Faculty of Resource Science and Technology

EFFECTS OF MRS-SAGO STARCH CONCENTRATION ON DIRECT LACTIC ACID PRODUCTION WITH CONTROLLED pH BY *Enterococcus faecium* NO.78

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ABSTRACT

The lactic acid production from starch can be made much more economical by using *Enterococcus faecium* No.78 to directly produce lactic acid from sago starch. The effects of different MRS-sago starch concentrations on direct lactic acid production were investigated in this study. The sago starch of 20 g/L, 30 g/L and 40 g/L were used as substrate in this study, with 0 g/L was used as control. Fermentation was conducted at 30°C, pH6.5 and agitation rate of 500rpm which were controlled constantly throughout fermentation. The increase in sago starch concentration had negative effect on the microbial growth. The highest microbial growth, 9.3 ($\log_{10}$ viable cells/mL) was achieved in fermentation by using 20 g/L sago starch. The maximum consumption of sago starch, 87.3% was observed in fermentation by using 20 g/L sago starch. The glucose consumption in all three fermentation trials were not different significantly. The highest lactic acid production, 22.9 g/L was achieved in fermentation by using 40 g/L sago starch. This shows that the lactic acid production increased in accordance with the utilization of higher sago starch concentration. The fermentation efficiency decreased slightly despite the usage of higher sago starch concentrations. Therefore, the optimum sago starch concentration for the highest lactic acid production was 40 g/L in consideration of the highest lactic acid production (22.9 g/L) while consuming the same amount of electricity, time and labour as in fermentation by using 20 g/L and 30 g/L sago starch.

Key words: *Enterococcus faecium* No.78, direct lactic acid production, sago starch concentration.

ABSTRAK

Penghasilan asid laktik secara terus daripada kanji sagu adalah lebih ekonomi dengan menggunakan mikroorganisma <u>Enterococcus faecium</u> No.78. Kajian ini bertujuan untuk melihat kesan perbezaan kepekatan kanji sagu ke atas penghasilan asid laktik. Kanji sagu yang berkepekatan 20 g/L, 30 g/L dan 40 g/L digunakan dalam kajian ini dengan 0 g/L dijadikan sebagai kawalan. Fermentasi yang dijalankan adalah dikawal sepanjang masa dengan menetapkan suhu operasi pada 30°C, pH6.5 dan 500rpm untuk kadar pengadukan. Peningkatan kepekatan kanji sagu didapati tidak mempengaruhi pertumbuhan mikrob kerana nilai tertingginya iaitu 9.3 ($\log_{10}$ sel hidup/mL) adalah daripada kanji sagu yang berkepekatan 20 g/L. Kanji sagu yang berkepekatan 20 g/L didapati tertinggi penggunaanya iaitu 87.3%. Penggunaan glukosa dalam ketiga-tiga fermentasi adalah tidak jauh perbezaannya. Penghasilan asid laktik yang maksimum pula iaitu 22.9 g/L dicapai dalam fermentasi menggunakan 40 g/L kanji sagu. Ini menunjukkan penghasilan asid laktik bertambah sejajar dengan peggunaan kepekatan kanji sagu yang lebih tinggi. Kecekapan fermentasi berkurangan meskipun kepekatan kanji sagu yang lebih tinggi digunakan. Oleh itu, kepekatan kanji sagu yang optimum untuk penghasilan asid laktik yang tertinggi kepekatannya adalah 40 g/L kanji sagu dengan mengambil kira penghasilan asid laktik yang berpekatan tertinggi (22.9 g/L) di samping menggunakan kuantiti elektrik, masa dan tenaga pekerja yang sama seperti di dalam fermentasi menggunakan kanji sagu yang berkepekatan 20 g/L dan 30 g/L.

Kata kunci: <u>Enterococcus faecium</u> No.78, penghasilan asid laktik secara terus, kepekatan kanji sagu.

1.0 INTRODUCTION

Nowadays, the applications of lactic acid have been widely incorporated into various industries including food, pharmaceutical and cosmetic industries. Lactic acid is being used as preservative, flavouring agent and emulsifying agent in food industries. More recently, biodegradable plastics are produced from the polymers of lactic acid which also have various medical and agricultural applications (Datta *et al.*, 1995; Narayanan *et al.*, 2004). Lactic acid can be produced by chemical synthesis or microbial fermentation. The fermentative production of lactic acid accounts for about 90% of 80000 tonnes of lactic acid produced worldwide annually while the remainder is produced synthetically by the hydrolysis of lactonitrile (Hofvendahl and Hahn-Hligerdal, 2000). This shows that there is a great potential in the lactic acid production through microbial fermentation. Therefore, studies should be conducted to discover potential lactic acid bacteria and to obtain the optimum conditions for high yield of lactic acid in lactic acid fermentation.

Sago starch is abundant in Sarawak and it is a low cost starch which makes this starch economically attractive to be used as substrate for lactic acid production. However, conventional fermentative production of lactic acid from starch requires pre-treatment by enzymatic gelatinization and liquefaction of the starch at an elevated temperature of about 90-130°C for at least 15 minutes, followed by enzymatic saccharification of starch to glucose and later the conversion of glucose to lactic acid by lactic acid fermentation using *lactobacili* (Linko and Javanainen, 1996). These steps require enzymes, electricity and time. The producers need to spend more for purchasing enzymes and electricity. These steps are also time consuming. These certainly would increase the cost of production and decrease the revenue obtained. However, fermentative production of lactic acid from starch can be made

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much more economical by combining the enzymatic hydrolysis of starch and microbial fermentation of the derived glucose, into a single step to eliminate the cost of commercial enzymes, energy and labour (Anuradha *et al.*, 1999). This approach is possible by using the amylase-producing amylolytic lactic acid bacteria which can directly convert starch into lactic acid. In this direction, this study involves the utilization of an amylolytic lactic acid bacteria strain, *Enterococcus faecium* No.78, which can directly convert starch to solely lactic acid. Sago starch is used as fermentation substrate in this study as Shibata *et al.* (2006) had reported that sago starch was superior substrate compared to other starches for direct L-lactic acid production by *E. faecium* No.78.

There was a study on the effects of sago starch concentration on direct lactic acid production without pH control by *E. faecium* No. 78. However, the satisfactory results were not obtained as pH was not controlled during fermentation. The previous studies on the lactic acid concentration, yield and productivity with controlled pH and uncontrolled pH showed that titration to a constant pH resulted in higher or equal lactic acid concentration, yield and productivity compared with uncontrolled pH (Hofvendahl and Hahn-Hligerdal, 2000). Therefore, in this study, the lactic acid production with pH controlled within optimum limits would be expected to result in high lactic acid yield. In this study, the fermentation pH is controlled at pH 6.5 (which is the optimum pH for lactic acid production from sago starch by *E. faecium* No.78) so that clearer effects of sago starch concentration can be identified.

This study aims at obtaining the optimum sago starch concentration for the direct lactic acid production with controlled pH by *Enterococcus faecium* No.78, so that the highest possible lactic acid concentration, yield and productivity can be achieved.

1.1 Objectives

(i) To study the effects of sago starch concentration on direct lactic acid production with controlled pH by *Enterococcus faecium* No.78.

(ii) To obtain the optimum sago starch concentration for the highest lactic acid production from sago starch by *Enterococcus faecium* No.78.

2.0 LITERATURE REVIEW

2.1 Lactic acid

Lactic acid or 2-hydroxypropionic acid ($CH_3CHOHCOOH$) is the most commonly occurring carboxylic acid in nature. Lactic acid was first produced commercially in 1881 by Charles E. Avery (Narayanan *et al.*, 2004). There are two isomeric forms of lactic acid which are L(+)-lactic acid and D(-)-lactic acid. Lactic acid can be produced by either chemical synthesis or microbial fermentation. Fermentative production of lactic acid is preferable compared to chemical synthesis due to the use of cheap renewable resources as raw materials and the production of stereospecific lactic acid product. Renewable resources such as whey, molasses, starch waste, beet, cane sugar and other carbohydrate rich materials can be used as raw materials in the fermentative production of lactic acid (Altaf *et al.*, 2006). Renewable resources are environmentally friendly as these resources do not give any significant contribution of carbon dioxide to the atmosphere, as do the limited oil- and fossil-fuel-based sources. Fermentative production can produce an optically pure lactic acid product which is more preferable, by choosing a suitable lactic acid bacteria strain whereas synthetic production always results in a racemic mixture of lactic acid (Hofvendahl and Hahn-Hligerdal, 2000).

Lactic acid is widely used in food industries. Lactic acid is used as preservative, inhibitor of unfavorable bacteria spoilage or works as flavouring/pH buffering agent in a wide variety of processed food (Narayanan *et al.*, 2004). Lactic acid is used as emulsifying agents in foods, especially for bakery goods (Datta *et al.*, 1995). Lactic acid is also used in pharmaceutical and cosmetic applications and formulations, particularly in topical ointments, lotions and parenteral solutions. Polymers of lactic acids are environmentally friendly alternative biodegradable thermoplastics whereby their degradation can be monitored by adjusting the

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composition and the molecular weight (Narayanan *et al.*, 2004). These biodegradable polymers are used in medical applications as surgical sutures, controlled-release drugs and prostheses, and also in agricultural applications as biodegradable mulch films (Datta *et al.*, 1995)

2.2 Sago starch

The carbon sources for the lactic acid fermentation process can be glucose, maltose, lactose and sucrose from cheap raw materials such as molasses, whey, cane and beet sugar and starch (Hofvendahl and Hahn-Hligerdal, 1997). The starch from different origins such as wheat, barley, potato, corn and sago has been utilized for the lactic acid production (Narayanan *et al.*, 2004). Among the starch from various origins, the use of sago starch is more economically attractive due to the abundance of sago palm in Sarawak. Therefore, sago starch is used in this study.

Sago palm (*Metroxylon sagu*), is a starch crop which is called 'rumbia' by local people in Sarawak. Sago palm grows well in the swampy areas (over 75% of Sarawak) where cannot sustain other crops. Sago starch is used in food industries and non-food industries. Sago starch is used in food industries for the production of high fructose syrup, glucose, monosodium glutamate (MSG), sago pearl, noodles, caramel and crackers. Besides that, sago starch is used for producing non-toxic paper glue in non-food industries. The productivity per land area of sago palm is the highest compared to other starchy crops of the world. Sago palms can generate 24t/ha of starch annually, compare to the lower productivity of rice (6t/ha), corn (5.5t/ha), wheat (5t/ha) and potato (2.5t/hs) (Bujang and Ahmad, 2000). Sun and Tomkinson (2003) reported that the predominant constituent of sago pith was sago starch whereby 83.9% dry weight of the palm pith is consisted of sago starch. In Sarawak, a fully cultivated sago

estate has about 138 palm/ha/year which produce about 185 kg starch/palm and therefore a total of 25.53 tonnes starch/ha/year can be estimated.

Between the years 1988-1990's, the annual export of sago starch from Sarawak fluctuates between 30,000 to 50,000 tonnes, earning incomes of between RM14 million to RM43 million (Dept of Statistics, 2000). The state can definitely gain more benefits by the exports of sago starch's value-added products such as lactic acid and ethanol (Bujang *et al.*, 2001).

According to Narayanan *et al.* (2004), there are studies being conducted to develop microbial processes for the production of high purity L (+) lactic acid at low cost from sago starch. Nolasco-Hipolito *et al.* (2002) had reported the production of lactic acid from hydrolysed sago starch using *Lactococcus lactis* IO-1.

2.3 Lactic acid bacteria

Lactic acid bacteria (LAB) consist of the genera: *Carnobacterium*, *Enterococcus* (*Ent*), *Lactobacillus* (*Lb*), *Lactococcus* (*Lc*), *Leuconostoc* (*Leu*), *Oenococcus*, *Pediococcus* (*Ped*), *Streptococcus* (*Str*), *Tetragenococcus*, *Vagococcus*, and *Weissella* (Stiles *et al.*, 1997, cited in Hofvendahl and Hahn-Hligerdal, 2000). Some of the lactic acid bacteria can produce amylase and therefore are grouped into amylolytic lactic acid bacteria. Amylolytic lactic acid bacteria can directly convert starch into lactic acid without requirement for prior enzymatic hydrolysis of starch. The utilization of amylolytic lactic acid bacteria such as *Lactobacillus amylovorus* (Zhang and Cheryan, 1991), *L. amylophilus* (Yumoto and Ikeda, 1995), *L. fermentum* Ogi E1 (Santoyo *et al.*, 2003), *Streptococcus bovis* 148 (Narita *et al.*, 2004), *L. manihotivorans* LMG18011 (Ohkouchi and Inoue, 2005) and *L. amylophilus* GV (Naveena *et al.*, 2005) for direct fermentation of starch to lactic acid have been reported.

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This study involves the utilization of *Enterococcus faecium* No.78, one of the amylolytic lactic acid bacteria strain. *Enterococcus faecium* No.78 belong to the *Enterococcus* genus. Members of the genus *Enterococcus*, or enterococci, are Gram-positive, facultatively anaerobic cocci which are arranged in pairs or short chains. The enterococci are normal inhabitants of the gastrointestinal tract of human and most animals (Franz *et al.*, 2003). These bacteria can also colonize various niches due to their high heat tolerance and the ability to adapt to adverse environment conditions (Psoni *et al.*, 2005). The enterococci can produce solely lactic acid from hexoses by homofermentative lactic acid fermentation. The enterococci play important roles in environmental, food and clinical microbiology. These bacteria involve in the ripening and aroma development of certain traditional cheeses and sausages. The enterococci are also used as probiotics (Franz *et al.*, 2003).

E. faecium are spherical to ovoid cells, mainly in pairs and short chains. These bacteria can survive in temperature ranging from 4°C to 45°C, capable of growing in 6.5% NaCl and can survive for 30 minutes at 60°C. *E. faecium* give final pH of 4.0-4.4 in glucose (Manero and Blanch, 1999). The *E. faecium* strain which will be used in this study is *Enterococcus faecium* No.78. *E. faecium* No.78, like other *E. faecium* strains, are Gram positive, facultatively anaerobic cocci which occur in singles or pairs. *E. faecium* No. 78 were first isolated from fermented rice cake known as "puto" in Philippines. This strain was identified by conducting 16S rDNA sequence analysis and sugars fermentative test. *E. faecium* No.78 are amylolytic lactic acid bacteria which are able to hydrolyze and ferment starch lactic acid. The optimum pH and temperature for *E. faecium* No.78 in lactic acid production are pH 6.5 and 30°C, respectively (Shibata *et al.*, 2006).

2.4 Effects of substrate concentration

Vishnu *et al.* (2002) had reported that starch to lactic acid conversion efficiency was more than 90% by *Lactobacillus amylophilus* GV6 at low substrate concentrations (<10%). Linko and Javanainen (1996) reported that the rate of lactic acid fermentation decreased when high initial sugar concentration (143-187 g/L) was used due to glucose inhibition especially in the early stages in their study on simultaneous liquefaction, saccharification, and lactic acid fermentation on barley starch by using *Lactobacillus casei* strain. There was a study on the direct lactic acid production from sago starch by *E. faecium* No.78 with 15 g/L, 20 g/L, 30g/L and 40 g/L of starch. That particular study had showed that the highest lactic acid production (10.62 g/L) was obtained when 30 g/L of sago starch concentration was used as substrate while the lowest lactic acid production (0.36 g/L) was obtained when 15 g/L of sago starch concentration was used. In this study, 20 g/L, 30 g/L and 40 g/L of sago starch are used to evaluate the effects of sago starch concentration.

2.5 Effects of pH

The fermentation pH is controlled by base titration, or by extraction, adsorption, or electrodialysis of lactic acid. The fermentation without pH control results in an incomplete substrate conversion and subsequently lower yield of the product (Peeva and Peev, 1997). The optimum pH for lactic acid production differs between 5.0 and 7.0. Ohkouchi and Inoue (2005) reported the yield of lactic acid from starch with pH control at 5.0 was 2.5-times higher than without pH control in their study. Therefore, in this study, the fermentation pH will be controlled at pH 6.5 (the optimum pH for lactic acid production by *E. faecium* No.78). The lactic acid production with pH controlled within optimum limits would be expected to result in high lactic acid yield.

2.6 Modified MRS medium

MRS (de Mann Rogosa and Sharpe) medium was developed for the cultivation and enumeration of *Lactobacillus* species. It is developed as substitution of tomato juice agar. Most lactic acid bacteria can be grown on this medium. This medium contains mixed peptone, yeast extract, beef extract, glucose, potassium phosphate, sodium acetate, ammonium citrate, magnesium sulphate, manganese sulphate and Tween 80. Tween 80, sodium acetate, magnesium sulphate and manganese sulphate act as growth stimulants. MRS medium, which contains yeast extract, peptone and meat extract, is superior to yeast extract and malt extract (Hofvendahl and Hahn-Hligerdal, 2000). In this study, modified MRS medium is used whereby the glucose content of the MRS medium is replaced with sago starch at 20 g/L, 30 g/L or 40 g/L concentration for different fermentation trials.

2.7 Batch fermentation

In batch fermentation, the feed is introduced only once before the starting of the fermentation process. In batch fermentation, all nutrients (except the molecular oxygen in an aerobic process and ammonia or other chemicals for pH adjustment) required for one run, are added to the medium before the fermentation process started, and the broth containing products can only be withdrawn at the end of each batch run (Yamane, 1995). Batch system is ideal as this system only needs low maintenance and the risk of contamination is very low. The batch fermentation mode was used by Zhang and Cheryan (1991) in a study on direct fermentation of starch to lactic acid by *Lactobacillus amylovorus*, and Oh *et al.* (2005) in a study on lactic acid production from agricultural resources by *Enterococcus faecalis* RKY1.

3.0 MATERIALS AND METHODS

3.1 MATERIALS

3.1.1 Sago starch

Sago starch was used as substrate or carbon source in this study. Commercial food grade sago starch powder was obtained from local supermarkets and stored at room temperature until used.

3.1.2 Microorganism

Enterococcus faecium No.78 (provided by Prof. Kenji Sonomoto, Head of Department of Agriculture, Kyushu University, Fukuoka, Japan) was used in this study. The prepared stock culture was kept in 20% glycerol at ultra low temperature of -80°C.

3.1.3 Media

3.1.3.1 Growth medium

Modified MRS (de Mann Rogosa and Sharpe)-sago starch medium with 20 g/L sago starch concentration was used for inoculum preparation.

3.1.3.2 Fermentation medium

Modified MRS-sago starch medium was used in the fermentation process. Modified MRS-sago starch medium with concentration of 20 g/L sago starch was used in the first trial. Modified MRS-sago starch medium with concentration of 30 g/L and 40 g/L sago starch was used as substrate in the subsequent trials, respectively. Modified MRS-sago starch medium with concentration of 0 g/L sago starch was used as control. The sago starch in the medium was gelatinized by heating the medium.

3.1.4 Enzymes

Two types of enzymes was used for sago starch hydrolysis which were Termamyl-120L (thermostable amylase from *Bacillus licheniforms,* 120 KNU/g) and AMG 300L (Amyloglucosidase from *Bacillus acidopullulyticus* 300) supplied by Novo Nordisk.

3.1.5 Fermentor

The fermentation process was performed in a 2L labscale benchtop fermentor (Biostat-B, B. Braun Biotech International, Germany) with 1L working volume as shown in **Figure 1**. The agitation rate, temperature and pH were controlled automatically during fermentation.
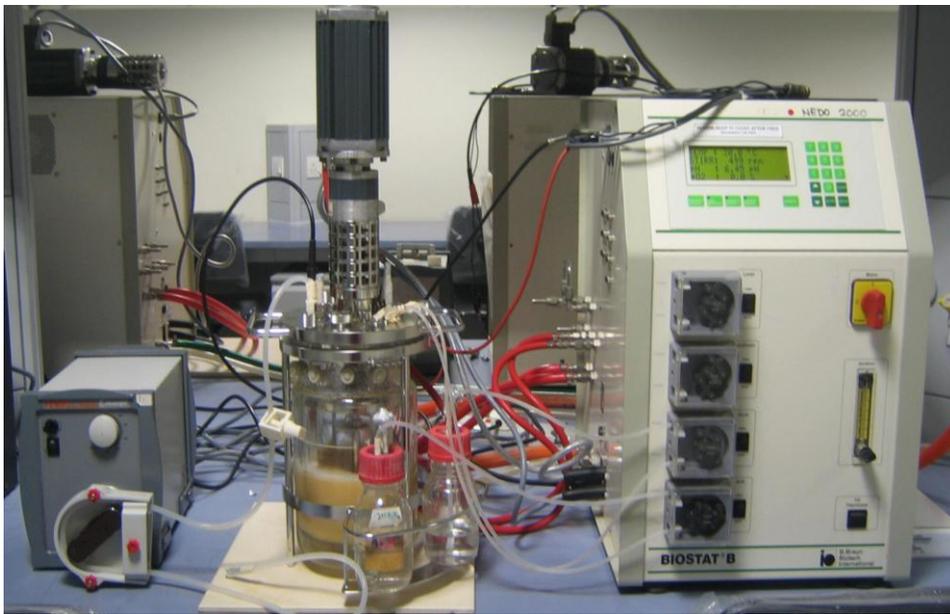
Figure 1: The 2L labscale benchtop fermentor with 1L working volume.

3.2 METHODS
3.2.1 Activation of *Enterococcus faecium* No.78

The stock culture of *E. faecium* No.78 at 1 mL was revived in 9 mL growth medium at 30°C for 24 hours.

3.2.2 Inoculum preparation

The revived bacteria were cultured in a fresh growth medium and were incubated at 30°C for 9 hours for inoculum preparation. The inoculum of 10% (v/v) or 100 mL was inoculated into the fermentation medium for each fermentation trial.

3.2.3 Batch fermentation

The fermentation process was performed in a 2L labscale benchtop fermentor (Biostat-B, B. Braun Biotech International, Germany) with 1L working volume. The fermentation process was conducted at constant pH of pH 6.5, with temperature at 30°C and agitation rate of 500 rpm. The pH was maintained by automatic addition of 10M NaOH and 2M H_2SO_4. For the first trial, modified MRS-sago starch medium with 20 g/L sago starch was used. The experiment was repeated with 30 g/L and 40 g/L of sago starch in modified MRS-sago starch medium, with 0 g/L sago starch as control.

3.2.4 Sampling

An aliquot of 20 mL sample was aseptically withdrawn from the fermentor at every 6 hours for analysis. The samples were kept at 4°C prior to analysis.

3.2.5 Enzymatic hydrolysis of sago starch

Sago starch in the samples was hydrolyzed prior to residual glucose analysis. Sago starch was hydrolyzed according to the method reported by Bujang *et al.* (1999). The pH of 10 mL sample was adjusted to pH 6.5 by adding 1M NaOH and 1M H_2SO_4. The gelatinization of samples were achieved by heating the samples at 90-100°C for 5-10 minutes. The liquefaction step was performed by adding Termamyl-120L and ion calcium to the sample. The samples were heated at 80-90°C for 2 hours. After 2 hours, the pH of samples was adjusted to pH 4.5 by using 1 M NaOH and 1 M H_2SO_4. The saccharification step was performed by adding AMG 300L (Novozymes) to the samples and the samples were incubated at 60-65°C for 2.5 to 4 hours. Then, the samples were centrifuged for 15 minutes at 8000 rpm to remove insoluble materials. In this experiment, 10μL of Termamyl-120L, 0.6mg of ion calcium and 12μL of AMG 300L was added to hydrolyse samples of fermentation with 20 g/L sago starch; 15μL of Termamyl-120L, 0.9mg of ion calcium and 18μL of AMG 300L was added to hydrolyse samples of fermentation with 30 g/L sago starch; 20μL of Termamyl-120L, 1.2mg of ion calcium and 24μL of AMG 300L was added to hydrolyse samples of fermentation with 40 g/L sago starch. The reducing and total sugar content in the samples' supernatant was determined by using Di-nitrosalicylic Acid (DNS) method (Miller, 1959).

3.2.6 Analytical techniques

3.2.6.1 Measurement of microbial growth

The growth of microorganism in the fermentation system was determined by viable cells count. An aliquot of 1 mL sample was added to 9 mL of 0.1% peptone water for diluting the sample. Serial dilutions were performed until appropriate dilution factor was achieved. Diluted sample of 100 μL was spread uniformly on the modified MRS-sago starch agar surface. The agar plates were incubated for 24-48 hours at 30°C. The colonies formed on the medium were counted. The results were presented as $\log_{10}$ of viable cells/mL.

3.2.6.2 Residual starch analysis

The residual starch in the fermentation medium was determined by using iodine-starch colorimetric method. The iodine solution was added at 0.1 mL to 1 mL of appropriately diluted sample. Distilled water was added to the mixture to bring the volume to 10 mL. Absorbance of the sample was measured at 590 nm by using a spectrophotometer. The residual starch concentration was calculated through a starch standard curve relating the absorbance at 590 nm (OD) to starch concentration (g/L) (Appendix A, **Figure 1**).

3.2.6.3 Hydrolyzed starch analysis

The hydrolyzed starch analysis was performed by using Di-nitrosalicylic Acid (DNS) method (Miller, 1959) to determine the concentration of residual glucose in the fermentation medium. DNS reagent of 3 mL was added to 3 mL of appropriately diluted sample. The mixture was heated in boiling water for 15 minutes. The 40% Rochelle salts solution was added at 1 mL to stabilize the colour formed after the mixture was cooled in ice water. Absorbance of the sample was measured at 575 nm by using a spectrophotometer. The residual glucose

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concentration was calculated through a glucose standard curve relating the absorbance at 575 nm (OD) to glucose concentration (g/L) (Appendix A, **Figure 2**).

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3.2.6.4 Determination of lactic acid concentration

Lactic acid production was analysed by High Performance Liquid Chromatography (HPLC) system (Waters) equipped with Waters 2487 dual λ absorbance detector set to 210nm and Waters 1515 isocratic HPLC pump. A 150 x 7.8mm fermentation monitoring column (Biorad, USA) was used. An aliquot of 20 μL sample was injected into the system at detection temperature of 60°C and flow rate of 0.6 mL/min. The mobile phase used was 1 mM H_2SO_4 (filtered through Whatman 0.45μm membrane filters using Millipore filtration system and degassed for 4 hours). Lactic acid concentration was calculated through the lactic acid calibration curve relating the area of peak against known concentration of 99.9% pure L(+)-lactic acid (g/L) (Supelco, USA) (Appendix A, **Figure 3**).

4.0 RESULTS AND DISCUSSION

In this study, the effects of sago starch concentration on direct lactic acid production with controlled pH by *Enterococcus faecium* No.78 were investigated by using three different sago starch concentrations, 20 g/L, 30 g/L and 40 g/L. The results of analyses for addressing effects of different sago starch concentrations were reported below.

4.1 RESULTS
4.1.1 Overall results of analyses for batch fermentation by using 0 g/L sago starch

Table 1: Results of analyses for fermentation by using 0 g/L sago starch.

Time (h)	Lactate (g/L)	Residual glucose (g/L)	Residual starch (g/L)	Log_{10} Viable cells/mL
0	0.633	0.000	0.000	8.2041
6	1.235	0.000	0.000	8.7924
12	1.543	0.000	0.000	8.8261
18	2.153	0.000	0.000	8.8692
24	2.288	0.000	0.000	8.7709
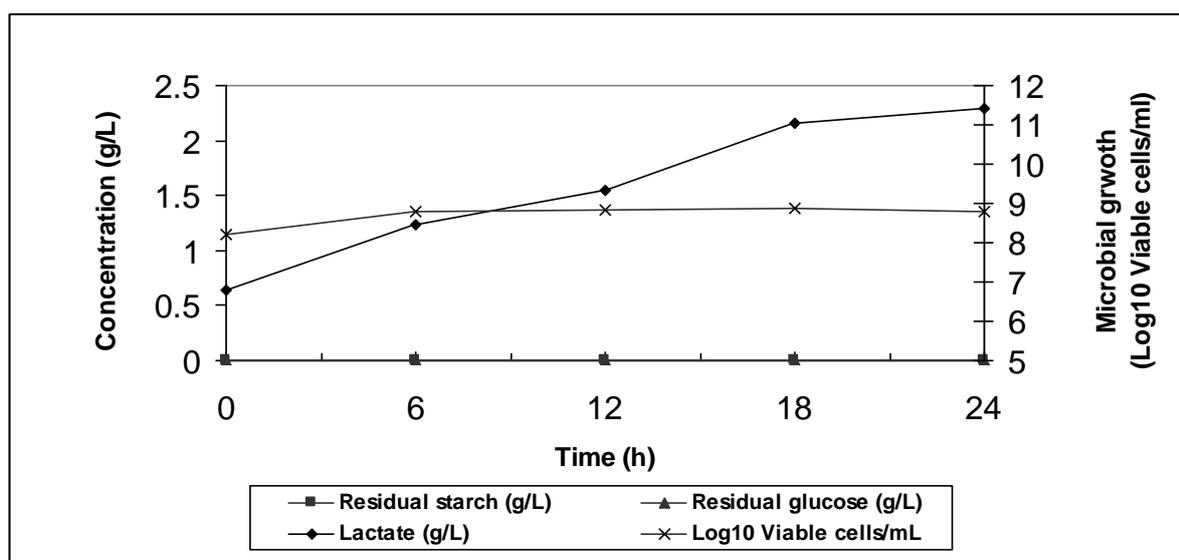
Figure 2: Results of analyses for batch fermentation by using 0 g/L sago starch.