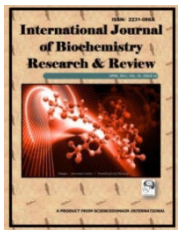

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Optimization of Fermentation Parameters for Ethanol Production from *Ziziphus mauritiana* Fruit Pulp Using *Saccharomyces cerevisiae* (NA33)

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Research Article

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ABSTRACT

Total demand for ethanol due to fear of crude oil depletion and the need to mitigate global warming due to green house gas emissions is increasing year after year. The present study was undertaken to investigate optimum parameters for ethanol production from *Ziziphus mauritiana* by *Saccharomyces cerevisiae* (NA33) strain. Various parameters, yeast concentration, pH and temperature were considered. A control experiment (without *Saccharomyces cerevisiae* (NA33) strain) was also set up for results comparison. The optimized conditions for ethanol production were established as pH 6, temperature 30°C and yeast concentration of 8.0g per 20g fruit pulp. Under these conditions an ethanol concentration of 63 g/L was achieved. The control vessel showed not much rate of fermentation and hence it was shown that addition of *Saccharomyces cerevisiae* (NA33) was necessary to increase the rate and yield.

Keywords: Ziziphus mauritiana; Saccharomyces cerevisiae; fermentation parameters; optimization.

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1. INTRODUCTION

Research for new substrates for ethanol production remains a worthwhile activity due to the debate on renewable energy, particularly with respect to bio-fuels production technologies (Lin and Tanaka 2006). Bio-fuels such as ethanol are increasingly gaining attention. They are very important tools for fighting against global warming because of their carbon neutrality. Bio-fuels are increasingly an important weapon in the armory against rising emissions of the greenhouse gas and the battle against global warming. In Zimbabwe *Ziziphus mauritiana* are wild fruits that are traditionally fermented into ethanol through spontaneous and uncontrolled processes (Nyanga et al., 2007). The ethanol is consumed as a beverage by local people and its alcohol content varies from producer to producer. *Ziziphus mauritiana* is a fruit that has a fruit pulp with a total carbohydrate content ranging from 14 to 16% at maturity (Nyanga et al., 2007). It has been reported to be rich in vitamins A and C, minerals, fibers and antioxidant (Szeto et al., 2002). Because of its drought tolerance, high carbohydrate content and wide availability*Ziziphus mauritiana* fruits are a suitable substrate for fermentation. Muchuweti and other coworkers reported that *Ziziphus mauritiana* fruit contains the following soluble sugars, galactose, fructose and glucose using Thin Layer Chromatography (Muchuweti et al., 2005). Generally it is known that the fermentation process performance is affected by operational conditions such as temperature, stirring rate, initial inoculum and substrate concentrations, dissolved oxygen among others (Dragone et al., 2011). Traditionally fermentation of *Ziziphus mauritiana* fruit relies on the microbiota from the fruit surfaces and to some extent from the utensils used during fermentation (Nyanga et al., 2007).In the absence of oxygen, yeasts can convert sugars into alcohol and carbon dioxide and continue living until they make so much alcohol that they die (about 12-13%). At concentrations greater than 13%, the enzymes of the yeast are deactivated (Solomons and Fryhle, 2002). The accumulation of a metabolic end product in the medium surrounding a fungus can represent a chemical stress to the organism. Several studies have been carried out on the kinetics of batch ethanol fermentation of various substrates at varying conditions of initial sugar concentration, pH and biomass. Ozmihci and Kargi (2007) found out that at an initial pH value of 5 and oxidation-reduction potential (ORP) of -250mV, the rate and extent of ethanol formation increased with increasing cheese whey powder (substrate) concentration up to $15gL^{-1}$ and then decreased for larger concentrations due to substrate inhibition at higher sugar levels. The results indicated that the initial sugar concentration should be below 75 g L^{-1} and initial biomass should be above 850 mg L^{-1} to obtain high rates and yields of ethanol formation and avoid substrate inhibition. However it does not necessarily follow that use of yeasts in fermentation yields high alcohol solutions in any chosen substrate. In a study carried out by Fraile etal. (2000), the influence of a selected strain of *S. cerevisiae* in the volatile composition of rose wines yielded different results. A comparison was made between rose wine prepared by inoculating with *S. cerevisae* (NA33) and by natural yeasts that are present in the grape (control sample). The results showed that the control sample contained higher concentrations of alcohols and esters than the inoculated wines. In the present study we investigated optimum conditions for ethanol production from *Ziziphus mauritiana* fruit pulp using *Saccharomyces cerevisiae*. Three factors were selected as process (independent) variables: initial yeast concentration, pH and temperature while initial rate of fermentation and ethanol concentration were selected as responses (dependent) variables.

2. MATERIALS AND METHODS

2.1 Sample Preparation

Z. mauritiana fruit were collected in Bindura, Zimbabwe. The fruit was sun-dried to drive off the water and hence concentrate the sugars (Ozminci and Kargi, 2009). The drying took 6 days, after which the sample was gently crushed using pestle and mortar to separate the fruit pulp from the seeds. The fruit pulp was then stored in a cool, dry cupboard until needed.

2.2 Fermentation

A sample mass of 20g of ground *Z. mauritiana* pulp were accurately measured out into each of nineteen 500 ml filter flasks. Respective amounts of *Saccharomyces cerevisiae* (Anchor yeast, Zimbabwe) were first dissolved in lukewarm water and left for 10 minutes before being used. Different pH conditions were set using 200ml. of distilled water of appropriate temperature and added to the pulp. A control sample was prepared in the same manner except that no yeast was added. The filter flasks were stoppered, balloons attached tightly to the arms of the flasks and the flasks were subjected to appropriate temperature conditions for 7 consecutive days.

2.3 Production of Carbon Dioxide

The balloons were marked at similar positions along their lengths. The pressure produced by the gas was exerted on the walls of the balloon, causing it to inflate. The circumference of the each balloon was measured using a piece of string which was then measured against a ruler. The measurements were done at hourly intervals for 5 consecutive hours and the readings were tabulated. Thereafter the fermentation was left to continue unhindered for the remainder of the seven days.

2.4 Effect of pH

Different pH solutions, pH 2, pH 4, pH 6, pH 8 and pH 11 were established in 5 beakers with distilled water. A pH meter was used to measure the pH of each solution during and after establishment. Some amounts of water were drawn from each pH solution and added to each appropriately labeled filter flasks already containing 20g of fruit pulp and 1.5g of yeast. A balloon was attached to the arm of each flask, and the vessels were stoppered and left at room temperature for seven days.

For pH 2, pH 4 and pH 6:

Distilled water at room temperature was placed in each of three beakers. The pH of the water was tested using a pH meter. Drops of 0.1M acetic acid were added to each beaker in turn and the pH of the water tested again after stirring. The process was repeated in each beaker until respective pH values were established in the beakers.

For pH 8 and pH 11:

Distilled water at room temperature was placed in each of two beakers. The pH of the water was tested using a pH meter. Small amounts of solid sodium carbonate (reagent grade)

were added gradually with effective stirring and measurement of the pH before successive additions, until pH 8 and pH 11 were established in the two beakers.

2.5 Effect of Temperature

Distilled water was set at 6 different temperatures in 6 beakers (5, 15, 25, 30, 37 and 50ºC). Water baths were prepared for 5ºC and 15ºC. Amounts were drawn from each of the different temperature ranges and poured in filter flasks appropriately labeled and already containing 20 g of fruit pulp and 1.5 g of yeast each until the 200ml mark was reached. The pH of each mixture was measured using a pH meter. After being stoppered and attaching a balloon at the rim of each flask, the reaction vessels were subjected to respective temperatures using either prepared water baths or incubators.

2.6 Effect of Yeast Concentration

Seven different amounts of yeast (0.6g, 1.8g, 2.1g, 2.7g, 5g, 8g and 10g) were weighed out and added to seven appropriately labeled filter flasks already containing the fruit pulp. Distilled water was added to the flasks until the volume of 200ml was obtained in each flask. The pH of each mixture was kept at 6. Balloons were attached to the arm of each filter flask, and the flasks were tightly closed and left at 30ºC for seven days.

2.7 Determination of the Amount of Ethanol

The ethanol concentrations were determined using the potassium dichromate (Analytical grade) redox titrations with ferrous ammonium sulphate (Analytical grade) being used as the reducing agent (Mendham et al., 2000). The determinations were done indirectly by finding the chemical oxygen demand of the ethanol solutions and then inferring the ethanol concentration from the stoichiometric relationship between potassium dichromate and ethanol (Boehnke and Delumyea, 2000). Ethanol solution was added to a known volume of potassium dichromate and left to react. The unreacted potassium dichromate was then back-titrated with ferrous ammonium sulphate (Christian, 1994). A 25ml-aliquot of potassium dichromate was placed in a 250ml conical flask. 1ml of ethanol solution (previously placed in a thermostatically-controlled bath for 30 minutes at 60ºC) was added. Ten drops of indicator solution were added to the mixture, followed by 150ml of distilled water. The solution was then titrated with ferrous ammonium sulphate until the end point was reached (light green colour). The volume used was noted. The procedure was repeated two more times to find the average. The process was done for all the samples of ethanol and the results were recorded.

3. RESULTS AND DISCUSSION

3.1 Increases in Balloon Circumference with Time for pH, Yeast Amount and Temperature

The increase in the circumference of the balloon was recorded on an hourly basis for five consecutive hours, for different conditions of pH, temperature and yeast concentration.

3.2 Effect of pH, Temperature and Yeast Amount on Ethanol Production after Seven Days

The rate of ethanol production was maximum at pH 6 (Fig. 1). This is due to the fact that proteins function in an environment that reflects this pH (Berg, 2007). A of pH 2 had the lowest carbon dioxide production presumably because the low pH encourages the production of acid instead of alcohol (Jennings, 1995). The trend was not linear because *Saccharomyces cerevisiae*, like any other enzyme, works at a particular pH range and rate does not gradually increase or decrease. (Fig. 5) also showed maximum ethanol concentration at pH 6.

For the yeast concentration the rates increased rapidly with the increase in the amount of yeast added, up to the yeast concentration of 8 g/20 g fruit pulp (Fig. 3). Beyond that point the rates no longer significantly increased, as indicated by the close proximity of the 8 g and 10 g profiles. At this point the substrate (fruit pulp) becomes limiting and increasing the yeast amount does not increase the rate of reaction.

The ethanol concentration for the variable, yeast concentration also followed the same trend as that for rate (Fig. 7), with the graph reaching its maximum at yeast concentration of 8g/20g fruit pulp. The present results differ from those reported in similar studies. (Karthikeyan et al, 1996) reported 10g as the maximum inoculum while in (Dragonei et al, 10thInternational Chemical and Biological Engineering Conference, 2008) 1-3g was the maximum inoculums.

A maximum rate was achieved at a temperature of 30°C (Fig. 2 and 6). This could be attributed to the behavior of enzymes at different temperature mediums. The rates of enzyme catalyzed reactions increase with temperature up to the temperature at which the enzyme begins to denature. Above that temperature, reaction drops precipitously as the enzyme denatures (Southerland, 1990). At very low temperature the enzyme is deactivated and reaction slows down or stops altogether. The rate was shown to be prominent from 25°C to 35°C, being more distinguished at 30°C.

The control vessel showed not much rate of fermentation (Fig. 4) and hence it was shown that fermenting using only microbes present in the fruit pulp reduces fermentation rate and yield.

It emerges then, from the above illustrations that the initial rate of reaction using *Saccharomyces cerevisiae* can be increased by;

- Increasing the amount of enzyme, generally.
- Employing a temperature of around 30°C.
- Setting the pH between 5and 6.

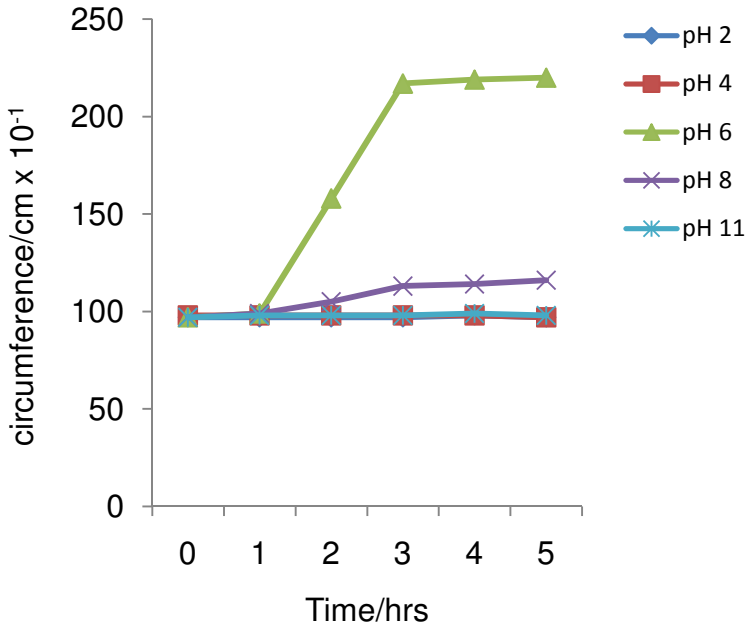

Fig. 1. Increase in balloon circumference with time for the variable pH temperature 30°C yeast 1.5g

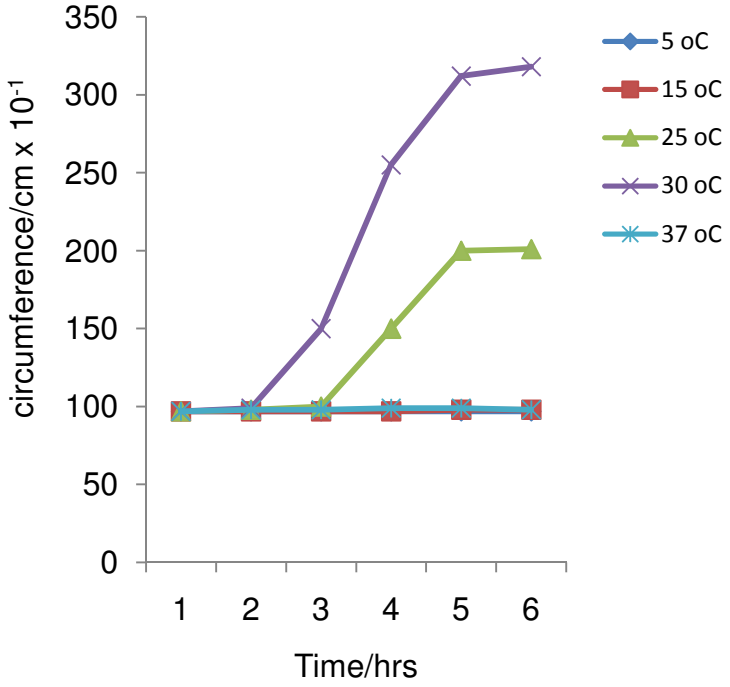

Fig. 2. Increase in balloon circumference with time as a function of temperature pH 6, yeast 1.5 g

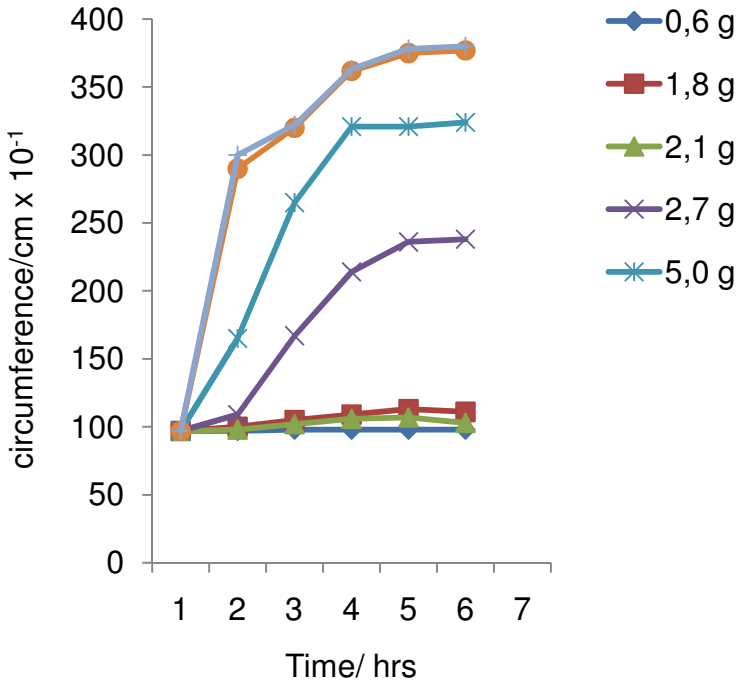

Fig. 3. Increase in balloon circumference with time for the variable yeast amount, at 30°C and pH 6.

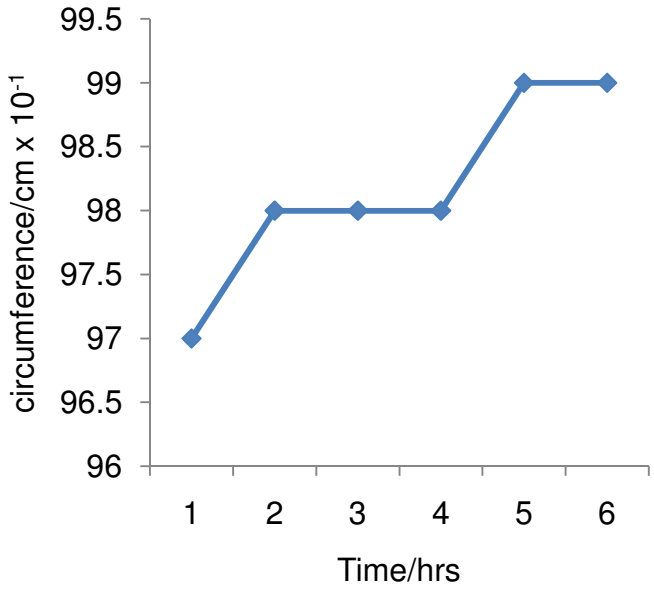

Fig. 4. Circumference of balloon with time for the control (Temperature 30°C, pH 6 no yeast added)

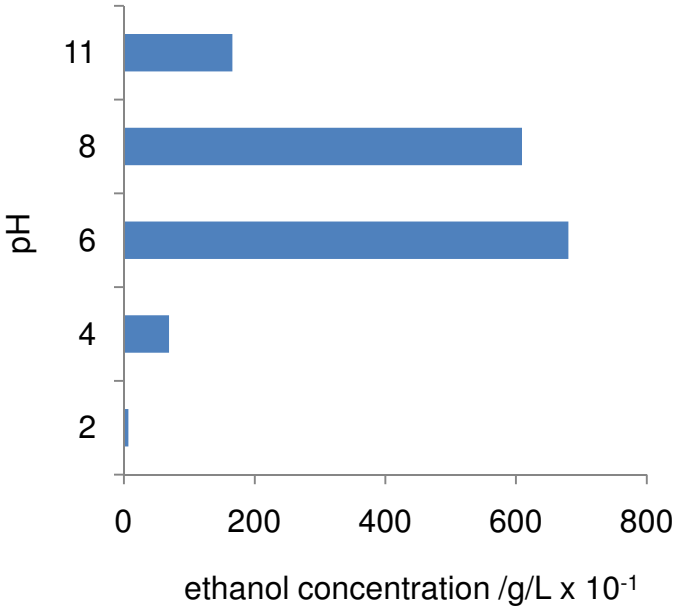

Fig. 5. Effect of pH on ethanol production

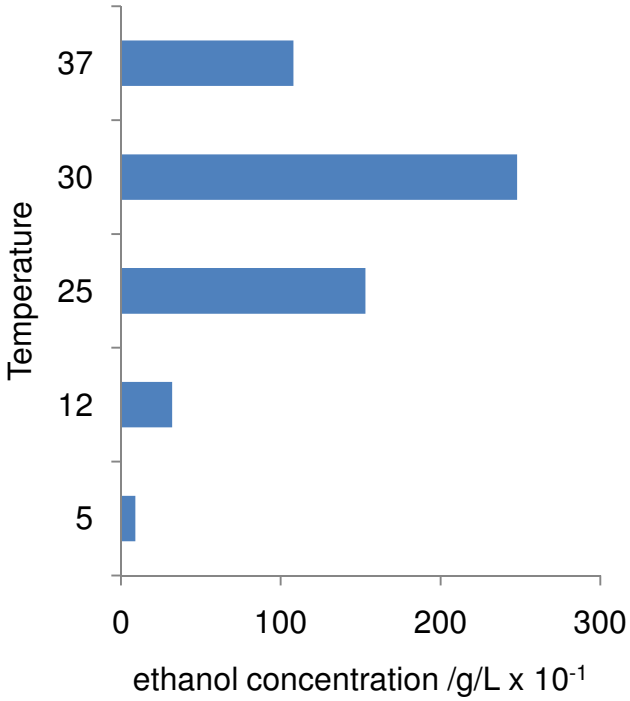

Fig. 6. Effect of temperature on ethanol production

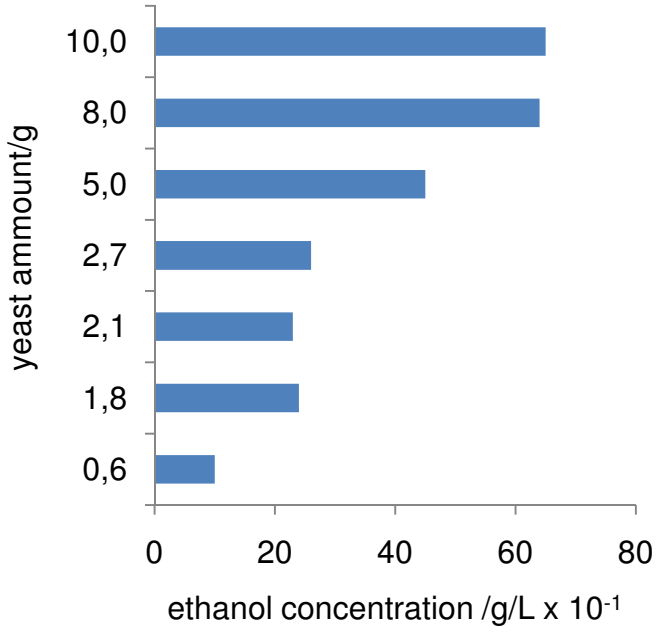

Fig. 7. Ethanol concentration against yeast amount

4. CONCLUSION

Dried *Z. mauritiana* fruit pulp is a suitable raw material for ethanol production by fermentation. A pH of 6 yielded the highest rate of fermentation, and the highest ethanol concentration after the stipulated seven days of fermentation. A temperature range of 30^{o}C was found to be the optimum temperature at which both rate of fermentation and ethanol concentration were highest. The yeast concentration of 8g/20g fruit pulp yielded the optimum rate of fermentation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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