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Wenny Irawaty

Wenny Irawaty

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Conference Management
Centre for Continuing Education
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Telephone: +64 9 373 7599
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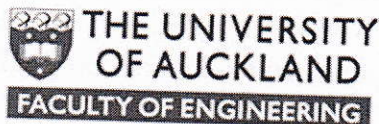
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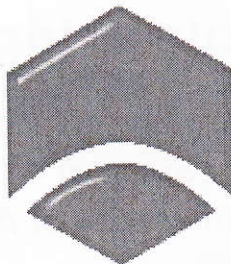


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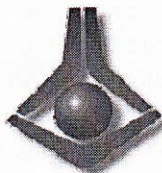
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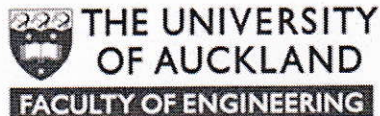
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Time	Room 1	Room 2	Room 3	Room 4	Room 5	Room 6
8:00 – 8:50	Registration					
8:50 – 9:00	Welcome and House keeping					
9:00 – 9:45	Professor William Svrcek Process Simulation – From Large Computers and Small Solutions to Small Computers and Large Solutions - 005 <i>Arawa Room</i>					
9:45 – 10:30	Professor Anton Middelberg Biomolecular Engineering - 006 <i>Arawa Room</i>					
10:30 – 11:00	Morning Tea					
11:00 – 12:40	Energy: Oil and Gas / Energy Saving <i>Arawa Room</i> Chair Shusheng Pang, New Zealand	Food and Beverage: General <i>Whitaven Room</i> Chair Jim Jones, New Zealand	Process Simulation <i>Murak Room</i> Chair William Svrcek, @hada	Separations: Adsorption <i>Tamak Room</i> Chair Darrell Patterson, New Zealand	Biotechnology I <i>Aarana Room</i> Chair Conan Fee, New Zealand	Particle Technology I <i>Greys Room</i> Chair Clive Davies, New Zealand
11:00 – 11:20	201 An upstream oil and gas processing energy model. Colley, D	206 Colour removal from a sugar process solution by adsorption onto ion-exchange resins: study of equilibrium, kinetics, decolorization rate, and ph and purity change. Arias, V	211 An exergy calculator tool for process simulation. Montelongo, J	216 The potential application of natural zeolite for greywater treatment: a review. Widiastuti, N	221 Peptide surfactants (Pepfactants [®]) for switchable foams and emulsions. Malcolm, A	226 Fluidization characteristics of nanoparticle agglomerates. Wang, S
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Continued

Recovery of Lactic Acid from Traditional Market Wastes Fermentation Broth

Wenny Irawaty, Widya Mandala Catholic University Surabaya, Indonesia

Ery Susiany R., Widya Mandala Catholic University Surabaya, Indonesia

Filicia Wicaksana, Widya Mandala Catholic University Surabaya, Indonesia

Ratia W., Widya Mandala Catholic University Surabaya, Indonesia

Cynthia W., Widya Mandala Catholic University Surabaya, Indonesia

This research was aimed to investigate the effect of temperature and water addition on lactic acid produced from fermentation using *Lactobacillus plantarum*. The effect of pH on lactic acid recovery from resin IRA-400 was also studied. The fresh organic wastes were collected from traditional market and then processed to be a media for fermentation. The vegetables wastes juice was sterilized, added nutrients and microorganisms. The mixtures were incubated at anaerobic condition at 30 and 40°C for 16 days. Following the fermentation process, the lactic acid in fermentation broth was recovered using resin at pH 2 and 5. The results showed that there was no significant difference in quantity of lactic acid recovered from resin at pH 2 and 5. Increasing temperature to 40°C increased the lactic acid produced. The highest concentration of lactic acid (0.6 g/100 mL) was obtained from fermentation of pure vegetables juice at 40°C for 12 days.

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Wenny Irawaty, Ery Susiany R., Filicia Wicaksana, Ratia W. and Cynthia W.

Chemical Engineering Department

Widya Mandala Catholic University Surabaya, Kalijudan 37 Surabaya 60114, East Java, INDONESIA

E-mail: irawaty@mail.wima.ac.id

Abstract

This research was aimed to investigate the effect of temperature and water addition on lactic acid produced from fermentation using *Lactobacillus plantarum*. The effect of pH on lactic acid recovery from resin IRA-400 was also studied. The fresh organic wastes were collected from traditional market and then processed to be a media for fermentation. The vegetables wastes juice was sterilized, added nutrients and microorganisms. The mixtures were incubated at anaerobic condition at 30 and 40°C for 16 days. Following the fermentation process, the lactic acid in fermentation broth was recovered using resin at pH 2 and 5. The results showed that there was no significant difference in quantity of lactic acid recovered from resin at pH 2 and 5. Increasing temperature to 40°C increased the lactic acid produced. The highest concentration of lactic acid (0.6 g/100 mL) was obtained from fermentation of pure vegetables juice at 40°C for 12 days. Keywords: lactic acid, recovery, IRA-400.

1. INTRODUCTION

Traditional markets in Surabaya, Indonesia produce a significant amount of solid waste (approximately 5,000–8,000 m³ a day). These solid wastes are selected for further processing according to the types of wastes, such as organic and inorganic, with the majority is biodegradable organic waste. A common treatment of organic wastes is by processing them to compost. Unfortunately, a number of accidents occurred during these waste treatment processes due to lack of professional training and safety concern (Surya, 2004).

Biodegradable organic wastes can be converted to various products such as ethanol, lactic acid and citric acid through fermentation process. Lactic acid is widely used in foods, pharmaceuticals, cosmetics, feedstock and plastics industries. A summary of previous studies that use liquid or

solid wastes as fermentation media to produce lactic acid is listed in Table 1.

Results from literature study suggested that there was no research that utilized organic wastes obtained from traditional markets for lactic acid production. Therefore, our research focuses on the processing of organic wastes to produce lactic acid. In addition, it is expected that the transformation of organic wastes to lactic acid could overcome the environmental problem, particularly in Surabaya, a highly populated second largest city in Indonesia.

Table 1. Various Media Used in Lactic Acid Fermentation

Media	Microorganism	Reference
Scampi waste	<i>L. paracasei</i> A3	Zakaria <i>et al</i> , 1998
Effluent of mussel processing wastes	<i>L. plantarum</i> A6	Pintado <i>et al</i> , 1999
Wood hydrolysate	<i>Rhizopus oryzae</i> NRRL 395	Woiciecho wski, 1999
Vegetable juice	<i>L. plantarum</i> NK-312	Gardner <i>et al</i> , 2001
Office paper pulp	<i>Rhizopus oryzae</i> NRRL 395	Park <i>et al</i> , 2004
Cassava bagasse	<i>L. delbrueckii</i> NCIM 2025	John <i>et al</i> , 2006
Kitchen garbage	<i>Lactobacillus</i>	Wang <i>et al</i> , 2006
Food waste	<i>L. salivarius</i>	Yang <i>et al</i>

1.1. Lactic Acid Fermentation

There are two different types of lactic acid fermentation: homolactic (pure lactic) and heterolactic (mixed lactic) fermentations (Chalal, 1990).

1. Homolactic Fermentation

The main product of this fermentation process is lactic acid. The species of bacteria involved in this fermentation (Homolactic Lactobacteriaceae) are *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Lactobacillus bulgaricus*, *Lactobacillus casei* and *Lactobacillus salivarius*.

2. Heterolactic Fermentation

This fermentation produces various substances such as lactic acid, acetic acid, ethanol, carbon dioxide and other components depending on the raw materials used. Heterolactic Lactobacteriaceae are *Lactobacillus brevis*, *Lactobacillus buchneri*, and other bacteria such as *Staphylococcus* *Bacillus* as well as *Rhizopus* fungi species.

1.2. Lactic Acid Recovery

Lactic acid produced by fermentation process requires further treatment in order to improve its purity. A conventional method of lactic acid recovery is by adding calcium hydroxide to precipitate calcium lactate upon separation of microorganisms. The salt is filtered and then treated with sulfuric acid to produce free acid and calcium sulfate. The disadvantages of this conventional technique are the requirement of additional purification to achieve a commercial standard of lactic acid, high product loss during crystallization and the handling of a large quantity of calcium compounds produced from this process.

Other techniques that have been investigated by several researchers for recovery of lactic acid from fermentation broth are as follows:

1. Liquid-liquid extraction

Different types of amines and diluents have been used for lactic acid extraction. The common types of amines used are tri-*n*-octyl-amine (Choudhury and Swaminathan, 1998), alamine 336: mixture of tri-*n*-octyl-amine and tri-*n*-decylamine (Wasewar, 2002), Hostarex A 327 (tri-*n*-octyl and *n*-decylamine), Cyanex 923 (tri-*n*-octyl and tri-*n*-hexylphosphinoxides) (Frieling and Schügerl, 1999). While the diluents are octanol, decanol, chlorobenzene, chloroform, methylene chloride, methyl isobutyl ketone (MIBK). A mixed extractants can also be used to improve the extraction. These mixtures are tri-*n*-octyl-amine and tri-*n*-butylphosphate in hexane (Matsumoto *et al*, 2001), tri-*n*-octyl-amine in decanol and dodecane (Yankov *et al*, 2004). The polar solvents yield high degree of extractions. For application with toxic solvents, the addition of kerosene may be necessary to neutralize the toxicity. The kerosene addition causes the formation of a third phase that can be avoided by adding some modifiers (Siebold *et al*, 1994). However, the cost of this extraction method is high.

2. Membrane Processes

Choi *et al* utilized an electrodialysis process for recovery and purification of lactic acid produced by fermentation (Choi *et al*, 2002). Despite no lactic acid loss was reported, this membrane separation process has the drawbacks of membrane price and installation, particularly for large scale production.

3. Adsorption

The use of adsorbent is able to reduce the chemicals addition during extraction step. Different types of adsorbents have been used for extracting lactic acid, such as activated carbon (Chen and Ju, 1998) and zeolite (Aljundi *et al*, 2005). The problem in using activated carbon is a wide pore size distribution. Zeolite has pore sizes suitable for this adsorption, but it has a lower adsorption capacity than other polymeric adsorbents. The advantage of this process is simple in operations (Aljundi *et al*, 2005).

4. Ion exchange resin

This technique involves the sorption of lactic acid on ion exchange resins and is widely used in bio-separation. Several types of ion exchangers have been used by various researchers, such as IRA-92 (Tong *et al*, 2004), IRA-400 (Cao *et al*, 2002), IRA-900, 400, 96, 97 (Moldes *et al*, 2001). IRA-400 is more frequently used than other resins due to its proper pore size and higher adsorption capacity for recovery of lactic acid. The resin can adsorb lactic acid in a wide range of pH through acid-base interaction. Furthermore, the resin can be generated by sulfuric acid (Cao *et al*, 2002).

The production cost of lactic acid depends on numerous factors such as the type of the process, and the final product quality. Although the exact costs of lactic acid recovery by different methods as described above have not been reported, it was suggested that the lactic acid production cost ranged from 0.1 to 2 US\$/kg (Akerberg, 2000).

As mentioned previously, the formation of lactic acid involves fermentation and purification processes. As the purification of lactic acid can be performed by various means, the production cost of lactic acid is more governed by the purification costs rather than its fermentation cost. Therefore, the method for purifying lactic acid has to be precisely selected. Among various purification techniques that have been reported, it seems that ion exchange resin is the most economical method for lactic acid recovery. In addition, the process is

relatively simple and feasible for industrial scale operation. In this research, the adsorption of lactic acid was carried out at pH above and below the pKa (3.87) of lactic acid (pH 5 and 2, respectively).

2. MATERIALS AND METHOD

2.1. Materials

The type of resin utilized in this research was IRA-400 (Sigma-Aldrich). The fresh resin was firstly treated with 1 M Sodium Hydroxide solution (10 times of the resin volume), followed by water, 1 M sulfuric acid and finally, rinsed with water. All reagents used in the research were analytical grade and were used without further purification.

2.2. Microorganism

Lactobacillus plantarum FC09 employed in this research was grown on Lactobacillus MRS Agar medium at 37°C for 3 days and then preserved at 4°C for future use.

2.3. Media Preparation

Fresh vegetables wastes: carrots, cabbage, spinach/broccoli and tomatoes were collected from traditional market in Surabaya. They were washed and cut into 1 mm-slices. Each type of vegetable was processed separately in a juice extractor for 30 minutes. The pulp was then separated from the juice. The fermentation media was prepared by mixing all vegetable extracts at the same composition, i.e. 25 % volume each. The mixture was sterilized at 121°C for 15 minutes. Salt mixture was added as nutrients with the following compositions: 0.8% KH₂PO₄; 0.3% MgSO₄; 0.06% ZnSO₄ and 0.01% Fe₂(SO₄)₄ (w/v).

2.4. Methods

Fermentation of Lactic Acid

Starter for fermentation was prepared by putting 50 mL of sterilized medium in 100-mL flasks and inoculated with 3 loops of *Lactobacillus plantarum* freshly picked from MRS agar. The flasks were incubated at 37°C for 5 hours. For fermentation step, 150 mL of sterilized medium was placed in 250-mL flasks and mixed with 50 mL of initially prepared starter. The mixtures were incubated at

anaerobic condition and at two different temperatures: 30 and 40°C. The effect of water addition into the media was also investigated. The ratios of added water to the media were 0, 5 and 10 (v/v). Following the fermentation process, the lactic acid in fermentation broth was recovered using resin IRA-400.

Recovery of Lactic Acid

In order to purify lactic acid from the fermentation broth, a 22/200 (22 mm x 200 mm) chromatography column packed with IRA-400 resin was used. The recovery processes were investigated at pH 2 and 5.

For adsorption at pH 2:

The pH of fermentation broth was set to 2. 30 mL of broth was passed through a resin column at a flow rate of 0.3 mL/min. The lactic acid can be recovered by eluting the column using 60 mL of water at an identical flow rate. During these processes, the pH of the column remained stable at approximately 2. The concentration of lactic acid in the eluate was then analyzed.

For adsorption at pH 5:

30 mL of fermentation broth at pH 5 was passed through a resin column at a flow rate of 0.3 mL/min. Then the column was rinsed with 60 mL of 50% methanol at the same flow rate. The pH of the column was maintained at 5. The column was eluted with 60 mL of 1 M sulfuric acid solution at a similar flow rate as stated above. The concentration of lactic acid in the eluate was analyzed.

The concentration of lactic acid in the eluate was determined using a simple acid-base titration with sodium hydroxide solution as a titrant and phenolphthalein as an indicator. Quantitative analysis of reducing sugar was performed according to the method of Luff Schoolt (Sudarmadji, 1997).

3. RESULTS AND DISCUSSION

The effect of pH on lactic acid recovery is shown in Figure 1. The fermentation media employed in this study was pure vegetable wastes juice, no added water, and incubated at 30°C.

The figure indicates that the quantities of lactic acid recovered from resin at pH 2 and 5 are almost identical. It appears that the pH level does not affect the lactic acid recovery process. In contrast to our results, Cao *et al* (2002) reported that 92%

of lactic acid could be recovered at pH 2 and 86% at pH 5. Cao *et al* used higher lactic acid concentration in the sample (5 to 11 g/100 mL), while the maximum lactic acid concentration obtained from present research is 0.45 g/100 mL. Therefore, the effect of pH on lactic acid recovery may be undetectable since the concentration of lactic acid obtained in present study is low. Based on the effectiveness of the process and economical factor, the adsorption of lactic acid at pH 2 is better than the one at higher pH. Thus, for the purification of fermentation broth, the sorption of lactic acid is carried out at pH 2.

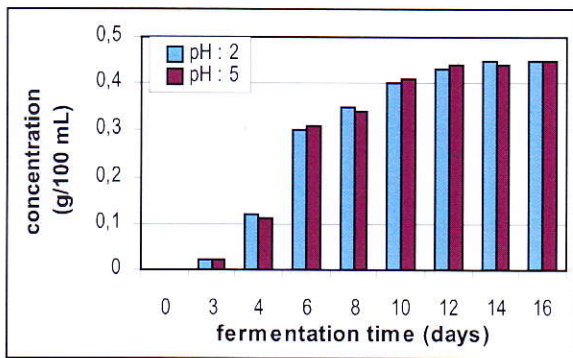


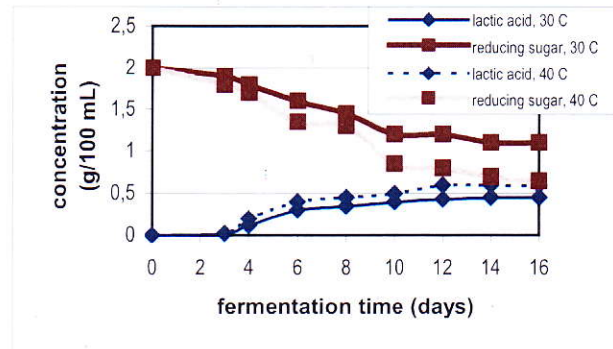
Figure 1. Recovered lactic acid at various pH values

Figure 2 shows the variation of lactic acid and reducing sugar concentrations during fermentation at various temperatures and added water.

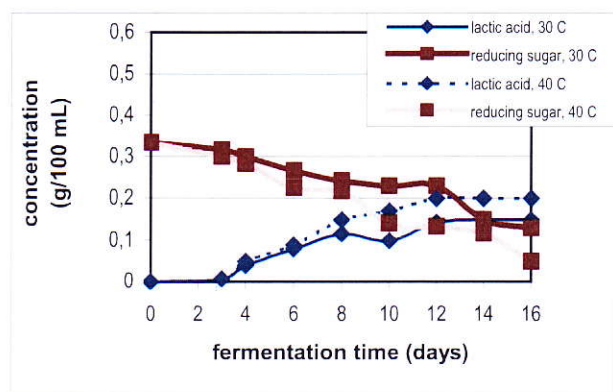
It can be seen in Figure 2 that the lactic acid produced at 30 and 40°C showed identical trends. During the first three days of fermentation using pure vegetable juice (no added water), the amount of lactic acid produced is low (Figure 2a). The explanation is that the microorganisms could be at the initial growth phase (lag phase) thus the carbon source has not been effectively consumed. After three days, the concentration of lactic acid increases and reaches approximately 0.45 g/100 mL media on the 12th day of fermentation at 30°C. After that, the amount of lactic acid remains constant. As the concentration of lactic acid increases smoothly, the concentration of reducing sugar decreases with minor fluctuation. The explanation is that the sugar is consumed by microorganisms as a carbon source.

After 12 days of fermentation, the process was terminated due to the following reasons: the increasing concentration of lactic acid produced during fermentation caused the decrease in pH level. Low pH environment and limited supply of sugar may cause the mortality of the

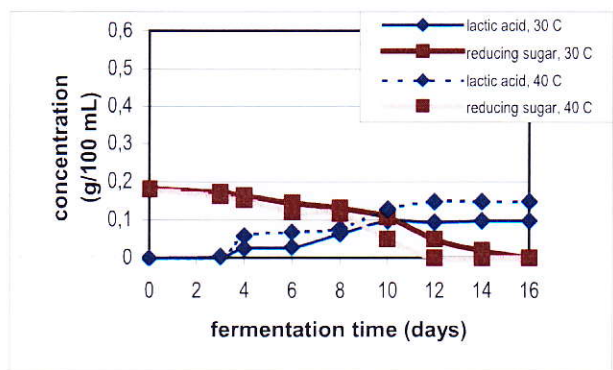
microorganisms. Figure 2 also shows that higher temperature (40°C) increases the activities of the microorganisms resulting in higher lactic acid produced.



(a)



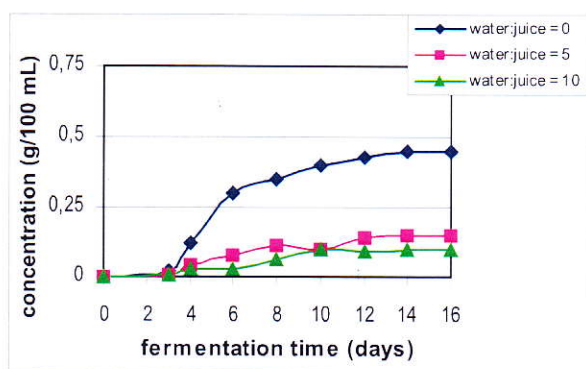
(b)



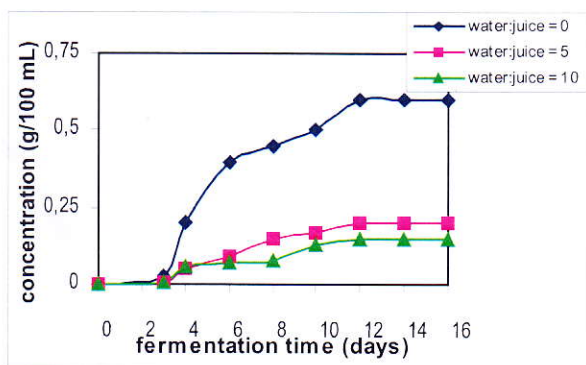
(c)

Figure 2. The variations of lactic acid and reducing sugar concentrations during fermentation at 30°C and 40°C and the ratio of added water:juice (v/v) : 0 (a), 5 (b), 10 (c)

The effect of water addition on fermentation broth can be seen in Figure 3.



(a)



(b)

Figure 3. Concentration of lactic acid during fermentation at 30°C (a) and 40°C (b) at various ratio of added water:juice (v/v)

Figure 3 indicates that the increase in water addition inhibits the production of lactic acid. Since identical volume of fermentation broth is used in all experiments, higher ratio of added water to vegetable juice means lower amount of vegetable juice. As microorganisms required carbon source for their growth, less amount of carbon source will affect their activities causing the decrease in lactic acid production.

4. CONCLUSIONS

The findings from this study suggested that organic wastes could be transformed to valuable lactic acid by fermentation and purification. It is to be hoped that the study could offer an alternative means to overcome the growing environmental concern of waste handling problem.

The recovery of lactic acid from fermentation broth was not affected by pH of the recovery system. Based on the simplicity process and economic factor, recovery of lactic acid at pH 2 was better than at pH 5. In fermentation using *Lactobacillus plantarum*, the pure vegetables wastes juice used as media gave the highest

content of lactic acid (0.6 g/100 mL). The optimum conditions for producing lactic acid were at 40°C and 12 days of fermentation.

5. ACKNOWLEDGEMENTS

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