Studies on Production of Lactic Acid Using Fermentation Route and Ascertaining its Process Variables

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***Abstract*–Lactic acid plays a vital role in several biochemical processes. In this present age of development of food and biotechnological products being the state of the art the application of lactic acid is ever increasing. The present investigation aims at the use of sugar-cane juice as an alternative carbon source for production of lactic acid. The mode of production of Lactic Acid by fermentation route was studied in a laboratory scale glass bio-reactor following the methodology of few published literatures. The optimization of process parameters like temperature and reaction time were done, the optimized range being 42-43C and 5-6 days for reasonable conversion. The deterministic process model was attempted using Monod equation, the kinetic parameters were evaluated as K_s = 0.36 gm/100 ml and μ_{max} =3.16 (1/hr) at aerobic condition. The model was tested with experimental backup: the goodness of fit was found to be in the regime of 0.85 to 0.95.**

Keywords: lactic acid production, lactic acid process modeling, fermentation route.

I. INTRODUCTION

Lactic Acid plays a vital role in several biochemical processes. It has number of applications as polymer precursor, emulsifying agent, acidulate and preserving food items. In this present age of development of food and biotechnological products being the state of the art the application of lactic acid is ever increasing.

Most commonly and commercially used process for production of Lactic Acid is hydrolysis of lactonitrile derived from acetaldehyde and hydrocyanide which are produced by petrochemical processes. But the manufacturing cost of such process is very high and several effluents released during this process affect the surrounding.

An alternative process is carbohydrate fermentation through which stereo specific lactic acid can be obtained depending on the strain being used. ***Lactobacillus sp.*** Constitutes the major part of lactic acid bacteria group because of their ability to convert lactose and other sugars to lactic acid.

Over the years, authors have suggested a large number of carbohydrates and nitrogenous materials for production of lactic acid on the basis of high lactic acid yields, optimum biomass production, fast fermentation rate, less pretreatment, low cost, ease of availability. In this regard molasses, bagasses are studied as cheap source of raw material. This is presented in the foregoing section.

II. LITERATURE REVIEW

Conversion of food waste into Lactic Acid is an important route. Kim et al. (2003) investigated the conversion of food wastes into lactic acid by simultaneous saccharification and fermentation (SSF) by lactobacillus delbreukii. The highest observed overall theoretical yield of lactic acid in the SSF was 91%.

Milind Patel (2006) reported Fermentation of sugar cane bagasse hemicelluloses hydrolysate to L(+)- lactic acid by thermo tolerant acidophilic Bacillus sp. Sugar cane bagasse hemicelluloses, hydrolyzed by dilute H_2SO_4, supplemented with mineral salts and 0.5% corn steep liquor, was fermented by the researchers to L(+)-lactic acid using a newly isolated strain of Bacillus sp. in batch fermenter. In batch fermentation at 50 ºC and pH 5 , over 5.5% (w/v) L(+)-lactic acid was produced (89% theoretical yield; 0.9 g lactate per g sugar) with an optical purity of 99.5%.

In the same way Laopaiboon et. al (2010) investigated the optimum conditions for acid hydrolysis of the bagasses for lactic acid production. The products of fermentation obtained by the researchers were lactic acid, 36.16%, acetic acid, 26.23%, formic acid 20.13% and ethanol 17.48%. However, enrichment of Lactic Acid percentages was yet to be investigated in this sequence. This was supplemented by Sikder et. al (2012) by using membrane-integrated bioreactor system consisting of sterilization, fermentation, microfiltration, nano filtration and final concentration to 95% Lactic Acid by vacuum evaporation.

Sakdaronnarong et. al (2014) developed a methodology for production of polylactic acid precursor. On careful analysis of all such investigations revealed that Production Lactic Acid using fermentation route was quite feasible. Depending upon the needs of the present day there is ample scope of studying further considering the modeling and simulation aspect of the process. The evolution of mechanistic models with adequate experimental back up is also required to be done.

III. AIMS & OBJECTIVES

The present investigation is therefore directed towards:

1. To stabilize the methodology of using sugarcane juice for lactic acid production using fermentation route.
2. To find out the optimum temperature for growth of Lactobacillus acidophilus on the substrate (sugar cane juice).
3. To determine the optimum time for fermentation.
4. To develop mathematical models for formation of lactic acid and to establish parameters associated with the process. The detailing of such process parameters would include maximum growth rate, rate constant.

IV. MATERIALS AND METHODOLOGY

A. *Micro-organism used:*

Capsules of ***Lactobacillus*** **acidophilus (**Lactobacillus caps.). Each capsule contains 10 million bacteria cells.

B. *Fermentation medium:*

The fermentation medium for each of the experimental setups consists of 10g yeast extract, 5gm peptone, 0.12g of $MgSO_4.7H_2O$, 0.005g of $MnSO_4.4H_2O$, 0.9g Sodium Acetate, 1.8g K_2HPO_4. The sugarcane juice was obtained from a vendor. This is charged in a 1.5 liter bioreactor with stirring arrangement by magnetic stirrer. The temperature is controlled using a digital incubator.

C. *Method:*

The procedure adopted to estimate the amount of Biomass concentration (by dry weight method) is as follows:

1. In this method, 1ml broth, enriched with bacterial biomass are centrifuged at 1000 rpm, 4^0C for 15 minutes individually.
2. The separated bacterial biomass are washed with phosphate buffer (pH 7.0) solution.
3. The washed biomass is transferred into a pre-weighed aluminum cup individually and is dried at 80^0C for 24 hours and the exact weight of biomass is then measured.

The procedure adopted to estimate the amount of lactic acid is as follows:

1. 50 ml of the aliquot was isolated and centrifuged.
2. The clear liquid was then decanted and fused $CaCl_2$ was added to it.
3. The weight of the calcium lactate precipitate was then measured.

D. *Theoretical Analysis*

Simulation has been carried out using following assumption:

1. Microorganisms follow the same intrinsic growth kinetics in Erlenmeyer flasks.

2. Liquid volume in the reactor is kept constant.

The material balance equations for biomass (C) in an unsteady state Bioreactor are as follows:

$$V\frac{dC}{dt} = F_{C_{in}} C_{in} - F_{C_{out}} C + \mu CV \quad (1)$$

or, $$\frac{dC}{dt} = \frac{F_{C_{in}}}{V} C_{in} - \frac{F_{C_{out}}}{V} C + \mu C \quad (2)$$

Where, $F_{C_{in}}$ = Flow of biomass entering into Bioreactor

$F_{C_{out}}$ = Flow of biomass leaving from Bioreactor

V = Volume available for reaction

As V remains constant through the reaction period

So, $$\frac{dV}{dt} = 0$$

So, $$F_{C_{in}} = F_{C_{out}} = F \quad (3)$$

By putting the value of equation 3 into equation 2, equation 4 will be

$$\frac{dC}{dt} = \frac{F}{V} C_{in} - \frac{F}{V} C + \mu C$$

or, $$\frac{dC}{dt} = DC_{in} - DC + \mu C \quad (4)$$

Where, $D = \frac{F}{V}$ = Dilution rate. Dilution rate is the reciprocal of time.

In most of the system the entering biomass concentration is zero, i.e. C_{in}=0. Then the equation 4 will be as follows:

$$\frac{dC}{dt} = -DC + \mu C$$

$$\frac{dC}{dt} = (\mu - D)C \qquad (5)$$

As the reaction took place in very dilute solution the Monod Equation was assumed to be valid. The specific growth rate of biomass (μ) is given by

$$\mu = \frac{\mu_{max} S}{K_S + S} \qquad (6)$$

By putting the value of μ into equation 5,

$$\frac{dC}{dt} = \left[\frac{\mu_{max} S}{K_S + S} - D\right]C \qquad (7)$$

At steady state:

$$\frac{dC}{dt} = 0$$

Then the equation 4 will be

$$DC_{in} - DC + \mu C = 0$$

or, $(\mu - D)C = DC_{in}$

or, $C = \frac{DC_{in}}{(D - \mu)}$ (8)

Substituting the value of μ from equation 6 into equation 8, the expression will be

$$C = \frac{DC_{in}}{D - \frac{\mu_{max} S}{K_S + S}} \qquad (9)$$

At steady state equation 5 will be

$(\mu - D)\, C = 0$

As, $C \neq 0$

So, $\mu - D = 0$

Or, $\mu = D$ (10)

V. RESULTS AND DISCUSSION

The progress curve for biomass concentration with respect to temperature was obtained following the methodology discussed. It is shown in Figure 1. It was evident from the curve that biomass growth is maximum at 42-43ºC which is 0.1(gm/ml).

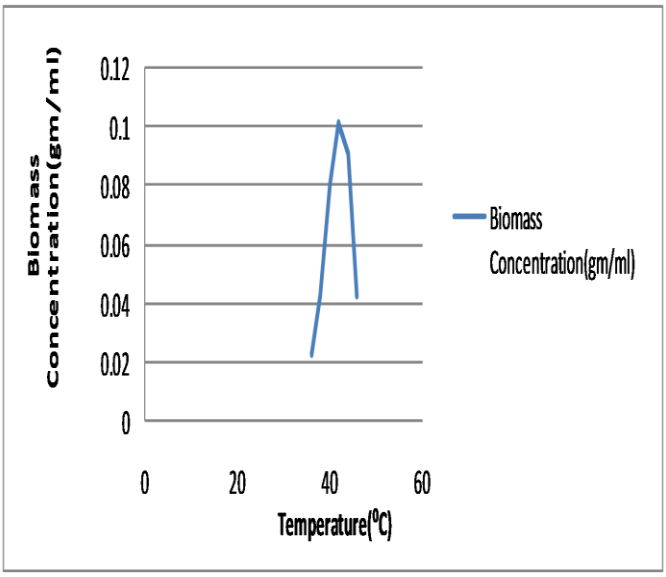

Fig 1. The progress curve for biomass concentration (g/ml) with respect to temperature(ºC)

At this optimized temperature level the biomass growth was studied and this is represented in Fig 2 as a rising curve.

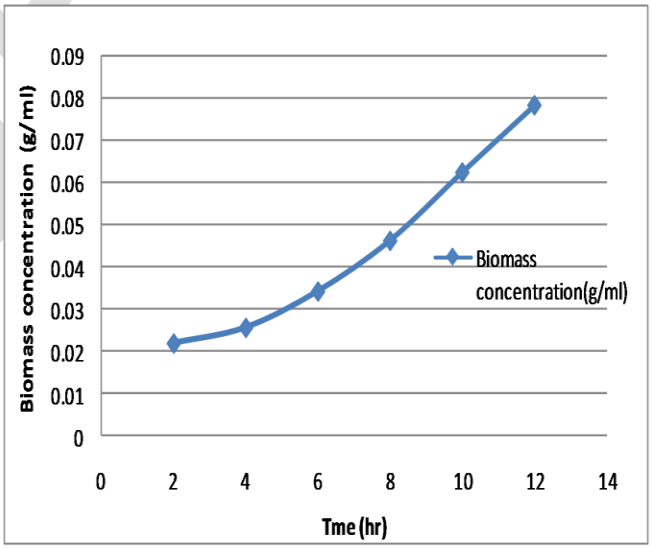

Fig 2. The progress curve for biomass concentration (g/ml) with respect to time(hr)

The progress curve showing the extent of lactic acid formation measured in terms of calcium lactate precipitated by weight versus Time(days) was obtained as shown in Figure 3.

The experimental results are shown in Table 1. It was evident that the optimum number of days for fermentation were around 5-6 days.

Table 1: Experimental results

Time (days)	Weight of calcium lactate Ppt. (gm)	pH
1	0.18	6.7
2	0.3	6.6
3	0.38	6.2
4	0.42	5.6
5	0.45	5.2
6	0.45	5.2
7	0.46	5.1

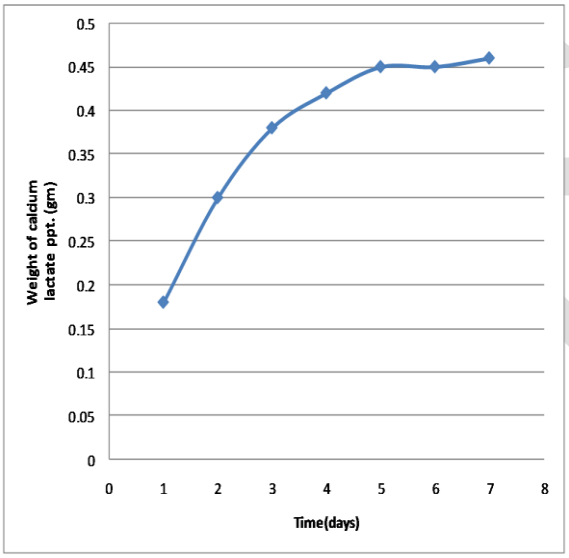

Fig 3. The progress curve showing the extent of lactic acid formation measured in terms of calcium lactate precipitated by weight versus Time(days).

The kinetics parameters μ_{max} and Ks were obtained by plotting a standard curve (Line weaver-Burk plot, Eadie-Hofstee plot, Hanes-Woolf plot). The values of maximum specific growth rate (μ_{max}), Monod constant (K_s) were 3.16 (1/hr), 0.36 gm/100 ml respectively at aerobic condition.

The differential equations were solved numerically using standard packages. These were further tested by experimental back up. The adequacy of the model were justified using higher (range varied from 0.85– 0.95) regime of correlation coefficient

VI. CONCLUSION

The details of production of lactic acid by fermentation route were studied using Sugar cane as raw material. The optimized parameters e.g. optimum temperature for the growth of Lactobacillus acidophilus, optimum number of days for fermentation, maximum specific growth rate, μ_{max} and saturation constant, K_s were also obtained. The model equations were solved numerically tested using experimental backup with higher values of correlation coefficient.

The mechanistic parameters like Dilution factor and working volume of the reactor would be obtained in future investigation to approach the scale up concepts of the particular type of bioreactor needed to carry out the process as wanted by the industries.

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