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RESEARCH ARTICLE

CITRUS SINENSIS (L) PEELS; POTENTIAL FOR BIOFUEL PRODUCTION

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Abstract

This research work focused on the production of biofuel from orange peels using *Bacillus subtilis* as a fermentor. The orange peels were collected from Ekeukwu market and Ihiagwa fruit center, Owerri Nigeria, sun dried and grinded manually to powder. The sample was further fermented for 8days using *Bacillus subtilis*, after which it was distilled for 6 hours in order to extract the biofuel.Analysis of the ethanol compostion of orange peels was carried out using gas chromatography.The parameters measured include pH, concentration of reducing sugar before and after fermentation as well as the characterization of biofuel produced. Results shows that 200g of orange peels produced 400ml of biofuel on the 8th day of fermentation and the percentage component of the biofuel produced are Ethanol (48.73%), Methane (26.89%), Methanol (17.22%), Carbon dioxide (7.15%), Phenol (0.005%) while the control did not produce any biofuel. From the study it was observed that ethanol has the highest percentage in the biofuel produced from orange peels while phenol was the least. The reducing sugar concentration of the orange peel before and after fermentation were 11.678 mg/l and 73.899 mg/l respectively. It was also observed from this research that the ideal pH to obtain a reasonable quantity of biofuel from orange peels ranges from 5.0 – 5.3. The production of ethanol from orange peels presents an alternative, cheap, environmental friendly and readily available option to use in place of fossil fuels.The result from this study showed that liquid fuel (bioethanol) can be produced from the fermentation and distillation of orange peelsI therefore recommend that further studies be carried out on finding the efficient processes and methods of increasing the yield of biofuel from agro-waste.

Introduction:-

Global energy consumption and dependence on non-renewable energy resources have increased the cost of transportation and highlighted the environmental problems by affecting the durability of ecosystems, global climate as well as oil reserves that result in greenhouse effect (Manasa and Narasimhulu, 2015). Hence, the need for an alternative energy source arises. A new wave of technologies is on the edge of producing energy that is clean, renewable, and most importantly economical (Manasa and Narasimhulu, 2015). There has been an urgent need for a

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renewable, sustainable energy sources. Ethanol had been a promising renewable source (Farrell *et al*., 2006). The increasing demand for ethanol for various chemical and motor-fuel industrial purposes such as alternative source of energy, industrial solvents, clean-sing agents, preservatives and its important role in reduction of greenhouse gas emissions has necessitated increased production of this alcohol (Edgardo *et al.*, 2008).

Citrus sinensis is one of the most important and widely grown fruit crops, with total global production reported to be around 120 million tons (Parle *et al.*, 2012). Orange trees are widely cultivated in tropical and subtropical climates for its tasty juice and medicinal value. In worldwide trades citrus fruits generate about 105 billion dollars per year all over the world. Orange fruit is cultivated in more than 130 countries including India, UK, France, Germany, Holland, Brazil, China, USA and Spain. Oranges are generally available from winter through summer with seasonal variations depending on the variety (Parle *et al.*, 2012).

Citrus fruits comprise an important group of fruit crops manufactured worldwide. In the fruit processing industry large amounts of waste materials are produced, in the form of peel, pulp, seeds, etc. [Dhillon *et al.*, 2014]. The waste material presents significant disposal difficulties, and when not used in any way it causes odor and soil pollution [Ma *et al.*, 2013; Dhillon *et al*., 2014]. Since the 1980s the worldwide production of citrus has increased drastically. Estimations show that in 2010 the orange production will reach 66.4 million metric tons, which is an increase with 14% compared with that of 1997-1999. Almost half, 30.1 million metric tons of the produced orange will be manufactured to yield juice, essential oils and other by-products [Mamma *et al.*, 2008].When dried citrus peels are rich in cellulose, hemicelluloses.

The term biofuel is referred to liquid, gas, and solid fuels that are predominantly produced from renewable biomass. Biofuels include bioethanol, biomethanol, vegetable oils, biodiesel, biogas, bio-synthetic gas (bio-syngas), bio-oil, bio-char, Fischer-Tropsch liquids, and biohydrogen. There are several reasons for biofuels to be considered as relevant technologies by both developing and industrialized countries. They include energy security reasons, environmental concerns, foreign exchange savings, and socio-economic issues related to the rural sector. Examples of biofuel some examples of biofuels production from food-processing lignocellulosic residues are reported below. Biodiesel is not reported in this list as it is produced through a chemical modification of vegetable oils and is not derived from lignocellulose.

Proteins and pectin, the fat content is however low [Ma *et al.*, 2013; Dhillon *et al*., 2014].

Advantages of biofuels include biofuels are easily available from common biomass sources; their combustion is included in a carbon dioxide cycle; biofuels have a considerable environmentally friendly potential; biofuels use positively impinges on the environment, economy and consumers; they are biodegradable and contribute to sustainability (Demirbas, 2010).

Materials and Methods:-

Sample Collection

Orange peels are waste from orange fruit. The peels were collected from Ekeukwu market and Ihiagwa fruit center in owerri, Imo State Nigeria. They were washed to remove dirt. The orange peels collected were transported to the laboratory for analyses.

Physiochemical Analysis of Sample

This was carried out to determine the reducing sugar (glucose) content of the sample before and after fermentation. The reducing sugar (glucose) was determined by various methods as described by AOAC, 1990.

Test Isolates

Bacillus subtiliswas the test isolates used in this study. Itwas reconstituted in malt extract broth.

Development / Standardization of Inoculum

One milliliter of the broth cultures of *Bacillus subtilis* were aseptically transferred into 200ml of appropriate freshly prepared broth and incubation followed immediately at room temperature under shaken conditions (120rpm) for 18-48hours. After which, cells were harvested by centrifugation at 4500rpm for 30minutes using an 800D model centrifuge. Harvested cells were washed thrice in sterile phosphate buffered saline and reconstituted in 10 ml sterile

deionized water producing 2.57 x106 cfu/ml *Bacillus subtilis.* These served as standard inocula for various fermentation procedures carried out in this work.

Starch Hydrolysis
This is the breaking apart of starch (polysaccharides) in reaction with water molecule to produce reducing sugar (monosaccharide, disaccharides or trisaccharides). Amylolytic fungal were employed for the hydrolysis (AOAC, 1990).

Amylolytic fungal hydrolysis (AOAC, 1990)
Amylolytic fungal hydrolysis entails the pre-treatment of the sample with amylase producing fungi which were indigenous to the sample. These amylolytic fungi comprised Aspergillus nijer, Aspergilus fumigatus and Geotrichum candidum. They were isolated and screened for amylase production usingthe method of Barnet and Hunter (1972). A consortium of these amylolytic fungi wasthen obtained and inoculated into 500ml of orange peel contained in a 500ml Erlenmeyer flask. This was allowed to stand for 48hours at room temperature for starch hydrolysis to take place. At the end of the incubation period, the reducing sugarconcentration and pH were determined by the AOAC (1990) method.

Fermentation Procedure
The orange feels were first sun dried and grinded into powdered form. Then respective standardized inocula (10ml) were inoculated into 500ml of the pre-treated samplecontained in 500ml Erlenmeyer flask as follows:
1. *Bacillus subtilis* + 500ml of amylolytic fungal pre-treated orange peel
2. The content of the flask was allowed to ferment for 8days.

Ph Determination
pH was determined using a pH meter. This was carried out on daily basis during the fermentation process to observe when the sample has reached ideal pH level for distillation. AOAC (1990).

Distillation procedure
Biofuel Production/ Estimation Distillation method was used to obtain the ethanol produced or to separate ethanol produced from the fermentation mixture. This was done by transferring the fermented sample into a ground bottom flask and distilled using soxhlet apparatus with heat energy source.
Thedistillate obtained was re-distilled and percent ethanol was estimated using the method of Berry, 1960

$$\% \text{ fuel (v/v)} = \frac{\text{Volume of distillate}}{\text{Volume of fermentation mixture}} \times 100 \%$$

Preparation of Samples for GC Analysis (AOAC 1990)
10ml of orange peel was extracted with 200ml of dichloromethane. The mixture was separated using separating funnel and the dichloromethane layer was concentrated in rotary evaporator.1ml of acetonitrile was added into the concentration and transferred into a vial ready for analysis.

Fixed Setting
Generally, the operator must adjust gas flows to the columns, the inlets, the detectors, and the split ratio. In addition, the injector and detector temperatures must be set. The detectors are generally held at the high end of the oven temperature range to minimize the risk of analyte precipitation. All of these parameters should have been set to the correct values, but double check all the instrument: Buck 530 gas chromatograph equipped with an on – column, automatic injector, Flame ionization dectector, HP 88 capillary column (100m x 0.25µm film thickness) CA, USA,Detector Temperature A: 250^0c Injector temperature: 22^0c
Integrator chart speed: 2cm/min.The temperature of oven was set to 180^0c and the GC was warmed set:

Temperature Condition

Initial Temp	Hold	Ramp	Final Temp
70^0c	5min	10min	220^0c
220^0C	2min	5min	280^0C

When the instrument is ready, the ‘’NOT READY’’ light will turn off, 1microliter of the sample was injected onto a column A using proper injection technique (syringe).

Calorimetric Determination of reducing sugar by the 3,5-dinitrosalicylic acid Method Several reagents have been employed which assay sugars by using their reducing properties. This method tests for the presence of free carbonyl group (C=O), the so-called reducing sugars.

This involves the oxidation of the aldehyde functional group present in the sample. The 3,5-dinitrosalicylic acid (DNS) is reduced to 3-amino-5-nitrosalicylic acid under alkaline conditions. The calorimetric determination of reducing sugars was done using the method described by Anamaria *et al.*, 2012.

Results:-

Concentration of Components from Orange Peels

Figure1 shows the concentration of components of biofuel produced from orange peels. Ethanol had the highest concentration with 14.0370 ppm (48.73%).

It was followed by methane with 7.7476 ppm (26.89 %), then methanol with 4.9597 ppm (17.22%). The concentration of carbon dioxide is 2.0594 ppm (7.15%). There was minute concentration of phenol 0.0015 (0.005%). This is result was obtained using gas chromatogram machine.

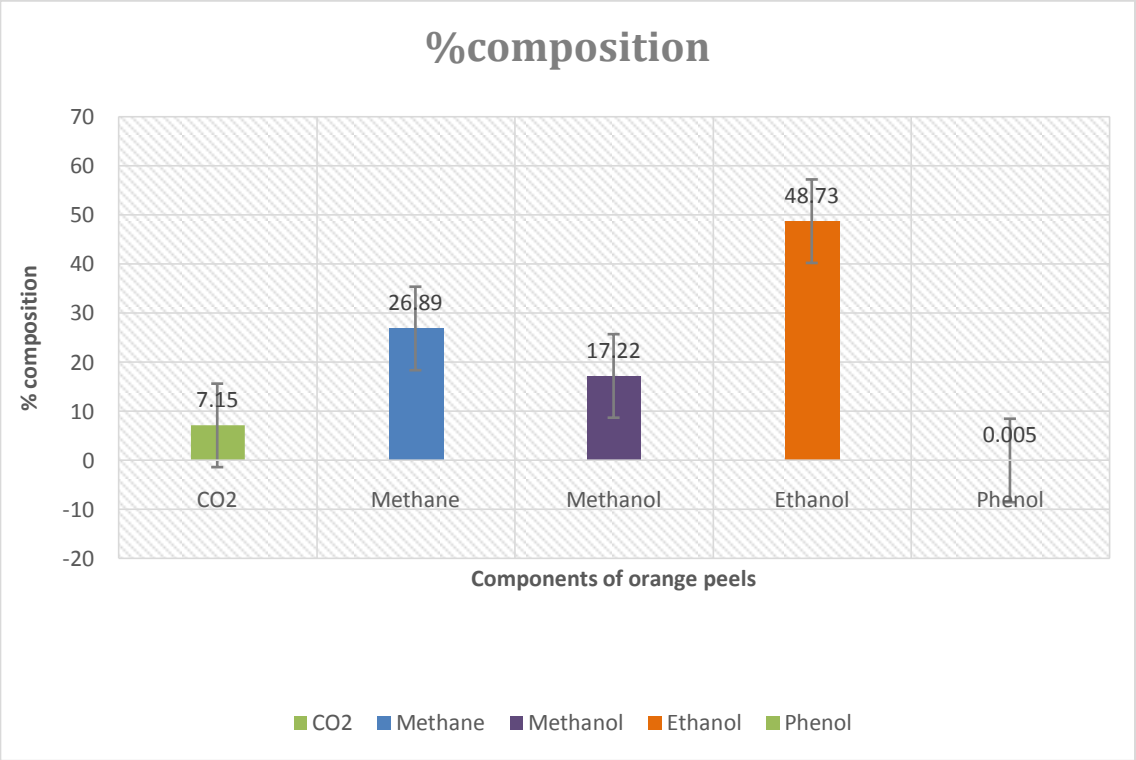

Fig 1:- Graph showing % composition of components from Orange peels

Presentation of pH and Day

Figure 2 shows the result of pH during days of fermentation of orange peels. There was increase in pH with increase in time.

pH value for Day 1 was 4.47.pH value of Day 2 was 4.54. pH value of Day 3 was 4.78. pH value of Day 4 was 5.01. pH value of Day 5 was 5.28. pH value of Day 6 was 5.48. pH value of Day 7 was 5.30, while pH value of Day 3 was 5.35.

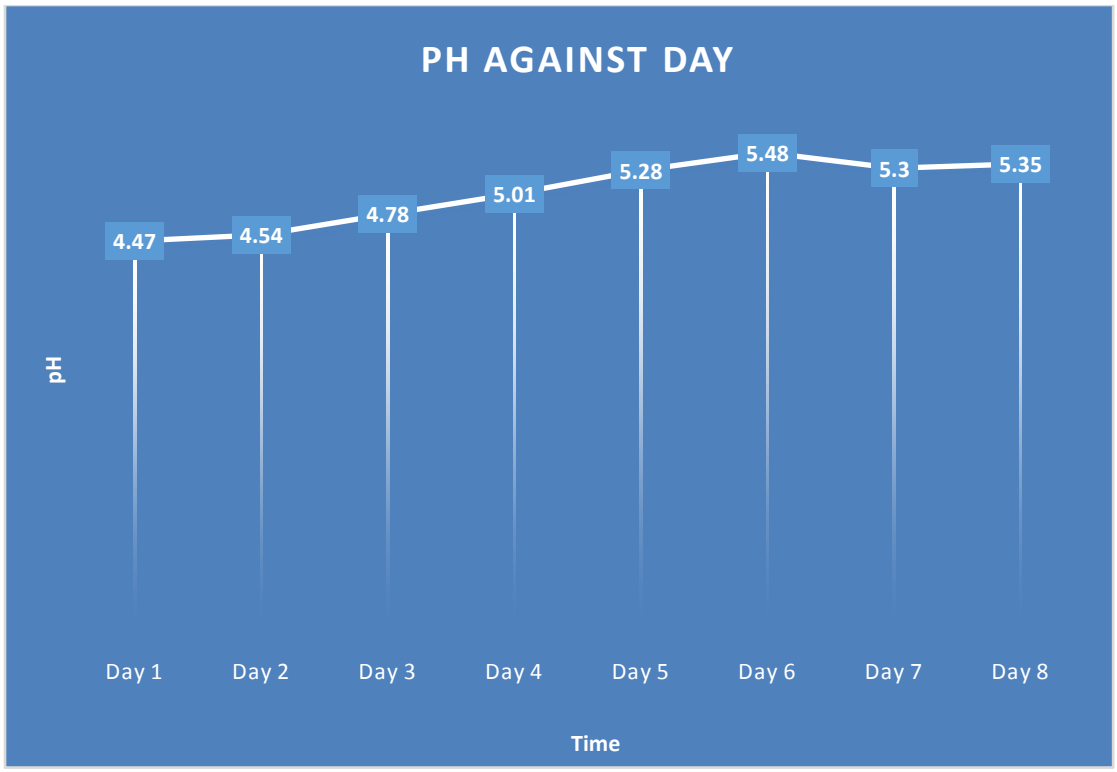

Figure 2:- Graph showing pH against Day.

Presentation of Reducing Sugar Concentration

The reducing sugar concentration of orange peels before and after fermentation is given in figure 3. The value before fermentation is 11.678 mg/l, while the value after fermentation is 73.899 mg/l.

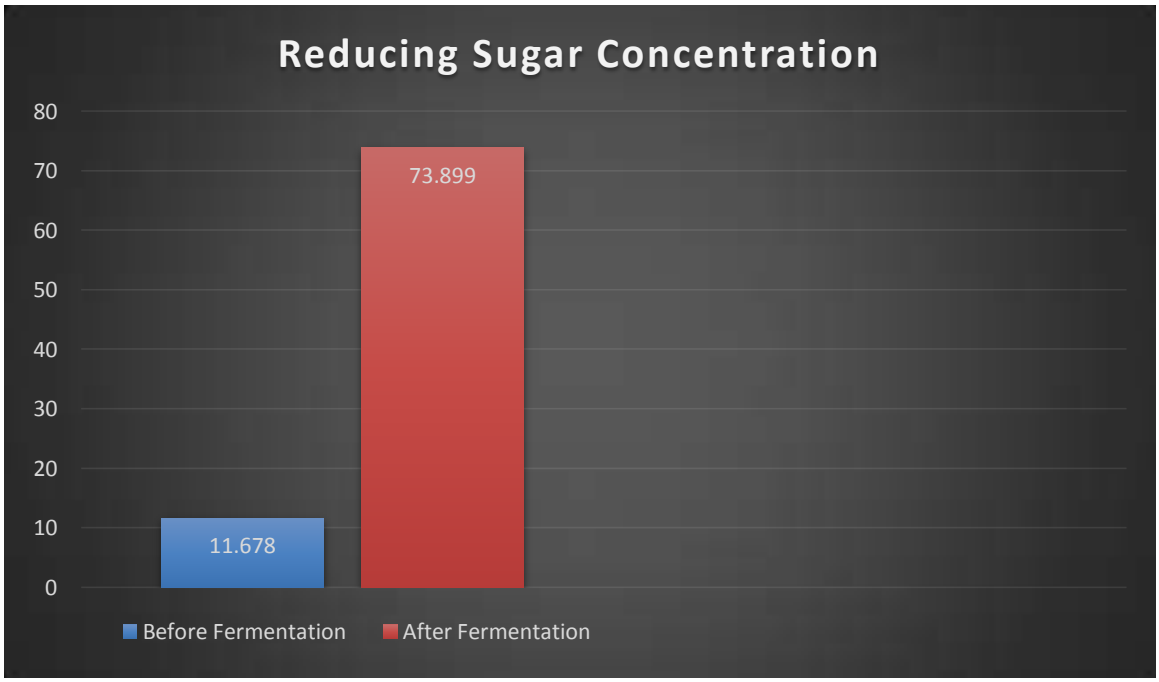

Figure 3:- Graph showing reducing sugar concentration before and after fermentation.

Presentation of Chromatogram Result of Orange Peels constituents.

Table . 4:- Gas chromatogram analysis.

Component	Retention	Area	Height	External	Units
CO_2	0.243	2040.6125	105.685	2.0594	Ppm
Methane	8.526	3059.8120	114.126	7.7476	Ppm
Ethanol	12.680	3818.6950	142.385	14.0370	Ppm
Methanol	18.446	3574.8432	133.658	4.9597	Ppm
Phenol	38.053	3515.0554	131.427	0.0015	Ppm
		16052.8432		28.8053	

Table 4 shows the result from the gas chromatograph analysis. The table shows that ethanol had the highest concentration (14.0370ppm) followed by methane (7.7476ppm). The table also showed the retention, area, height and external as shown in the figure above

Discussion:-

Production of bio-ethanol from fermentable sugars in orange peels is an alternative to utilize industrial orange peel waste and avoid disposal-associated problems, thereby making wealth out of waste. The orange peels have to undergo a period of fermentation, Patil and Dayanand (2006) reported that the period of fermentation depends upon the nature of medium, fermenting organisms, concentration of nutrients and the process physiological conditions. On studying the ability of *Bacillus subtilis* to utilize hydrolyzed orange peel wastes with no need of supplying additional nutrients to produce bioethanol in this work, it was observed that an optimum concentration of 48.73% of ethanol (biofuel), 26.89% of methane, 17.22% of methanol, 7.15% of carbon dioxide and 0.0015% of phenol was produced on the 8th day of fermentation with pH of 5.35 using *Bacillus subtillis* (Figure 4) which collaborates with the result of Manasa and Narasimhulu (2015).

It is evident from the results represented in Figure 4 that pH increased gradually with time from 4.47 to 5.35 as the fermentation process went on. This is at variance with the study of (Wilkins *et al.*, 2007) in which*Saccharomyces cerevisiae* was usedto hydrolyzed orange peel waste. The reason might be due to the microorganisms utilized for the production.

The *Bacillus subtilis* used aided the fermentation process of the orange peels by catalyzing the breakdown of the cellulosic biomass by the enzyme Cellulase. It has been observed that many bacteria could be used for fermentation. However, in this work, *Bacillus subtilis* was used and on the 8th day of the fermentation process, 48.73% of biofuel was distilled from the orange peels. A similar work was done by Wilkins *et al.*(2007) involving production of biofuel from orange peels. In his experiment, he used *Saccharomyces cerevisiae* and obtained37.1% of biofuel showing that *B substilis* was better fermenter.

Sugar concentration is also critical in fermentation process and influencing the rate of bio-ethanol production. Initial sugar concentration has also been found to determine the amount of alcohol produced (Mariam *et al.*, 2009). Orange peels contain different carbohydrate polymers which make it attractive as a raw material for production of metabolites such as ethanol by suitable microorganisms. The initial reducing sugar concentration as reported in the (Figure 4) was 11.678mg and after fermentation increased to 73.899mg/l. This collaborates with the work of Mrudula and Anitharaj (2011), in their experiment, they used *Bacillus subtilis* to produced biofuel from orange peels and obtained an initial reducing sugar of 10.989mg/l which increased upon fermentation to 62.978mg/l.

The starch hydrolysis performed was to aid the breaking down of the starch content of the orange peels in reaction with molecule containing water. A similar work was done by Manasa and Narasimhulu (2015), involving production of bio-ethanol from orange peels. In their experiment they used fungus *Mucor indicus* to produce bio-ethanol from both acid hydrolyzed and enzymatic hydrolyzed orange peels. The maximum ethanol yields were 0.36 g/g and 0.33 g/g respectively. The maximum ethanol yield was achieved after about 24 h in cultivations performed on both acid hydrolyzed and enzymatic hydrolyzed orange peels. However, in this study, the maximum ethanol yield is 14.0370 ppm and this was achieved within 8 days of fermentation and 6h distillation in cultivation performed in amylolytic fungal hydrolysis.

Conclusion:-

Orange peels have the potential to produce biofuel which is cheap, environmental friendly, readily available and also has the capacity of providing employment for youth to make wealth out of waste.

Biofuels are a reliable alternative energy resource but more research is needed to make them suitable for widespread consumption. With the right technology and production method, large quantity of biofuel will be produced from wastes like orange peels, and consumers can then begin to enjoy all the benefits of this sustainable, renewable energy source.

It is therefore recommended that further investigations be carried out on finding efficient processes and methods to increase yield of biofuels from orange peels. Government and other multinationals should invest in turning waste like orange peels to wealth like bioethanol. Government should provide means of educating farmers on the potentials of making biofuel from their waste Government should build recycling plants to enhance the biofuel production.

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