KINETIC STUDY OF THE UTILISATION OF DIFFERENT SUBSTRATES TO LACTIC ACID USING *Lactobacillus delbrueckii*

Abdullah

Department of Chemical Engineering, Faculty of Engineering,
Diponegoro University, Semarang. Telp: 7460058, e-mail: abd_busairi yahoo. com

Hanapi bin Mat[*)] and Widayat[**)]
[*)]Department of Chemical Engineering, Faculty of Engineering and Natural Resouerces,
Universiti Teknologi Malaysia 81310 UTM Skudai, Johor
[**)]Department of Chemical Engineering, Faculty of Engineering,
Diponegoro University, Semarang.

Abstract

Lactic acid fermentation includes several reactions in association with the microorganism growth. A kinetic study was performed of the utilisation of multiple substrates to lactic acid using lactobacillus delbrueckii. Batch fermentation was performed to study effect of different substrates such as glucose, fructose and sucrose. The objective of this research is to study kinetics grow microbial. A anaerobic fermentation were studied in 3 litres stirred fermentor (Biostat B Model) with working volume of 1 liter, temperature = 40°C, pH = 6.0, inoculums size = 5%, sugar concentration = 20 g/l. During the first hours of fermentation, glucose and fructose accumulated in the medium and the rate of hydrolysis of sucrose to glucose and fructose was faster than conversion of these substrate. The maximum concentration of glucose and fructose was 5.82 and 5.14 g/l respectively. The sucrose, glucose, and fructose consumption completely utilized at 56, 68, and 104 hours, respectively. Kinetic parameter for maximum specific growth rate in glucose, fructose and sucrose is 0.103, 0.0448, and 0.0398 (h^{-1}), respectively. The saturation constant is 2.13, 2.67, and 3.32 g/l.

Key Words: Kinetic Study, Sugars, Lactic Acid, Maximum Specific Growth Rate and Saturation Constant

Introduction

The methods for the preparation of lactic acid are divided in two groups, biochemical and chemical process. Commercial production has until recently only been performed by lactic acid fermentation, but some chemical method's have also been discussed for the manufactured of lactic acid (Holten, 1971). Lactic acid is generally produced from glucose, maltose, sucrose, or lactose. Starches, especially those from corn and potatoes, are hydrolysed by enzymes or by acid to maltose and glucose before the lactic acid fermentation (Atkinson and Mavituna, 1991). Sucrose from cane and beet sugar, whey containing lactose and maltose, and dextrose from hydrolysed starch are presently used commercially (Vickroy, 1983).

Starch or sugar containing substances can be used as raw material. Starch raw materials have to be degraded first enzymatically or by means of acid, because the *lactobacilli* do not have amylolytic. This again means a higher cost for addition step which also brought in impurities (Buchta, 1983). In general, lactic acid bacteria utilise common carbon sources such as glucose, fructose, lactose, maltose, and sucrose for growth and lactic acid production. Starch can not be utilised but there are several reports that certain members of lactic acid bacteria can use liquefied starch. *Lactobacillus delbrueckii* is preferred organism to production lactic acid using glucose, fructose, and sucrose but lactose can not be utilised (Atkinson and Mavituna, 1991).

Two different types of lactic acid fermentation from carbohydrates are known to be homolactic fermentation and heterolactic fermentation. Pure lactic or homolactic fermentation is essentially performed by the homolactic *lactobacteriaceae.* The *lactobacilli* has the enzyme aldolase and lack of the enzyme phosphoketolase, by glycolytic pathway (Embden-Meyerhof pathway) more than 85% glucose convert to lactic acid (Freeman, 1985). Many *lactobaciili* species produce levans sucrase in response to growth on sucrose. Depending on the enzymes present in a given strain, the disaccharide sucrose (α-glucose-1,2-β-fructose) can be cleaved by two alternatives namely hydrolysis by enzymes invertase or levansucrase yield glucose and fructose (Zubay, 1984; Moat, 1985).

The knowledge of the kinetics of fermentation is necessary in order to size the fermentor and its associated equipment, and this information is normally obtained from laboratory experiment using one to three litres fermentor (Russel, 1987).

Equation 1 Malthus' law (De Man, et al, 1986), which in the form of gives one of the simplest models belonging to the general form

$$\frac{dX}{dt} = \mu.X \qquad (1)$$

In batch fermentation, the specific growth rate is constant and independent of the changing of the nutrient concentration. It is to be expected that growth rate, as any chemical reaction rate, will depend on the concentration of chemical nutrients. Monod-Type relationship is usually expressed the specific growth rate, μ is usually expressed as a function of the limiting substrate concentration (S),

$$\mu = \mu_m \left[\frac{S}{K_s + S} \right] \qquad (2)$$

The Monod equation only applies to the growth phase and in the present case production of lactic acid diverts substrate

and inhibition restricts growth. Therefore, eqn (1) must be extended to include the lactic acid concentration P, i.e.

$$dP/dt = Y_{P/X}\, dX/dt \qquad (3)$$

In many fermentations, especially those involving secondary metabolites, significant product formation does not occur until relatively late in batch cultivation, perhaps approaching or into the stationary phase. Occasionally, a simple 'non growth-associated' model suffices for product formation kinetic in such cases. In these models, production rate is proportional to biomass concentration rather than growth rate (Monteagudo, et.al, 1997)

In this research work, the kinetic study of the utilisation of glucose, fructose and sucrose to lactic acid using *lactobacillus delbrueckii* is studied.

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Materials and Methods

Chemical

The sugars used were analytical grade and supplied by Merck, BDH and Fluka. All chemicals used were analytical grade and used as received.

Strain

The micro-organism used in this study was *Lactobacillus delbrueckii subsp. delbrueckii ATCC 9649* obtained from DSMZ, Germany.

Inoculum Media

The preparation of Inoculum media starts with transferring of the lyophilised culture (Freeze Dried) to a liquid MRS medium. The culture was transferred to solid MRS medium after a visible growth of microorganism was observed which normally takes one day (Mercier *et al.*, 1992; Sakamoto and Komagata, 1996).

Batch Fermentation

The fermentation was carried out in 3-litre fermentor (Biostat B Model). The fermentor was equipped with pH, temperature and dissolved oxygen controllers. The fermentor containing 950 ml substrate was first sterilised at 121°C for 15 minutes. 50 ml of Inoculums was sterilised separately and added aseptically to the fermentor. Anaerobic system were produced by sparging the fermentor by nitrogen 6.5 ml/minute and speed at 50 rpm (Lund *et al.*, 1992). Samples of 10-20ml were withdrawn from the fermentation broth at regular time interval. The microbial cells were separated by centrifugation for dry biomass determination. The supernatant was immediately frozen for further determination of the lactic acid, glucose, fructose and sucrose concentrations (Mercier *et al.*, 1992).

Dry Cell Weight

Cell concentration was measured by constructing a calibration curve of optical Density as a function of dry cell weight. Dry weight was determined by centrifugation at 4000 rpm for 15 minute, washed twice with distilled water and dried at 103°C for 24 hours and weighied (Aeslichmann and Stockar, 1987). The optical density was measured on spectrophotometer UV-1601 at 620 nm.

Chemical Analysis

The Organic acid content was measured by HPLC (Waters TM 600). A 250 mm X 4.6 mm ID Spherisob Octyl column (Waters) with UV detector (210 nm) were used. The eluent used was 2 M phosphoric acid at flow rate of 0.8 ml per minute and temperature 25 °C. The sugar content was also measured by the same HPLC, using a 300 mm X 4 mm ID.μ Bondapak/ Carbohydrate column (Waters) with RI detector. The eluent used was a mixture acetonitrile : water (80:20) at flow rate of 2 ml per minute and temperature 25 °C.

Evaluation Methods

This research focused in kinetics of growth microbial. For the determination of the kinetic parameters such as μ_{max} and K_s as a functional of the initial concentration (S) and cell density (X) like eq. 2. The kinetics parameters were obtained from regression analysis. The equation 2 turned into;

$$\frac{1}{\mu} = \frac{1}{\mu_{max}} + \frac{K_s}{\mu_{max}} \frac{1}{S} \qquad (4)$$

Result and Discussion

In order to understand the fermentation characteristics of different carbon sources, three types of sugars such as glucose, fructose and sucrose were chosen. The results of the microbial growth, sugar utilisation and lactic acid production are given in Figures 1, 2, 3 and 4.

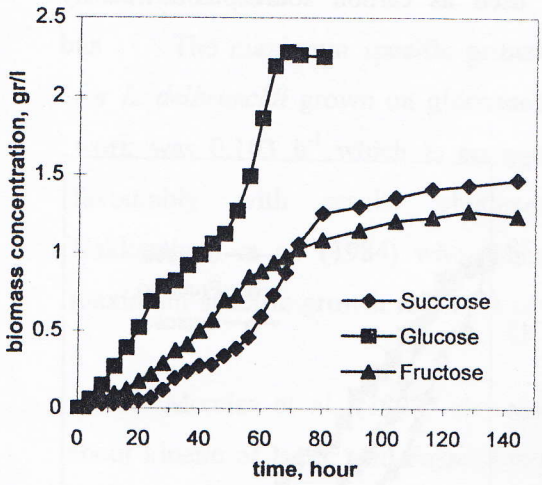

Figure 1. Time Dependence of Biomass Concentration during Lactic Acid Fermentation of Glucose, Fructose and Sucrose. Experimental Conditions: T, 40°C; pH, 6.0; Inoculum, 5%; and Stirring Speed, 50 rpm

Bacterial Growth

The profiles of dry cell weight concentration with fermentation time on several of sugar types are given in Figure 1.

The profiles of biomass concentration with time of fermentation show that the lag phases were up to 4 hours for all types of sugars, which are an adaptation period of bacteria to fermentation environment. The exponential growth phase of glucose medium is seen to last 68 hours followed by sucrose and fructose at 116 and 128 hours, respectively. After the exponential phase is stationary phase with the maximum biomass concentration achieved was at 2.28, 1.44 and 1.36 g dry cell weight/l, respectively. This indicates that the best growth of *L. delbrueckii* was obtained when the glucose was used as carbon source, followed by sucrose and fructose.

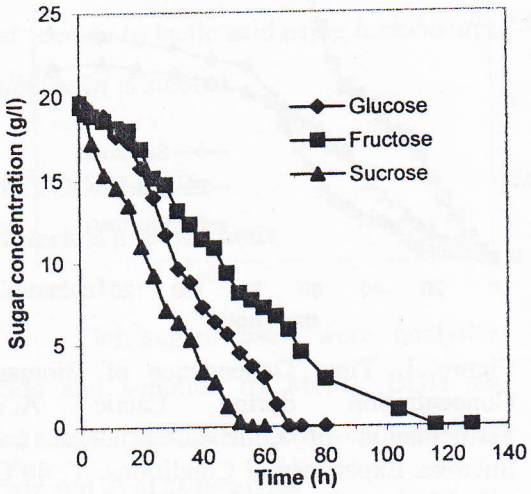

Figure 2: Time Course of Glucosa, Fructosa and Sucrosa Concentration during Lactic Acid Fermentation. Experimental Conditions: T, 40°C; pH, 6.0; Inoculum, 5%; and Stirring Speed, 50 rpm.

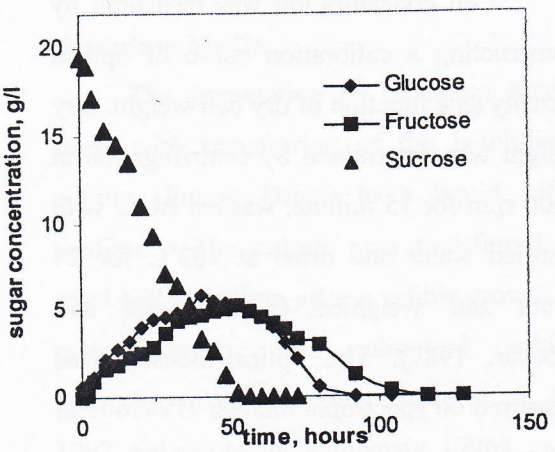

Figure 3: Time Course of Sugar Concentration during Lactic Acid Fermentation of Sucrose. Experimental Conditions: T, 40°C; pH, 6.0; Inoculum, 5%; and Stirring Speed, 50 rpm.

Sugar Utilisation

The glucose, fructose and sucrose concentrations used were 20 g/l. Figure 2 shows that the sucrose consumption completely utilised at 56 hours, followed by glucose and fructose at 68 and 104 hours, respectively. The consumption pattern of the sucrose during the first 8 hours of fermentation indicates that the glucose and fructose concentrations increased in the medium due to the rate of hydrolysis of sucrose to glucose and fructose was faster than the conversion of these substrate (Figure 3). The maximum concentration of glucose and fructose was 5.82 and 5. 14 g/l, respectively. The sucrose, glucose and fructose consumption on sucrose fermentation completely utilised at 56, 92 and 116 hours.

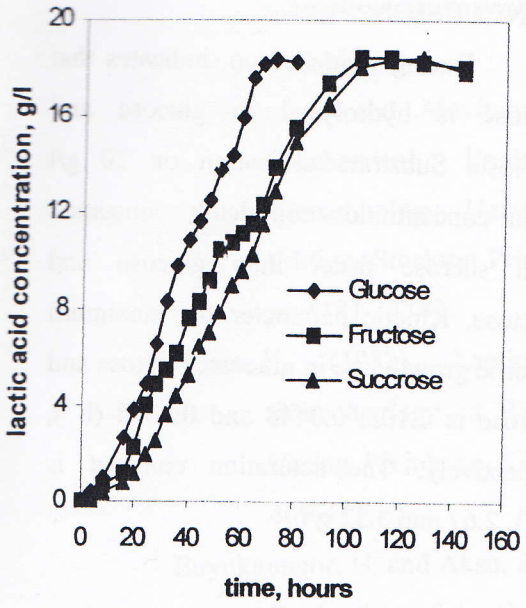

Figure 4: Time Course of Lactic Acid Concentration during Lactic Acid Fermentation of Single and Mixed Sugar. Experimental Conditions: T, 40°C; pH, 6.0; Inoculum, 5%; and Stirring Speed, 50 rpm. Lactic Acid Production

Effect of the sugar typxes used on the lactic acid production is given in Figure 4. The maximum concentration of lactic acid obtained for glucose medium was 18.25 g/l or 92 % of yield at 68 hours. The maximum concentration of lactic acid (yield) for fructose and sucrose obtained were , 18.3 g/l (93 %) and 18.22 g/l (92%) at 104 and 116 hours, respectively. Although the maximum lactic acid concentration were produced almost similar but the productivity was different. The maximum lactic acid productivity for glucose, fructose and sucrose were 0.27, 0.19 and 0.17 g /l.h, respectively.

Table 1. The Parameters Kinetics Lactic Acid Fermentation for Different Types of Sugar

Type of Sugars	K_s (g/l)	μ_{max} (h^{-1})
Glucose	2.13	0.103
Fructose	2.67	0.0448
Sucrose	3.32	0.0398

Evaluation Monod Parameters

The kinetic parameters such as μ_{max} and K_s were obtained from regression analysis of Eqs 1 and 2, and the results are shown in Table 1.

The maximum specific growth rate for *L. delbrueckii* grown on glucose in this work was 0.103 h^{-1} which is comparative favourably with results obtained by Hakkarainen et al. (1984) who found the maximum specific growth rate of 0.150 h^{-1}.

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Mercier et al. (1992) also studied about kinetic of lactic acid fermentation on glucose by *L. amylophilus* which they obtained the maximum specific growth rate of 0.29 h^{-1} at pH, 6; temperature, 30 °C; and stirring speed, 350 rpm. But Tyree et al. (1990) obtained higher maximum specific growth rate than other authors which is 0.722 h^{-1} using *L. xylosus* with operation conditions of pH: 6.0, temperature: 30 °C and stirring speed, 150 rpm. The different results might be due to difference of

operation conditions and types of strain used.

The saturation constant (K_s) of glucose fermentation are lower than those of fructose and sucrose and the the comparison of the saturation constant (K_s) on glucose and sucrose utilisation in lactic acid fermentation by the different authors were given in Table 2 and 3.

Table 2. Comparison of the Saturation Constant (K_s) on Glucose Utilisation in Lactic Acid Fermentation.

Strain	pH	T (°C)	Speed (Rpm)	K_s
L. delbrueckii	6.00	40.0	50	2.31
L. delbrueckii[*)]	6.20	44.0	400	10.50

*) : Buyukgungor and Aksu. (1984)

Table 3. Comparison of The Saturation Constant (K_s) on Sucrose Utilisation in Lactac Acid Fermentation.

Strain	pH	T (°C)	Speed (Rpm)	K_s
L. delbrueckii	6.00	40.0	50	3.32
L. delbrueckii[*)]	6.00	49.0	800	4.47
L.bulgaricus [**)]	5.60	45.0	400	1.80

*) : Suscovic et al. (1992)

**) : Gadgil and Venkatesh (1997)

CONCLUSION

During fermentation, indicates that sucrose is hydrolysed to glucose and fructose. Substrate utilisation on 20 g/l sugar concentration completely consumed with sucrose faster than glucose and fructose. Kinetic parameter for maximum specific growth rate in glucose, fructose and sucrose is 0.103, 0.0448 and 0.0398 (h^{-1}), respectively. The saturation constant is 2.13, 2.67 and 3.32 g/l.

Notations

α

β

μ_{max} = Maximum specific growth rat, h^{-1}

μ = Specific growth rate, $hour^{-1}$

$\frac{dX}{dt}$ = Microbial growth rate, g l^{-1} h^{-1}

K_s = Saturation constant, g/l

P = Lactic acid concentration, g/l

S = Substrate concentration, g/l

t = Time, h

X = Biomass concentration, g/l

$Y_{p/x}$ = Product yiel on the formed biomass, g P/g X

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