Journal of Dry Zone Agriculture, 2021, 7(2):53-76

Production and optimization of bioethanol from over ripen sour banana fruit wastes (*Musa sapientum)* using *Saccharomyces cerevisiae*

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Received 27th February 2021, Accepted 10th November 2021

Abstract

This study was carried out to produce bioethanol from low quality over ripen *Musa sapientum* (sour banana) fruit wastes to enhance the yield of bioethanol. When the sour banana juice was inoculated with *Saccharomyces cerevisiae* (2 g/L) in the fermentation media (100 mL, 8° Brix) composed of 10 g/L yeast extract, 10 g/L KH_2PO_4, 2 g/L $(NH_4)_2SO_4$, 2 g/L peptone, and 0.5 g/L $MgSO_4 \cdot 7H_2O$ and fermented for 24h at 30 °C and 100 rpm, the ethanol yield was 0.8% v/v. When nitrogen sources urea, ammonium sulphate, ammonium carbonate, and ammonium nitrate were used in the fermentation media (2.0 g/100mL), significantly higher ethanol yield ($p<0.05$, 0.90%) was produced with ammonium carbonate. When yeast inoculum was increased to 5 g/L, the ethanol yield was significantly higher ($p<0.05$, 1.00%, 1.11 times) than the control. When the temperature was 25 °C, the ethanol yield was significantly increased ($p<0.05$) by 1.2 times the control temperature of 30 °C. When the rotation speed was 150 rpm, the ethanol yield was significantly higher ($p<0.05$) than the control (100 rpm). Ethanol yield was significantly higher ($p<0.05$, .15 times - 4.10 %)

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with 90% of banana juice. With 0.1 g/100mL of ammonium carbonate, ethanol yield was significantly increased by 1.1 times ($p<0.05$, 40 %) than the non-optimized control (0.2 g/100mL). Sucrose significantly stimulated ethanol yield than the other sugars. Fifteen grams per hundred milliliters of sucrose yielded significantly higher ethanol ($p<0.05$, 2.33 times) than the non-optimized control (2 g/100mL). When the pH of the medium was optimized at 6.0, the ethanol yield was significantly higher ($p<0.05$, 12.60%). Therefore, *Musa sapientum* could be an effective substrate for bioethanol production and optimization process increased the bioethanol yield significantly by 15.75 times (12.60% - 1.6°Brix).

Keywords: baker's yeast, bioethanol, sour banana fruit waste, fermentation, incubation period

INTRODUCTION

Since petroleum-based fossil fuels are exhausted super-fast to meet the demands of the rapidly increasing human population, the energy crisis has become an important global concern nowadays (Prasad *et al*., 2007). Greenhouse gas emissions from fossils fuels cause adverse effects on the nature. Increase in the CO_2 level by the burning of petroleum-based fuels causes global warming (Naik *et al*., 2010). Disruption of oil supply in the Middle East countries where major field of petroleum-based fossil fuels are found would cause a huge struggle for fuel consumption and fuel-based essential sectors (Nagashima *et al*., 1984; Ogbonna *et al*., 2001). Scientists have shown great interest in finding out a sustainable and environmentally friendly energy sources for our industrial needs and for regular consumption for the vehicles of public all over the world (Mabee *et al*., 2005). As a solution, bioethanol is considered as one of the best options as a sustainable and renewable energy source.

The merits of bioethanol are higher octane number, evaporation enthalpy, flame speed and wider range of flammability, that make them suitable as a fuel source (Balat, 2007; Balat *et al*., 2009; Dias de Oliveira *et al*., 2005). Since the bioethanol is an eco-friendly oxygenated fuel containing oxygen of more than 35%, it is highly suitable to reduce the emission of particulate and other greenhouse gases during combustion (Demirbas, 2008; Malca *et al*., 2006; Searchinger *et al*., 2008). In addition to the above, bioethanol reduces the interference on ozone due to its lower ambient photochemical reactivity (Lynd *et al*., 1991; McCarthy *et al*., 2006).

Bioethanol can compete with petroleum in terms of sustainability and economic viability, only when it is produced from cheaper natural sources (Cysewski *et al.,*1978; Maiorella *et al*., 1984). At present, starch from cereal crops and juice and molasses from a wide range of crops are the two types of primary feed stocks employed in large scale biofuel production (Balat *et al.,* 2009; Mojovic *et al*., 2006; Salassi *et al*., 2007; Wilkie *et al*., 2000). Bioethanol production from diverse lignocellulosic biomasses has been studied widely but this type of research study is confined to the laboratory level. Usage of free sugar containing juice as feedstock for ethanol production than starch or lignocellulosic biomass has been cheaper and easily available. This may be due to the non-requirement of costly steps such as pretreatment of the lignocellulosic biomasses and hydrolysis step to obtain fermentable sugars (Bryan *et al*.,1990; Ganesh *et al*., 1995; Nilkolovv *et al*., 2000; Rolz *et al*., 1980). Microbial involvement in fermentation of sugars would be sometimes possible in the absence of oxygen with glucose and this results in ethanol and carbon dioxide (Deesuth *et al*., 2012; Ingram *et al*., 1998). Fermentation of yeast to produce alcoholic beverages such as beer and wine has been a prominent practice in the past, and this step is still efficiently used to produce bioethanol from renewable energy sources (Dien *et al*., 2003; Kosaric *et al*., 1995). *Saccharomyces cerevisiae* (de Mancilha *et al*., 1984; Liang *et al*., 2008; Sheoran *et al*., 1998; Yu *et al*., 2009). *Saccharomyces diastaticus* (Maruthai *et al*.,2012), *Kluyveromyces marxianus* (Limtong *et al*., 2007; Nonklang *et al*., 2008). *Escherichia coli* and *Klebsiella oxytocastrain* (Da silva *et al*., 2005) and *Zymomonas mobilis* (Cazetta *et al*., 2007; Gunasekaran *et al*., 1999; Rogers *et al*., 1982; Rodriguez *et al*., 1986) have been widely used for ethanol production from sweet sugary juices. Among these, *S. cerevisiae* has been the best choice for alcoholic fermentation because of the following reasons: efficient capacity to convert sugar into alcohol, capability of producing loosely clumped mass of fine particles during growth, easier to settle or suspend in the fermentation chamber (Kosaric *et al*., 1995) and higher tolerance to the ethanol present in the growing media (Olsson *et al*., 1993).

The optimum temperature range for the efficient function of *S. cerevisiae* for ethanol production is 30–35 °C and slightly alkaline media is highly preferable for effective fermentation. The heterotrophic microorganisms are generally used in fermentation process, they need at least a carbon and a nitrogen source for their survival and their growth. The direct bioethanol production from the free sugar containing juices of some plants is conducted by this yeast and they convert sucrose or mono

saccharides present in the raw materials into ethanol through the direct fermentation process (Cardona *et al*., 2007; Hossain *et al*., 2010). Banana, pineapple, orange, mango, sugarcane, and some fruits are the potential crops yield free sugar containing juices (Ensinas *et al*., 2009). These plants contain free sugars such as sucrose, glucose, and fructose (Dhaliwal *et al*., 2011). Sucrose is the major sugar in fermentable juices and it can be easily converted into glucose and fructose during fermentation process by using the enzyme invertase, found in yeast (Dodic *et al*., 2009; Sanchez *et al*., 2008).

A trend of converting staple paddy fields into banana cultivations has been increasing during the last decades due to the advantage that diverse banana plants grow very well in the dry zones of Sri Lanka. Among the different types of banana cultivars grown in Sri Lanka, most varieties are very popular because of their taste, low price, and nutritional qualities. Sour type banana is one of the very unpopular types of banana fruits produced in excessive quantities in northern Sri Lanka due to its sour taste. Large quantities of this variety of banana type are wasted without human consumption and considerable amount is allowed to deteriorate due to lower human attention and very poor taste. The shelf-life of this type of banana is also very short and it is easily susceptible to microbial invasion. Due to its small size, irregular-shaped black lesions formed frequently on the skin and poor taste, it is discarded into the garbage or used as feed for cows in large farms in the Jaffna peninsula. Sometimes, farmers choose not to harvest this type of banana from their cultivation land. Usage of plant juices as feedstocks would cause low storability and subjected to microbial decomposition and these are the demerits of the usage of sugary juices for fermentation (Dodic *et al*., 2009). To purify the juices, the conventional liming-carbonation method that uses more energy and produce waste and $CO_2$is replaced by the usage of membrane technology nowadays (Lipnizki *et al*., 2006). Method using membrane filtration of sugar juice is highly preferred over the conventional liming-carbonation method for yielding higher sucrose concentration (Hakimzadeh *et al*., 2006; Kawa-Rygielska *et al*., 2013; Regiec *et al*., 2004; Shahidi *et al*., 2006). Further, the sour variety of banana is very cheap, easily available, and grows excessively all over Sri Lanka. Therefore, the objective of the study was to determine the bioethanol production from the poor quality sour type banana fruit waste and to optimize the conditions to enhance the yield.

MATERIALS AND METHODS

Source of microbial strain and fruit

Baker's yeast (*Saccharomyces cerevisiae*) was purchased from the local market. Sour banana fruits (*Musa sapientum*) were grabbed from the Botanical Garden of the Department of Botany, University of Jaffna, and juice was prepared. Compared to other types of microorganisms, yeasts especially *Saccharomyces cerevisiae* is the common microbe employed in ethanol production due to its high ethanol productivity, high ethanol tolerance and ability to ferment a wide range of sugars (Azhar *et al*., 2017)

Chemicals and media

All the chemicals used were obtained from Sigma-Aldrich Chemicals PVT LTD, 301/2, Galle Road, Colombo-00300, Western province, Sri Lanka. Basal medium containing 10 g/L yeast extract, 10 g/L KH_2PO_4, 2 g/L $(NH_4)_2SO_4$, 2 g/L Peptone, and 0.5 g/L $MgSO_4 \cdot 7H_2O$ was prepared. After the autoclaving of the conical flask containing 100 mL media, it was inoculated with 0.2 g of *Saccharomyces cerevisiae* (2 g/L).

Production of biofuel and measurement

To the fermentation medium (100 mL), (2 g/L) inoculum was added and incubated at room temperature (30 °C) in a rotatory shaker (100 rpm), provide a smooth uniform circular motion with an orbit of 16mm, speed range 30-300 rpm, load bearing capacity 10 kg, depth (metric) 420 mm, height (metric) 270 mm. Each flask was cultured at room temperature (30 °C) under oxygen limited condition up to 24 h. The oxygen limited condition was provided by sealing the flask tightly with parafilm and keeping it in an Himedia glass anaerobic chamber, supplied with transparent, unbreakable polycarbonate jar of 3.5 L capacity with sturdy, aluminium lid clamp and sealing ring. Medium was mixed with distilled water and the suspension was mixed and the extract was centrifuged in Hermle. Z 306 model centrifuge with rotor, rotor's radius 8 cm, speed range: 200 to 14,000 rpm, max. capacity 4x100 mL. The supernatant was used for bioethanol measurement.

Analytical methods

Sugar concentration was measured by using dinitrosalicylic acid method (Miller, 1959) and refractometer method before and after the fermentation process. The suspension was mixed and the extract was centrifuged for 20 min at 3000 rpm (Relative centrifugal force = 805 x g) in a Hermle. Z 306 model centrifuge with rotor, rotor's radius 8 cm, speed range :200 to 14,000 rpm, Max. capacity 4x100 mL. The supernatant was used for bioethanol measurement in percentage using ebulliometer (Wahab *et al*., 2005).

Optimization of conditions for bioethanol production

Production of bioethanol in sour banana medium

Fermentation medium (100 mL) was inoculated with *Saccharomyces cerevisiae* (0.2 g) and incubated at 30 °C for 24 h. Ethanol production was measured by using Salleron ebulliometer, electric heating system 220V-125W, Analysis time 5 minutes approximately, range 0-18% alcohol.

Effect of nitrogen source

Fermentation media were prepared by taking different nitrogen sources (ammonium sulphate, ammonium nitrate, ammonium carbonate and urea) in a concentration of 0.2 g/100mL. The experiment was continued and ethanol production was measured by using Salleron ebulliometer electric heating system 220V-125W, Analysis time 5 minutes approximately, range 0-18% alcohol.

Effect of inoculum size

Media were prepared by mixing the optimized nitrogen source (ammonium carbonate) with liquid fermentation media. Different amounts of yeast inoculum (0.4, 0.5, 0.6, 0.8, 1.0 g/100 mL) was added in the media and incubated at room temperature (30 °C).

Effect of temperature

Media were prepared by mixing the optimized nitrogen source (ammonium carbonate) with liquid fermentation media. Optimized amount of yeast

inoculum (5 g/L) was added to the media and incubated at different temperatures (10, 20, 25, 30, 35, 40 °C) ranging from 10 – 40 °C.

Effect of rotation speed

Media were prepared by mixing the ammonium carbonate with liquid fermentation media. Yeast inoculum (5 g/L) was added to the media and incubated at the optimized temperature (25 °C) at different rotation speeds (50, 100, 150, 200, 250 rpm) by using Stuart orbital shaker, provide a smooth uniform circular motion with an orbit of 16 mm, speed range 30-300 rpm, load bearing capacity 10 kg, depth (metric) 420 mm, height (metric) 270 mm.

Effect of substrate concentration

Fermentation media were prepared by mixing all the substances with different concentration of substrate (5%, 10%, 25%, 50%, and 90%) of liquid fermentation media. The fermentation medium was inoculated with yeast inoculum (0.5 g/100mL) and incubated at 25 °C at 150 rpm.

Effect of amount of nitrogen source (ammonium carbonate)

Media were prepared by mixing all the substances with different amount of ammonium carbonate (0.1, 0.2, 0.5, 1.0, 1.5, and 2.0 g/100 mL) with 90% of banana juice concentration of liquid fermentation media. The medium was inoculated with yeast inoculum (0.5 g/100mL) and incubated at 25 °C at 150 rpm.

Effect of carbon source

Fermentation media were prepared by mixing all the substances with 90% of banana juice concentration and 0.1 g/100mL of ammonium carbonate of liquid fermentation media. Different carbon sources such as glucose, sucrose, maltose, and dextrose (2 g/100mL) were added to the media and inoculated with yeast inoculum (0.5 g/100mL) and incubated at 25 °C at 150 rpm.

Effect of amount of carbon source

Media were prepared by mixing already optimized substances at the appropriate level in the liquid fermentation media. Different amount of

carbon source (sucrose – 1 g, 2 g, 4 g, 6 g, 8 g, 10 g, 15 g and 20 g) was added to the media and inoculated with yeast inoculum (0.5 g/100mL) and incubated at 25 °C at 150 rpm.

Effect of pH of the medium

Media were prepared by mixing already optimized substances at the appropriate level in the liquid fermentation media. The medium was set at different pH values such as 4.0, 5.0, 6.0, 7.0, and 8.0 and inoculated with yeast inoculum (0.5 g/100mL) and incubated at 25 °C at 150 rpm.

Effect of incubation period

Media were prepared by mixing already optimized substances at the appropriate level in the liquid fermentation media. The medium was set at pH 6.0 and inoculated with yeast inoculums (0.5 g/100mL) and incubated at 25 °C at 150 rpm. The set ups were incubated at different incubation periods (24 h, 48 h, 72 h and 96 h).

Statistical analysis

All the experiments were carried out in triplicate and the average values were used to plot the graphical representation. Statistical analyses were performed using Minitab 16.0 Version. The data were analyzed using one way ANOVA. Tukey's multiple comparison test was used to determine significant differences at $p < 0.05$.

RESULTS AND DISCUSSION

Production of bioethanol in sour banana medium

The amount of ethanol produced from the banana juice was 0.8% under non- optimized conditions initially at room temperature after 24 h of fermentation. There were significant differences in the sugar content values obtained before fermentation and after the optimization of fermentation (Table 1).

Effect of nitrogen source

When different nitrogen sources such as urea, ammonium sulphate, ammonium carbonate, and ammonium nitrate were used in the

fermentation media, significantly higher ethanol production ($p<0.05$, 0.90%) was obtained in the medium containing ammonium carbonate (Figure 1) than the other nitrogen sources. Ammonium carbonate as a weak base can provide alkaline environment that facilitates the fermentation process. Hence, ammonium carbonate was chosen as nitrogen source for further studies.

Table 1: Sugar concentrations before and after the fermentation using dinitrosalicylic acid method (Miller,1959) and refractometer method.

	Sugar concentration	
	Before the fermentation	After the optimization of fermentation conditions
Dinitrosalicylic acid method (at 540nm)	0.55 ± 0.011 moldm^{-3}	0.08 ± 0.005 moldm^{-3}
Refractometer method	8 ± 0.577 ^{o} Brix	1.6 ± 0.057 ^{o} Brix

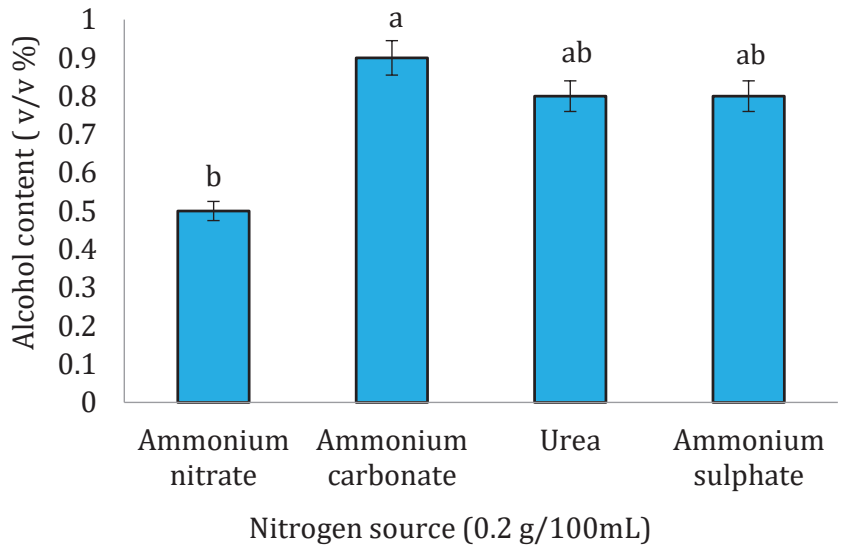

Figure 1: Effect of different nitrogen sources on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae.* Different alphabets show significant differences between the mean values.

Effect of inoculum size

When the size of yeast inoculum was 0.5 g/100mL, ethanol yield was significantly increased by 1.11 times (0.90% to 1.00 %, $p<0.05$) than the non-optimized control (0.2 g/100mL) (Figure 2). Hence 0.5 g/100mL of yeast inoculum was chosen for further studies. The concentration) of added inoculum in the fermentation media does not have a significant influence ($p<0.05$) on final ethanol production but also it affects sugar consumption rate (Laopaiboon *et al*., 2007). When the inoculum concentration increased within a certain range that can causes a reduction in the fermentation time due to the rapid growth of the yeast cells in the fermentation media they immediately consume fed sugars producing ethanol.

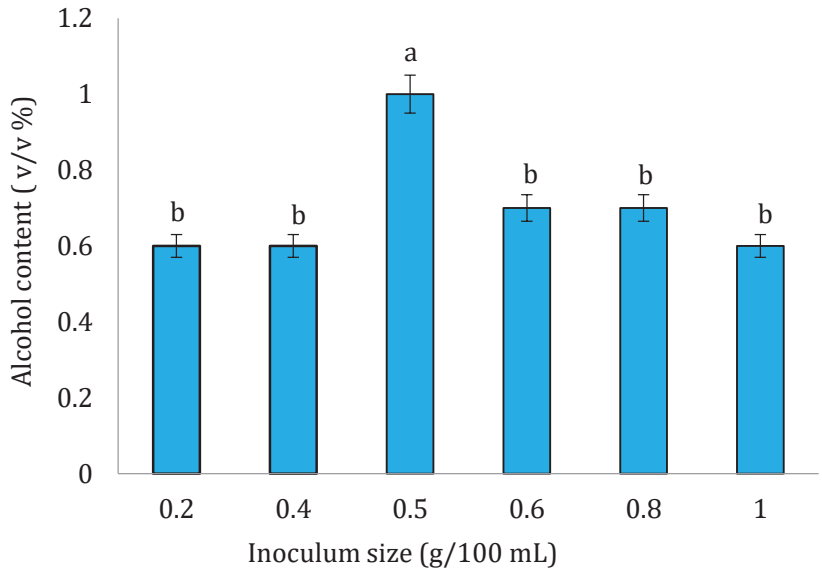

Figure 2: Effect of different size of inoculum on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae*. Different alphabets show the significant differences between the mean values.

Effect of temperature

The bioethanol production after 24 hours at 10, 20, 25, 30, 35 and 40 °C was 0.60%, 1.00%, 1.20%, 0.90%, 0.70% and 0.60% respectively (Figure 3). Even though yeast grew well at temperatures between 30 – 60 °C, the bioethanol production was significantly higher at 25 °C (1.20%,

p<0.05) than the non-optimized temperature 30 °C. When the culturing temperature was optimized as 25 °C, ethanol production was increased by 1.20 times (from 1.00% to 1.20%) than the non-optimized condition (30 °C). At 30 °C and above the bioethanol yield showed a decreasing trend and this decrease may be due to the stress factor on microorganisms, which is unfavorable for their growth. Microorganisms produce heat-shock proteins in response to the high temperature and inactivate their ribosomes. In addition, microbial activity and fermentation process are regulated by different enzymes which are also sensitive to high temperature since it denatures their tertiary structure eventually inactivating them (McMeekin *et al*., 2002; Phisalaphong *et al*., 2006). Microorganisms employed in the fermentation method have optimum temperature range for their better growth. Therefore, it is necessary to predetermine an optimum temperature during fermentation for proper microbial growth as well as a higher yield of ethanol. It is generally believed that the ideal fermentation temperature range is between 20 and 35 °C and high temperature in almost all fermentation processes creates uncontrollable issues (Ballesteros *et al*., 2004; Phisalaphong *et al*., 2006). Hence 25 °C temperature was chosen for further studies.

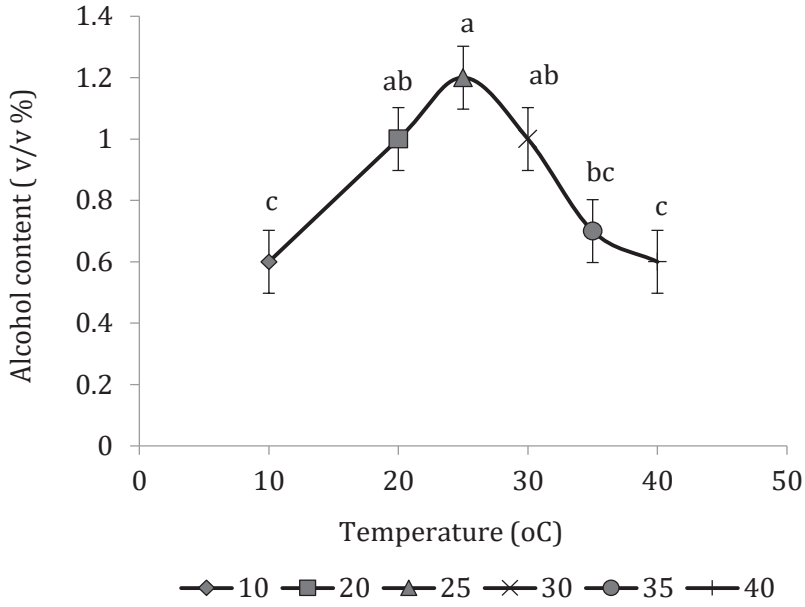

Figure 3: Effect of different temperatures on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae*. Different alphabets show the significant differences between the mean values.

Effect of rotation speed

When different rotation speeds (50, 100, 150, 200, 250 rpm) were used, significantly higher ethanol production (1.30 %, $p<0.05$, Figure 4) was obtained when 150rpm was used. When the rotation speed of the media was optimized as 150 rpm, ethanol yield was increased by 1.08 times than the speed at non-optimized condition (100 rpm). Agitation enlarged the porosity of nutrients from the fermentation broth to inside the cells and in the same way removing ethanol from the cell interior to the fermentation broth. It conjointly will increase the sugar consumption of microbial cells and reduces the inhibition of ethanol on cells. Commonly 150–200 rpm is usually rotation speed is employed for the surplus bioethanol production by yeast cells (Liu *et al*., 2008). Once the surplus agitation is given it ends up in the restricted metabolic activities of microbial cells within the media. Hence 150 rpm rotation speed was chosen for further studies.

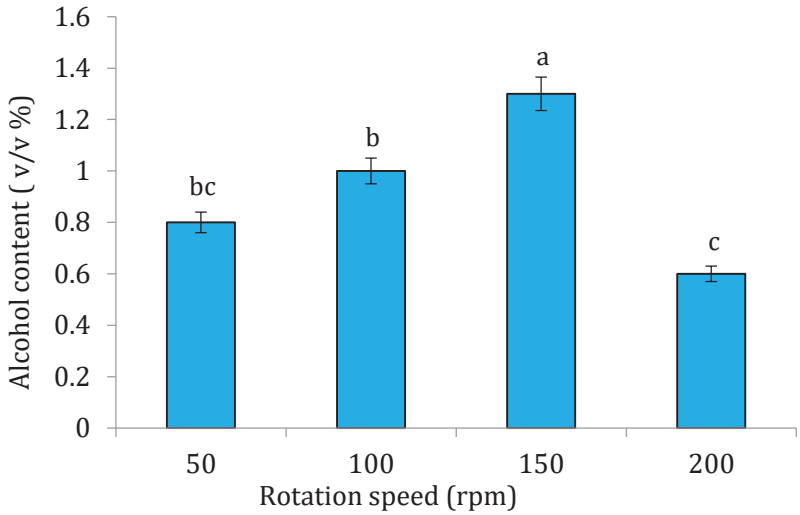

Figure 4: Effect of different rotation speeds on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae*. Different alphabets show the significant differences between the mean values.

Effect of substrate concentration (raw fruit juice)

When different concentrations of raw fruit juice (5%, 10%, 25%, 50%, and 90%) were chosen, significantly higher ethanol production was obtained at 90% of substrate concentration (3.15 times, from 1.30% to 4.10%,

p<0.05) than the non-optimized substrate concentration of 25% (Figure 5). Substrate concentration has the direct effect on fermentation rate and microbial cells. Generally, fermentation rates are going to be enlarged with the rise in substrate concentration up to a definite level. However, the surplus sugar concentration can exceed the uptake capability of the microorganisms cells resulting in a gradual rate of fermentation. Higher ethanol production can get at higher initial sugar concentration (Laopaiboon *et al*., 2007). Hence 90% substrate concentration in the fermentation media was chosen for further studies.

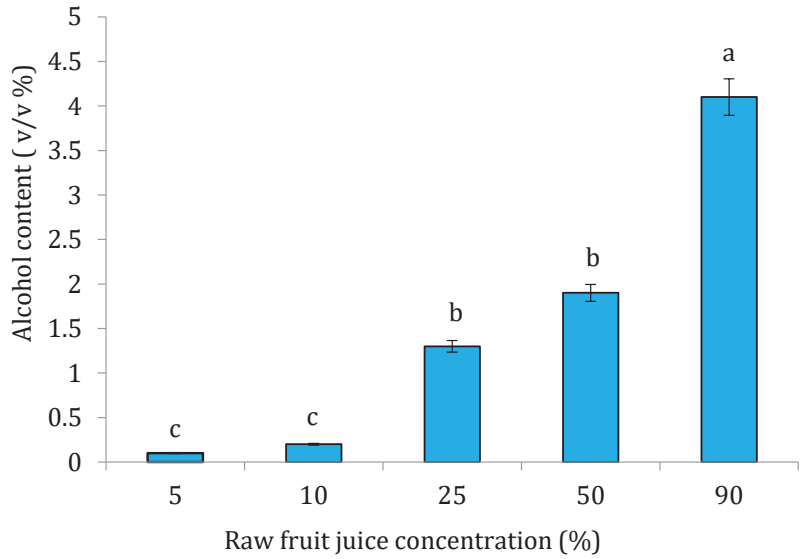

Figure 5: Effect of different concentrations of raw fruit juice on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae*. Different alphabets show the significant differences between the mean values.

Effect of amount of ammonium carbonate

When the amount of ammonium carbonate was used as 0.1 g/100mL, the ethanol yield was significantly increased by 1.07 times (from 4.10% to 4.40%, Figure 6, p<0.05) than the non-optimized amount of ammonium carbonate (0.2 g/100mL). Fermentation medium containing 0.1g of ammonium carbonate yielded significantly higher ethanol production than the other concentrations except for 0.2 g/100 mL. Higher concentration of nitrogen sources may inhibit the growth of yeast in the fermentation

medium and this will lead to a decrease in the ethanol production. Hence 0.1 g/100mL of nitrogen source (ammonium carbonate) in the fermentation media was chosen for further studies.

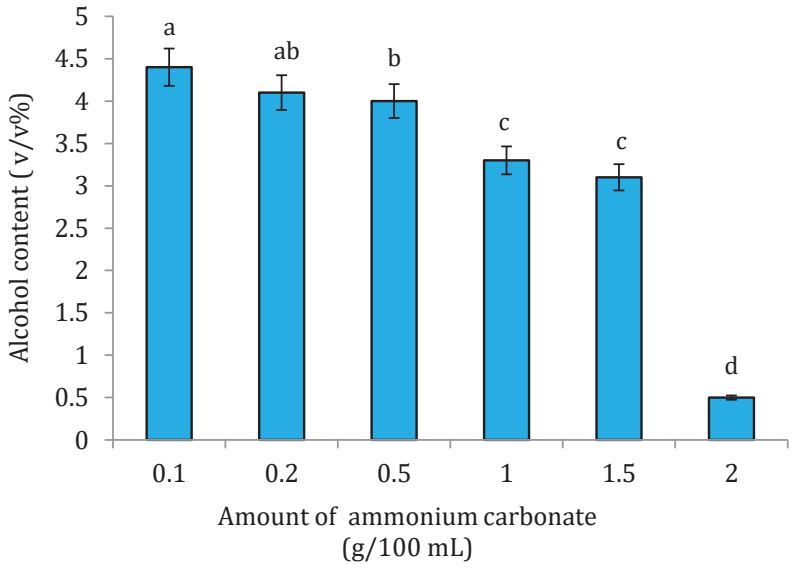

Figure 6: Effect of different amounts of nitrogen sources on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae.* Different alphabets show the significant differences between the mean values.

Effect of carbon source

When different carbon sources such as glucose, sucrose, maltose, and dextrose (2 g/100mL) were separately added in the media setups, significantly higher ethanol production (4.80%, $p<0.05$) was obtained in the medium containing sucrose (Figure 7) than the other media. Sucrose was the best among the carbon sources used for bioethanol production and it may be due to its ability to make the yeast cells develop a foam surface for efficient fermentation than the other carbon sources. Hence sucrose was chosen as the carbon source for further studies.

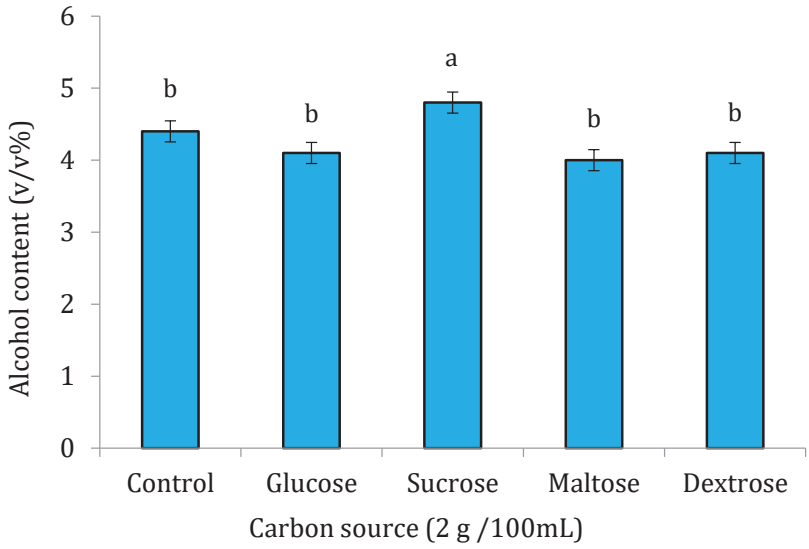

Figure 7: Effect of different carbon sources on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae*. Different alphabets show the significant differences between the mean values.

Effect of amount of carbon source (sucrose)

When the amount of sucrose in the media was optimized as 15 g/100mL, the ethanol yield was significantly increased by 2.33 times (from 4.80% to 11.20%, $p<0.05$) than the non-optimized amount 2 g/100mL (Figure 8). An increase in the concentration of sucrose increases the rate of anaerobic respiration in the yeast cells. An increase in substrate availability allows more cells to use up the substrate for respiration, thereby increasing the amount of its by-product CO_2, High concentration of ethanol is toxic to yeast and it can retard the rate of cell respiration in yeast, or even lead to cell death. Higher concentrations of sucrose in the fermentation media might lead to decrease in the bioethanol production. Hence 15 g/100mL of sucrose in the fermentation media was chosen for further studies.

Effect of pH of the medium

When the pH of the media was kept at6.0, ethanol yield was significantly increased by 1.13 times (from 11.20% to 12.60%, $p<0.05$) than the non-optimized control pH 7.0 (Figure 9). The management of pH has a direct influence on the growth of microorganisms used for fermentation process and co-jointly on their cellular processes (Kasemets *et al*., 2007; Pirselove

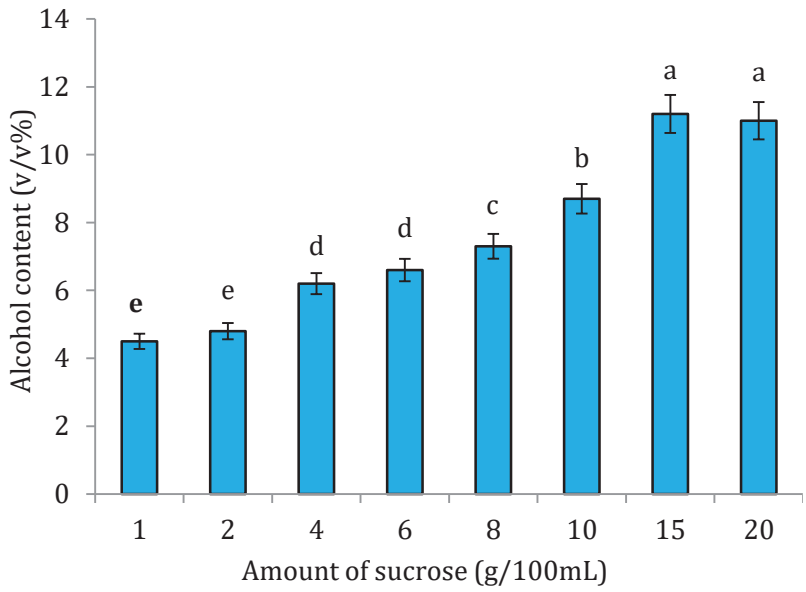

Figure 8: Effect of amount of carbon sources used in the fermentation media on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae.* Different alphabets show significant differences between the mean values.

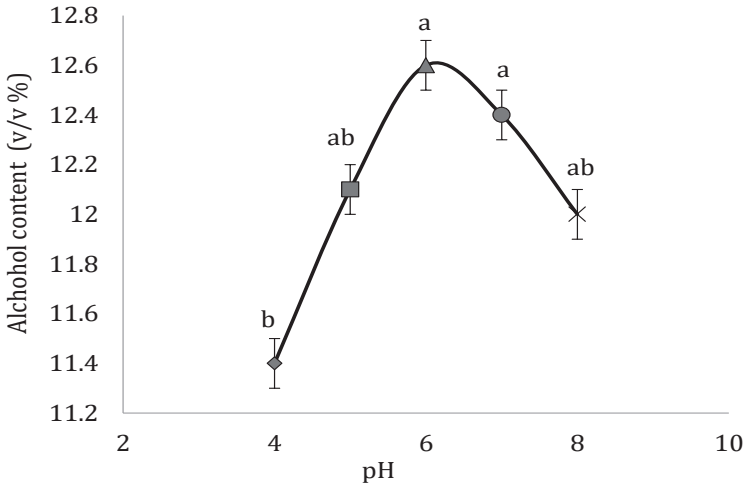

Figure 9: Effect of different pH on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae*. Different alphabets show significant differences between the mean values.

et al., 1993). The H^+ concentrations in fermentation broth will be ready to amendment the entire charge of plasma membrane so moving the porosity of some essential nutrients into the cells. Once fermentation medium becomes more acidic, the fermentation rate conjointly will increase. This might ensue to enzymes made by yeast to ferment aldohexose and these enzymes might need custom made to acidic conditions. Yeast cells are more tolerant to acidic conditions than basic conditions. The organic and inorganic chemicals employed in the media may be responsible for the change in the pH of the media due to the different ions released. Hence pH of the fermentation media was chosen as 6.0 for further studies.

Effect of incubation period

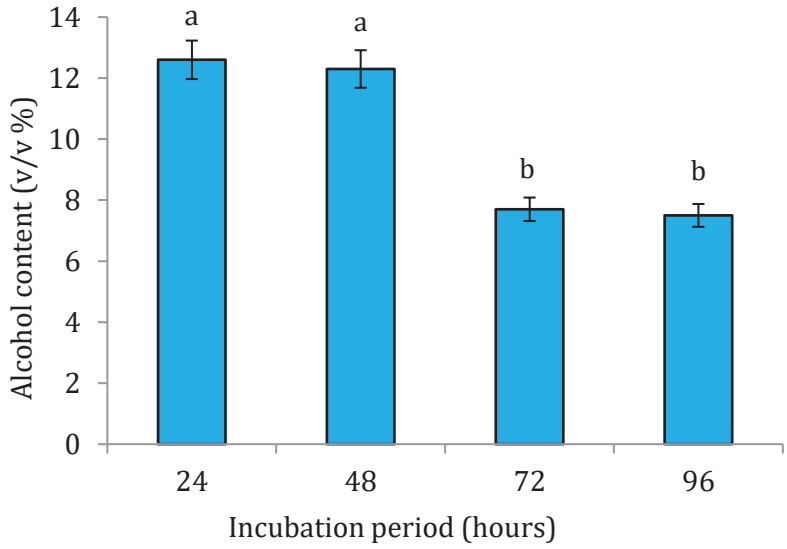

Figure 10: Effect of different incubation periods on bioethanol production from banana fruit juice using *Saccharomyces cerevisiae.* Different alphabets show significant differences between the mean values.

The bioethanol production after 24, 48, 72 and 96 hours of fermentation were 12.6%, 12.3%, 7.7% and 7.5% respectively (Figure 10). Since there was no significant difference in the alcohol yield between the different incubation periods of the media, it was decided to use 24 h as the incubation period for future experiments. Short fermentation time causes inadequate growth of microorganisms within the fermentation media that ends up in inefficient fermentation. Long fermentation time causes toxic

impact on microorganisms growth particularly in batch fermentation due to the presence of a higher concentration of ethanol in the fermented broth (Asmamaw *et al*., 22014; Hossain *et al*., 2011; Nadir *et al*., 2009).

CONCLUSIONS

The *Musa sapientum* (sour) banana juice is an effective substrate for ethanol production using yeast. After optimization of carbon and nitrogen sources, culture conditions, and media composition, the bioethanol yield was significantly increased (15.75 times, from 0.8% to 12.60%) than the non-optimized conditions. Large scale fermentation study should be carried out in order to determine whether this finding could be commercialized.

ACKNOWLEDGEMENT

The authors sincerely express their gratitude to the University of Jaffna research Grant 2016 for their financial support.

DECLARATION OF CONFLICT OF INTEREST

Authors have no conflict of interest to declare.

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