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Optimization of Bioconversion of Sugarcane Bagasse to Ethanol using improved strain of Saccharomycescerevisiae developed through Ultraviolet Radiation.

Adetuwo,Olagunju Johnson
Institution: AdekunleAjasin University, Akungba-Akoko, Nigeria.

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ABSTRACT
Ethanol provides an alternative to the current use of liquid fossil fuels and it could be able to sustain the current energy consumption globally. Lignocellulosic residues such as sugarcanebagasse could be used to produce large quantity of ethanol because it is abundant and sustainable, using bagasse as a raw material for bioethanol production could be helpful in reducing environmental waste that are causing by bagasse. **Aim of this study:** This research work was carried out in order to increase bioethanol production from bagasse using mutant strain of Saccharomycescerevisiae developed through ultraviolet radiation. **Methods:**Bagasse were collected from main market in Okitipupa, dried, blended and sieved. Chemically pretreated with 1% NaOH in 100ml of distilled water. Solution was filtered and the filtrates were neutralized with appropriate dilute HCl. Saccharification of the pretreated bagasse was carried out using Aspergillusniger isolated from garden soil. The hydrolysed samples were inoculated with Saccharomycescerevisiae isolated from raphia palm wine that has been treated with ultraviolet light accordingly. **Result**: Bioethanol production in the mutant strain of Saccharomycescerevisiae SUV_5 and SUV_{10} were three and two fold increased respectively than wild-type (12.7g/ml and 8.9g/ml against 4.21g/ml). Also the mutants strain tolerated harsh operational parameters, low and high temperatures and pH, than the wild-type. The mutant strain still produced ethanol at 45^0C and at pH 7.5. **Recommendation:** The result of this study can be of a better application in the large production of bioethanol from bagasse which is renewable and highly abundant, it is saving costs by recycling of wastes, and it also helps to alleviate environmental problem such as an excessive release of greenhouse gases from combustion of non-renewable fossil fuel.

Key words: Sugarcane bagasse, Saccharomycescerevisiae, ethanol, pretreatment, fossil fuel

I. INTRODUCTION

Ethanol provides an alternative to the current use of liquid fossil fuels and it could be able to sustain the current high energy consumption globally (Abiodun, 2007; Abu, et al., 2005). Furthermore, ethanol serves as a major raw material for biomedical and pharmaceutical companies. The demand for ethanol has increased steadily over the last century as the world population has grown and more countries have become industrialized. Abundant and sustainable energy resources could be considered as one of the cornerstones of the prosperity of the humanity (Abila, 2010) The increasing human population is constantly exerting more pressure on the world's natural resources, which include natural fossil fuels that are non-renewable (Abiodun, 2007). There are concerns regarding the use of fossil fuels due to its growing scarcity and its negative impact on our environment. Therefore, there is a growing need in the world for energy sources that are renewable, sustainable, and eco-friendly. Renewable energy is currently being derived from the wind, water and sun, but to a limited extent. These forms of natural resources are very attractive for the production of renewable energy, but the technologies needed to make them readily available for use are difficult to get by. Ethanol on the other hand could serve as an alternative to the current use of liquid fossil fuels because it is cheaper, cost effective, renewable and

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the raw materials to produce it, is abundance. There are mainly two processes involved in the conversion: hydrolysis of cellulose in the lignocellulosic biomass to produce reducing sugars, and fermentation of the sugars to ethanol (Oyeleke and Jibrin, 2009). The cost of ethanol production from lignocellulosic materials is relatively high based on current technologies, and the main challenges are the low yield and high cost of the hydrolysis process (Ander and Erikson, 1997; Abila, 2010). However, one of the primary goals of Industrial Microbiology research and development is the establishment of economically viable processes through increasing product yield and reduced operating cost in order to maximise profit. The most important means of achieving this has been by strain improvement, using a variety of techniques (Singh and Sharma, 2015). Improvement of the productivity of industrial strains by exposing microorganism to UV radiation is one cheaper and quick methods of strain Improvement technique in industrial microbiology (Okafor, 2007; Rabbani, 2017). Wild-type strains isolated from natural environment usually produce only a low level of products.

II. MATERIALS AND METHODS:

Collection and pretreatment of sugarcane bagasse: Bagassewere collected from Sabo in Okitipupa town in a clean polythene bag and was blended and sieved using sieve of 0.2mm. The bagasse was pretreated with 1% of NaOH for five days, then filtered with Whatman filter paper. The filtrate was neutralized with appropriate dilute HCl.

Saccharification of pretreated sugarcane bagasse: The enzymatic Saccharification of pretreated sugarcane bagasse was done using Aspergillusniger isolated from garden soil to produce fermentable sugar (Oyeleke and Jibrin, 2009).

Isolation and screened of Saccharomyces cerevisiae for ethanol production: Saccharomyces cerevisiae using for this study were Isolated from Raphiaraphia palm wine that has been stored for five days using standard microbiological methods, then isolates were screened for bioethanol production by inoculated them into 100ml hydrolysedbagasse, and then incubated the mixtures at 28^0C for 48 hours (Oyeleke and Jibrin, 2009).

Saccharomyces cerevisiae mutants strains development: Saccharomyces cerevisiae culture was serially diluted appropriately. 1ml of culture streaked on Petri plates having solidified agar medium. The plates was exposed to UV light at a distance of 50cm for various time intervals (5, 10, 15, 20, 25 and 30 mins). The treated Petri plates were covered with dark nylon and incubated at 30°C for 3 days. Different colonies on agar plates were picked up with inoculating needle and placed into 30ml of hydrolysed bagasse (hydrolyzates) media and incubated for 5days. Liquid samples were collected and solid biomass was separated with centrifugation (8000rpm) for 20 minutes. Ethanol content was analyzed in the supernatant (Singh and Sharma, 2015).

Effect of pH on ethanol production of the wild, and the mutant strains of Saccharomyces cerevisiae: The broth media were prepared (30ml of hydrolysed bagasse) and sterilized for fifteen minutes at 121^0C. The pH of the media was varies from 4.5 to 7.0 using H_2SO_4 and NaOH and inoculated with the wild-type and mutants strains of Saccharomyces cerevisiae. Cultures were incubated at 30^0C for three days. Ethanol concentration was determined by spectrophotometer by taking O.D at 650nm against the media as blank (Singh and Sharma, 2015).

(ii) Effect of temperature on ethanol production of the wild, and the mutant strains of Saccharomyces cerevisiae: The broth media were prepared (30ml of hydrolysed bagasse) and sterilized forfifteen minutes at 121^0C. Later inoculated with the wild-type and mutants strains of Saccharomyces cerevisiae. Cultures were incubated at varies temperature ranged from 20^0C to 45^0C for three days. Ethanol concentration was determined by spectrophotometer by taking O.D at 650nm against the media as blank (Singh and Sharma, 2015).

Extraction of Ethanol from the broth by Distillation Method: Three grams of calcium oxide powder was added to 150ml of distillate before distillation was carried out with a distillation apparatus set up for each of the fermented broth. The fermented broth was transferred into round bottom flask and placed on a heating vessel fixed to a distillation column enclosed in running tap water. Another flask was fixed to the other end of the distillation column to collect distillate at 78ºC which is the standard temperature for ethanol production (Oyeleke and Jibrin, 2009).

Determination of Concentration of Bioethanol Produced: Determination of concentration of bioethanol produced was carried out using the method described by Oyeleke and Jibrin (2009). 1ml of standard ethanol was diluted with 100ml of distilled water to give a concentration of 5%. From this stock solutions 0.2, 0.4, 0.6, 0.8 and 1.0% of the ethanol was prepared by diluting it with distilled water. To each of the varying ethanol concentrations, 1ml of chromium reagent was

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added and allowed to stand for an hour for colour development. The absorbance of each concentration was measured at 650nm using UV-VIS spectrophotometer and the readings sued to developed standard ethanol curve. A quantity of one millilitre (1ml) of each bioethanol samples were put in test tubes and treated with 1ml of chromium reagent. The mixture was allowed to stand for an hour and the absorbance was measured as for standard curve.

III. RESULTS

Ultraviolet mutagenesis were performed by exposing the isolates to ultraviolet light, and screened for the ethanol production. Only two mutants SUV_5 and SUV_{10} produced higher ethanol, 12.6g/ml and 8.9g/ml respectively as compared to wild-type 4.2g/ml (table 1, figure 1). All other isolated cultures exhibited decrease in ethanol production (table 1). Petriplates with exposure time of 25 min shows no significant ethanol 0.2g/ml. Bioethanol production in the mutant strain of Saccharomycescerevisiae exposed to ultraviolet radiation for five and ten minutes were three-fold increased than wild-type (12.7g/ml against 4.21g/ml). Also the mutants strain tolerated harsh operational parameters, low and high temperatures and pH, than the wild-type. The mutant strain still produced ethanol at 45^0C and at pH 7.0 (figure 2 and figure 3).

Table 1. Strain improvement of Saccharomycescerevisiae by ultra-violent radiation Exposed

Time in minutes	Saccharomycescerevisiae strains	Ethanol produced in g/ml
0	Wild type	4.2
5	SUV_5	12.6
10	SUV_{10}	8.9
15	SUV_{15}	3.1
20	SUV_{20}	1.5
25	SUV_{25}	0.2

SUV: Means Saccharomycescerevisiae"ultraviolet" mutants strain developed through ultraviolet radiation the subscript indicates the number of time in minutes exposed to ultraviolet radiation.

Figure 1. Ethanol produced by wild-type and the UV mutants strains of Saccharomycescerevisiae in g/ml.

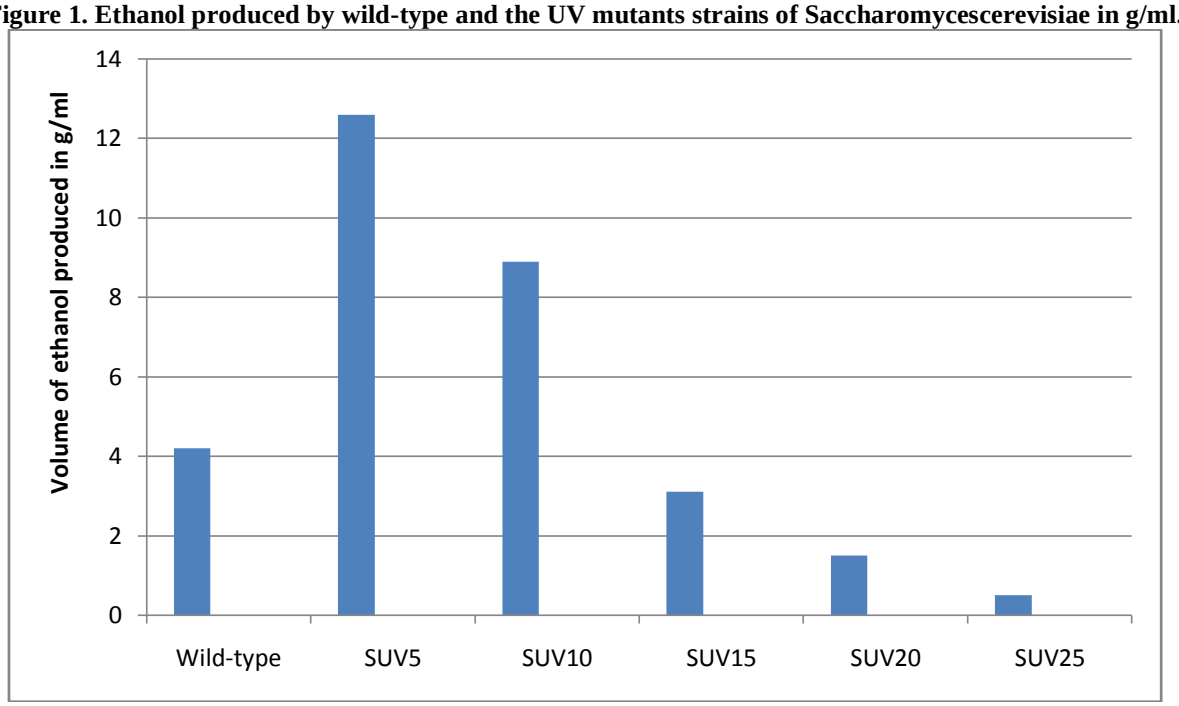

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Figure 2. Effect of temperature on ethanol produced by the wild-type and the selected mutants strain of Saccharomycescerevisiae

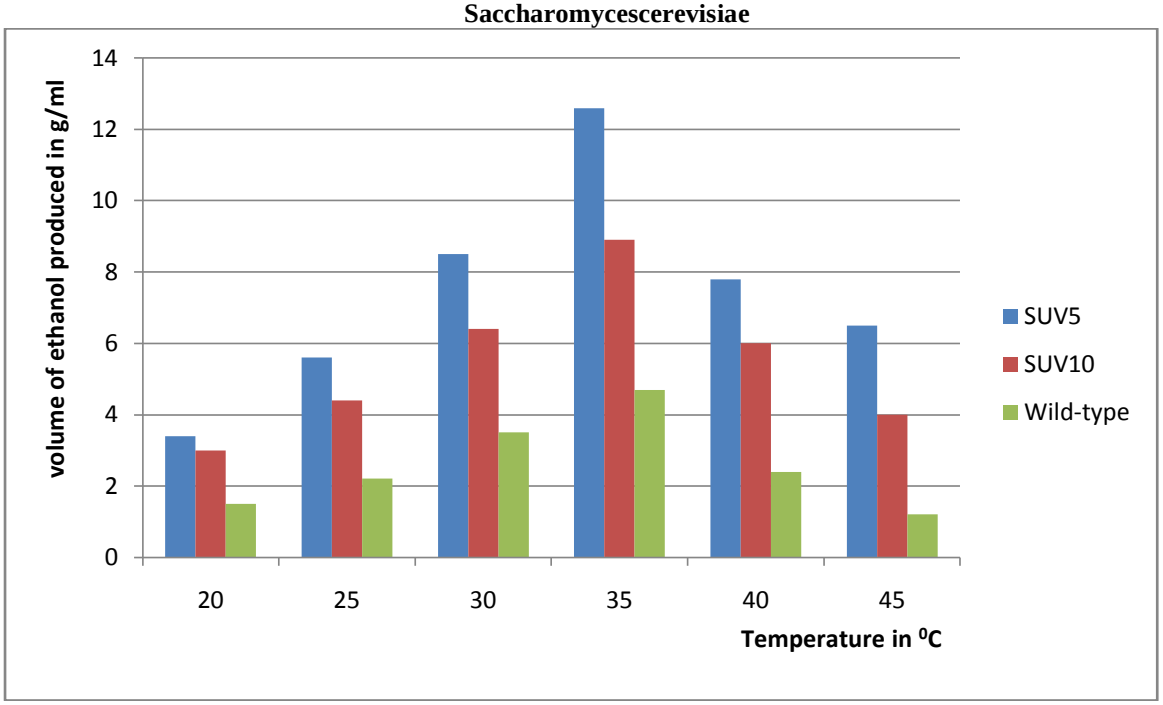
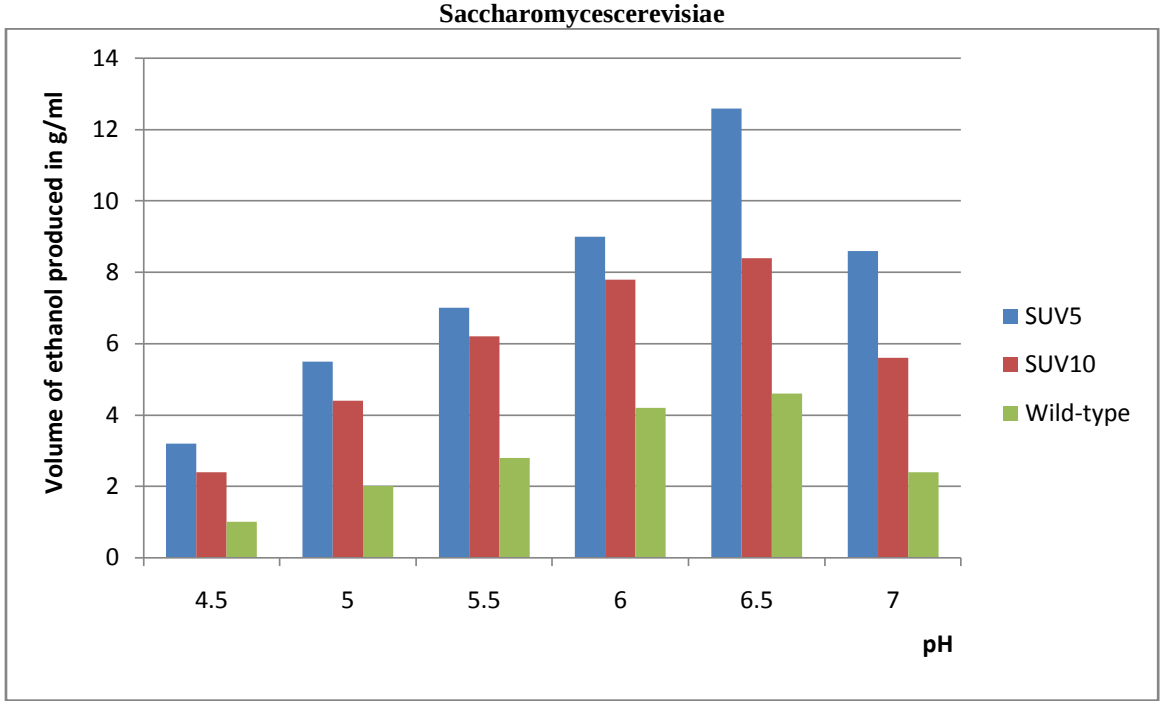


Figure 3. Effect of pH on ethanol produced by the wild-type and the selected mutants strains of Saccharomycescerevisiae



IV. DISCUSSION AND CONCLUSION:

Pretreatment ofbagasse with NaOH removed lignin and hemicellulose, reduce cellulose crystallinity, and increase the porosity of the bagasse. The presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis (Sun and Cheng, 2002). Removal of lignin and hemicellulose, reduction of cellulose crystallinity, and increase of porosity in pretreated Lignocellulosic biomass can significantly improve the hydrolysis, as noted by many researchers (Ado,

et al., 2009; zhang, et al., 2007). Ultraviolet mutagenesis; cultures of Saccharomycescerevisiae were exposed to UV radiation, the radiation generated different mutants of Saccharomyces cerevisiae with varies ethanol production abilities (figure 1). ExposedSaccharomycescerevisiae to ultraviolet radiation for 5 and 10 minutes respectively improved the bioethanol production capacity from sugarcane bagasse, while longer time above 15 minutes brought out undesirable traits in Saccharomyces cerevisiae as bioethanol production capacity is decreased as noted in this research. Singh and Sharma (2015) demonstrated the influence of the ionizing radiations to induce mutation in S. cerevisiae. In this research, the use of Improved strains Saccharomycescerevisiae gives a better yield because there is significance difference between ethanol produced by wild-type and, the Improved strains (mutants) of Saccharomycescerevisiae.

Recommendation: The result of this study can be of a better application in the large production of bioethanol from bagasse which is renewable and highly abundant, it is saving costs by recycling of wastes, and it also helps to alleviate environmental problem such as an excessive release of greenhouse gases from combustion of non-renewable fossil fuel.

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