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Research article

ASSESSING THE EFFICIENCY OF SACCHAROMYCES CEREVISIAE AND SACCHARAMYCES CARLSBERGENSIS IN THE FERMENTATION OF AQUATIC WEEDS

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Abstract

In a batch fermentation experiment, the efficiency of yeast: *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* was determined using *Eichhornia crassipes, Pistia stratiotes* and *Salvinia molesta* as fresh water biomass. The yeasts were activated before inoculation and fermentation. The result show that ethanol yield of the *E. crassipes, P. stratiotes* and *S.molesta* fermented with the composite mixture of *S. cerevisiae* and *S. carlsbergensis* were 3.0g/L, 2.3g/L and 2.0g/L respectively, indicating higher yield compared to when the biomass were fermented with either *S. cerevisiae* or *S. carlbsergensis.* However, slight variation were observed when *S. cerevisiae* was used, resulting to 0.8g/L for *E. crassipes*, 0.5g/L for *P. stratiotes and* 0.4g/L for *S.molesta.* Similarly, the productivity rates of the biomass fermented with the same mixture were 0.05g/L/h, 0.004g/L/h and 0.003g/L/h. And the values change to 0.001g/l/h, 0.0009g/l/h and 0.0007g/l/h when *E. crassipes, P. stratiotes* and *S.molesta* were fermented with *S. cerevisiae* respectively. The same trend was observed with *S. carlbsergensis* when used alone. Generally, the paper shows that synergistic effect of the yeast influences an increase in the ethanol yield. The study concludes that *S.cerevisiae* and *S.carlsbergensis* are more efficient when used in synergy for the fermentation of fresh water biomass than when single yeast was used.

Key words: Fermentation, Aquatic weed, Freshwater, Yeast.

1.0 Introduction

Fossil fuel combustion continues to cause significant build-up of carbon dioxide in the atmosphere (Sunita and Narayan, 2010). Global warming and climate change has been attributed to high concentration of carbon dioxide in the atmosphere (Dominic and Rainer, 2007; Nelson, 2011). The eminent ecological threat resulting from human addiction to oil and other non renewable fuels has necessitated a search among governments and academia for alternatives (Dominic and Rainer, 2007). One of the innovative technologies is the use of bio-ethanol as an alternative fuel in the transportation sector (World Energy Council, 2007). Bio-ethanol is a fuel made from plants and is a clean burning fuel that makes no net contribution to global warming (Dominic and Rainer, 2007). This is because the carbon dioxide produced from the combustion for ethanol is consumed by plan growth and thus, maintains the carbon cycle balance in nature (Macedo, 2004).

A number of plants species are used for the bio-conversion to ethanol, among them are; marine and freshwater weeds and terrestrial plants (Dominic and Rainer, 2007). Aquatic weeds or freshwater biomass: waterhyacinth (*Eicchornia crassipes),* water lettuce (*Pistia stratiotes)* and water fern (*Salvinia molesta*) are considered for this study. Aquatic weeds are plants which grow and complete their life cycle on water and causing harm effects to the aquatic environment (Sooknah and Wilkie, 2004). They are found on the surfaces of water bodies and are readily available in northern part of Nigeria and as such do not compete with crops grown on land for space and nutrients. While they are considered as noxious weeds in many part of the world as they grow fast, depletes nutrients and oxygen from water bodies affecting flora and fauna, converting them to produce bio-ethanol could significantly address the environmental issues and stimulate the buildup of a local renewable energy supply chain.

In the light of this, this paper seeks to use two micro organisms: *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* for the production of bio-ethanol from waterhyacinth (*Eicchornia crassipes),* water lettuce (*Pistia stratiotes)* and water fern (*Salvinia molesta*). The paper also evaluated the efficiency of the composite mixture of the micro organisms on the fermentation processes with a view to determine the most effective one.

Saccharomyces species are facultative anaerobes and under anaerobic condition can ferment glucose to ethanol (Sherman, 2002). *Saccharomyces cerevisiae* is the world's most important yeast and has been a very useful fungus in bread making and other purposes (Alves and Castilho, 2005). It is also known as top yeast and is known to be efficient in fermenting hexoses (Alves and Castilho, 2005). *Saccharomyces carlsbergensis* is considered as lager yeast or bottom yeast, often found in areas where fermentation occurs, such as the surface of fruits (Sherman, 2002). Infrared spectroscopy is an extremely effective method for determining the presence or absence of a wide variety of functional groups in a molecule. (John, 2000). This technology was used in this study evaluated the by-products of the fermentation to determine the presence of alcohol group and other important organic compounds present in the fermented samples.

2.0 Materials and Methods

The fresh water Biomass: *Eichhornia crassipes, Pistia stratites* and *Salvinia molesta* were sampled from Ahmadu Bello University Dam and Hanwa Dam within Kaduna State. The aquatic plants were thoroughly washed with tap water to remove adhering dirt and were chopped into small pieces using sharp knife. The plants were dried separately in an oven at 105^0C for six hours and subsequently pulverized using motar and pestle (Galbe and Zacchi, 2007).

2.1 Hydrolysis

10 g of each dried pulverized plant sample was weight separately using electronic weighing balance and placed into a 250 cm^3 conical flask, 10% sulfuric acid was added and made up to 150 cm^3. The mixture was autoclaved at 121^0C for 15 minutes and was then filtered using whatman filter paper to remove the unhydrolysed materials (Carvelheiro *et al.,* 2008).

2.2 Hydrolysate Detoxification and Fermentation

The hydrolysate of each Biomass sample was heated to 60^0C (for dissolution) then basified with Na0H by adding 2.0 g starting with 0.5 g at interval and measured with a pH meter till it reaches pH 9.0 - 9.5. 1.0 g of $Ca(OH)_2$ was added to the solutions to detoxity harmful materials present in the hydrolysate and filtered to remove insoluble

residues. The filterate was used as fermentable sugars (Martinez *et al.*, 2000). 2.0 g of peptone water was added to the previously detoxified hydrolysate and the pH was adjusted to 5.6 by adding 10% sulfuric acid (H_2S0_4). The medium was sterilized by autoclaving at 121^0C for 15mins. The yeast (*Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis)* were inoculated into the medium and fermented by incubating for 3 weeks at 30^0C (Standbury and Whittaker, 1984). The fermented medium was aliquoted after 7 days, 14 days and 21 days interval and distilled to assay ethanol content. Ethanol content was determined by Dichromate assay: 7.5 g of potassium dichromate was dissolved in dilute sulfuric acid and the final volume was adjusted to 250 cm^3 with deionized water; and the maximum absorbance was recorded at 590nm with a multiwavelenths spectrophotometer.

2.3 Fourier Transform Infrared (FTIR) Analysis

The fresh water biomass (*E. crassipes, P. stratiotes* and *S. molesta)* were subjected to infrared spectrophotmetric analysis to detect functional groups other than –OH of the ethanol that may be present in the weeds fermented samples. Fourier Transform Infrared Spectrophotometer (FTIR) Analysis: The functional groups present in all the three aquatic weeds were characterised by a Fourier transform infrared (Perkin Elmer FTIR Spectrometer: Spectrum RX1). The determination of the types and distribution of functional groups present in the all the samples was carried out to facilitate a better understanding of the nature of the sample and to possibly aid identification of the functional groups (Jin and Bai, 2002). This was achieved by matching the wavelength of light absorbance to the type of bond or functional group that absorbed light energy at the standard wavelength. The FTIR spectrometry readings were carried out at specific wavelengths and slits for each sample under study. The spectral range varied from 4000 to 400 cm^{-1}.

3.0 Results and Discussions

The various functional groups detected based on peak absorbance from FTIR analysis of *Eichhornia crassipes*, *Pistia stratiotes* and *Salvinia molesta* are presented in figure 1, 2 and 3 respectively. The peak at 3400 cm^{-1} indicates the presence of alcohol functional group. This was done to further underpinned and corroborate the results obtained. The FTIR analysis indicates that other functional groups are present in the aquatic weeds other than OH group. Other key functional groups such as alkenes, aromatics were detected in the all the samples.

The synergistic effect of *S. cerevisiae*, *S. carlsbergensis* for the bioconversion of *Eichhornia crassipes, Pistia stratiotes and Salvinia molesta* to bioethanol is presented in Table 1. The results show maximum ethanol yield and productivity rate after 21 days of fermentation with *Eichhornia crassipes* to be: (3.0 g/L, 0.005 g/L/h), *Pistia stratiotes:* (2.3g/L, 0.004 g/L/h) and *Salvinia molesta:* (2.0 g/L, 0.003 g/L/h). Higher yield of ethanol were obtained compared to when the biomass were fermented with either *S. cerevisiae* or *S. carlbsergensis* as indicated in Table 2 and 3. There was significant drop from 3.0 to 0.8 g/L in the ethanol yield when *E.crassipes* was fermented with *Saccharomyces cerevisiae* (Table 2). However, the yield increased to 1.0 g/L when Saccharomyces *carlsbergensis* was used (Table 3). Comparable tendencies were observed for the productivity rate showing decreased from 0.005 g/L (Table 1) to 0.002 g/L in Table 3 and 0.001 g/L in Table 2. Similar trends were recorded when *P.stratiotes* and *S.molesta* fermented with the composite mixture of the two yeasts and independently using either *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis* for 21 days as depicted in Table 2 and 3 respectively. This is supported by Badger (2002).

E.crassipes, *P.stratiotes* and *S.molesta* fermented with *S.cerevisiae* and *S.carlsbergensis* in synergy are more efficient in terms of ethanol yield after day 21 of fermentation compared to fermentation with either *Saccharomyces cerevisiae* or *Saccharomyces carlsbergensis*. This shows that the synergistic effects of the two organisms improve ethanol yield. The three aquatic weeds *E. crassipes, P. stratiotes* and *S. molesta* have great potentials in bioconversion to ethanol using the two yeast strains: *S. cerevisiae* and *S. carlsbergensis.*

Table 1: Ethanol Yield Using *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis*

SN	Aquatic Weed	Ethanol Yield *(g/L)*	Productivity (g/L/ h)

1	*E.crassipes*	3.0	0.005
2	*P.stratiotes*	2.3	0.004
3	*S.molesta*	2.0	0.003

Table 2: Ethanol Yield Using *Saccharomyces cerevisiae*

SN	Aquatic Weed	Ethanol Yield *(g/L)*	Productivity (g/L/ h)
1	*E.crassipes*	0.8	0.001
2	*P.stratiotes*	0.5	0.0009
3	*S.molesta*	0.4	0.0007

Table 3: Ethanol Yield Using *Saccharomyces carlsbergensis*

SN	Aquatic Weed	Ethanol Yield *(g/L)*	Productivity (g/L/ h)
1	*E.crassipes*	1.0	0.002
2	*P.stratiotes*	0.6	0.001
3	*S.molesta*	0.5	0.0009

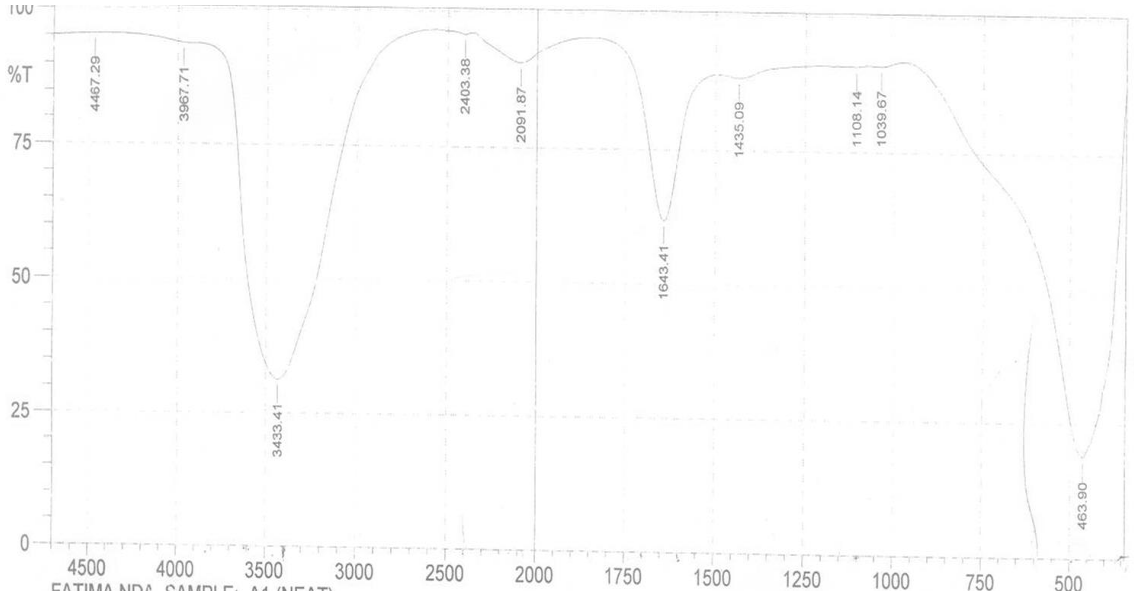


Figure 1: FTIR Analysis showing *Eicchornia crassipes* peak absorbance

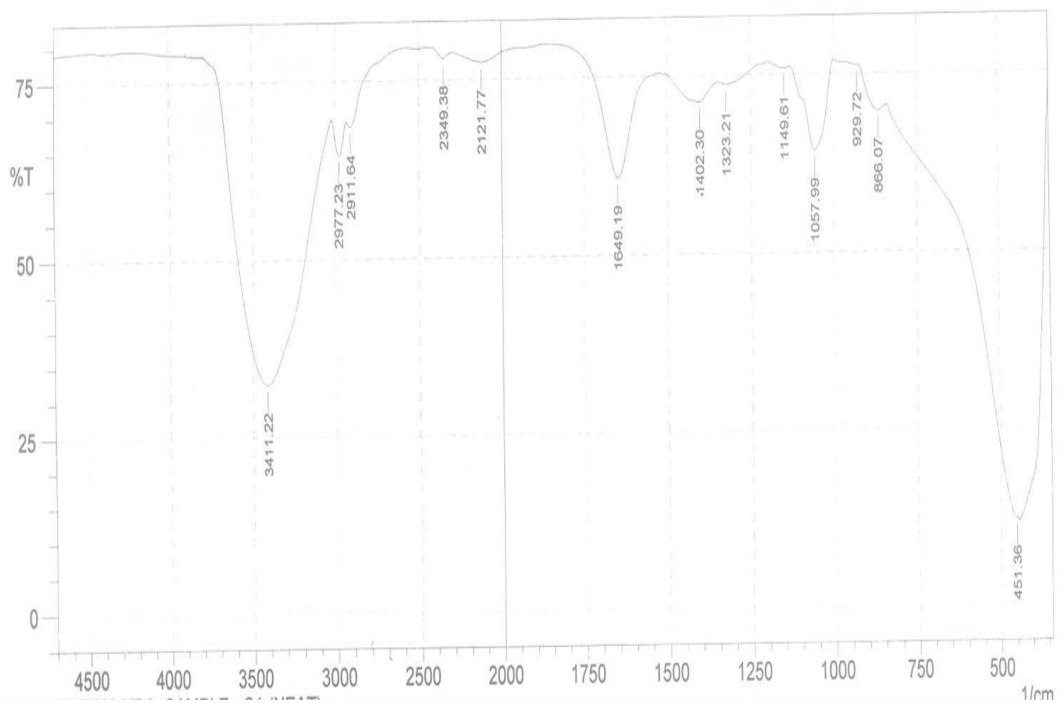
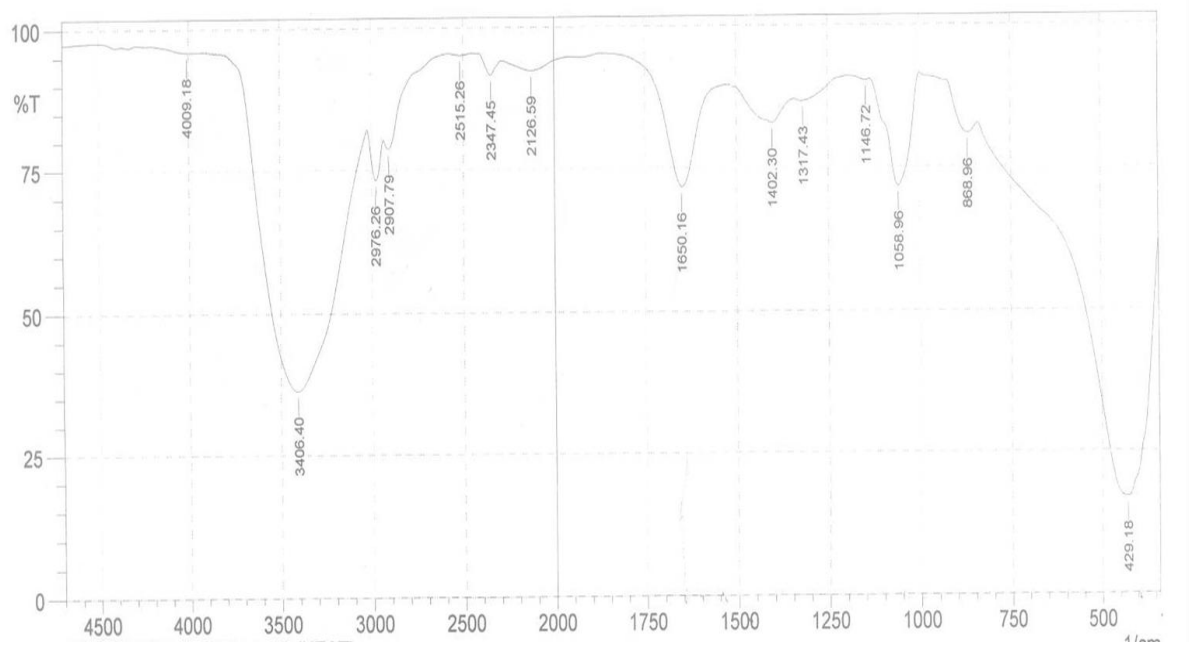


Figure: 2: FTIR Analysis showing *Pistia stratiotes* peak absorbance



4.0 Conclusions

The synergistic effect of *Saccharomyces cerevisiae* and *Saccharomyces carlsbergensis* in this study contributes for the ethanol production efficiencies of the three aquatic weeds. While *Saccharomyces carlsbergensis* has highest efficiency than *Saccharomyces cerevisiae*,the composite mixture of the two yeasts prove to be better than when

either of them was used independently. The study concludes that *S.cerevisiae* and *S.carlsbergensis* are more efficient when used in synergy for the fermentation of fresh water biomass than when single yeast was used.

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