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Research

Developing a Green Method for the Production of Bioenergy from Organic Wastes

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Abstract: Background: Bio-ethanol is a renewable, clean-burning liquid fuel with minimal environmental impact compared to petroleum-based alternatives. It can be produced from various organic wastes using either chemical or biological methods. While ethene from fossil sources can be hydrated to form ethanol, fermentation of sugar-rich feedstocks remains a viable alternative.

Purpose: This study aims to produce bio-ethanol from agricultural residues and organic wastes, specifically rice husk, biochar, Spirogyra, and seaweed using acid and base hydrolysis.

Methods: Rice husks were sourced from Effium Rice Mill, Ohaukwu (Ebonyi State, Nigeria), with a portion converted into biochar via pyrolysis at the Alex Ekwueme Federal University Ndufu-Aliki Ikwo Biotechnology Laboratory. Spirogyra and seaweed were collected from Okpuitumo Dam, Ikwo (Ebonyi State), thoroughly washed, oven-dried, pulverized, and sieved to uniform particle size (250 mm). Samples were characterized to determine lignin, cellulose, hemicellulose, and lignocellulose content. Pretreatment, hydrolysis, saccharification, and fermentation processes were conducted to evaluate ethanol yield.

Results: Acid hydrolysis using 1 mol/dm^3 hydrochloric acid yielded the highest ethanol output (59.53%) compared to 1 mol/dm^3 sodium hydroxide. Among the substrates, seaweed and Spirogyra showed the highest bio-ethanol yields at 59.53% and 41.88%, respectively.

Conclusion: Algal biomass, particularly seaweed and Spirogyra, represents a high-potential, underutilized resource for bio-ethanol production. Acid-mediated hydrolysis is especially effective and should be further explored for sustainable biofuel generation.

Keywords: Bio-ethanol, Hydrolysis, Saccharification, Pretreatment, Biomass, Fermentation.

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Introduction

Biomanufacturing is an aspect of biotechnology, particularly white biotechnology that involves the manufacturing of products of value such as biofuels, biofertilizers and biopesticides which is usually mediated by biological agents.[1] Biological systems are used in biomanufacturing to create economically viable biomaterials for use in industrial applications, biotechnology, medicine, and the food and beverage sector.[2] The last century has witnessed a dramatic increase in population and consequently rises in energy demand simultaneously. The rising issues of carbon (iv) oxide emissions which is as a result of energy consumption, together with the predicted future scarcity of petroleum-based fuels, has raised some concerns and strengthened the interest in alternative, non-petroleum- based sources of energy that are renewable, sustainable, efficient, cheap, easy and safe.[3] Utilising sustainable lignocellulosic biomass to produce biofuels is one potential solution to energy and environmental issues.[1] Like other matters biofuels exist in three phases, they can be solid, liquid or gaseous and are obtained directly or indirectly from biomass, which may be of plant or animal origin.[1] Bioethanol is a renewable and sustainable fuel which does not create negative environmental impact, unlike conventional fuel.[4] Bioethanol production requires a lot of application of technologies such as fermentation; which involves biological conversion of biomass and organic matter such as energy-rich crops, and lignocellulosic biomass.[5] Greenhouse gas emissions have significantly decreased the use of sustainable biomass resources to produce biofuel, such as bioethanol; they also present appealing solutions to reduce greenhouse gas emissions, which will lessen dependency on petroleum-based oil imports, address the issue of energy security, boost rural and agricultural economies, and increase the sustainability of the global transportation system.[6] Microbial fermentation can be used to produce a variety of valuable products for the chemical and pharmaceutical industries from organic by-products, in addition to biofuel.[5] Bioethanol feedstock’s can be divided into three major groups: (1) sucrose containing feedstock’s (e.g. corn, Milo, wheat, sorghum and fruits), (2) starchy materials (e.g. corn, Milo, wheat, rice, potatoes, cassava, sweet potatoes and barley), and (3) lignocelluloses biomass (e.g. wood, straw, and grasses).[7] According to Ballesterosa et al., the majority of first-generation bioethanol production processes currently in use sugarcane juice and cereals (corn or grain) as more readily degradable biomass feedstock.[7] However, there is a significant conflict between the production of food and feed and the use of these crops only for energy.[6] Lignocellulosic biomass, such as agricultural crop residues, waste from forests and wood industry, organic materials found in municipal solid trash, and energy crops, can be converted into second-generation biofuel using thermochemical or biochemical processes.[7] Cellulosic ethanol (CH_3CH_2OH) and cellulosic butanol ($CH_3CH_2CH_2CH_2OH$) are the common examples produced from organic wastes through biochemical processes.[3]

Bioethanol can be used as an alternative source to conventional fuel for transportation.[4] A distilled colorless liquid fuel, bioethanol is made from a variety of possible feedstocks, including sugar beet, wheat, corn, cassava, fruit, barley, molasses, potatoes, sorghum, switch grass, and cellulose biomass like straw, wood paper, and other cellulosic wastes like grasses.[3] Apart from ethanol being a potential fuel alternative it also offers a sustainable economy by reducing the reliance on petroleum-based fuel, and more importantly, it emits neutral carbon (iv) oxide and ethanol boost economy by providing value-added market opportunities for the agricultural sector.[8] Therefore, this study sought to fill the research gap for ethanol production from agricultural wastes and residues (rice husk, biochar, spirogyra, and seaweed) using chemical methods (acid and base hydrolysis).

Bioethanol Production Processes Overview

The four main unit operations are; pre-treatment; hydrolysis, fermentation, and distillation/purification are needed to produce ethanol from cellulose feedstocks.[9] In order to convert lignocellulosic biomass into bio-ethanol, pre-treatment is an essential step. In order for the enzymes that transform the carbohydrate polymers into fermentable sugars to reach cellulose, it is necessary to alter the structure of cellulosic biomass.[10] A successful biomass pre-treatment breaks down the lignocellulose structure (Fig. 2), increasing the accessibility of the carbohydrates during the enzymatic saccharification process.[11]

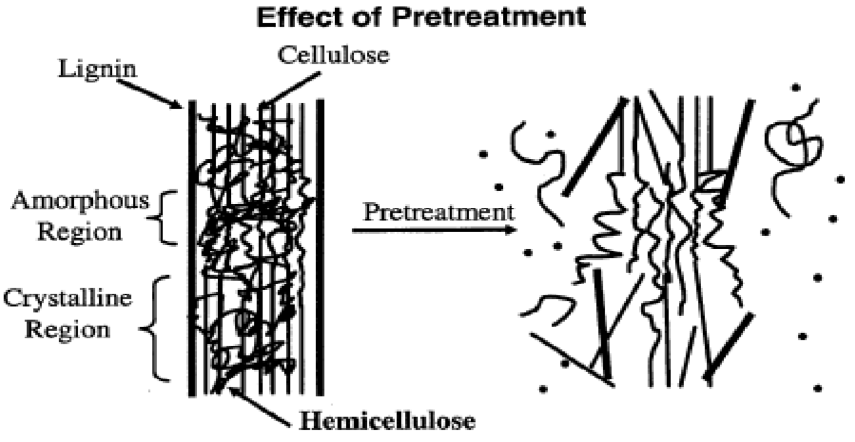

Figure 3: Schematic representation of effect pretreatment on lignocellulosic biomass, source.[12]

Biological, physical, and chemical procedures are the three types of pre-treatment technologies for lignocellulose biomass.[13] Hemicellulose and lignin are typically broken down by microorganisms like white, brown, and soft rot fungus in biological pre-treatment.[9] According to Kirk, the benefits of biological pre-treatment include low energy costs, no chemical usage, minimal capital costs, and gentle operating conditions.[14] Comparing the biological pre-treatment procedure to other pre-treatment technologies, the primary disadvantage is the sluggish rate of hydrolysis that is achieved.[9] Despite its examination on various lignocellulosic biomass, fungi like *Pleurotus ostreatus* and *Cyathus stercoreus* are far from being a viable pre-treatment method for commercial use due to their slow growth rate and low efficiency.[15] The process of hydrolysing cellulosic materials results in the production of monomeric sugars from the carbohydrate polymers cellulose and hemicellulose.[16] Cellulases or acids like sulphuric acid can catalyse the cleavage of these polymers chemically or enzymatically.[17] The amount of lignin and hemicellulose present, cellulose fiber crystallinity, and porosity or accessible surface area all influence how easily cellulosic biomass hydrolyses.[18]

Materials and Methods

The chemicals utilized included: Sodium Hydroxide (NaOH), Hydrogen Chloride (HCl), Sulphuric acid (H_2SO_4, (98%) (PVS Chemical Inc), Dinitrosalicyclic acid (DNS) (Thermo Scientific Chemicals), Sodium Metabisulphite ($Na_2S_2O_5$), Potassium Sodium Tartrate (Univar Solutions and E&C Chemicals Inc), Dextrose sugar (Cargill), Yeast extract (Lallemand Inc), baker's yeast *(Saccharomyces cerevisiae*).

The equipments utilized included: Weighing balances (S.METTLER), Sieves (250mm), Shaking Incubator (MaxQ^{TM}), Vertical Autoclave (Systec GmbH & Co. KG), pH indicator (MilliporeSigma), Electro-thermal Ovens Spectrophotometer, Burette (DWK Life Science), Conical flasks (Pyrex), Beakers (TLG^{TM}), Funnels, Test tubes, volumetric flask, Whatman filter paper, vacuum pump (KNF), Petri dish and Buckner's flask

Methods

Sample collection and preparation

The waste(s) were collected from rice husks at Effium rice mill Ohaukwu in Ebonyi, Eastern Nigeria and biochar was obtained from the biology laboratory AE-FUNAI, seaweed and spirogyra were collected from Okpuitumo Dam Ikwo, in Ebonyi Eastern Nigeria. The samples were taken to biotechnology laboratory AE-FUNAI. The seaweed and the spirogyra were thoroughly washed and then oven-dried, then a clean dry mortar and pestle was used to pound the samples followed by sieving with a 250mm mesh size sieve to obtain uniform sizes of the samples.

Characterization of Wastes

To identify and examine the extracts, approximately 1g of sample filtrate was weighed, boiled with 110mL for 90 minutes, filtered and dried overnight at 60˚C. It was then soaked with ethanol and boiled for 90 minutes, filtered and washed with ethanol and water, then dried again overnight at the same temperature.

Determination of Cellulose Content

The sample extracts were treated under reflux for one hour in four cycles using an alcoholic nitric acid solution. Following each cycle, the solution was removed for a fresh volume. To generate the alcoholic nitric acid solution, four litres of 97% pure alcohol were mixed with one volume of 68% (w/w) nitric acid solution. Finally, the cellulose was dried, cleaned, and weighed. Cellulose (%) = W1-W2/W1*100

W1 = oven-dried sample

W2 = extractable residue

Determination of Lignin Content of the Substrate

A flask containing an extractive-free sample was filled with 72% sulfuric acid. The flask was placed in a water bath set at 30 degrees Celsius for an hour while the substance was being dispersed. After that, add deionized water and autoclave for one hour at 121 degrees Celsius. The insoluble substance (lignin) was then purified using vacuum filtration. After washing the lignin with hot water until it was acid-free, it was dried and weighed[15].

Lignin (%) = W_1-W_2/W_1*100

W_1 = oven-dried sample

W_2 = extracted residue

Determination of Hemicellulose Content

The calculation of the cellulose content was based on the assumption that the sole components of the total biomass are cellulose, hemicellulose, lignin, ash, and extractives [17].

$100 = W_C + W_H + W_E + W_L$

$W_H = 100 - W_C + W_E + W_L$

Determination of Moisture Content of the Substrate

After weighing an empty Petri dish, 1g of samples was put within. The dish was kept in a drying oven set to 105°C for 90 minutes. The dish was taken out of the oven with a tong and allowed to cool in a desiccator before being weighed. Following weighing, the moisture content was calculated using the following procedure. Moisture content (%) =

$$\frac{(W_1 - W_2) \ \text{x}100}{W_s}$$

Where W_1 = Weight of Petri-dish before oven drying + weight of sample

W_2 = Weight of Petri-dish after oven drying + weight of sample

W_s = Weight of sample

Reagent Standardisation

- Preparation of Acid Concentration (0.5M and 0.1M) of HCl

  To create 0.5mol/dm3 and 1mol/dm3 of HCl, respectively, 10.74mL and 21.48mL of the stock of HCl were measured into a 250mL volumetric flask after a little amount of distilled water was added.

a. Preparation of 0.5M HCl in 250 mL volumetric flask. The concentration of the stock solution was calculated as follows

$$\text{Molarity} = \frac{\text{Specific gravity x \% purity x 100}}{\text{Molar mass x volume of the flask}}$$

Given, specific gravity of HCl = 1.18

Percentage purity = 36%

Molar mass = 36.5g/mol^{-1}

$$\text{Molarity} = \frac{1.18 \text{ x } 36 \text{ x}1000}{36.5 \text{ x } 250}$$

$$= 11.64\text{mol/dm}^3$$

Therefore, the concentration of the solution was 11.64mol/dm^3; dilution formula was applied to get the volume required to prepared 0.5mol/dm^3 of HCl in 250 mL volume flask from the stock solution.

$C_1V_1=C_2V_2$

Where; C_1 = 11.64mol/dm^3, V_1 =volume of the stock?

$C_2=0.5\text{mol/dm}^3$, V2=250mL

$$\text{Therefore, V1} = \frac{0.5 \text{ x } 250}{11.64}$$

= 10.74mL

For the 1 mol/dm^3 of HCl

C_1 = 11.64mol/dm^3, V_1= volume of the stock?

C_2 = 1 mol/dm^3, V_2= 250mL

Therefore, $$V_1 = \frac{1 \times 250}{11.64}$$

= 21.48mL

In a 250 mL volumetric flask, 10.7 mL of the HCl stock solution was diluted with distilled water and built up to the appropriate level with distilled water to create 0.5mol/dm3 of HCl. In a 250 mL volumetric flask, 21.48 mL of the HCl stock solution was diluted with distilled water to create 1mol/dm3 of HCl.

- Preparation of 5% hydrochloric acid (HCl) Solution

In order to make 5% HCl from the stock solution, 5 mL of the HCl stock solution was measured into a volumetric flask, and it was thoroughly agitated for approximately 5 minutes after a little amount of distilled water was added.

Solution = solution + solvent

5% HCl = 5mL of HCl + 95mL of distilled water

- Preparation of 0.5mol/dm^3 of Sodium Hydroxide Solution

$$\text{Mass of NaoH} = \frac{\text{Molarity x molar mass x required volumetric flask}}{\text{Volume (dm3)}}$$

$$= \frac{0.5 \times 40 \times 250}{1000}$$

= 5g of NaoH pellet

- To prepare 1mol/dm^3 of sodium hydroxide (NaoH) solution

$$\text{Mass of NaoH} = \frac{\text{Molarity x molar mass x required volumetric}}{\text{Volume (dm3)}}$$

$$\frac{1 \times 40 \times 250}{1000}$$

= 10g of NaoH pellets

- Weighing out 5g and 10g of NaOH pellets, we poured tiny amounts of water to each beaker to dissolve them. The solutions were built up to the proper level after being moved into two distinct volumetric flasks.
- Preparation of 5% Sodium Hydroxide Solution

A 100mL volumetric flask was filled with around 5g of sodium hydroxide pellets. A little amount of water was then added, and the mixture was shaken vigorously until it was completely dissolved. More distilled water was then added until the desired level was attained**.**

Aspergillus niger Screening and Isolation from Soil Samples to Produce a Clean Culture of the Fungus

Approximately 3.9g of potato dextrose agar (PDA) was dissolved in 100mL of distilled water and sterilized at temperature of 121°C psi

a. Approximately 2g of the soil sample was dissolved in 10mL of distilled water and followed by serial dilution of you solution formed.
b. The sterilized medium was poured into sterilized Petri dishes and allowed to gelled
c. Approximately 0.1mL of the 10^{-4} and 10^{-5} was pipetted into the center of the surface of the gelled PDA and L-shaped glass spreader was dipped into alcohol and then flamed over burden burner
d. The glass spreader was used to spread the sample on the surface of the medium
e. Plates were incubated at 25°C for 72 hours
f. To isolate the pure culture of *Aspergillus niger* by streak plate technique.

Approximately 3.9g of Potato dextrose agar was dissolved in 100mL of distilled water in 250mL conical flask.

a. The medium was autoclave for 20 minutes at 121°C psi along side with glass Petri dishes
b. The medium was poured into the aseptically into the Petri dishes and allowed to gel
c. A flamed wireloop was used to scoop a colony and streak it on the surface until it thins out the fungi and allowed to incubate for 4 days.

Enzyme Dosage Fermentation

It is possible to stimulate Aspergillus niger to enhance cellulase production extracellularly by adjusting the media, even though the fungus intrinsically produce this enzyme but can be induced for effective production. Within a 500mL conical flask (fermenter), 0.2g of yeast extract, 0.5g of sodium trioxonitrate (v), 0.1g of potassium orthophosphate, 0.05g of magnesium sulfate, 0.0001g of ferric chloride, and 1g of carboxymethyl cellulase were added. The mixture was then homogenized and sterilized for 15 minutes at 121oC psi. After adding about 40 milliliters of distilled water to the microbial culture to create a submerged solution, it was syringed into my medium and cultured for seven days. Buckner's apparatus, which included a vacuum pump and a filter, was used to harvest the enzyme. The filtrate was then collected in a sterile container and placed in a refrigerator.

Sample Saccharification

Various quantities of sodium hydroxide (0.5M and 1M), cellulase (a hydrolytic enzyme), and hydrochloric acid (0.5M HCl and 1M HCl) were used to saccharify the samples. For each acid and base concentration, about 70 mL were measured and appropriately labeled in a separate conical flask (the volume was selected so that it will not dry out on heating). It was filled with around 5g of each sample, and it was shaken vigorously to dissolve the samples. Before the reactors were put into the oven, which was preheated to 100˚C, they were corked with aluminum foil. It was removed after the allotted time of one, two, and three hours and neutralized using 12% NaOH for NaOH and 12% Na0H for HCl. Whatman filter paper was used to filter the solution before it was transferred into various conical flasks. Using 1 milliliter of the filtrate and 9 milliliters of water, the samples were diluted (dilution factor = 10). Place around 3 mL of each of the diluted solutions into test tubes with clear labels. Then, add 3 mL of DNS reagent, along with the blank (control). After covering the samples with aluminum foil, they were submerged in a water bath set at 90˚C for ten minutes. The pure isolate of Aspergillus spp. was reconstituted using distilled water and injected with the hot liquid water pre-treated samples during biological hydrolysis. The samples were then cultured for seven days at 25 °C. Conical flasks, the fermenter, and every piece of equipment used for fermentation were autoclaved to ensure they were sterile. For fifteen minutes, the sterilizing process was conducted at 121 degrees Celsius under pressure.

Each acid's undiluted filtrates were kept in a separate container based on the kind of acid. The amount of simple sugar in these filtrates was measured by diluting 0.2 mL of the filtrate in 10 mL of water (dilution factor = 50) after they had been heated to concentrate them.

It was filled with around 3 mL of DNS and heated to 900 °C for 10 minutes. At 540 nm, the absorbance was measured with the UV spectrophotometer. For the fermentation procedure, the yeast Saccharomyces cerevisiae was employed. After aseptically adding 2.74g of baker's yeast to each sample, along with a pinch of glucose dissolved in warm water, and vigorously stirring it, the yeast was immediately transferred into the concentrated extracts and stirred some more. It was then covered with aluminium foil and incubated for three days at 25°C.

With the heating mantle securely attached to the power supply, the distillation apparatus was put together. In the setup, the heating mantle was set at 60 degrees Celsius, and the sample was contained in a round-bottom flask.

Statistical Analysis

The impact of the different types of acids and their concentrations on the synthesis of bioethanol was compared using IBM Statistical Package for Social Science (SPSS) version 23.0. A 5% threshold for statistical significance (p value < 0.05) was established.

Results

3.1. Table 4: Waste Characterisation

Waste	Moisture	Lignin	Cellulose	Hemicellulose
Rice husk	9.0	5.0	61.3	18.0
Biochar	7.0	4.3	24.9	41.0
Seaweed	0.05	5.0	61.3	18.0
Spirogyra	9.5	4.1	29.3	32.5

According to the results of the gravimetric characterisation of the samples in Table 4, the highest cellulose concentration is found in rice husk and seaweed, whereas the highest hemicellulose content is found in charcoal and spirogyra. The amount of simple sugar generated under the experimental conditions was determined using a visible UV spectrophotometer, as shown in Table (5). 59.53% bioethanol was obtained from 1M HCl seaweed hydrolyzate, which had the maximum absorbance of 1.598.

Table 5: Levels of glucose in the samples in relation to acid and waste source Concentration

S/N	**WASTE(S)**	Hydrochloric acid		Sodium hydroxide	
		0.5M	**1 M**	**0.5M**	**1 M**
1.	Rice husks	1.335	1.579	1.069	1.105
2.	Spirogyra	1.568	1.299	1.055	1.696
3.	Seaweed	1.449	1.597	1.190	1.233
4.	Biochar	1.211	1.487	1.019	1.426

Using 1mol/dm^3 and 0.5 mol/dm^3 of hydrochloric acid and 1 mol/dm^3 and 0.5 mol/dm^3 of sodium hydroxide, the analysis's findings (Fig. 4) examined the impact of acid and base concentration on the hydrolysis-based synthesis of bio-ethanol. Seaweed was found to create the most bioethanol at varying doses utilizing the chemical approach (acid and base). GC-FID was used for additional measurements on the samples (see appendix 1). The GC result verified that the samples contained ethanol.

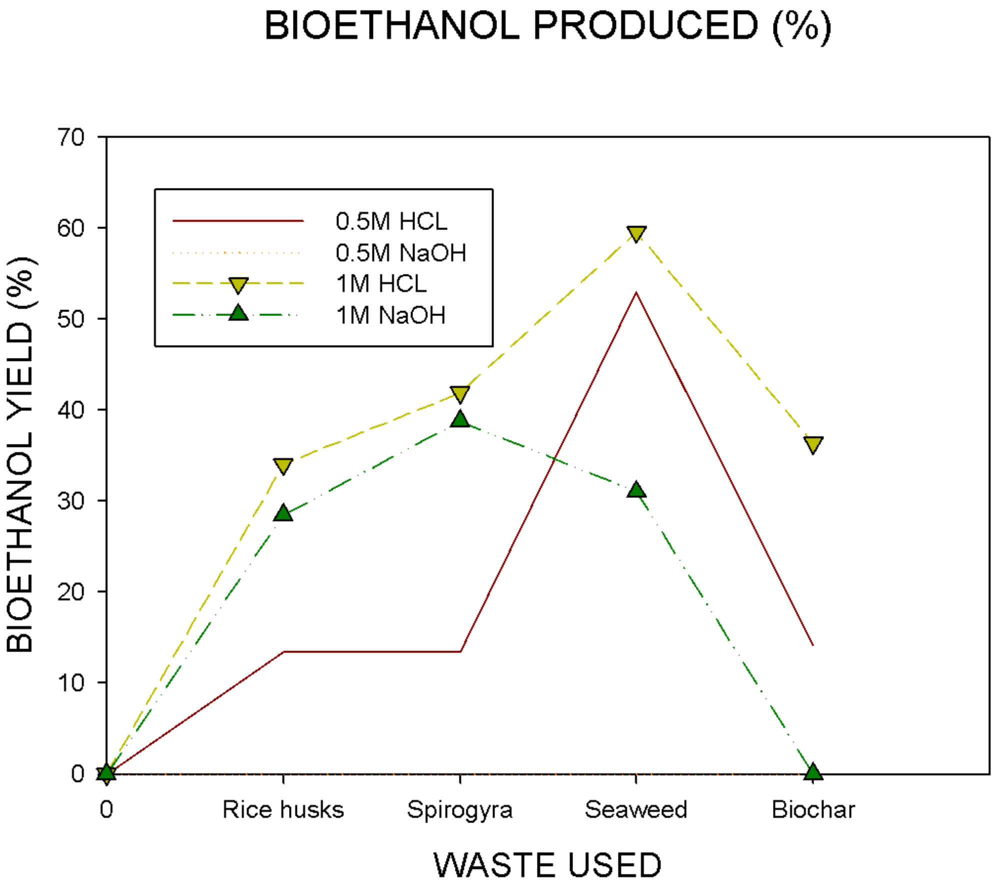

Figure 4: The percentage of ethanol produced from organic waste

Discussion

Using chemical techniques of acid and base mediated hydrolysis, the study sought to produce ethanol from agricultural wastes and residues (rice husk, charcoal, Spirogyra, and seaweed). An alga (seaweed and Spirogyra) was found to have the best production of bio-ethanol among the organic wastes employed in this study, and the acid (1 mol/dm^3 HCl) produced the largest percentage of bio-ethanol when compared to the base (1 mol/dm^3 NaOH). Of the four wastes employed in this investigation, the maximum concentration of glucose was seen at 1M of acid at 160°C. Additionally, the analysis of variance showed that ethanol generation rose as the concentration of acid (HCl) increased. The results are consistent with those of Krassig and Schurz, who found that employing Spirogyra as the substrate and pre-treating and hydrolyzing it with hydrochloric acid produced the highest percentage yield of ethanol from organic wastes of glucose.[1] This shows that complex carbohydrates in organic biomass can be broken down into fermentable sugars more easily in acidic environments. The generation of various intermediate and inhibitory products, which results in a decreased glucose yield, is likely the reason why the glucose concentration was shown to decrease at lower acid concentrations as time and temperature increased.[2] The various sample components found in the lignocellulose biomass used in this study were identified by successive chemical extraction using Sun and Cheng's method in order to perform gravimetric analysis.[3] Agricultural wastes and residues have cellulose, hemicelluloses, and lignin concentrations that are consistent with the previous thorough report published in the literature by Shi et al.[4] However, the cellulose and hemicellulose contents in this study are comparatively larger than the lignin concentration. The highest bio-ethanol yields were produced by algae, namely seaweed and Spirogyra, out of all the organic waste kinds that were tested. Therefore, seaweed had the largest potential for producing ethanol (59.53%), followed by charcoal and Spirogyra, whereas rice husk produced the least amount. This suggests that algal biomass that is rich in polysaccharides such as cellulose starch and agar is ideal for producing ethanol, particularly when hydrolyzed in an acidic environment.[1] The comparatively low lignin content of algae in comparison to lignocelluloses such as rice husk, which can obstruct hydrolysis and subsequent fermentation, may also be responsible for this high yield. The findings of this study shed important light on how well the chemical hydrolysis process produces bio-ethanol from various organic wastes. Investigations on the synthesis of bio-ethanol from various wastes, particularly micro-algae, are necessary. Additionally, research should be done on producing bio-ethanol using the biological process (alone). This is due to the biological method's lower cost and lack of an inhibitory intermediate.

Conclusion

Acid hydrolysis is a more effective pre-treatment technique than base hydrolysis for turning organic waste into bio-ethanol, according to this study. Additionally, compared to more lignocellulose feedstock like rice husk and charcoal, algal biomass has better potential for producing bio-ethanol sustainably. This implies that expanding the focus of biofuel research and development to include freshwater and marine algae as renewable feedstock sources could be beneficial.

Declaration of competing interest

The author affirms they have no conflicting interests.

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Authors' contributions

Nwankwo, Samuel Chiemerie prepared the study's design, the idea and statistical analysis, and also collected the data; the literature search, manuscript preparation, and editing was completed by Nwankwo Samuel Chiemerie. The author examined and approved the manuscript.

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Availability of data and materials

The primary information bolstering the study's conclusions was the GC FID result, of which the sample was supplied at Spring Board Awka and the analysis was done at the Project Development Institute Emene in Enugu.

Consent for publication

Not applicable.

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