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Microwave Assisted Bioethanol Production from Sago Starch by Co-Culturing of Ragi Tapai and Saccharomyces Cerevisiae

N. Saifuddin and Refal Hussain
Department of Engineering Science and Mathematics, College of Engineering,
University Tenaga Nasional, Jalan IKRAM-UNITEN 43000, Kajang, Selangor, Malaysia

Abstract: Problem statement: Environmental issues such as global warming and recent events throughout the world, including the shortage of petroleum crude oil, the sharp increase in the cost of oil and the political instability of some crude oil producing countries, have demonstrated the vulnerability of the present sources for liquid fuel. These situations have created great demand for ethanol from fermentation process as green fuel. A main challenge in producing the ethanol is the production cost. A rapid and economical single step fermentation process for reliable production of bioethanol was studied by co-culturing commercialized ragi tapai with *Saccharomyces cerevisae* using raw sago starch. **Approach:** Enzymatic hydrolysis of sago starch by various amylolytic enzymes was investigated to reveal the potential coupling mechanism of Microwave Irradiation-Enzyme Coupling Catalysis (MIECC). **Results:** It was shown that enzymatic hydrolysis of starch using typical enzymes may successfully be carried out at microwave condition. The MIECC resulted in increasing initial reaction rate by about 2 times. The results testify on specific activation of enzymes by microwaves and prove the existence of non-thermal effect in microwave assisted reactions. Low power microwave irradiation (80W) does not increase the temperature beyond 40^{o}C and hence denaturation of the enzyme is avoided. The maximum ethanol fermentation efficiency was achieved (97.7% of the theoretical value) using 100 g L^{-1} sago starch concentration. The microwave assisted process improved the yield of ethanol by 45.5% compared to the non-microwave process. Among the other advantages of co-culturing of ragi tapai with *S. cerevisiae* is the enhancement of ethanol production and prevention of the inhibitory effect of reducing sugars on amylolytic activity and the reaction could be completed within 32±1 h. **Conclusion:** The present study have demonstrated the ability of using cheaply and readily ragi tapai for conversion of starch to glucose and the utilization of sago starch as a feed stock, which is cheaper than other starches like corn and potato. The present study has highlighted the importance of well controlled microwave assisted enzymatic reaction to enhance the overall reaction rate of the process.

Key words: Sago starch, bioethanol, co-culture, enzymatic hydrolysis, *Saccharomyces cerevisiae*, microwave assisted reaction, non-thermal effects, enzyme denaturation, Simultaneous Saccharification and Fermentation (SSF)

INTRODUCTION

Heavy reliance on the use of fossil resources for the generation of transportation fuels and materials, has cause a rising concern over their cost, sustained availability and impact on global warming and pollution. Motor vehicles account for a significant portion of urban air pollution in much of the developing world. It is projected that there will be 1.3 billion light duty vehicles, automobiles, light trucks, SUVs and minivans, on roadways around the world by 2030 (Balat and Balat, 2009). According to Goldemberg (2008), motor vehicles account for more than 70% of global carbon monoxide (CO) emissions and 19% of global carbon dioxide (CO_2) emissions. CO_2 emissions from a gallon of gasoline are about 8 kg. Bio-fuels are liquid or gaseous fuels made from plant matter and residues, such as agricultural crops, municipal wastes and agricultural and forestry by-products. Liquid bio-fuels can be used as an alternative fuel for transport, besides other alternatives such as Liquid Natural Gas (LNG), Compressed Natural Gas (CNG), Liquefied Petroleum Gas (LPG) and hydrogen (Semin *et al*., 2009). Bioethanol produced from renewable biomass,

Corresponding Author: N. Saifuddin, Department of Engineering Science and Mathematics, College of Engineering, University Tenaga Nasional, Jalan IKRAM-UNITEN 43000, Kajang, Selangor, Malaysia

such as sugar, starch, or lignocellulosic materials, is one of the alternative energy resources that are both renewable and environmentally friendly (Balat *et al*., 2008; Baras *et al*., 2002). It can be blended with petrol (E5, E10, E85) or used as neat alcohol in dedicated engines, taking advantage of the higher octane number and higher heat of vaporisation and it is also an excellent fuel for future advanced flexi-fuel hybrid vehicles (Chum and Overend, 2001; Kim and Dale, 2005). Internal combustion engines operating on ethanol also produce fewer greenhouse gas (GHG) emissions since ethanol is less carbon-rich than gasoline.

Fermentation-derived ethanol can be produced from sugar, starch or lignocellulosic biomass. Sugar and starch-based feedstocks are currently predominant at the industrial level and they are so far economically favorable. Starch-based materials are currently most utilized for the ethanol production in North America and Europe. The hydrolysis of starch may be considered as a key step in the processing of starch-based feedstock for the bioethanol production. The main role of this step is to effectively provide the conversion of two major starch polymer components: amylose, a mostly linear α-D-(1-4)-glucan and branched amylopectin to fermentable sugars that could subsequently be converted to ethanol by yeasts or bacteria. The most commonly used distillers yeast *S. cerevisiae* is unable to hydrolyse starch. Traditional production of ethanol from starch requires a three-stage process; liquefaction of starch by α-amylase, saccharification of liquefied starch by enzymes to sugars followed by fermentation using *S. cerevisiae.* Amylolytic enzymes from bacteria and fungi are used for the saccharification of starch and this adds to the overall cost of the bioethanol production process. Simultaneous saccharification of starch with an amylolytic yeast or mold and fermentation of saccharified starch by distillers yeast is an effective method for direct fermentation of starch (Somda *et al*., 2011; Nadir *et al*., 2009).

Sago palm *Metroxylon sagu* is an important economic species and is now grown commercially in Malaysia, Indonesia, the Philippines and New Guinea for the production of sago starch. Sago Palm has a great potential to be a Malaysian leading producer of starch. From less than 20,000 hectares in 1991, sago plantations in Malaysia, have grown to around 53,000 hectares in 2010. One of the potential uses of the sago palm is for the production of bioethanol. More importantly, the sago starch is of such a quality that ethanol conversion efficiencies of up to 72% can be obtained (for hydrated ethanol). Taking an optimistic yield of 20 tons of clean starch per hectare, this comes down to an alcohol yield of 14,400 liters, (1540 gallons) per acre, making sago one of the most productive energy crops.

Current world bioethanol research is driven by the need to reduce the costs of production. For example, improvement in feedstock pretreatment, shortening of fermentation time, lowering the enzyme dosages, improving the overall starch hydrolysis and integration of the Simultaneous Saccharification and Fermentation (SSF) process could be the basis of cutting down production costs. Many microorganisms including *Saccharomyces cerevesiae* (yeast) are not able to produce ethanol from starch due to lack of starch-decomposing enzyme. Specific enzymes such as amylase, amyloglucoamylase and pulluanase are needed for hydrolyzing starch (Nurachman *et al*., 2010; Jamai *et al*., 2007). Tapai is a traditional fermented food popular in Malaysia and Indonesia. To prepare tapai, a carbohydrate source and an inoculum containing the microorganisms is necessary. The inoculum is called ragi tapai and is cheaply available in local market. Microorganisms found in the traditional ragi tapai are moulds (*Rhizopus oryzae*, *Amylomyces rouxii*, *Mucor sp. and Candida utilis*) and yeasts (*Saccharomyces cerevisiae*, *Saccharomycopsis fibuliger*, *Endomycopsis burtonii*). The moulds are strong amylolytic (Gandjar, 2003). Microwave Irradiation-Enzyme Coupling Catalysis (MIECC) has also been proven as a useful tool for many enzymatic transformations in both aqueous and organic solutions (Leadbeater *et al*., 2007; Yadav and Sajgure, 2007; Roy and Gupta, 2003). It has been proposed that in case of low power of high-frequency electromagnetic field the nonthermal activation of enzyme may be observed (Yadav and Lathi, 2007; Saifuddin *et al*., 2009). Enzymatic hydrolysis of starch is a very important not only for bioethanol production but many other industrial process and study of amylolytic enzyme working at microwave conditions is of great importance from both the scientific and industrial interest.

The objective of this study is to improve the bioethanol production from raw sago starch by using co-culturing approach and microwave irradiation. Microwave pretreatment will be carried on the starch solution as some previous studies have shown that application of microwave irradiation pretreatment may significantly increase the conversion of starch materials to glucose (Zhu *et al*., 2006; Palav and Seetharaman, 2007). The bioconversion of the microwave treated sago starch in single step process will be performed by co-culturing of commercial ragi tapai and *Saccharomyces cerevisiae*.

MATERIALS AND METHODS

Microorganisms, culture conditions and reagents: *Saccharomyces Cerevisiae* ("Angel" Super dry Yeast for fuel ethanol) was purchased from Angel Yeast Co., Ltd. China. Bacto-Peptone and Yeast extract was purchased from BD Diagnostic Systems USA. The other culture used was commercial ragi tapai (which provides the amylolytic enzymes). It was obtained from the local market.

Sago based on starch flour used in this experiment is from one brand and was obtained from local market in Malaysia. *Saccharomyces cerevisiae* was used for the fermentation of hydrolyzed sago starch. Before using as inoculums, both, the dry *S. Cerevisiae* (5 g) and ragi tapai (10 g) were aerobically propagated separately in 250 mL flasks containing 100 mL YEP broth media (10 g L^{-1} yeast extract, 10 g L^{-1} Peptone and 5 g L^{-1} NaCl) at 37°C and 250 rpm for 3 h. The liquid media was autoclaved at 121°C for 15 min before the aerobic propagation.

The chemical reagents were of analytical grade and used without further purification. Sodium hydroxide was purchased from Merck; acetic acid, sulfuric acid, calcium chloride, ammonium sulphate, magnesium sulphate, anhydrous ethanol, calcium hydroxide and anhydrous glucose were purchased from J.T. Baker.

Optimizing microwave irradiation duration and sago starch concentration: In a typical experiment 25 g of sago starch and 1 mg of $CaCl_2$ were dispersed in 250 mL of deionized water placed in glass flasks (10% w/v sago starch slurry). The pH was adjusted to 7.2. The mixture was heated up to 60°C for 5 min to obtain starch slurry. The slurry was heated to 80°C for 45 min to reduce viscosity. The slurry was allowed to cool to 40°C before addition of the 10 mL of 10% ragi tapai culture suspension. The mixture was then subjected to the microwave treatment in a microwave oven (Sanyo, EM-S9515W). Output power was set at 80 W and the effects of heating between 1-10 min were investigated. The control samples were not subjected to the microwave irradiation. After the microwave treatment at each time interval, the flasks were kept in a water bath with shaker at 45°C with agitation at 60 rpm for up to 20 min. At the end of 20 min, the amount of glucose released was determined for each flask. The result will indicate the optimal time of microwave exposure to achieve the highest amount of glucose.

For optimization of sago starch concentration, similar experiment was conducted but using sago starch slurry of 20% (50 g/250 mL deionized water) and 30% (75 g/250 mL deionized water). The microwave output power was set at 80 W and the exposure time was set at 5 min (optimal time). The amount of glucose was measured as mentioned previously after 20 min incubation at 45°C with agitation at 60 rpm. The result will indicate the optimal concentration of starch slurry to achieve highest amount of glucose and the highest percentage conversion to glucose. Percentage conversion to glucose was calculated using the following equation. The theoretical amount of glucose produced is 1110 g glucose for 1 kg starch (100% efficiency):

$$\text{Percentage conversion to glucose} = \frac{\text{amount of glucose produced } (\frac{g}{kg}\text{starch})}{\text{Theorctical amount of glucose produced } (\frac{g}{kg}\text{starch})} \times 100\%$$

For optimization of level of ragi tapai concentration, another similar experiment was conducted using fix concentration of sago starch slurry at 10% (25 g/250 mL deionized water) and three levels of the ragi tapai concentration of 10%, 20% and 30%. The microwave output power was set at 80 W and the exposure time was set at 5 min (optimal time). The amount of glucose was measured as mentioned previously after 20 min incubation at 45°C with agitation at 60 rpm. The result will indicate the optimal concentration of ragi tapai to achieve highest the highest percentage conversion to glucose. Percentage conversion to glucose was calculated using the previously described equation.

Microwave assisted simultaneous saccharification and fermentation of sago starch: The hydrolysis of sago starch followed by yeast fermentation in single step was performed by sequential co-culture process. The yeast (*S. cerevisiae)* was added 2 hours after the microwave assisted saccharification process for the initiation of the fermentation process. The starch slurry was prepared as mentioned above. The sago starch slurry after addition of 10 mL of 10% ragi tapai was subjected to microwave treatment at 80 W for 5 min (optimal time). After the microwave treatment, the flasks were kept in a water bath with shaker at 45°C with agitation at 60 rpm for 2 h, to facilitate enzymatic hydrolysis (saccharification) of starch to sugar (glucose). At the end of each saccharification period, the flask were individually fermented by addition of 10 mL of 5% *S. Cerevisiae* culture suspension (having an absorbance of 3.8 - 4.0 at 450 nm) along with $(NH_4)_2SO_4$ (1.3 g L^{-1}), $MgSO_4.7H_2O$ (0.01 g L^{-1}) and $CaCl_2$ (0.06 g L^{-1}).The pH was adjusted to 5.5. The

mixture was then subjected to the microwave treatment in a microwave oven with output power 80 W for 5 min. After the microwave treatment, the glass flasks were kept in an incubated shaker at 100 rpm and 37°C for 30 h. Ethanol concentration was measured after the 30 h fermentation period.

Conventional saccharification and fermentation was also performed according to the method mentioned previously but without the use of microwave irradiation. This served as the control process.

Analytical methods: The ethanol and glucose concentration in the samples was measured at several intervals. Samples were collected at every 2 h interval for the first 12 h which consist of 6 data and another 10 data at every 6 h for the next 60 h. A total of 16 data were collected for the entire run of 72 h. During the sago starch hydrolysis and fermentation, the content of reducing sugars, calculated as glucose, was determined by 3,5-dinitrosalicylic acid (DNS) method (Miller, 1959). A standard curve was drawn by measuring the absorbance of known concentrations of glucose solutions at 570 nm.

The ethanol concentrations of samples were determined using a spectrophotometric method with potassium dichromate reagent (Caputi *et al*., 1968). The supernatant taken at various interval was added with 25 mL of chromic acid (potassium dichromate reagent) followed by incubation at 80°C for 15 min. After incubation 1mL of 40% sodium potassium tartarate was added. The absorbance was measured in a UV-Vis spectrophotometer (spectronic 20) at 600 nm. A standard graph was plotted by taking different concentration of absolute ethanol (10-100%) and measuring its absorbance at 600 nm. The concentration of ethanol from the various intervals was determined by reading off from the standard graph.

The fermentation efficiency was computed from the theoretical ethanol yield and that obtained in the various treatments using the equation:

$$\text{fermentation efficiency} = \frac{\text{Ethanol produced } (\frac{g}{kg}\text{starch})}{\text{theorctical yeild of thanol } (\frac{g}{kg}\text{starch})} \times 100\%$$

Theoretical yield of ethanol is 567 g kg^{-1} starch and was calculated using the equation:

$$\text{Theoreticalyield } \left(\text{g kg}^{-1} \text{ starch}\right) = \frac{51^{a} \times 1.11^{b}}{1.0}$$

Where:

a = Theoretical yield (g) of ethanol from 1.0 kg glucose

b = Yield of glucose (kg) from 1.0 kg starch

RESULTS AND DISCUSSION

Optimization of Microwave treatment for hydrolysis of starch: Hydrolysis of starch prior to fermentation to ethanol is a very important step because the yeast, *S. cerevisiae*, is non-amylolytic and was reported to be unable to hydrolyze starch (Jamai *et al*., 2007). It is however, a very good candidate for fermentation of sugar to ethanol. Figure 1 presents the influence of the duration of the microwave treatment (80 W) on the concentration of glucose achieved after the liquefaction of the sago starch slurry. The low level of MW power was used in order to avoid the enzyme denaturation and to minimize the thermal effects of the process. As shown in Fig. 1, the duration of 5 min was the optimal exposure time for the microwave treatment at 80W output power. Therefore microwave treatment of 5 min was selected for further experiments since during that time maximal glucose concentrations were attained. The microwave experiments were carried out at low power level and constant temperature. The reason is the well known phenomenon of enzyme denaturation at high temperature which decreases the catalytic activity of the enzymes. Microwave treatment helps in destroying the crystalline starch structure and hence makes it easier for the enzymes to convert it to glucose. Similarly, relatively short duration of the microwave treatment was also selected by other investigator as appropriate for destroying the starch crystalline arrangement (Palav and Seetharaman, 2007). Khanal *et al*. (2007) reported that ultrasound pretreatment (2 kHz; 20 and 40 s) enhanced glucose yield due to reduction in particle size and better mixing. It may also help in the release of starch from its complex with lipids. However, Nikolic *et al*. (2010) found that ultrasound treatment consumed a large amount of energy, adding further towards the cost of bioethanol production. On the other hand, similar increased in efficiency of the hydrolysis process probably through the same phenomenon can be obtained by using microwave irradiation which consumes much lesser energy than ultrasound.

The results from starch concentration optimization experiments (10, 20 and 30%) indicated that as the starch slurry concentration increased, the amount of conversion of starch to glucose also increased. Even though the quantity of glucose released was more from 30% (w/v) slurry (Fig. 2), the percentage conversion to glucose was the highest with 10% (w/v) slurry (Fig. 3).

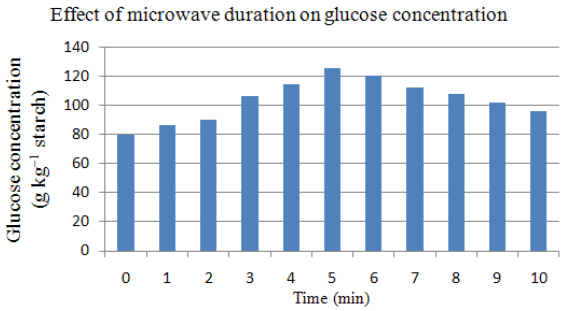

Fig. 1: The effect of time of microwave exposure on glucose concentration obtained after hydrolysis of sago starch (10%) at 80 W with 10 mL of 10% ragi tapai. The control sample is the 0 min

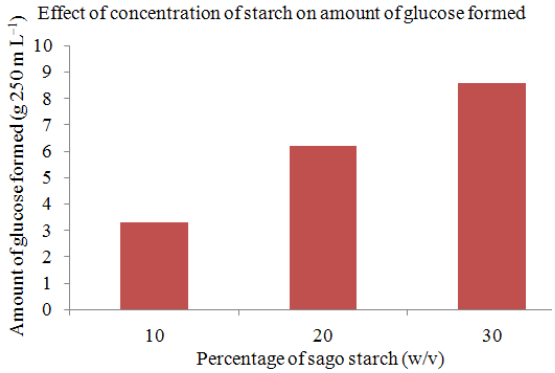

Fig. 2: All the three starch slurries (10, 20 and 30%) were added initially added with 10 mL of 10% ragi tapai and irradiated with microwave at 80W for 5 min. The amount of glucose released was monitored after 20 min incubation at 45°C

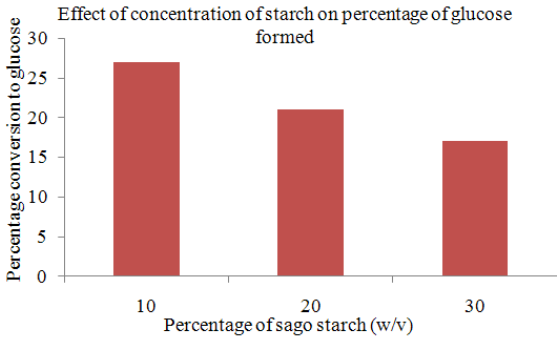

Fig. 3: The percentage conversion to glucose after 20 min incubation at 45°C for three different concentrations of starch slurry (10, 20 and 30%) was calculated from the data obtained from Fig. 2 using the equation given in the text

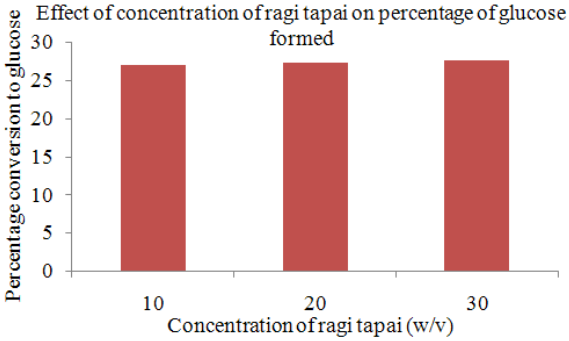

Fig. 4: The percentage conversion to glucose after 20 min incubation at 45°C for three different concentrations of ragi tapai (10, 20 and 30%) at starch slurry concentration of 10% was calculated using the equation given in the text

When 20% (w/v) and 30% (w/v) starch slurry were used the rate of conversion to glucose did not increase proportionally with the increase in starch content. In fact the rate was fastest with 10% slurry. In high viscous environment i.e. higher starch concentration the inactivation of amylase and other enzymes during the microwave irradiation may be observed because of hot spots generated in the system arising from poor heat exchange. On the other hand at lower viscosity i.e. lower starch concentrations heat exchange is better and hot spots are not created. It is anticipated that at low power level of microwave irradiation non-thermal effects of microwave play a role. At low power level the active site of the enzyme molecules may undergo conformational changes to favor the cleavage of the glycosidic bonds. This will enhance the efficiency of the enzyme. Non-thermal effects or microwave effect has been observed in a number of microwave assisted catalytic or enzymatic reactions (La Hoz *et al*., 2007; Yadav and Lathi, 2007; Saifuddin *et al*., 2011). Previous study on ethanol production from fresh cassava mash also showed that high viscosity caused resistance to solid–liquid separation and lower fermentation efficiency (Srikanta *et al*., 1992). High viscosity also causes several handling difficulties during processes and may lead to incomplete hydrolysis of starch to fermentable sugar (Wang *et al*., 2008).

There was minimal increase in the percentage conversion of starch, when the ragi tapai concentration levels were increased from 10-20 and 30% with the starch slurry at 10% (Fig. 4). Increasing the level of concentration of ragi tapai did not result in any further increase in the starch hydrolysis. Most studies have shown that *S. cerevisiae* is non-amylolytic yeast and

was reported to be unable to hydrolyze starch (Jamai *et al*., 2007). However study by Azmi *et al*. (2010) has shown that *S. cerevisiae* has the ability of hydrolysing starch but at very low rate. The study also reported that ragi tapai is more efficient in hydrolyzing raw starch to glucose compared to the fungi *Candida tropicalis* (Azmi *et al*., 2010). This will subsequently produce higher ethanol.

Microwave assisted simultaneous saccharification and fermentation of sago starch: Microwave assisted fermentation by yeast was performed by sequential co-culture process in a single step. The *S. cerevisiae* (yeast) was added 2 h after the microwave assisted saccharification treatment. This was done in order to bring sufficient amount of sugar before the start of fermentation. Two sets of experiments (microwave assisted saccharification and fermentation and conventional saccharification and fermentation) were performed. In microwave assisted sacchrification and fermentation samples were subjected to 80W microwave irradiation for 5 min after addition of ragi tapai. After saccharification period of 2 h at 45°C the fermentation process was started by adding the yeast and subjected to microwave irradiation at 80W for 5 min. After which it was incubated at 37°C for up to 36 h. Figure 5 shows the result of both the microwave assisted saccharification and fermentation and conventional saccharification and fermentation. The microwave assisted process showed higher amount of ethanol production with 553 g ethanol per kg starch produced after 30 h of fermentation. In the conventional process the amount of ethanol produced was 296.1 g per kg starch at 30 h of fermentation. The co-culture after 2 h allows the mixed culture to hydrolyze the raw starch into glucose and made them available for *S. cerevisiae* to subsequently ferment them into ethanol. Simultaneous saccharification and fermentation has the advantage that high sugar concentrations are never achieved in the system, which will facilitate the enzymatic hydrolysis of starch to be carried in the forward direction. Microwave aided ragi tapai hydrolysis followed by yeast fermentation for 36 h showed that the residual reducing sugar in the fermented broth after 30 h was about 2.25 ± 1.50 g L^{-1}. Hence the total amount of utilized glucose was 98% indicating the end of fermentation. This indicated that most of the sugars formed were simultaneously fermented to ethanol before it accumulate and correspondingly inhibit the fermentation process by osmotic pressure on the cells (Bai *et al*., 2008). For the non-microwave process residual reducing sugars in the fermented broth after 30 h was about 10.60 ± 1.50 g L^{-1}.

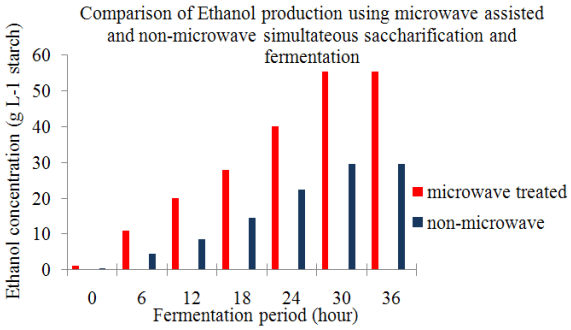

Fig. 5: The starch slurry concentration used was 100g L^{-1}. Both saccharification and fermentation were subjected to 80W microwave irradiation for 5 min. For non-microwave saccharification and fermentation microwave irradiation was not applied

Fermentation efficiency for microwave added saccharification and fermentation was very high with 97.7% efficiency. The non-microwave process had fermentation efficiency of about 52.2%. Previous study on enzyme catalysed liquefaction and saccharification of sweet potato starch showed that liquezyme X (Novo Industries, Denmark) could liquefy the starch at pH 7.0 and 90°C within 1 h, followed by saccharification by Dextrozyme GA at pH 4.0 and 60°C. However, approximately 95-96% conversion of starch only could be achieved during a saccharification period of 48 h.

When we compared the results obtained in the simultaneous saccharification and fermentation of corn meal with microwave (Nikolic *et al*., 2008) and ultrasound pretreatment (Nikolic *et al*., 2010) to the control sample, the ethanol concentration was increased by 13.4% by microwave and 11.15% by ultrasound pretreatment. It was mentioned that this may be due to the mechanism of microwave action on swelling and gelatinisation of starch granules and destruction of the starch crystalline arrangement was probably different compared to the ultrasound. However in this study the improvement recorded with microwave treatment for both the processes (saccharification and fermentation) gave a total efficiency improvement of about 45.5%. As stated earlier low power microwave irradiation does not increase the temperature beyond 40°C and hence denaturation of the enzyme is avoided. The significant contributor is proposed to be the non-thermal effects, while the thermal effect plays only a minor role under these conditions. The "thermal" effects refer to interactions resulting in increased random motion of particles (e.g., atoms, molecules, ions, or electrons)

where the kinetic energy statistics of such fluctuations are represented by a single thermodynamic equilibrium distribution (i.e., Maxwell-Boltzmann, Bose-Einstein, or Fermi-Dirac). "Non-thermal" effects refer to interactions resulting in non-equilibrium energy fluctuation distributions or deterministic, time-averaged drift motion of matter (or both) (Kuhnert, 2002; Booske *et al*., 1997). This provides the molecule collision under microwave irradiation extra driving force compared to that under conventional heating, which results in higher rate of reaction under mmicrowave irradiation as long as the enzyme is not deactivated by microwave. The other contribution of the non-thermal effects is that microwave energy can also modulate the configuration of enzyme molecules by accelerating the molecular rotation and electron spin oscillation of the active site of the enzyme, which can provide more chance to make the substrates fit to the enzyme in unit of time (Saifuddin *et al*., 2011). The specific nature of this enzyme increases yield tremendously. Li and Yang (2008) in the production of zeolite membranes, have speculated on the non-thermal effects of microwave reaction. Apparently, microwave heating could result in different membrane morphology, orientation, composition for the zeolite membranes. Huang *et al*. (2005) reported that there is microwave effect on substrate specificity of alcohol. The reaction rate altering can be explained in respect of two important parameters-polarity and steric hindrance effects.

CONCLUSION

Ragi tapai was chosen based on its ability to produce glucose and ethanol yields from starch directly as presented previous by other researches, but with low yields (Azmi *et al*., 2010). Since the yields are low, co-culturing with yeast had been proposed as a way to increase the yield. Maximum processing time needed was 2 hr of hydrolysis and 30 hours of fermentation for the ragi tapai-yeast system. Rapid utilization of the glucose by yeast also prevented bacterial contamination in the broth, permitting an almost complete conversion of glucose to ethanol. Simultaneous single step bioconversion from unhydrolyzed sago starch into ethanol will not only reduce the cost of enzymes that is used in liquefaction and saccharification steps but will also reduce the substrate inhibition, especially on yeast cells. Besides the advantage of using cheaply and readily ragi tapai for conversion of starch to glucose, the feed stock is also cheaper than other starches like corn and potato. The present study had highlighted the importance of well controlled microwave assisted enzymatic reaction to enhance the overall reaction rate of the process. It is worth to point out some general statements: (1) Enzymatic hydrolysis of starch using typical enzymes may successfully be carried out at microwave condition (2) The effect of microwave irradiation strongly depends on: (a) Microwave power level - higher levels of MW may cause denaturation of the enzyme; (b) Viscosity of the reaction system that is the function of starch concentration-in less concentrated slurry the diffusion of heat is uniform with no hot spots and hence allowed the increase the reaction rate without denaturation of the enzyme (3) The dominant factor in the microwave assisted reaction in this study may be treated as non-thermal effects; (4) The Microwave Irradiation-Enzyme Coupling Catalysis (MIECC) effect on ethanol production had shown an reaction rate increase of close to two times.

In the future work, the ethanol produced could be tested on a spark-ignition engine to monitor the emission and other thermodynamic parameters. The ethanol can be tested to see its compliance with ASTM D4806, which is a standard for anhydrous denatured fuel ethanol for blending with gasoline and ASTM D5798, which is a standard specification for fuel ethanol (Ed75-Ed85) for automotive spark-ignition engine.

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