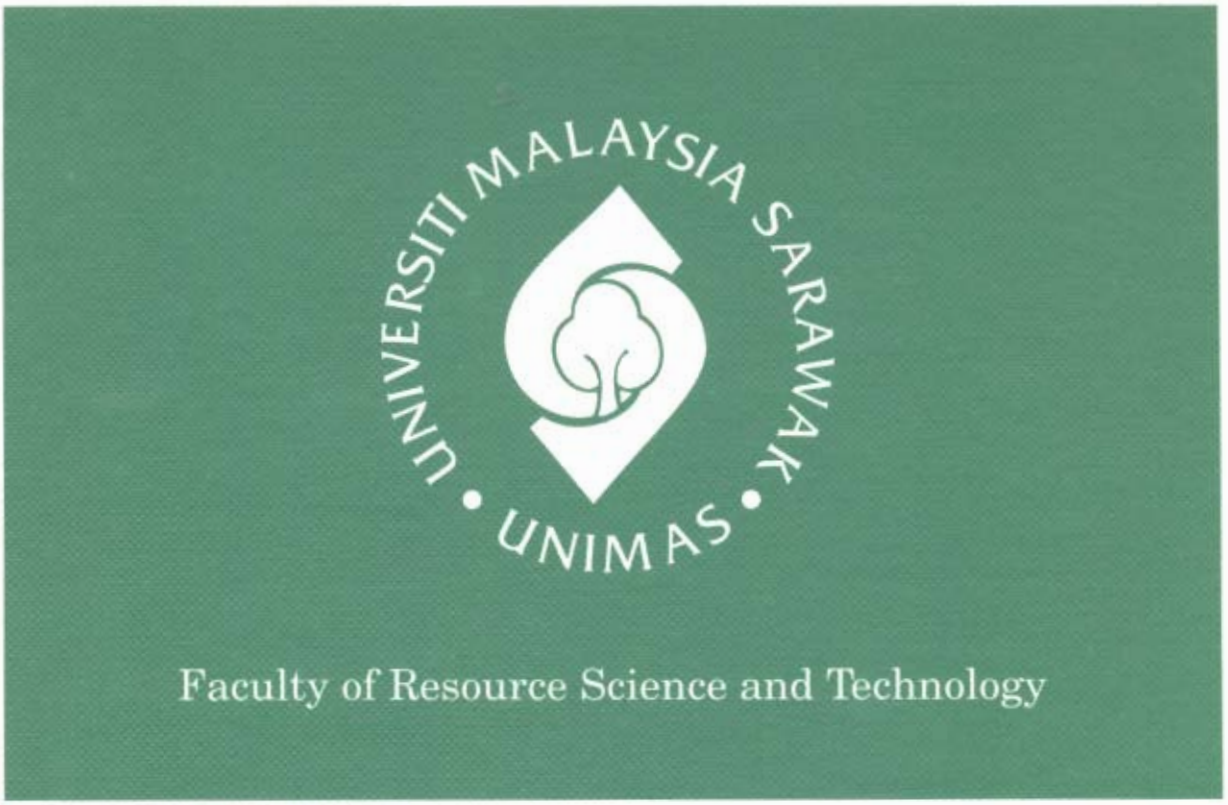

EFFECTS OF POLYPEPTONE CONCENTRATION ON LACTATE FERMENTATION

Shazrina Satar

Bachelor of Science With Honours
(Biotechnology Resource)
2004

Faculty of Resource Science and Technology

EFFECTS OF POLYPEPTONE CONCENTRATION ON LACTATE FERMENTATION

Shazrina Satar

Bachelor of Science with Honours
(Biotechnology Resource)
2004

- Sago palm
- Starch
- batch fermentation

EFFECTS OF POLYPEPTONE CONCENTRATION ON LACTATE FERMENTATION

SHAZRINA SATAR

This project is submitted in partial fulfillment of
the requirements for the degree of Bachelor of Science with Honours
(Biotechnology Resource)

Faculty of Resource Science and Technology
UNIVERSITI MALAYSIA SARAWAK
2004

Table of Contents

Effects Of Polypeptone Concentration On Lactate Fermentation

Shazrina Satar

Program of Resource Biotechnology
Faculty of Resource Science and Technology
University Malaysia Sarawak

ABSTRACT

Lactate production from fermentation of hydrolyzed sago starch by *Lactococcus lactis* IO-1 was studied at four different concentrations of polypeptone: 0g/L, 5g/L, 10g/L and 15g/L where 10g/L polypeptone act as control. This research has been done in order to find out the minimal concentration of polypeptone which can produce the optimal amount of lactic acid. Hence, the medium cost for the lactic acid fermentation can be minimized. From observation, batch fermentation trials utilizing 5g/L polypeptone and 10g/L polypeptone exhibit only minimal differences in the amount of lactate and biomass produced. Lower concentration of polypeptone results in higher residual glucose, thus needed longer period for complete consumption. 15g/L polypeptone was found to produce higher amount of lactate, 4.315g/L compared to 3.605g/L lactate from 10g/L polypeptone (control), 3.325g/L lactate from 5g/L polypeptone and 3.053g/L lactate from 0g/L polypeptone. All fermentation takes about 18 hours to produce maximum concentration of lactate.

Key words: Batch fermentation, *Lactococcus lactis* IO-1, Polypeptone, Lactate production.

ABSTRAK

Penghasilan laktat daripada proses fermentasi kanji terhidrolisis oleh Lactococcus lactis IO-1 telah dikaji pada empat kepekatan polipepton yang berbeza iaitu 0g/L, 5g/L, 10g/L dan 15g/L dimana 10g/L polipepton adalah sebagai kawalan. Kajian ini dijalankan untuk mengenalpasti kepekatan polipepton yang minimum tetapi dapat menghasilkan Asid laktik dalam jumlah yang optimum. Maka, kos media fermentasi Asid laktik dapat dikurangkan. Daripada pemerhatian, didapati fermentasi kelompok yang menggunakan samada 5g/L polipepton atau 10g/L polipepton hanya menunjukkan perbezaan yang minima dalam penghasilan laktat dan biomas. Kepekatan polipepton yang lebih rendah menyebabkan baki glukosa adalah lebih tinggi, sehingga memerlukan masa yang lebih lama untuk digunakan sepenuhnya. 15g/L polipepton didapati menghasilkan jumlah laktat yang paling tinggi iaitu 4.315g/L berbanding 3.605g/L laktat daripada 10g/L polipepton (kawalan), 3.325g/L laktat daripada 5g/L polipepton dan 3.053g/L laktat daripada 0g/L polipepton. Semua fermentasi yang dijalankan mengambil masa lebih kurang 18 jam untuk menghasilkan kepekatan laktat yang maksimum.

Kata kunci: Fermentasi kelompok, Lactococcus lactis IO-1, Polipepton, Penghasilan laktat

1.0 INTRODUCTION

Basically, Fermentation is the term used by Microbiologist to describe any process for the production of a product by means of the mass culture of a microorganism (Pumphrey and Julien, 1996). Fermentation can also defined as an energy-yielding metabolic pathway that involves no net change in oxidation state which means it is not completely oxidized. There are three types of fermentation, which are Batch fermentation, Fed batch fermentation and Continuous culture.

In this research, we study the effect of different concentration of polypeptone on lactic acid production with hydrolyzed sago starch (HSS) as substrate during batch fermentation process by *Lactococcus lactis* IO–1. Polypeptone previously reported as the nitrogen source for the growth of *Lactococcus lactis* IO–1 in fermentation media (Peterson and Pigford, 1984). However, it was an expensive source of nitrogen, the same as yeast extract. Thus, to make the production cost less than the present cost, the minimum concentration of polypeptone which can produce the optimum acid lactic have to be identified.

2.0 LITERATURE REVIEW

2.1 Sago

Sago palm which can produced sago starch is one of Genus *Metroxylon* belonging to Family Palmae and locally known as "Mulong" tree. According to Bujang and Ahmad (1999), sago palm is the only commodity that can grow under the harsh swampy environment, which occupies 75% of Sarawak with only minimum care. Confirmation have been done by Ishizaki *et al* (1997) that sago palm can produce 25t/hy of starch, and this is the highest productivity so far recorded compared to cereals including rice, and the other starchy crops. Hence, sago is a low-cost carbohydrate sources and plays an important role in nutritional development (Chanyavilas *et al*, 1999). Furthermore, starch is considered to be one of the most abundant plants products and a major source of energy in the human diet (Bujang *et al*, 2001). From the Department of Statistic (2001), sago managed to procure a total income of US$ 9.15 million for the state. Besides, sago starch potentially used in industries as foodstuff, manufacturing of food additives (sugar and flavoring), a recyclable source of energy through conversion of starch to biofuel (Bujang, 1998) and in the production of paper glue without toxin (Bujang and Ahmad, 2000). In this research, the usage of hydrolyzed sago starch (HSS) due to the inability of *Lactococcus lactis* IO–1 to ferment glucose to ethanol using dry sago starch directly.

2.2 Lactic acid

Lactic acid, $CH_3CHOHCOOH$ is commercially produced in fermentation since 1881 (Vickroy, 1985) and widely used in the food, chemical and pharmaceutical industries. It can be used as raw material for the production of polylactate (PLA), the basic substance for biodegradable plastic (Bujang *et al.*, 2001). Lactic acid fermentation is one of the world's most important commercial fermentation processes. It is a form of anaerobic respiration that has a glucose-consuming catabolic pathway and is used by both bacteria and animals to produce ATP in the absence of oxygen. Lactic acid fermentation is a most practical method of preservation and lactic acid bacteria are widely used as a low cost method for food preservation by fermentation and generally no or little heat is required during the fermentation. In lactic acid fermentation the following reaction takes place

Pyruvic acid + NADH <= > Lactic acid + NAD

This reaction does not generate carbon dioxide during biochemical reaction to produce lactic acid from glucose, thus it is the ideal bioconversion process with minimum impact on the environment (Ishizaki, 2000).

2.3 *Lactococcus lactis* IO–1

Microorganism that has been used for lactic acid fermentation of sago starch is*Lactococcus lactis* IO–1 (Ishizaki and Vonttaveesuk, 1996). *Lactococcus lactis* IO-1 grow in both anaerobic and microaerophilic conditions. In previous research, optimal pH for *Lactococcus lactis* IO–1 is pH 6.0 while the optimal temperature for growth is 37^0C. In TGC medium, the strain grew with peculiar filamentous shooting from the top the yellow zone (anaerobic zone) to the bottom. The strain was gram-positive ovoid coccus and catalase negative (Ishizaki *et al*, 1990). Ishizaki and Vonktaweesuk (1996) & Sirisansaneeyakul *et al.* (1998) reported that lactic acid fermentation by *Lactococcus lactis* IO–1 was a high potential process for the production of L-lactic acid and no other volatile fatty acid was detected (Ishizaki *et al*, 1990). With 1% of inoculate glucose, more than 90% were converted to lactic acid by *Lactococcus lactis* IO–1 (Ishizaki *et al*, 1990).

2.4 Batch Fermentation

Batch fermentation can be considered as a system that represents growth in a closed system using shake flask or bioreactor. In batch processes, the reactor is filled with a sterile nutrient substrate and inoculated with the microorganism. The culture is allowed to grow until no more of the product is being made. Besides that, all nutrients required during one run of cultivation except for molecular oxygen in an aerobic process and ammonia or other chemicals for pH adjustment, are added to the medium before cultivation started, and the broth containing the product is withdrawn only at the end of each batch run (Yamane, 1995). According to Jolhery (2002), biomass or dry cell weight is an important parameter in batch fermentation processes.

2.5 Polypeptone

Basically, polypeptone is a combination of casein peptone and meat peptone designed for incorporation into several formulas where abundant growth is desired. According to Ching and Leong (1990), polypeptone is a protein, which consists of longer sequence of amino acid, usually within 50 to a thousand molecule of amino acid. In this research, polypeptone is one of the nitrogen sources which play an important role in the growth of *Lactococcus lactis* IO–1 in fermentation medium (Peterson and Pigford, 1984).

3.0 OBJECTIVE

Objective of this research is to find out the minimal concentration of polypeptone which can produce the optimal amount of lactic acid. Besides, with the minimum concentration of polypeptone, we can minimize the medium cost for the lactic acid fermentation process.

4.0 MATERIALS AND METHODS

4.1 MATERIALS:

4.1.1 Sago Starch

Industrial grade sago starch powder obtained from local market

4.1.2 *Lactococcus lactis* IO–1

The microorganism used was *Lactococcus lactis* IO–1 JCM 7638 with optimum growth temperature at 37^0C, optimum pH 6.0 and stored at temperature -80^0C.

4.1.3 Enzymes for Hydrolysis

The enzymes used for hydrolysis were Termamyl-120L (thermostable amylase from Bacillus *lincheniformis*, 120 KNU/g) and Dextrozyme (a mixture of glucoamilase from *Aspergillus niger* and pullulanase from *Bacillus acidopullulyticus*, 225 AGU/ml) supplied by Novo Nordisk.

4.1.4 Culture Medium

TGC medium (Bacto thioglycolate w/o dextrose, Difco Laboratories U.S.A.) was generally used. Basal media was glucose broth consisted of yeast extract 10g/L, different concentration of polypeptone (0g/L, 5g/L, 10g/L and 15g/L), sodium chloride 5g/L and glucose 50g/L (from HSS). The same medium with 10g/L glucose, 10g/L yeast extract, 10g/L polypeptone and 5g/L sodium chloride was used for inoculum preparation. The 10% (v/v) inoculum was used as seed culture for all fermentation trials (Ishizaki and Ohta, 1989).

4.1.5 Shake Flask

Fermentation was carried out in shake flasks with a working volume of 200 ml for 30 hours, temperature maintained at 37^0C and agitation rate is 200rpm.

4.1.6 Polypeptone

Different concentrations of polypeptone (0g/L, 5g/L, 10g/L and 15g/L) was prepared in the early stage of the experiment.

4.2 METHODS :

4.2.1 *Lactococcus lactis* IO–1 Activation

Lactococcus lactis IO–1 was take out from stock culture (-80^{0}C) and was left at room temperature. The microorganism was transferred to Thioglycolate (TGC) medium without dextrose and was incubated for 18 hours at 37^{0}C and pH 6.0. The 1000µl starter culture was then transferred to 19ml (final volume: 20ml) seed culture at pH 6.0 in a universal bottle and incubated at 37^{0}C for another 6 hours.

4.2.2 Enzymatic Hydrolysis of Sago Starch

2 enzymes (Novo Nordisk) that were used in the hydrolysis process are 100µl Termamyl-120L and 120µl Dextrozyme. Hydrolysis was performed as reported before (Bujang *et al.*, 1999). Refer to appendix 1 for details.

4.2.3 Medium and Parameters for Fermentation

In the fermentation using shake flask, the medium in the shake flasks was autoclaved. The temperature was maintained at 37^{0}C and mild agitation of 200 rpm was provided in order to maintain a homogeneous culture. The initial pH was set at pH 6.0 and not controlled throughout the fermentation process. All the experiments were run parallel in triplicates.

4.2.4 Sampling

Each fermentation was operated until 30 hours. Sampling was done every 6 hours starting from 0 hour, 6 hours, 12 hours, 18 hours, 24 hours and 30 hours. For each sample, 10ml were taken manually for each time and were kept at lower temperature (4^0C) before been analyzed.

4.2.5 Analytical Techniques

4.2.5.1 Reducing Sugar Analysis

The reducing sugar assays was based on Dinitrosalicyclic acid (DNS) method (Miller, 1959). For further details, refer to appendix 2.

4.2.5.2 Cell Growth Determination

Dried cell weight (DCW) method was done to determine the growth of *Lactococcus lactis* IO–1. Refer to appendix 2 for further details.

4.2.5.3 Lactic Acid Determination

Determination of the concentration of lactate produced was analyzed using HPLC. Refer to appendix 2. The results were then compared to the standard curve of pure acid lactic.

5.0 RESULTS AND DISCUSSION

5.1 Effects of different polypeptone concentration

5.1.1 Batch fermentation utilizing 0g/L polypeptone concentration.

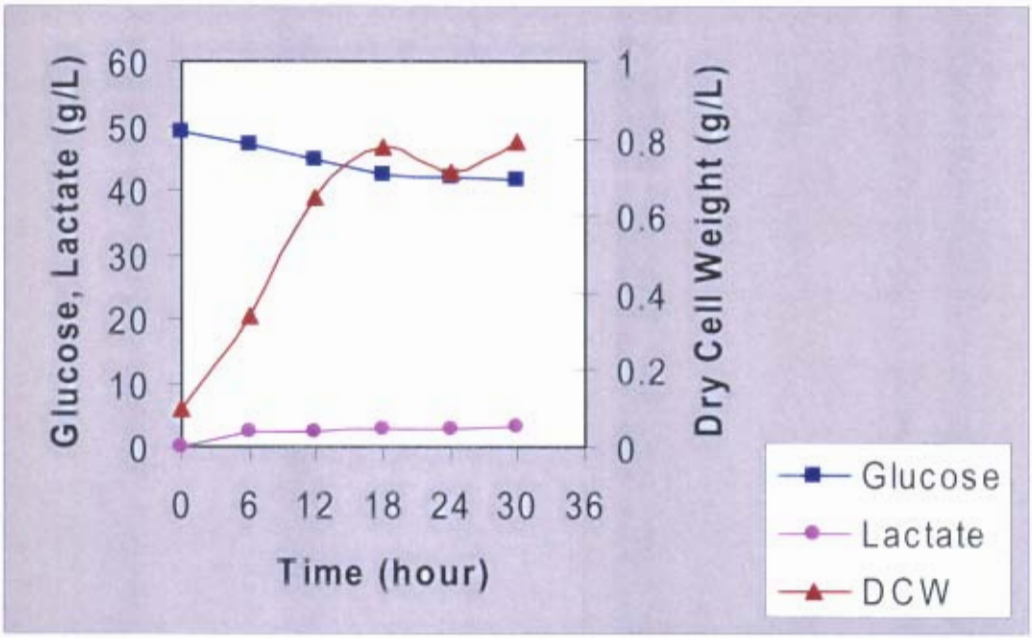

Figure 5.1.1: Batch fermentation utilizing 0 g/L polypeptone concentration

From Figure 5.1.1, the initial glucose concentration was 49.09g/L and this amount decline to 42.26g/L after 18 hours, a consumption of 6.83g/L or 13.9% and then become approximately constant. Lactate production accelerates for the first 6 hours, up to 2.319g/L, but the production was slower after the 12 hours. The maximum lactate production was 3.053g/L, achieved after 30 hours. Meanwhile, the biomass (DCW) increased for the first 18 hours and then became almost constant reaching the 30th hour. The amount of lactate produced was corresponding to the

excessive decline of glucose concentration and the increasing of the dry cell weight (the important parameter in batch fermentation). This is because the bacteria used the consumed glucose to build the cell and also for their growth instead of producing lactate. Furthermore, lower concentration of polypeptone results in higher residual glucose, thus needed longer period for complete consumption. The experiment was terminated at 30 hours at 41.56g/L of residual glucose concentration. The results pattern are the same with three others polypeptone concentration.

5.1.2 Batch fermentation utilizing 5g/L polypeptone concentration

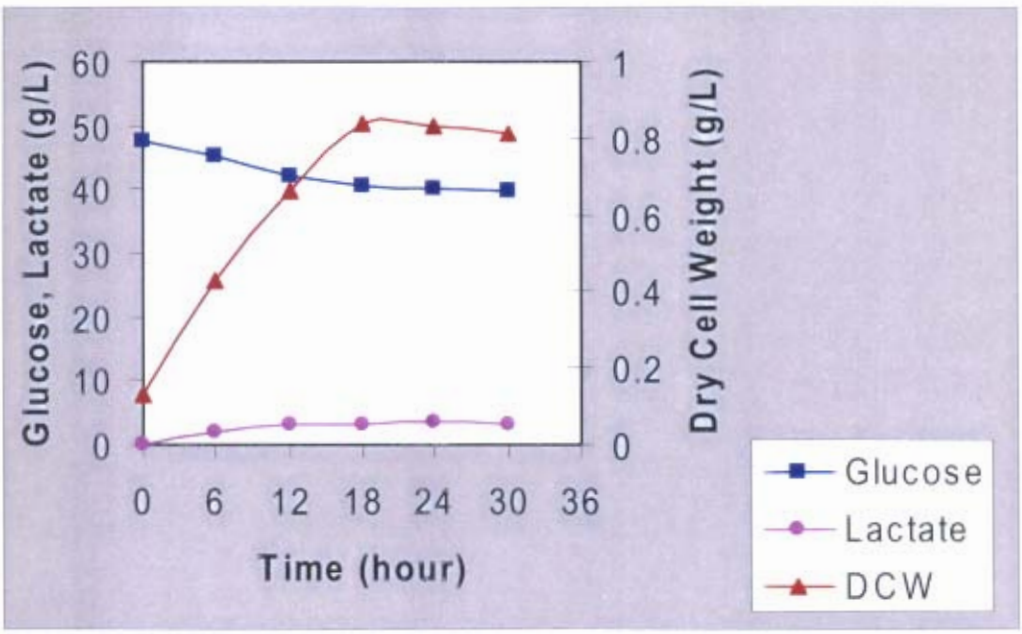

Figure 5.1.2: Batch fermentation using 5 g/L polypeptone concentration

From Figure 5.1.2, the initial glucose concentration was 47.69g/L and this amount decline to 40.5g/L after 18 hours, a consumption of 7.19g/L or 15.1% and then become approximately constant. Lactate production accelerates for the first 12 hours, up to 3.0g/L, but the production was slower after the 18 hours. The maximum lactate production was 3.325g/L, achieved after 24 hours. Meanwhile, the biomass (DCW) increased for the first 18 hours and then became almost constant reaching the 30th hour.

5.1.3 Batch fermentation utilizing 10g/L polypeptone concentration

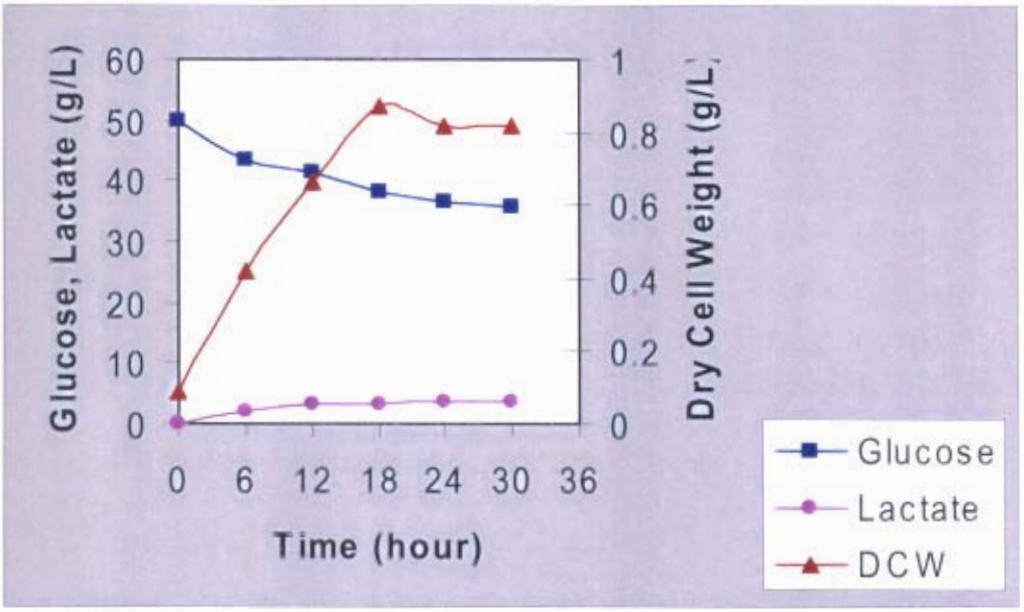

Figure 5.1.3: Batch fermentation using 10 g/L polypeptone concentration

From Figure 5.1.3, the initial glucose concentration was 49.97g/L and this amount decline to 36.47g/L after 24 hours, a consumption of 13.5g/L or 27% and then become approximately constant. Lactate production accelerates for the first 12 hours, up to 3.231g/L, but the production

was slower after the 18 hours. The maximum lactate production was 3.605g/L, achieved after 24 hours. Meanwhile, the biomass (DCW) increased for the first 18 hours and then became almost constant reaching the 30th hour.

5.1.4 Batch fermentation utilizing 15g/L polypeptone

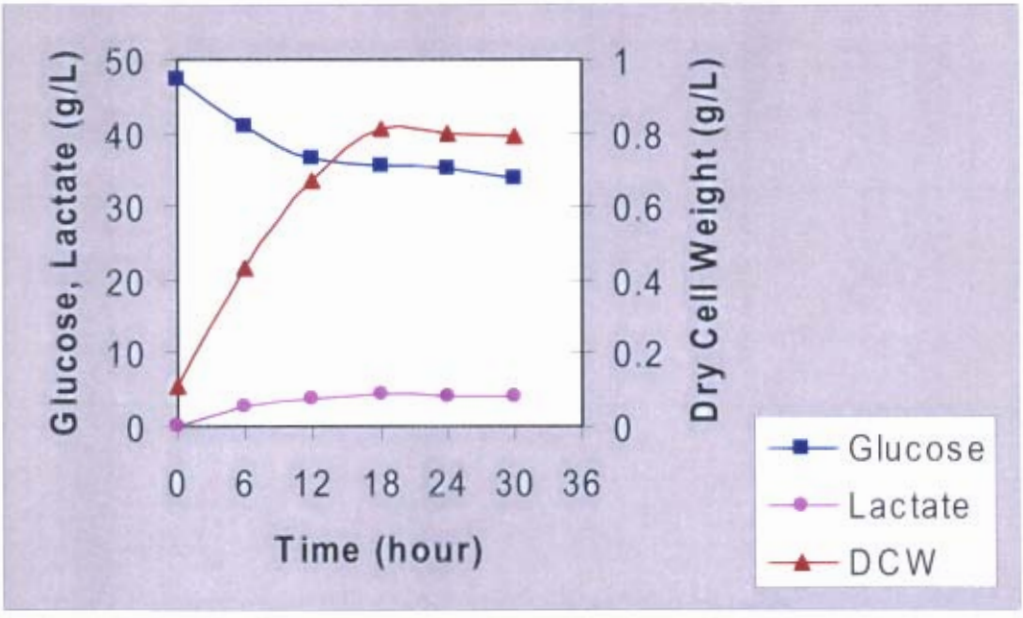

Figure 5.1.4: Batch fermentation using 15 g/L polypeptone concentration

From Figure 5.1.4, the initial glucose concentration was 47.17g/L and this amount decline to 35.59g/L after 18 hours, a consumption of 11.58g/L or 25% and then become approximately constant. Lactate production accelerates for the first 18 hours, up to 4.315g/L (maximum lactate production), but the production was slower after the 24 hours. Meanwhile, the biomass (DCW) increased for the first 18 hours and then became almost constant approaching the 30th hour.

5.2 Discussion on the comparisons of four different polypeptone concentrations utilizing batch fermentation.

Table 5.2.1: Batch fermentation utilizing different concentrations of polypeptone

Parameter	**0g/L**	**5g/L**	**10g/L**	**15g/L**
Initial glucose concentration (g/L)	49.09	47.69	49.97	47.17
Residual glucose concentration (g/L)	41.56	39.63	35.77	33.67
Glucose consumption (g/L)	7.53	8.06	14.2	13.5
Glucose consumption efficiency (%)	15.3	16.9	28.4	28.6
Lactate concentration (g/L)	3.053	3.325	3.605	4.315
Fermentation efficiency (%)	40.5	41.3	25.4	32.0
Dry cell weight (g/L)	0.78	0.84	0.87	0.81

Table 5.2.1 represents the summary of the results for lactate production using four different concentration of polypeptone.

The main objective of this research is to find out the minimal concentration of polypeptone which can produce the optimal amount of lactic acid. Besides, with the minimum concentration of polypeptone, the medium cost for the lactic acid fermentation process can be minimized. Polypeptone previously reported as the nitrogen source for the growth of *Lactococcus lactis* IO–1 in fermentation media (Peterson and Pigford, 1984). However, it was an expensive source of

nitrogen, the same as yeast extract. Referring to the expenditure for batch fermentation process provided by the State Level Sago Committee, the current price of polypeptone and yeast extract is RM 600 per kg respectively. Using the same amount of polypeptone and yeast extract in batch fermentation, we have to suffer a 96.4% loss of profit compared to 76.5% of profit gain from continuous fermentation process. Thus, to make the production cost less than the present cost, the minimum concentration of polypeptone which can produce the optimum amount of lactic acid have to be identified.

From Table 5.2.1, glucose consumption efficiency is corresponding to the used of polypeptone concentration where the higher polypeptone concentration, show the higher glucose consumption efficiency. However, in the amount of lactate concentration, it only shows minimal differences for each polypeptone concentration used, compared to lactate yield produced by 10g/L polypeptone concentration which act as a control in this experiment. This means that with the used of lower concentration of polypeptone, lactate can still be produced.

Referring to the fermentation efficiency, it show that 5g/L polypeptone has the highest fermentation efficiency that is 41.3% as compared to control, 10g/L polypeptone which only show the efficiency of 25.4%. This may be due to the lower glucose consumption of 5g/L polypeptone with almost similar amount of lactate yields of 10g/L polypeptone.

Lower glucose consumed by bacteria using broth media with different concentration of polypeptone shows that the tendency of using protein source in production of lactate rather that using the glucose source. Aiba *et al.* (1973) proved this situation by mentioning that the greater

chance of a high yield product can be obtained if the organism can assimilate the greater amount of nitrogen. Besides, lower concentration of polypeptone also results in higher residual glucose, thus needed longer period for complete consumption. Furthermore, according to Jolhery (2002), relatively slower consumption of glucose was observed under uncontrolled pH condition. Luedeking and Piret (1958) reported similar trend, where fermentation progressed more rapidly when the pH was controlled than when it was not controlled.

Instead of that, dry cell weight or biomass also plays an important role in analyzing lactate fermentation process. All experiments shows that the growth of *Lactococcus lactis* IO–1 were at the exponential phase in the first 18 hours processes where 0.78g/L, 0.84g/L, 0.87g/L and 0.81g/L achieved for each of the four polypeptone concentration respectively. Then, it become constant tills the end of the process. This show that during first 18 hours, *Lactococcus lactis* IO–1 multiply rapidly thus used higher amount of glucose and protein in order to build the cell and in the same time producing lactate. So, as the amount of dry cell weight become higher, the more consumption of glucose and the more yield of lactate produced.

6.0 CONCLUSION

The effects of different concentration of polypeptone on lactate fermentation have been studied in order to find out the minimal concentration of polypeptone that can produced optimal amount of lactate and in the same time minimized the medium cost. In this study, 10g/L polypeptone act as a control. Thus, the other three different concentration of polypeptone were then compared with lactate produced from 10g/L polypeptone concentration. With comparison to the yield of lactate by 10g/L polypeptone and also the fermentation efficiency, 5g/L polypeptone is preferable as the concentration that suits to the research objectives. Eventhough 15g/L polypeptone concentration produced the highest yield of lactate and the lactate yield of 0g/L is almost the same with 5g/L, it is still recommended that 5g/L polypeptone can be accepted as the minimum concentration of polypeptone that can produce optimal amount of lactate and in the same time, help to minimize the medium cost. This due to the cost of 5g/L polypeptone is much cheaper than 15g/L and also to the advantage of 5g/L that can produced lactate slightly faster than 0g/L polypeptone. As in large scale industries, the usage of yeast extract and polypeptone is in ratio 1:1 and this ratio makes us suffer for 96.4% loss of profit. Thus, in order to reduce the production cost, the suggesting ratio is 2:1 which when converted to g/L, it is 10g/L yeast extract with 5g/L polypeptone as the fermentation medium. However, to obtain more precise result, further study need to be done focusing on the concentration of polypeptone between 0g/L to 5g/L.

7.0 ACKNOWLEDGEMENT

I would like to express my sincere appreciation to my supervisor, Cik Dayang Salwani for her supervision and guidance throughout this study. I also wish to extend my sincere gratitude to Dr. Kopli Bujang as my co-supervisor for his advice and comments. Also big thanks to Staff and Faculty of Resource Science and Technology for their co-operation and also for providing necessary facilities and instruments. Last but not least, special thanks to all my friends, colleagues and my family for their supports and co-operation throughout this study.