solution gives a blue-black colouration which indicates the presence of starch.

Reducing sugar test using Benedict's solution:-

2ml of Benedict's solution was measured in a test tube and 5 drops of saccharified sugar sample solution was added and the mixture heated. A brick red precipitate was observed, indicating and confirming the presence of reducing sugar.

Preparation of yeast inoculums (activation of yeast):-

The baker's yeast contains purely saccharomyorscerevisal. 10grams of the baker's yeast was added to the 5% sugar solution and stirred until fumes were observed. This sugar solution activates the yeast for fermentation.

Preparation of fermentation medium:-

The enzyme hydrolyzed starch slurry was boiled for 1hour to precipitate any available protein. The slurry was filtered to remove the precipitate.

600ml of the filtrate was collected which contained soluble sugar. Six fermentation tubes A,B,C,D,E and F were set up properly for fermentation process. 100ml of the saccharified sugar was added to each 0.3g of ammonium sulphate and ammonium hydrogen phosphate was added to each tube as a source of nitrogen and phosphorus to provide nutrient for the growth of the yeast.

Then the activated yeast was added in the portion of 0.5ml, 1.0ml, 2.0ml, 4.0ml, 8.0ml, 10.0ml to tubes A,B,C,D,E and F respectively. This was to ferment for 3days.

Alcohol Determination:-

50ml of water was added to the fermented mixture. The flask was connected to a condenser and heated, 95ml of distilled water was collected. This was made up to 100ml with distilled water. The distillate was tested for percentage alcohol using an alcoholic meter.

Results and Discussions:-

Table 1 shows the fermentation rate of *Amorphophallusflavovirens,* from the table, the quantity of yeast added to tube E and F was in excess, so the carbon-dioxide was displaced at once. That is in E and F there fermentation rate was fast compared to other tubes. The rate of fermentation in tube A was very slow due to the quantity of yeast added. This shows that in the sugar of *Amorphophallusflavovirens,* the rate of fermentation depends on the quantity of yeast added.

Table 2, shows the percentage alcohol yield of *Amorphophallusflavovirens*and its specific gravity. The percentage alcohol yield increases as the quantity of yeast added increases.

Fermentation tubes E and F with 8ml and 10ml of yeast respectively gave the highest alcohol yield. Meanwhile, as the quantity of alcohol yield increases it's specific gravity decreases.

Table 3 shows the physiochemical parameter of the sample.

Table 1:-Fermentation rate results.

Days	Time	A	B	C	D	E	F	Temp.
	(S)	$(cm)^3$	$(cm)^3$	$(cm)^3$	$(cm)^3$	$(cm)^3$	$(cm)^3$	(0^0C)
0	1.00pm	90	15	30	40	100	160	26^0C
1	1.00pm	530	350	680	730	-	-	27^0C
2	1.00pm	770	300	790	910	-	-	27^0C
3	1.00pm	736	290	810	1050	-	-	27^0C

Table 2:-Percentage alcohol and specific gravity .

Yeast added	% alcohol	Weight of S.G bottle + distillate	Weightof distillate	S.G of the distillate
0.5ml	3%	70.70g	44.09g	0.9964g
1.0ml	3%	70.70g	44.09g	0.99644g
2.0ml	3.2%	70.67g	44.06g	0.9957g
4.0ml	3.5%	70.63g	44.02g	0.9948g
8.0ml	4.0%	70.59g	43.98g	0.9933g
10.0ml	4.5%	70.58g	43.97g	0.9937g

Table 3:-Physiochemical parameters of samples.

Sample	Weight/percentage
Wet weight of the sample	4053.74g
Dry weight of the sample	372.94g
Weight of water	3680.74g
Percentage dry starch in the dry sample	68.64%
Percentage chaff in the dry sample	31.36%
Percentage dry starch in wet sample	6.32%
Moisture content	90.8%

Conclusion:-

The major objective of this work was to carry out production of bio-ethanol from specie of non-edible tuber (*Amorphophallusflavovires)* and stop the use of edible tubers like cassava etc in ethanol production to avoid food shortage and ensure food security.

The ethanol produced from *Amorphophallusflavovires*was found to be chemically identical with ethanol produced from cassava starch .

Therefore, we suggest that ethanol producing industries all over the world should channel there resources more towards the usage of non edible plants in ethanol production instead of using edible plants so as to avoid food shortage and ensure food security.

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