PRELIMINARY STUDY ON LACTATE PRODUCTION UTILIZING THE TURBIDOSTAT SYSTEM

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Bachelor of Science with Honours (Resource Biotechnology) 2005
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Preliminary Study on Lactate Production Utilizing the Turbidostat System

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ABSTRACT

The possibility of utilizing the Turbidostat System in lactic acid fermentation was investigated in this study. Hydrolysed sago starch (HSS) was used as a carbon source of glucose to produce lactic acid by Lactococcus lactis 101. Batch fermentation system was conducted at pH 6.0 maintained at 37°C. The highest concentration of lactic acid, 33.0 g/L, was obtained at 24 hours fermentation and the turbidometer reading is 0.093. The biomass concentration and residual glucose is 2.35 g/L and 10.83 g/L respectively. Later, continuous fermentation was carried out with cell recycling using the same parameters as in batch fermentation and the flow rate of media is 50 mL/hour. The cell recycling was performed at 16 hours of fermentation. The lactate concentration and biomass concentration obtained during 24 hours of continuous fermentation was higher than in batch fermentation with 38.75 g/L and 3.44 g/L respectively. The turbidometer reading is 0.098. The highest lactate production was obtained at 48 hours of fermentation with the value 60.18 g/L and turbidometer reading is 0.28. The biomass concentration and residual glucose concentration is 13.9 g/L and 13.81 g/L respectively.

Keywords: Turbidostat System, cell recycling, lactic acid, batch fermentation, continuous fermentation.

ABSTRAK

Kajian ini bertujuan untuk melihat keupayaan penggunaan Sistem Turbidostat ke atas fermentasi asid laktik. Kanji sagu terhidrolisis (HSS) telah digunakan sebagai sumber karbon glukosa untuk menghasilkan laktik asid oleh Lactococcus lactis 101. Sistem fermentasi secara kelompok dijalankan pada pH 6.0 dan dikekalkan pada suhu 37°C. Kepekatan laktik asid yang tertinggi dicapai pada masa ke 24 proses fermentasi iaitu 33.0 g/L dengan bacaan turbidometer 0.093. Kepekatan biomass dan baki glukosa adalah 2.35 g/L dan 10.83 g/L masing-masing. Seterusnya, sistem fermentasi secara berterusan telah dijalankan bersama kitar semula sel dengan menggunakan parameter yang sama seperti di dalam fermentasi secara berterusan dan kadar aliran media yang digunakan ialah sebanyak 50 mL/jam. Kitar semula sel dijalankan pada masa ke 16 jam fermentasi. Kepekatan asid laktik dan kepekatan biomass yang dicapai pada masa ke 24 jam fermentasi dalam fermentasi berterusan adalah lebih tinggi berbanding dalam fermentasi secara kelompok dengan nilai 38.75 g/L dan 3.44 g/L masing-masing. Bacaan turbidometer ialah 0.098. Kepekatan biomass dan baki glukosa ialah 13.9 g/L dan 13.81 g/L masing-masing.

Kata kunci: Sistem Turbidostat, kitar semula sel, asid laktik, fermentasi secara kelompok, fermentasi secara berterusan.
1.0 INTRODUCTION

Within the last decade, lactic acid or lactate is widely used as a precursor in polylactic acid (PLA) synthesis to produce biodegradable plastic (Bujang et al., 2001). Lactic acid was first commercially produced by Charles E. Avery at Littleton, Massachusetts, USA in 1881 (Vickroy, 1985a). Lactic acid fermentation is one of the world’s most important fermentation processes (Fellows, 1990). In the food industries, lactic acid is used to acidify jams, jellies, confectionery, sherbets, soft drinks, extracts and other products. Lactic acid has also shown promise in inhibiting mycotoxinogenic fungi, and foot-and-mouth virus in sheep’s dung. Lactic acid is produced by the anaerobic fermentation of carbohydrates (Frazier and Westhoff, 1988). Lactic acid fermentation using sago starch as a carbon source which produces polylactate shows great potential (Ishizaki, 2000).

There are three types of fermentation systems; batch, fed-batch and continuous. This study attempts to utilize the Turbidostat System, one type of continuous culturing system, on the lactic acid fermentation by Lactococcus lactis IO1 using hydrolysed sago starch as a carbon source. The turbidostat is a system which is operated by maintaining a constant cell growth or cell density by supplying fresh medium as required (Scragg, 1991). In previous study by Ishizaki et al., (2000) about continuous L-lactic acid production using pH-dependent substrate system couple with cross flow filtration and turbidity control culture system, at a cell concentration of 15g/l and a feed glucose concentration of 53g/l, the highest lactate concentration in broth, 39.2g/L, was obtained at dilution rate of 0.21 1/h with the respective glucose residual concentration was 1.90g/l.

The objectives of this study are:

i. To observe possible utilization of Turbidostat System on the lactate production.

ii. To identify the turbidity that shows the highest lactate production.
2.0 LITERATURE REVIEW

2.1 Lactic Acid
Lactic acid, CH₃CHOHCOOH was produced by lactic acid bacteria in glucose fermentation (Ishizaki, 1997). Its molecular weight is 90.08g/mol with heating and melting point at 122°C and 17°C respectively. In fermentation, lactate is a secondary metabolite produced during the log-phase of Lactococcus lactis IO-1 growth (Bujang et al., 2001). Lactic acid (2-hydroxypropionic acid) is an organic hydroxy acid whose occurrence in nature widespread. (Vickroy, 1985a). Lactic acid is a weak acid which is non-corrosive and a good solvent (Vickroy, 1985b). Lactic acid is commercially produced either by chemical synthesis or by microbial fermentation. (Litchfield 1996 and Lunt 1998). Lactic acid is used directly in the production of some rye and sourdough bread. Lactic acid is mainly used as food ingredient, chemical and pharmaceutical applications (Vickroy, 1985a).

2.2 Lactococcus lactis IO-1
Lactic acid bacteria are used widely for the manufacturing of cheese, fermented milks, and other processed dairy products (Davidson et al, 1991). Lactococcus lactis IO-1 was lactic acid bacteria which important in fermentation industry. The bacterium is a gram positive and appears as an ovoid coccus, 0.8-0.9 μm width and 1.1-1.2 length (Ishizaki et al., 1990). The strain is a homofermentative with an ideal pH for glucose consumption at pH 6.0. Optimal temperatures for Lactococcus lactis is 37°C, however, it can survive in temperature between 10°C to 45°C. (Ishizaki & Ohta, 1989). According to Ishizaki et al., (1990), Lactococcus lactis IO-1 is a homolactic L-lactic acid-producing organism. The special characteristic of strain IO-1, it could convert more than 90% of the glucose to lactic acid by utilizing 1% of the glucose in inoculum. Due to the fact that it produces solely L-homolactic acid, such fermentation processes would not produce carbon dioxide, an important criteria for large-scale lactate production (Ishizaki et al., 1993).

2.3 Sago
Sago palm or Metroxylon spp. (Metroxylon sagu) along with some other species of Palmae family producing sago starch is one of the oldest tropical plants used by mankind (Takamura,
The local name for sago is ‘mulong’. In Sarawak, sago grows well in the vast area of peat swamps. These areas are located in coastal belts such as Mukah, Dalat and Igan (Sim, 1985). Sago palm consists mainly of energy-living starch with very little protein or minimal content. Sago palm is the highest starch producer among all grains and cereals, almost three times greater than corn, rice and wheat and about 17 times than tapioca (Ishizaki, 1997). In Malaysia, the used of sago starch has been increasing, and it’s presently been used for the production of glucose or food production. Sago starch is widely used in food industry especially in the production of Monosodium Glutamate (MSG), crackers and caramels and production of syrup with high fructose and glucose content. In industrial application, sago starch is also used in the production of paper glue without toxin (Bujang & Ahmad, 2000). Sago starch represents an alternative cheap carbon source for fermentation processes that is attractive out of both economic and geographical considerations (Aziz, 2002).

2.4 Turbidostat System

According to Walker and Gingold (1993), if the fresh medium is added continuously, at an appropriate rate, and the culture vessel is fitted with an overflow devise, such that culture is displaced by the incoming fresh medium, a continuous culture may be established. Continuous culture, on the other hand, is a system for growing cells in an open system where the microbial population is maintained in the continuous state of balanced growth by continuously removing some of the culture and replacing it with fresh medium at the same rate. A turbidostat (instead of chemostat) is one type of continuous culture system. Turbidostat is a continuous culturing system which is operated by maintaining a constant cell density by supplying fresh medium as required. A turbidostat, in addition, possesses a photocell system which continuously monitors the cell density (turbidity), and maintains this constant by controlling the medium inlet flow rate (Scrugg, 1991). If the turbidity tends to increase, the feed rate is increased to dilute the turbidity back to its setpoint. When the turbidity tends to fall, the feed rate is stopped for a while so that growth can restore the turbidity to its setpoint. There is no limiting nutrient in turbidostat system and the dilution rate are varies. The turbidostat operates best in high dilution rates. The turbidostat will be monitored using an appropriate detector (laser probe) and the liquid flow rate will be automatically adjusted so as to maintain the variable at constant level.
3.0 MATERIALS AND METHODS
3.1 Materials
3.1.1 Sago starch
Commercial food grade sago starch is obtained from local supermarket. Samples are kept free of moisture in an airtight container.

3.1.2 Microorganism
This study utilized the *Lactococcus lactis* IO-1 from the Japanese Collection of Microorganism (JCM) 7638. The prepared stock culture is kept in glycerol at ultra low temperature of -84°C. The stock culture is revived in Thioglycolate (TGC) medium without Dextrose (Difco, USA) and incubated for 18 hours at 37°C. This can be stored for up two weeks in the same medium prior to use.

3.1.3 Culture Media
Thioglycolate (TGC) media without dextrose is used to culture *Lactococcus lactis* IO-1. For fermentation and feeding media, please refer to Appendix A.

3.1.4 Enzyme for Hydrolysis
The enzymes that used for sago starch hydrolysis are Termamyl-120L (thermostable amylase from *Bacillus licheniforms*, 120KNU/g) and Dextrozyme (a mixture of glucoamylase from *Aspergillus niger* and pullulanase from *Bacillus acidopullulyticus*).

3.1.5 Cell Recycling
In this study, a hollow fiber membrane (HFM) is connected to the sampling tube from the fermentor. The hollow fiber cartridge Microza PSP-103) from Akashi is used to recycle cell back to the fermentor. This is to maintain high cell density and to minimize wash-out from the fermentor (Ishizaki et al, 2000).
3.1.6 Polystat cc2
Poly Compatible Control, Polystat cc2 (HUBER Version 1.0/02) is used in this study to maintain the broth temperature at 37°C throughout the fermentation.

3.2 Methods
3.2.1 *Lactococcus lactis* IO-1 Activation
The *Lactococcus lactis* IO-1 stock culture (-84°C) is revived in Thioglycolate (TGC) medium without Dextrose (Difco, USA) and incubated for 18 hours at 37°C. This is stored for up to two weeks in the same medium prior to use. Seed culture is prepared by inoculating *Lactococcus lactis* in Thioglycolate (TGC) without dextrose media into inoculum medium and incubated at 37°C for 6 hours. Seed culture volume is 10% of the working volume during fermentation.

3.2.2 Enzymatic Hydrolysis of Sago Starch (Bujang *et al*., 1999).
The processes involved in Enzymatic Hydrolysis of Sago Starch are shown in Appendix B.

3.2.3 Turbidostat System.
Turbidity control consisted of a laser probe (LA-300LT), Automatic System Research Co. Ltd., Tokyo, for online measurements of turbidity. It will be preset to control the cell density at specific concentrations as determined by a previous calibration between the cell concentration and the culture turbidity (Ishizaki *et al*., 2000). During fermentation, the laser probe will be utilized as a detector to determine the turbidity or cell growth where the optimum lactate is produce. A plot of turbidity versus cell dry weight (biomass concentration) is made for standard curve.

3.2.4 Fermentation System
Fermentation with batch system, was carried out in 1L fermentation jar with a working volume of 700ml, maintained at 37°C and stirred with magnetic stirrer without gas flow. The temperature was controlled by Polystat cc2 as shown in Figure 1. The culture pH (6.0) was controlled by addition of 10M NaOH throughout the culture. Later, the fermentation was initially started in batch mode switch to continuous mode. The flow rate of fresh media that was studied in this
experiment is 50ml/hour. The continuous fermentation was carried out with the same parameter used in batch fermentation but in addition of hollow fiber membrane (HFM) for cell recycling which was performed at 16 hours of fermentation as shown in Figure 2.

Figure 1: Batch fermentation system set-up.

Figure 2: The continuous fermentation system set-up with cell recycling.

3.2.5 Sampling
The sampling was performed every 4 hours until 24 hours in batch fermentation and the sample volume is 10 ml. While during continuous fermentation system, samples was extracted every 6 hours until 48 hours. The sample volume is 10mL. Samples are kept at 4°C prior to further analysis.
3.2.6 Analytical Methods

3.2.6.1 Determination of Biomass by dry cell weight (DCW)

Samples (10ml each time) is centrifuged (KUBOTA) in graduated centrifuge tubes at 7000 rpm for 15 minutes at 4°C. The supernatant is then discarded and kept at 4°C for reducing sugar analysis and lactate analyses. The cell is suspended in distilled water. The cell is then recentrifuged again at 7000 rpm for 15 minutes at 4°C following which the supernatant is discarded. The pellet is dried in oven at 70°C for 24 hours until the weight is constant. After drying, the centrifuge tube is reweighed and the dry cell weight (DCW) was determined as follows:

\[
\text{Dry cell weight (g/l)} = \frac{\text{wt. of centrifuge tube + cells (g)} - \text{wt. of centrifuge tube (g)}}{\text{Sample volume (mL)}} \times 10^3
\]

3.2.6.2 Reducing sugar analysis

The reducing sugar is analyzed using the Di-nitrosalicylic acid (DNS) method (Miller, 1959). For DNS reagent preparation, please refer to Appendix A.

3.2.6.3 Determination of lactate

Lactate is analyzed by using High Performance Liquid Chromatography (HPLC Water 2487), a method that modified and developed in this laboratory. Exactly 20μl of sample is injected into the system and detection temperature is 60°C with flow rate of 0.6mL/min. 1mM H₂SO₄ (filtered and degassed on Whatman 0.45μm membrane filter) is used as the mobile phase. Concentration of lactic is calculated from the lactic acid calibration curve, plotted between areas of lactic acid against known concentration of 99.9% pure L(+) lactic acid (Supelco, USA). (Refer to Appendix C: Figure 15)
4.0 RESULTS

The possible utilization of Turbidostat System in lactate production was investigated. The batch fermentation was first carried out in a working volume of 700ml, maintained at 37°C, and the culture pH is 6.0, controlled by the addition of 10M of NaOH. Later on, the continuous fermentation with cell recycling using flow rate of media, 50 ml/hr, was then performed with the same parameters as in batch fermentation.

4.1 Batch Lactate Fermentation

The batch fermentation system was first carried out to observe the possibility of utilizing the Turbidostat System on lactate fermentation. From the experiment, when there is an increased in biomass concentration or cell growth, the turbidometer reading also increased. This increment of turbidometer reading is parallel with the cell density (DCW). A plot of turbidometer reading (turbidity) versus dry cell weight (DCW) was then made for standard curve.

The standard curve for dry cell weight is shown in Figure 3.

![Figure 3: Standard curve for dry cell weight](image)
4.1.1 The turbidity graph and dry cell weight of *Lactococcus lactis* IO-1

The turbidity graph and dry cell weight of *Lactococcus lactis* IO-1 for batch lactate fermentation is shown in Figure 4.

![Turbidity graph and dry cell weight](image)

**Figure 4**: Turbidity graph (a) and dry cell weight of *Lactococcus lactis* IO-1 (b) for batch lactate fermentation

From Figure 4(a), the turbidometer reading in batch lactate fermentation was increased along with the fermentation time. During the first half period of fermentation, the turbidometer reading has shown great increment. From 0 hour fermentation, the turbidity is 0.005 increased to 0.081 at 12 hours fermentation, an increment about 93.83%. After 12 hours fermentation until 24 hours time, the increment is slower with the value 0.093 at 24 hours fermentation, increased about 12.9% from 12 hours fermentation. The increment in the turbidometer reading (turbidity) was caused by the growth of the cells. From Figure 4(b), the biomass concentration at 0 hour fermentation is 0.67 g/L increased to 1.97 g/L or about 66% during 12 hours fermentation with the turbidity 0.005 and 0.081 respectively. The highest biomass concentration was obtained during 16 hours of fermentation with the value 2.37 g/L with the turbidity 0.089. However, the biomass concentration was slightly decreased at 24 hours fermentation with the value 2.35 g/L. There is a linear relationship between turbidity and dry cell weight. This is because when there is an increased in turbidity the dry cell weight also increased. Turbidity can be used as on way for measurements of biomass concentration without the need to do the DCW analysis.
4.1.2 Lactate concentration and Glucose consumption

The lactate concentration and glucose consumption in batch fermentation is shown in Figure 5.

![Figure 5: Lactate concentration (a) and glucose consumption (b) in batch lactate fermentation](image)

Figure 5: Lactate concentration (a) and glucose consumption (b) in batch lactate fermentation

From Figure 5(a), the lactate concentration has shown an increment from 0 hour until 24 hours fermentation. During 12 hours fermentation time, lactate concentration increased from 1.37 g/L to 20.09 g/L for 0 hour and 12 hours fermentation respectively, an increment about 93.2%. The highest lactate concentration in batch fermentation was obtained at 24 hours of fermentation with the value 33 g/L and the turbidity is 0.093. The increment of lactate concentration from 0 to 24 hours of fermentation was 95.85%. From Figure 5(b), the starting glucose concentration was 60 g/L (55.93 g/L upon sampling at 0 hour) and is reduced to 26.74 g/L or 52.2 % after 12 hours of fermentation. The residual glucose is declined to 10.83 g/L during 24 hours fermentation, a consumption of 45.1 g/L or 80.64 %.

4.1.3 Overall result

The overall result for batch fermentation is shown in Figure 6.

![Figure 6: The overall results for batch lactate fermentation](image)
Tabulated data for batch fermentation is shown in Table 1 (Please refer to Appendix D)

4.2 Continuous Lactate Fermentation

The continuous lactate fermentation was performed with cell recycling (performed at 16 hours of fermentation) and the flow rate of media 50 ml/hour using the same parameters as in batch lactate fermentation.

4.2.1 The turbidity graph and Dry cell weight of *Lactococcus lactis* IO-1

The turbidity and Dry cell weight of *Lactococcus lactis* IO-1 for continuous lactate fermentation is shown in Figure 7

![Graph](image)

(a) Turbidity (b) Dry cell weight

**Figure 7:** The turbidity (a) and Dry cell weight of *Lactococcus lactis* IO-1 (b) for continuous lactate fermentation

From Figure 7(a), the turbidity in continuous lactate fermentation during 24 hours of fermentation was 0.098, higher 0.005 or 2.37% than to the turbidity in batch fermentation. The turbidity shows higher increment at 36 hours of fermentation (96% increment) at 0.15. The turbidity kept on increased until it reached the highest value during 48 hours fermentation with the value 0.28. The increment from 0 hour to 48 hours of continuous fermentation was 97.86%.

From Figure 7(b), the biomass concentration during 24 hours of continuous fermentation was 3.44 g/L, 1.09 g/L or 31.7% higher than in batch system. The highest biomass concentration was obtained in the 48 hours fermentation time with the value 13.9 g/L, an increment about 97.20% from 0 hour of fermentation.
4.2.2 Lactate concentration and Glucose consumption

The lactate concentration and residual glucose in continuous lactate fermentation is shown in Figure 8.

![Graphs showing lactate concentration and residual glucose over fermentation time.](image)

**Figure 8:** The lactate concentration (a) and residual glucose (b) in continuous lactate fermentation

According to Ishizakt et al. (2001), in continuous fermentation, a high cell concentration obtained through cell recycling enhances lactic acid production. The cell recycling that was performed at 16 hours of fermentation time resulted in an increased in biomass concentration and so to the lactate concentration. From Figure 8(a), the lactate concentration obtained during 24 hours of continuous fermentation was 38.75 g/L and turbidity is 0.098. The highest lactate concentration was obtained during 48 hours of fermentation time at 60.18 g/L, an increment about 98.15% from 0 hour of fermentation and the turbidity is 0.28. From Figure 8(b), the starting glucose concentration was 60 g/L (56.93 g/L upon sampling at 0 hour) and is reduced to 32.28 g/L, a consumption of 43.3 % after 24 hours fermentation. The glucose declined to 13.81 g/L during 48 hours of fermentation, a consumption of 43.12 g/L or 75.74%.
4.2.3 Overall result

Overall result for continuous lactate fermentation is shown in Figure 9.

![Figure 9: The overall result for continuous lactate fermentation](image)

Tabulated data for continuous lactate fermentation is shown in Table 2. (Please refer to Appendix D)
4.3 Overall result for all fermentation trials

Summary of tabulated data for all fermentation trials is shown in Table 3.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Batch lactate fermentation</th>
<th>Continuous lactate fermentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial glucose concentration (g/L)</td>
<td>55.93</td>
<td>56.93</td>
</tr>
<tr>
<td>Residual glucose concentration (g/L)</td>
<td>10.83 (24 hours)</td>
<td>32.28 (24 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.81 (48 hours)</td>
</tr>
<tr>
<td>Glucose consumption (g/L)</td>
<td>45.1</td>
<td>24.65 (24 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>43.12 (48 hours)</td>
</tr>
<tr>
<td>Glucose consumption efficiency (%)</td>
<td>80.64</td>
<td>43.3 (24 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75.74 (48 hours)</td>
</tr>
<tr>
<td>Lactate concentration (g/L)</td>
<td>1.39 (0 hour)</td>
<td>1.11 (0 hour)</td>
</tr>
<tr>
<td></td>
<td>33.0 (24 hours)</td>
<td>38.75 (24 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>60.18 (48 hours)</td>
</tr>
<tr>
<td>Dry cell weight (g/L)</td>
<td>0.67 (0 hour)</td>
<td>0.39 (0 hour)</td>
</tr>
<tr>
<td></td>
<td>2.35 (24 hours)</td>
<td>3.44 (24 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13.9 (48 hours)</td>
</tr>
<tr>
<td>Turbidometer reading</td>
<td>0.093 (24 hours)</td>
<td>0.098 (24 hours)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.28 (48 hours)</td>
</tr>
</tbody>
</table>

Table 3: Represents the summary of the result for batch and continuous lactate fermentation

Based on Table 3, the highest lactate production in batch fermentation was obtained after 24 hours fermentation with the value 33 g/L and the turbidity reading is 0.093, an increment about 95.85% from 0 hour of fermentation. The biomass concentration and glucose residual is 2.35 g/L and 10.83 g/L respectively. However, the biomass concentration at 24 hours fermentation is slightly lower than at 16 hours of fermentation. As reported, this may be due to the lack of nutrients or the accumulation of toxic material (Scragg, 1991) in broth due to accumulation of lactate (Jolhery, 2001). This was also may be due to contamination that occurred when sampling was performed. Later, the fermentation was initially operated in batch mode for about 16 hours switch to continuous mode together with cell recycling. The flow rate of fresh media used in this study was 50ml/hour. In continuous fermentation, a high cell concentration
obtained through cell recycling enhances lactic acid production (Ishizaki et al., 2000). The biomass concentration after 24 hours continuous fermentation was 3.44 g/L, 1.09 g/L or 31.7% higher than in batch fermentation, resulted in an increased in lactate production with the value 38.75 g/L. The glucose concentration decreased from 56.93 g/L to 32.28 g/L or 43.3% at 0 hour and 24 hours respectively. The turbidometer reading is 0.098. The highest lactate production was obtained after 48 hours fermentation with the value 60.18 g/L and the turbidometer reading is 0.28, an increment about 45.2% than lactate obtained in batch system. The glucose declined to 13.81 g/L during 48 hours of fermentation, a consumption of 43.12 g/L or 75.74%. The biomass concentration increased to 13.9 g/L at 48 hours fermentation.

This study has shown the possibility of utilizing the Turbidostat System in lactate production where it is important as an indicator to measure the cell growth in lactate fermentation without the need to do DCW analysis.
5.0 CONCLUSION
This study confirms the possibility of utilization of Turbidostat System in lactate production for the measurements of biomass directly without the need to do the Dry Cell Weight (DCW) analysis. In batch fermentation, the highest lactate concentration, 33.0 g/L, was obtained at turbidity 0.093 during 24 hours of fermentation. While during continuous fermentation with flow rate of media 50 ml/hour and cell recycling, the highest lactate production, 60.18 g/L, was achieved at turbidity 0.28 during 48 hours of fermentation, an increment about 45.2 % than in batch system. In future work, higher flow rates of media can be used to obtain more lactate production.

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