

Article

Ethanol Fermentation of Microwave-assisted Acid Hydrolysate of Cassava Pulp with *Saccharomyces cerevisiae* in the Presence of Activated Carbon

Euis Hermiati ^{1*}, Djumali Mangunwidjaja ², Titi Candra Sunarti ², Ono Suparno ², Bambang Prasetya ³, Sita Heris Anita ¹, Lucky Risanto ¹

¹ R & D Unit for Biomaterials, Indonesian Institute of Sciences, Jl. Raya Bogor Km 46, Cibinong, Bogor 16911, Indonesia

² Department Agro-industrial Technology, Faculty of Agricultural Engineering and Technology, Bogor Agricultural University, Kampus IPB Darmaga, Bogor, Indonesia

³ Deputy for Life Sciences, Indonesian Institute of Sciences, Sasana Widya Sarwono 3rd Floor, Jl. Jend. Gatot Subroto 10, Jakarta 12710, Indonesia

* Author to whom correspondence should be addressed; E-Mail: e_hermiati@yahoo.com; Tel.: +62-21-87914511; Fax: +62-21-87914510.

Article history: Received 12 June 2012, Received in revised form 7 August 2012, Accepted 13 August 2012, Published 15 August 2012.

Abstract: The use of activated carbon has been proved could increase glucose yield from microwave-assisted hydrolysis of cassava pulp in water medium, but not in acid medium. The aim of this research was to study the effects of activated carbon addition on the fermentation of cassava pulp hydrolysate. In this work the fermentation of cassava pulp hydrolysates produced from microwave-assisted acid hydrolysis, either of those with or without activated carbon, was performed. The results of the fermentation were also compared with those resulted from hydrolysates treated with activated carbon for inhibitors adsorption before the fermentation. The hydrolysis was conducted in 0.88% sulfuric acid at 20% substrate concentration, 50% or 550 W microwave power level for 9 min. The amount of activated carbon added was 25% (w/w). The fermentation was performed in a gyratory shaker (150 rpm) at room temperature with *Saccharomyces cerevisiae* LIPI MC 0070 for 120 h. The samples were taken every 24 h for analysis of pH, total sugar, reducing sugar, glucose and ethanol. At the end of the fermentation ethanol yield and concentration obtained in samples treated with activated carbon (27-28% of dry matter; 34-35 g/L) were lower than those without activated carbon or those with activated carbon added after hydrolysis (31-32% of dry matter; 38-39 g/L). However, the fermentation was completed

very much faster in the hydrolysates with activated carbon which has a high adsorption property than in those without activated carbon or with activated carbon of low adsorption property. Therefore, ethanol productivity (0.44-0.55 g/L/h) of the former was higher than the latter (0.28-0.34 g/L/h).

Keywords: acid hydrolysates; activated carbon; cassava pulp; ethanol; fermentation; microwave heating.

1. Introduction

Cassava pulp contains significant amount of carbohydrates, especially starch, so it is a potential raw material for glucose production. Our previous research shows that the hydrolysis of cassava pulp in sulfuric acid under microwave irradiation was able to produce a high glucose yield and a high glucose concentration in the hydrolysates, 87% of theoretical (77% of dry matter) and 140 g/L, respectively (Hermiati *et al.* 2012c). However, the glucose produced from acid hydrolysis of starchy or lignocellulosic materials might be difficult to be fermented by microorganisms due to the formation of some secondary products of carbohydrates degradation, such as furfural and hydroxymethyl furfural (HMF) (Larsson *et al.* 1999; Palmqvist and Hahn-Hagerdal 2000). These two compounds inhibit the growth of yeast cells of *Saccharomyces cerevisiae*, and consequently, slower the production of ethanol (Boyer *et al.* 1992a, 1992b). Our previous research shows that the addition of activated carbon in the hydrolysis of cassava pulp in water medium under microwave irradiation increases the glucose yield as well as reduces the concentration of HMF in the hydrolysates (Hermiati *et al.* 2012a), whereas it decreases the glucose yield when the hydrolysis is conducted in acid medium (Hermiati *et al.* 2012b). Activated carbon has been known as a useful material for a detoxification process of some acid hydrolysates, which usually contain some inhibitors or toxic compounds, so that the fermentation of the hydrolysates can be completed more rapidly (Musatto and Roberto 2004; Ge *et al.* 2011). Another report also mentions that the presence of activated carbon in the fermentation could enhance the fermentation process of glucose solution using *S. cerevisiae* (Ikegami *et al.* 2000).

To the best of our knowledge, there was not any report on the use of activated carbon in the fermentation of the hydrolysates produced from a microwave-assisted acid hydrolysis as well as the effects of the use of the activated carbon on the fermentation process. Therefore, in this work, the effects of the addition of two types of activated carbon having two different adsorption properties, on the results of ethanol fermentation of cassava pulp hydrolysates was studied. Each activated carbon was added at the same amount, either at the time of hydrolysis or after the hydrolysis, and kept in the

hydrolysates during the whole fermentation process, and then, the results of each fermentation process were evaluated.

2. Materials and Methods

4.1. Materials

Cassava pulp was collected from a home industry of tapioca in Bogor, West Java, Indonesia. The material was prepared as previously reported (Hermiati *et al.* 2011). Activated carbon 1 (Y-8/20 AW, previously Y-10S AW) was obtained from Ajinomoto Fine-Techno Co., Inc., Japan, and the other activated carbon was purchased from Bratachem, Bogor, Indonesia. Both types of activated carbon are granular types with particle sizes 10-20 mesh. Activated carbon 1 (AC1) has a iodine adsorption number of 1213 mg/g, methylene blue adsorption number of 264 mg/g and surface area of 982 m²/g, whereas those values for activated carbon 2 (AC2) are 1075 mg/g, 18 mg/g, and 67 m²/g, respectively. Sulfuric acid and other reagents are of analytical grades. The culture of *Saccharomyces cerevisiae* LIPI MC 0070 was obtained from Research Center for Biology, Cibinong, Bogor, Indonesia.

4.2. Cassava Pulp Hydrolysis under Microwave Irradiation

Suspension of cassava pulp in 0.88% sulfuric acid (4 g/20 mL or 20%) was put in a 100 mL Teflon tube and mixed with a stirrer bar to homogenize the suspension. Activated carbon (1 g) was added to the samples with activated carbon treatment. The samples were then subjected to hydrolysis using SHARP R-360J(S) kitchen microwave oven, having frequency of 2,450 MHz with output power of 1100 Watt. The irradiation was conducted at 50%, approximately equal to 550 W, power level for 9 min, which is the optimum condition obtained in the previous research (Hermiati *et al.* 2012c). After microwave irradiation, the sample was cooled immediately to room temperature in an ice bath.

4.3. Analysis of Soluble Fraction in the Cassava Pulp Hydrolysates

The soluble fraction was analyzed for its total soluble solid (TSS) using ATAGO Hand Refractometer N-20. Glucose content was determined by Glucose CII test kit (Wako Junyaku, Co., Osaka), and the glucose yield was estimated as starch-based theoretical yield. Phenol sulfuric acid and Somogyi-Nelson methods were used for the determination of total sugar and reducing sugar content, respectively (Wrolstad *et al.* 2005). The pH value was determined using pH meter, while the formation of brown compounds was determined by measuring absorbance at 490 nm (Warrand and Janssen, 2007; Whistler and Daniel, 1985) and measuring the HMF content according to AOAC (980.23-1999).

4.4. Preparation of Cassava Pulp Hydrolysates for Ethanol Fermentation

The preparation of cassava pulp hydrolysates is presented in Figure 1. After microwave-assisted acid hydrolysis, there were two types of hydrolysates, the one without activated carbon (H0) and the one with activated carbon (H1A, H2A). The hydrolysates were neutralized by the addition of NH₄OH 5% up to pH of 5.0-5.5. The neutralized hydrolysates were put in 50 mL centrifuge tubes. Some of the hydrolysates without activated carbon treatment in the hydrolysis were then treated with the same types and the same amount of activated carbons as the ones used in the hydrolysis (H1B, H2B). The treatment was conducted in a water bath shaker at 50 °C for 30 min. The aim of this process was for adsorption of fermentation inhibitors. The volume of the hydrolysates was made to 27 mL by the addition of distilled water.

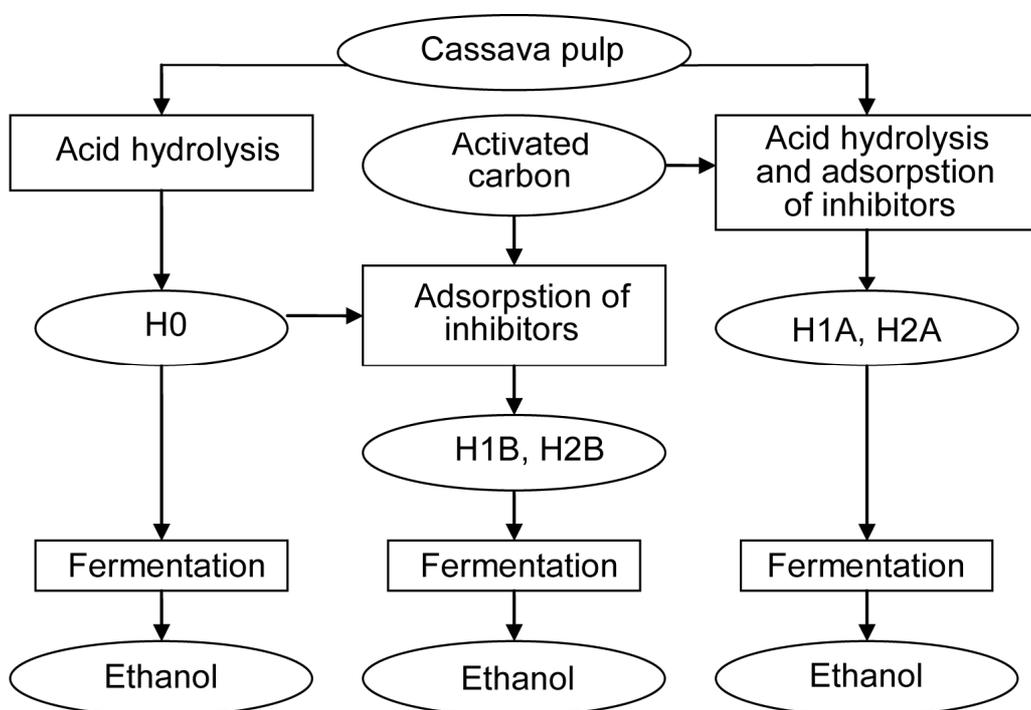


Figure 1. Flow diagram of the preparation of cassava pulp hydrolysates for ethanol fermentation. H0 = hydrolysates without activated carbon; H1A and H1B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis; H2A and H2B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis.

4.5. Preparation of *S. cerevisiae* *Innoculum*

The culture of *S. cerevisiae* was inoculated in a PDA (Potato Dextrose Agar) slant, then, it was incubated at room temperature for 5-7 days. Five mL YPG (yeast extract 10 g/L, peptone 20 g/L, glucose 50 g/L, pH 5) was sterilized, then it was added to the agar slant that contain *S. cerevisiae*. The yeast was dissolved into the YPG solution, after that it was transferred to another 25 mL YPG medium, and incubated in a water bath shaker at 30 °C for 24 h. Optical density of the yeast culture was

measured at 600 nm and the number of cells was estimated using haemocytometer. The yeast was ready to be transferred into the cassava pulp hydrolysates for the fermentation process.

4.6. Fermentation of Cassava Pulp Hydrolysates by *S. cerevisiae*

All of the hydrolysates prepared were sterilized at 121 °C for 15 min, and cooled down to room temperature. The culture of *S. cerevisiae* (10% v/v) was added to the sterilized hydrolysates, so that the total volume of each hydrolysate was 30 mL. The fermentation was performed in a gyratory shaker at 150 rpm, at room temperature. Samples were taken after 24, 48, 72, 96 and 120 h, except for H1A and H1B only until 96 h of fermentation. The fermentation broth was centrifuged at 8000 rpm, 5 °C for 20 min, and the supernatant was separated from the residue. The supernatant was analyzed for its pH, total soluble solids, glucose, total sugar, and reducing sugar content. Ethanol content (% v/v) in the hydrolysates was analyzed using a Shimadzu 14B gas chromatograph with Carbowax 20M column and FID (Flame Ionization Detector). The temperature of the column was 80 °C (isothermal), and an air, hydrogen and nitrogen pressures were 50, 70, and 150 kPa, respectively. Pure ethanol was used as an external standard.

3. Results and Discussion

3.1. Changes of pH during the Fermentation

The pH of the cassava pulp hydrolysates without activated carbon treatment (H0) showed a great decrease after 72 h of fermentation, whereas the pH of the hydrolysate treated with AC1 (H1A and H1B) had been decreased after 48 h of fermentation and those treated with AC2 (H2A and H2B) had been decreased after 24 h of fermentation (Figure 2).

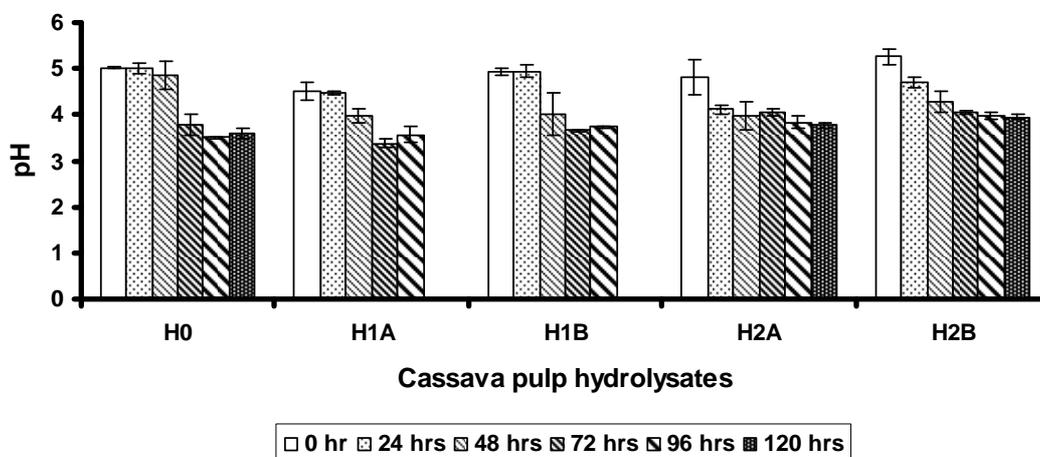


Figure 2. The changes of pH of cassava pulp hydrolysates during fermentation. Data points are mean values \pm SD (n = 3). H0 = hydrolysates without activated carbon; H1A and H1B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis; H2A and H2B = hydrolysates treated with AC2, added in the hydrolysis and after hydrolysis.

These changes of pH indicated that the activity of *S. cerevisiae* in converting glucose to ethanol was greater in the hydrolysates with activated carbon treatment. As we know, glycolysis is the first step in the ethanol fermentation, where glucose was converted to pyruvic acid. After that, pyruvic acid is oxidized to acetaldehyde. In this process carbon dioxide is produced. The acetaldehyde is then converted to ethanol. The pyruvic acid and the dissolved carbon dioxide might cause the reduction of pH value in the hydrolysates.

3.2. Changes of Sugar Concentration during the Fermentation

The glucose concentration in the cassava pulp hydrolysates was approximately 140 g/L. The concentration was decreased after the neutralization using NH_4OH 5% and the dilution of the hydrolysates using distilled water to make the same volume throughout the hydrolysates. The glucose concentration in the hydrolysates for fermentation was dropped to approximately 85 g/L. The decrease of total soluble solid as well as total sugar and reducing sugar concentration due to the dilution process were also observed. Hence, the ethanol concentration in the hydrolysates was also lower than it should be if there was not any dilution before the fermentation.

The results of the analysis of total soluble solids (Figure 3), total sugar, reducing sugar (Table 1) and glucose (Figure 4) are in agreement with the results of pH measurements, which indicated that there was a difference in the activities of *S. cerevisiae* in the five types of cassava pulp hydrolysates prepared. These analyses show that the ethanol fermentation was faster in the hydrolysates treated with AC1, either the activated carbon was added in the hydrolysis or after the hydrolysis. There was a great decrease in the total soluble solids, total sugar, reducing sugar and glucose concentration in the hydrolysates treated with AC1 (H1A and H1B) after 72 h of incubation. The decrease of those parameters was much slower in the hydrolysates treated with AC2 (H2A and H2B) or those not treated with activated carbon (H0). For example, almost all of glucose in H1A and H1B was consumed by the yeast after 72 h of incubation, while that in H0, H2A and H2B was consumed after 120 h of incubation (Figure 4). The difference in the adsorption properties of AC1 and AC2 might affect the difference in the fermentation performances of the hydrolysates treated with AC1 and AC2. AC1 which has higher adsorption capacities and larger surface area could adsorb more inhibitor compounds than AC2, so that the yeast could grow faster in the hydrolysates treated with AC1 than that treated with AC2. Thus, the glucose in the hydrolysates treated with AC1 was consumed more rapidly than that in the hydrolysates treated with AC2. The estimation of degree of polymerization in Table 1 shows that there was an increase in the degree of polymerization of the soluble carbohydrates in the hydrolysates due to an increase of incubation time. At the end of the fermentation the estimated degree of polymerization of the soluble carbohydrates was close to two. This suggests that *S. cerevisiae* only consumed the glucose

and did not consume the disaccharides, such as maltose. Therefore, there might be some maltose left in the hydrolysates.

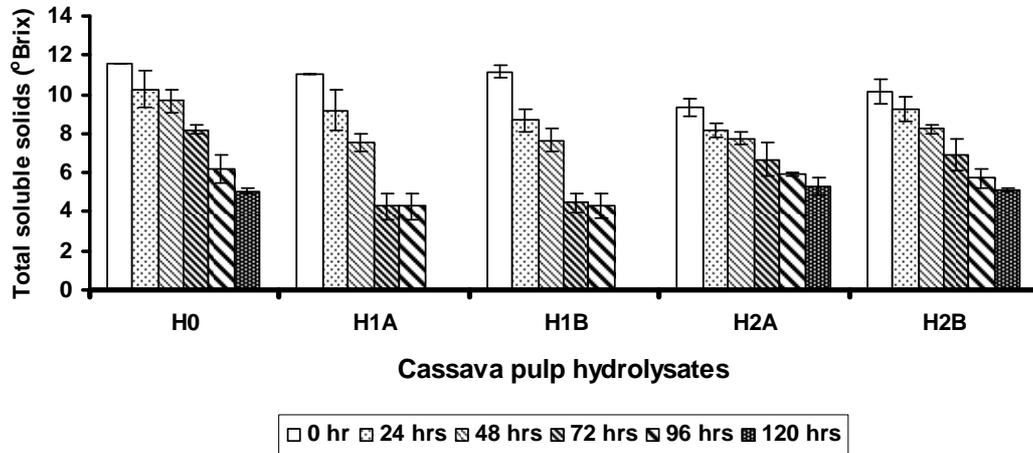


Figure 3. The changes of total soluble solids in the cassava pulp hydrolysates during fermentation. Data points are mean values \pm SD (n = 3). H0 = hydrolysates without activated carbon; H1A and H1B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis; H2A and H2B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis.

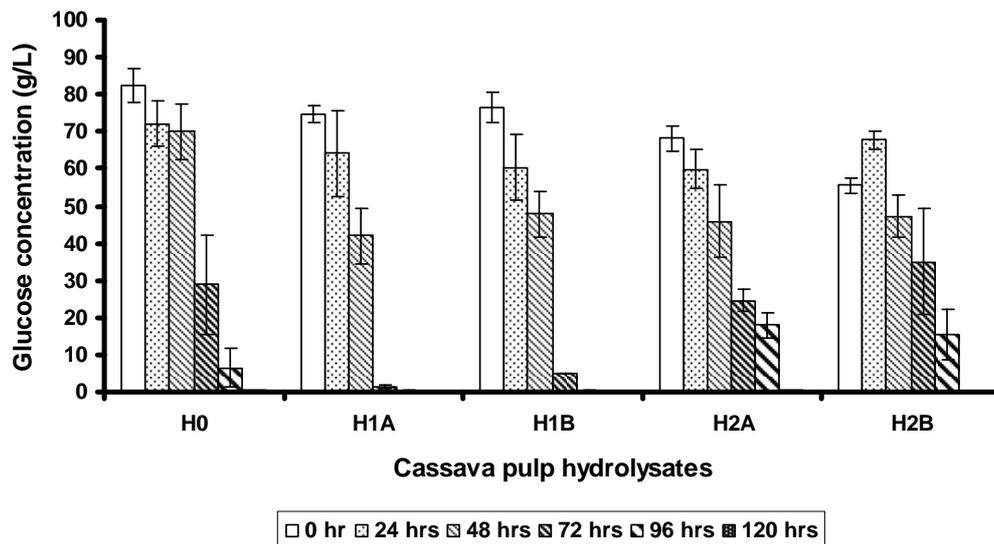


Figure 4. The changes of glucose concentration in the cassava pulp hydrolysates during fermentation. Data points are mean values \pm SD (n = 3). H0 = hydrolysates without activated carbon; H1A and H1B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis; H2A and H2B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis.

3.3. Ethanol Production from the Fermentation of Microwave-assisted Acid Hydrolysates of Cassava Pulp

Ethanol production from the fermentation of microwave-assisted acid hydrolysates of cassava pulp was in agreement with the results of pH and sugar concentration changes in the hydrolysates. Table 2 shows that the highest ethanol concentration in H1A (35.40 g/L) and H1B (39.58 g/L) was obtained after 96 and 72 h, respectively, while that from H0 (38.21 g/L), H2A (33.80 g/L) and H2B (39.40 g/L) was obtained after 120 h of incubation. It is also interesting to note that the highest ethanol production obtained from H0, H1B and H2B was higher than that obtained from H1A and H2A. This might be due to the adsorption of some maltooligosaccharides on the surface of activated carbon during the hydrolysis of H1A and H2A. In these samples, activated carbon was added to the cassava pulp suspension and involved in the hydrolysis using microwave heating. The maltooligosaccharides adsorbed on the surface of activated carbon could survive from further hydrolysis to glucose (Matsumoto *et al.* 2008 & 2011), so that there was lower glucose obtained in the hydrolysates, and consequently, there was lower ethanol concentration as well. An experiment using glucose solution (data not shown) indicates that some of the glucose was also adsorbed on the surface of activated carbon. The initial glucose data in Table 3 also shows that some glucose was adsorbed on the surface of activated carbon, which is indicated by the higher initial glucose concentration before the addition of activated carbon (82.4 g/L) than after the addition of activated carbon (76.6 g/L in H1B and 55.5 g/L in H2B). Ikegama *et al.* (2000) proposed that some glucose was adsorbed on the surface of activated carbon during the fermentation, but the yeast (*S. cerevisiae*) still could consume this adsorbed glucose. However, we propose that this phenomenon might be due to an equilibrium process in the fermentation system. During the fermentation the glucose in the solution was consumed by *S. cerevisiae*, and the glucose adsorbed on the surface of activated carbon might be released or desorbed to the solution in order to make an equilibrium concentration of glucose in the system. On the other hand, the yeast could not consume the maltooligosaccharides, which were adsorbed on the surface of activated carbon or freely available in the solution. Therefore, the ethanol produced from the hydrolysates without activated carbon treatment (H0) was almost the same as that from the hydrolysates treated with activated carbon after the hydrolysis (H1B and H2B), and the ethanol produced from the hydrolysates treated with activated carbon in the hydrolysis (H1A and H2A) was lower than the three other treatments.

Table 1. The changes of total sugar and reducing sugar concentration, and the estimated degree of polymerization of the soluble carbohydrates in the cassava pulp hydrolysates during fermentation

Sugar in the Hydrolysates	Incubation (hours)					
	0	24	48	72	96	120
Total Sugar (g/100 mL) ^a						
H0	10.01 ± 0.36	9.33 ± 0.34	9.49 ± 1.23	4.61 ± 0.46	2.11 ± 0.70	1.86 ± 0.28
H1A	9.20 ± 0.27	8.42 ± 0.89	6.48 ± 0.92	1.44 ± 0.32	1.54 ± 0.16	
H1B	9.44 ± 0.11	7.97 ± 0.49	6.23 ± 0.81	1.53 ± 0.09	1.70 ± 0.12	
H2A	8.92 ± 0.24	7.92 ± 0.50	7.25 ± 0.27	5.33 ± 1.14	3.89 ± 0.25	2.36 ± 0.67
H2B	10.20 ± 1.16	9.58 ± 0.32	7.50 ± 0.25	5.45 ± 1.04	3.86 ± 0.42	2.15 ± 0.27
Reducing Sugar (g/100 mL) ^a						
H0	8.68 ± 0.54	9.27 ± 0.64	7.98 ± 0.36	5.26 ± 1.60	2.64 ± 1.32	0.89 ± 0.14
H1A	8.48 ± 0.38	8.04 ± 0.45	5.66 ± 1.11	0.93 ± 0.10	0.78 ± 0.06	
H1B	8.82 ± 0.45	7.97 ± 0.36	5.85 ± 0.75	0.88 ± 0.05	0.84 ± 0.02	
H2A	7.86 ± 0.24	7.24 ± 0.21	6.16 ± 0.33	4.16 ± 1.18	2.79 ± 0.19	1.23 ± 0.60
H2B	8.06 ± 0.29	7.74 ± 0.27	6.03 ± 0.30	4.12 ± 0.89	2.54 ± 0.52	0.95 ± 0.10
Estimated Degree of Polymerization of Sugar						
H0	1.15	1.01	1.19	0.94	0.96	2.12
H1A	1.09	1.05	1.16	1.54	1.98	
H1B	1.07	1.00	1.06	1.74	2.03	
H2A	1.13	1.09	1.18	1.30	1.40	2.03
H2B	1.26	1.24	1.25	1.33	1.54	2.27

Note: ^a Values are means ± SD (n = 3); H0 = hydrolysates without activated carbon; H1A and H1B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis; H2A and H2B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis.

Table 2. Ethanol production (g/L) from the fermentation of microwave-assisted acid hydrolysis of cassava pulp

Hydrolysates	Incubation (hours)				
	24	48	72	96	120
H0	ud	ud	14,31 ± 1,80	33,09 ± 5,26	38,21 ± 3,19
H1A	ud	10,10 ± 3,47	32,01 ± 3,25	35,40 ± 1,02	
H1B	ud	13,06 ± 0,73	39,58 ± 1,00	39,06 ± 0,27	
H2A	4,92 ± 0,87	11,23 ± 1,76	19,01 ± 4,07	25,77 ± 1,91	33,80 ± 2,72
H2B	6,18 ± 1,21	11,41 ± 1,13	21,30 ± 4,03	28,61 ± 3,25	39,40 ± 0,40

Note: ud = undetected.

Values are means ± SD (n = 3); H0 = hydrolysates without activated carbon; H1A and H1B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis; H2A and H2B = hydrolysates treated with AC1, added in the hydrolysis and after hydrolysis.

Table 3. Ethanol productivity and ethanol yield from the fermentation of microwave-assisted acid hydrolysates of cassava pulp by *S. cerevisiae*

Fermentation parameters	H0	H1A	H1B	H2A	H2B	Kosugi <i>et al.</i> (2009)
Initial glucose (g/L)	82.4	74.6	82.4 (76.6)	68.2	82.4 (55.5)	105.8
Ethanol concentration (g/L)	38.21	35.40	39.58	33.80	39.40	32.9
Ethanol productivity (g/L/h)	0.34	0.44	0.55	0.28	0.33	0.27
Ethanol yield (g ethanol /g initial glucose)	0.46	0.47	0.48 (0.52)	0.50	0.48 (0.71)	0.31
Ethanol yield (% of theoretical – glucose based)	90.93	93.04	94.19 (101.32)	97.16	93.75 (139.19)	61
Ethanol yield (% of dry matter)	30.86	28.59	31.96	27.29	31.81	-
Ethanol yield (% of theoretical – starch based)	68.39	63.35	70.84	60.48	70.51	-

Note: H0 = hydrolysate without activated carbon; H1A and H1B = hydrolysates treated with AC1, added in and after the hydrolysis; H2A and H2B = hydrolysates treated with AC2, added in and after the hydrolysis; Values in parentheses are the values obtained based on glucose concentration after inhibitors adsorption process using AC1 or AC2 for the hydrolysates H1B and H2B.

The addition of activated carbon which has a larger surface area (AC1) resulted in a higher ethanol productivity than the one without the addition of activated carbon or the one with the addition of AC2, which has much smaller surface area. Since AC1 has larger surface area, this activated carbon can adsorb more fermentation inhibitors than AC2, and the lesser the inhibitors in the solution, the faster the growth of the yeast. Thus, the higher the ethanol productivity in the samples treated with AC1. This experiment shows that adsorption properties of activated carbon affected the performance of the fermentation using *S. cerevisiae*. Therefore, it is important to choose the activated carbon for the adsorption of fermentation inhibitors, such as furfural and HMF, so that high ethanol productivity can be obtained.

The maximum ethanol produced in this study (39.58 g/L) was achieved after 72 h of incubation, while some other studies on the production of ethanol from cassava pulp could obtain almost the same ethanol concentration in a shorter duration of incubation, for example 3.62% (w/v) in 24 h (Srinorakutara *et al.* 2006) and 30.31-34.68 g/L in 30 h (Thongchul *et al.* 2010). However, our results were comparable with the results of Kosugi *et al.* (2009) who used a combination of hydrothermal and enzymatic hydrolysis of cassava pulp, and resulted in the maximum ethanol concentration of 32.9 g/L after 120 h of incubation. The slower fermentation process in our study might be due to the presence of some fermentation inhibitors, such as acetic acid, furfural and HMF, in

the hydrolysates, whereas the enzymatic hydrolysates were free from these inhibitors. According to Harmsen *et al.* (2010), at low pH acetic acid is in the undissociated form, dissolved in fat and diffused into the cells of microorganisms. Inside the cells the acetic acid is dissociated, caused the drop in the pH of the cells, hence, the cell activities were inhibited. Furfural and HMF inhibit the growth of the cells of *S. cerevisiae*, especially in the lag phase, thus, inhibit the formation of ethanol (Boyer *et al.* 1992 a, 1992b); however, they did not affect the yield of ethanol (Klinke *et al.* 2004). The results of this study confirm these suggestions, which is indicated by the low ethanol productivity and the high ethanol yield obtained from the hydrolysates without activated carbon treatment. Ethanol yield obtained in this study (0.46-0.50 g ethanol/g glucose) was lower than that obtained by Thongchul *et al.* (2010) who used enzymatic hydrolysis and fermentation using *Rhizopus oryzae* (0.52-0.59 g ethanol/g glucose), but higher than that achieved by Kosugi *et al.* (2009) through hydrothermal and enzymatic hydrolysis and fermentation using *S. cerevisiae* (0.31 g ethanol/g glucose). The ethanol yield based on the weight of dry cassava pulp and the weight of starch in the cassava pulp shows that our results (27-32% and 60-71.5%) are higher than that reported by Kunhi *et al.* (1981), who got 27.050% and 42.936%, respectively.

4. Conclusion

The adsorption properties of activated carbon affect the performance of the fermentation of microwave-assisted acid hydrolysates of cassava pulp by *S. cerevisiae*. Activated carbon having larger surface area could adsorb more fermentation inhibitors, resulted in higher ethanol productivity. Even though the presence of the fermentation inhibitors affected ethanol productivity, it did not affect the ethanol yield. The addition of activated carbon after hydrolysis gave higher ethanol yield than the addition of activated carbon in the hydrolysis, which was due to the adsorption of some maltooligomers on the surface of activated carbon that was added in the hydrolysis. Further detail study was needed in order to elucidate the behavior of activated carbon during the microwave-assisted acid hydrolysis and the fermentation of the hydrolysates.

Acknowledgments

The authors would like to thank the Ministry of Research and Technology of the Republic of Indonesia for funding this research through Incentive Program for Researchers and Engineers 2011. The authors also thank Mr. RP Budi Laksana for helping in some laboratory works.

References

- Boyer, L. J., Vega, J. L., Klasson, K. T., Clausen, E. C., and Gaddy, J. L. (1992a). The effects of furfural on ethanol production by *Saccharomyces cerevisiae* in batch culture. *Biomass Bioener.*, **3**: 41-48.
- Boyer, L. J., Vega, J. L., Basu, R., Clause, E. C., and Gaddy, J. L. (1992b). The effects of furfural on ethanol production by *Saccharomyces cerevisiae* in a cross-linked immobilized cell reactor. *Biomass Bioener.*, **3**: 71-76.
- Ge, J. P., Cai, B. Y., Liu, G. M., Ling, H. Z., Fang, B. Z., Song, G., Yang, X. F., and Ping, W. X. (2011). Comparison of different detoxification methods for corn cob hemicellulose hydrolysate to improve ethanol production by *Candida shehatae* ACCC 20335. *African J. Microbiol. Res.*, **5**: 1163-1168.
- Harmsen, P. F. H., Huijgen, W. J. J., Lopez, L. M. B., and Bakker, R. R. C. (2010). *Literature Review of Physical and Chemical Pretreatment Processes for Lignocellulosic Biomass*. Energy Research Center of the Netherlands. <http://www.ecn.nl/docs/library/report/2010/e10013.pdf>. Access date: 1 May 2012.
- Hermiati, E., Azuma, J., Mangunwidjaja, D., Sunarti, T. C., Suparno, O., and Prasetya, B. (2011). Hydrolysis of carbohydrates in cassava pulp and tapioca flour under microwave irradiation. *Indo. J. Chem.*, **11**: 238-245.
- Hermiati, E., Azuma, J., Tsubaki, S., Mangunwidjaja, D., Sunarti, T. C., Suparno, O., and Prasetya, B. (2012a). Improvement of microwave-assisted hydrolysis of cassava pulp and tapioca flour by addition of activated carbon. *Carbohydr. Polym.*, **87**: 939-942.
- Hermiati, E., Mangunwidjaja, D., Sunarti, T. C., Suparno, O., and Prasetya, B. (2012b). Microwave-assisted acid hydrolysis of starch polymer in cassava pulp in the presence of activated carbon. *Procedia Chem.*, **4**: 238-244.
- Hermiati, E., Mangunwidjaja, D., Sunarti, T. C., Suparno, O., Prasetya, B., and Risanto, L. (2012c). Optimization of acid hydrolysis of cassava pulp under microwave irradiation for ethanol production. (in the process of publication).
- Ikegamai, T., Yanagishita, H., Kitamoto, D., and Haraya, K. (2000). Accelerated ethanol fermentation by *Saccharomyces cerevisiae* with addition of activated carbon. *Biotechnol Lett.*, **22**: 1661-1665.
- Klinke, H. B., Thomsen, A. B., and Ahring, B. K. (2004). Inhibition of ethanol-producing yeast and bacteria by degradation products produced during pre-treatment of biomass. *Appl. Microbiol. Biotechnol.*, **66**: 10-26.

- Kosugi, A., Kondo, A., Ueda, M., Murata, Y., Vaithanomsat, P., Thanapase, W., Arai T., and Mori, Y. (2009). Production of ethanol from cassava pulp via fermentation with a surface engineered yeast strain displaying glucoamylase. *Renew. Ener.*, **34**: 1354-1358.
- Kunhi, A. A. M., Ghildyal, N. P., Lonsane, B. K., Ahmed, S. Y., and Natarajan, C. P. (1981). Studies on production of alcohol from saccharified waste residue from cassava starch processing industries. *Starch/Stärke*, **33**: 275-279.
- Larsson, S., Palmqvist, E., Hahn-Hagerdal, B., Tengborg, C., Stenberg, K., Zacchi, G., and Nilvebrant, N. O. (1999). The generation of fermentation inhibitors during dilute acid hydrolysis of softwood. *Enzyme Microb. Technol.*, **24**: 151-159.
- Matsumoto, A., Tsubaki, S., Sakamoto, M., and Azuma, J. (2008). Oligosaccharides adsorbed on activated charcoal powder escaped from hydrolysis by microwave heating in water. *Proceedings of Global Congress on Microwave Energy Applications*. August 4-8, 2008, Otsu, Japan, pp. 785-788.
- Matsumoto, A., Tsubaki, S., Sakamoto, M., and Azuma, J. (2011). A novel saccharification method of starch using microwave irradiation with addition of activated carbon. *Bioresour. Technol.*, **102**: 3985- 3988.
- Mussatto, S. I., and Roberto, I. C. (2004). Alternatives for detoxification of diluted-acid lignocellulosic hydrolysates for use in fermentative processes: a review. *Bioresour. Technol.*, **93**: 1-10.
- Palmqvist, E., and Hahn-Hagerdal, B. (2000). Fermentation of lignocellulosic hydrolysates II: Inhibitors and mechanism of inhibition. *Bioresour. Technol.*, **74**: 25-33.
- Srinorakutara, T., Kaewvimol, L., and Saengow, L. (2006). Approach of cassava waste pretreatments for fuel ethanol production in Thailand. *J. Sci. Res. Chula Univ.*, **31**: 77-84.
- Thongchul, N., Navankasattusas, S., and Yang, S. T. (2010). Production of lactic acid and ethanol by *Rhizopus oryzae* integrated with cassava pulp hydrolysis. *Bioprocess Biosyst. Eng.*, **33**: 407-416.
- Warrand, J., and Janssen, H. G. (2007). Controlled production of oligosaccharides from amylose by acid-hydrolysis under microwave treatment: Comparison with conventional heating. *Carbohydr. Polym.*, **69**: 353-362.
- Whistler, R. L., and Daniel, J. R. (1985). Carbohydrates. Di dalam: Fennema OR, editor. *Food Chemistry*. New York: Marcel Dekker, pp 69-137.
- Wrolstad, R. E., Acree, T. E., Decker, E. A., Penner, M. H., Reid, D. S., Schwartz, S. J., Shoemaker, C. F., Smith, D., and Sporns, P. (2005), *Handbook of Food Analytical Chemistry: Water, Proteins, Enzymes, Lipids, and Carbohydrates*. Hoboken, New Jersey: John Wiley & Sons.