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A STUDY ON OPTIMIZATION OF LACTIC ACID PRODUCTION FROM WHEY BY *LACTOBACILLUS SP* ISOLATED FORM CURD SAMPLE

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ABSTRACT: The aim of this research was to use whey as a substrate for lactic fermentation by using isolated *Lactobacillus* from curd sample. The effect of different process parameters such as pH of the medium, temperature, inoculums size, incubation time and shaking speed were optimized to enhance the conversion of whey sugar into lactic acid. The optimum condition was found for fermentation with the process conditions of pH 6.5, temperature 37°C and inoculum size 4% (v/v) with an incubation of 120 h and effective rotation speed of 150. The above mentioned optimized process parameters can be used in large scale production of lactic acid fermentation in further investigations by using whey as a substrate .

KEYWORDS: Fermentation, whey, optimum conditions, shaking speed, inoculum size.

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1.INTRODUCTION

Lactic acid is produced by humans, animals, plants and microorganisms [1-4].Lactic acid is an organic acid which has plenty of industrial applications. The most important application of it as a food preservative and acidulate in foods [5-9]. Lactic acid also be used as a prosthetic device and controlled delivery of drugs in pharmaceutical process. Biochemically lactic acid used as a precursor of polylactic acid and as moisture agents in cosmetics [10-12]. Lactic acid is produced by both chemical synthesis process as well as by microbial fermentation process. Only disadvantage of chemical synthesis method is formation of a racemic (DL) mixture. Major advantage of microbial

fermentation method is either L(+) or D(-) lactic acids can be produced which is depending on the type of fermentative microorganism, whether they possess homofermentative or heterofermentative reactivity. Whey is a byproduct of the dairy industry, which serves as an inexpensive medium for lactic acid fermentation. It contains approximately (w/v) 5 % lactose, 1 % protein, 0.4 % fat, and some minerals. Certain types of microorganisms such as *Lactobacillus spp*. [13-14] and *Rhizopus spp.* [15] are capable of producing lactic acid in high concentrations. *Rhizopus spp*., especially *R. oryzea*, produce L(+)-lactic acid from glucose , starch [16] and molasses, in the presence of $CaCO_3$ .The lactic acid bacteria usually metabolize glucose via glycolysis, since glycolysis results only in lactic acid as a major end product of glucose metabolism and that is why only the fermentative lactic acid bacteria are used for the industrial production of lactic acid [17]. The present research activity was carried out to investigate the potential capability of Lactic acid bacteria, isolated from Curd sample from Local dairy products vendors of Kalyani Dist. Nadia, West Bengal to ferments lactic acid using whey, supplemented with different components of media.

2. MATERIALS AND METHODS

Preparation of whey

Calcium lactate was added at boiling condition to coagulate proteins and that coagulated protein was separated from whey by using filtration.

Microorganisms

Potential Lactic acid producing bacteria from genus *Lactobacillus* were isolated on MRS medium from the Local dairy products vendors of Kalyani, Dist. Nadia, West Bengal.

Preparation of Starter Culture

The bacterial culture was grown in 50 mL of MRS medium in 250 mL Erlenmeyer flask. After sterilization, the medium was inoculated with a loopful of culture from agar slant and incubated at 37°C for 24 h under stationary conditions. Further after making successive pure culture from grown colonies, actual fermentation process continued [18].

Fermentation medium

Whey was sterilized at 121°C for 15 minute autoclave. Media supplemented along with sterilized whey, yeast extract (0.75%, w/v), manganese sulphate (20 mg/L), and calcium carbonate (1.5%, w/v) [19]. This medium was utilized for the lactic acid fermentation by using isolated strain.

Optimization of process parameters for lactic acid Production

Various influencing parameters such as varying pH, inoculum size, temperature, carbon sources at different concentrations, composition of fermentation medium, incubation period and influence of shaking were optimized to accelerate lactic acid fermentation by isolated strain.

Lactic acid estimation

Lactic acid was estimated by modified Barker and Summerson (1941) method [11]. At first, lactic acid was oxidized with strong sulphuric acid solution into acetaldehyde and then it was coupled with

p-hydroxydiphenyl in the presence of cupric ions to yield a purple compound complex. At 560 nm the absorbance of purple compound was measured using spectrophotometer.

Effect of pH on fermentation

The fermentation medium was adjusted to different pH (4.0, 5.0, 6.0, 6.5 and 8.0, 9.0) for optimizing and kept in shaker incubator at 37°C with rotating speed of 150 revolutions per minute. Lactic acid production was checked after 24 h. The optimized pH was maintained for further study.

Effect of Temperature on fermentation

The optimum pH was maintained at five different temperatures (20, 25, 30, 37, 45 and 50°C) by keeping them with rotating speed of 150 revolutions per minute and the lactic acid production was estimated after 24 h.

Effect of inoculum size on fermentation

To study the influence of inoculum concentration on the lactic acid fermentation, different inoculum concentration (1-5%, v/v) were added sequentially to the fermentation medium and the lactic acid production was measured after 24 h.

Effect of Incubation Period on fermentation

To find out the optimal time required for incubation to get maximal lactic acid production, the fermentative medium inoculated with bacterial culture was incubated for 24h, 48h, 72h, 96h, 120h, 144h and 168h respectively under the found above optimized conditions. At the end of each incubation period, lactic acid produced was estimated.

Effect of rotation speed on fermentation

To study the influence of rotation speed on the lactic acid fermentation, different rpm at (50 to 200) were set sequentially to the fermentation process respectively under the found above optimized conditions and the lactic acid production was measured after 24 h.

3. RESULTS AND DISCUSSION

Effect of pH

The effect of pH on lactic acid production was estimated by using fermentation medium having a pH range of 4.0 -9.0 (Figure 1). The maximum lactic acid production (42.8 gm/L) was obtained at pH 6.5 on 24h of incubation. From pH 4.0 to 6.5 drastically increase the fermentative product, whereas after optimum pH 6.5, the lactic acid production sharply decreased. Krischke *et al*., repotted that by using *L. casei* strain a pH range of 6.0-6.5 has been optimal for lactic acid production, which is supported to our obtained result [20]. However, Ha *et al*., (2013) suggested pH 5.5 has been optimum for lactic acid production by using the strain *L. helveticus* [12]. Similarly, Hofvendahl *et al*., (2000) reported that almost 95 % lactose conversion (w/v) corresponding to 33.48 gm/L lactic acid production was suitable at pH 6.5 [13]. All the above findings, concluding that a pH 6.5 would be the optimal for maximum lactic acid production.

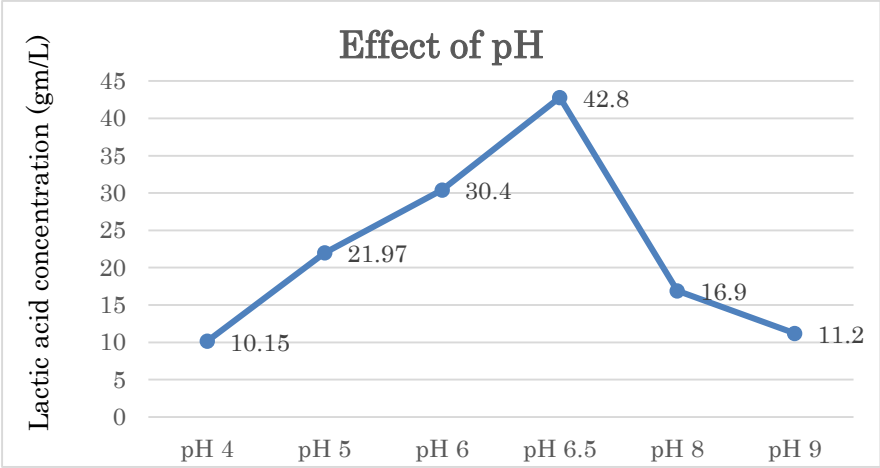

Figure 1: Effect of pH on lactic acid production

Effect of temperature

To find the optimum temperature for lactic acid production, after adding whey into medium, inoculation was incubated at a temperature range of 25-50°C(Figure 2). The lactic acid production increased sharply with increase in the temperature from 25°C up to 37°C; and maximum production was found at 37°C(43.6 mg/L)however, an decrease in at 45°C (34.2 gm/L) and much lower of lactic acid production was found at 50°C (20.2gm/L). The temperature is also one of the important factors, which influences the activity of metabolic cell enzymes and every enzyme are most active at optimum temperature. In optimum temperature enzymatic reaction shows maximum reaction velocity. However, below and above the optimal temperature, reaction rate is slow down, which may effect on the cellular metabolism process. According to Ha *et al.*, (2013) the optimal temperature for growth of lactic acid bacteria varies between the 20 to 45°C and obviously it varies on species to species [12,21-23]. Krischke *et al.*, (1991) used 37°C temperature for lactic acid production using *L. casei* [20]. Ilmen *et al.*, (2007) reported maximum lactic acid production of 33.72 gm/L at 37°C by *L. casei* [18]. From the above observations, it is cleared that a temperature range of 37-40°C was considered optimal for lactose conversion to lactic acid using bacterial cells.

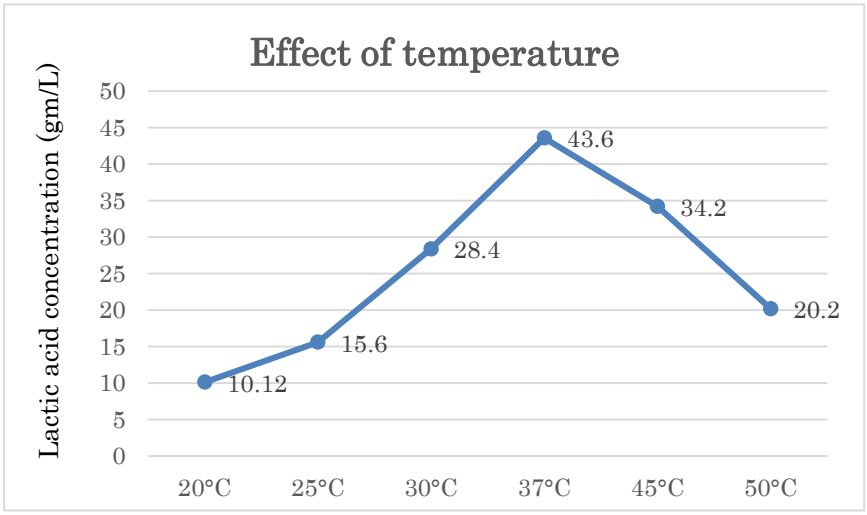

Figure 2: Effect of temperature on lactic acid production

Effect of Inoculum Size

To find out the influence of inoculum concentration on the lactic acid production, different inoculum levels (1-5%, v/v) were added to the fermentation medium (Figure 3). The lactose utilization and lactic acid production increased sharply with the rise in inoculum concentration up to 4% (v/v), thereafter no improvement in both the functions was observed by increasing the concentration of inoculum. The maximum lactic acid production of 43.4gm/L was observed with the adding of 4% (v/v) inoculums and later on production was lower down though increasing the inoculums concentration. At low density of starter culture (1%, v/v), the lowest lactic acid production was observed (9.6gm/L). The use of 2% (v/v) inoculum for the lactic acid production has been reported in earlier studies by Ha*et al*., (2003); and Gandhi *et al.*, (2000) [12,14,24-27]. Chiarini *et al*., (1992) reported 3%, v/v inoculum for lactic acid production[13]. Guha *et al*., (2013) confirmed maximum lactic acid production of 2.52 gm/L with 4% (v/v) inoculum of bacterial culture which is supporting to our obtained result [15,28]. From the above observations, an inoculum of 4% (v/v) could be considered optimal for achieving maximum lactic acid production using 24 h old bacterial culture and 4% (v/v) inoculum concentration was used in the subsequent studies.

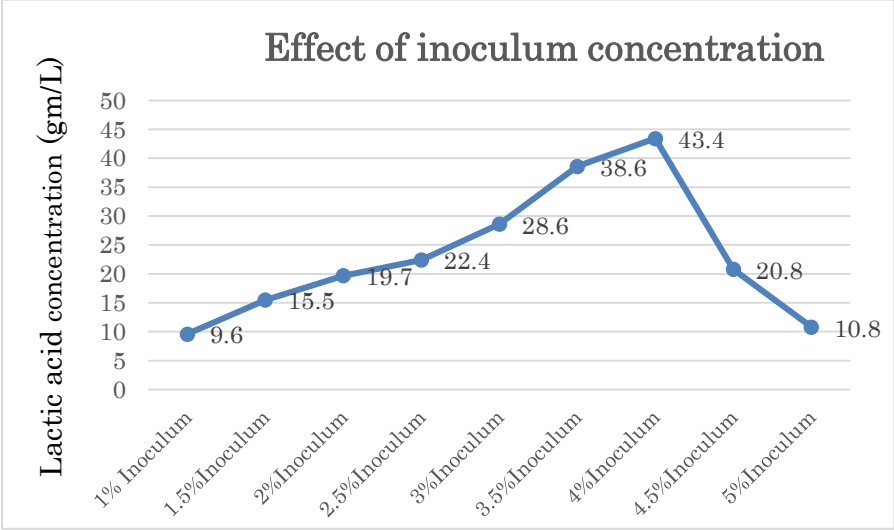

Figure 3: Effect of inoculum size on lactic acid production

Effect of Incubation Period

To find out the optimal incubation time for the maximal lactose utilization and lactic acid production, the whey medium inoculated with bacterial culture was incubated for different time at 24, 48, 72, 96, 120, 144 and 168 hour under the above optimized conditions. The samples were taken out at specified time intervals and the results obtained are presented in Figure. 4. As evident from the results, an increase in lactose utilization and subsequent lactic acid production was found increased till 120 h and thereafter sharply decrease both the activity was reported. This is due to the growth of the culture entered to the stationary phase and as a consequence of slow down the metabolic activity [16-17,29-31]. A maximum lactic acid production of 41.7 gm/L was observed after 120 h of incubation. Therefore, an incubation time of 120 h was considered optimal for lactic

acid production in our case. Many researcher reported that incubation period of 48 h has been generally used for lactic acid production using different lactobacilli cultures [18-19,32-35].Though the reduction in fermentation period is additionally advantageous to improve the economics of the process, according to our obtained result, still we used, an incubation time of 120 h as optimal for maximum lactose conversion to lactic acid.

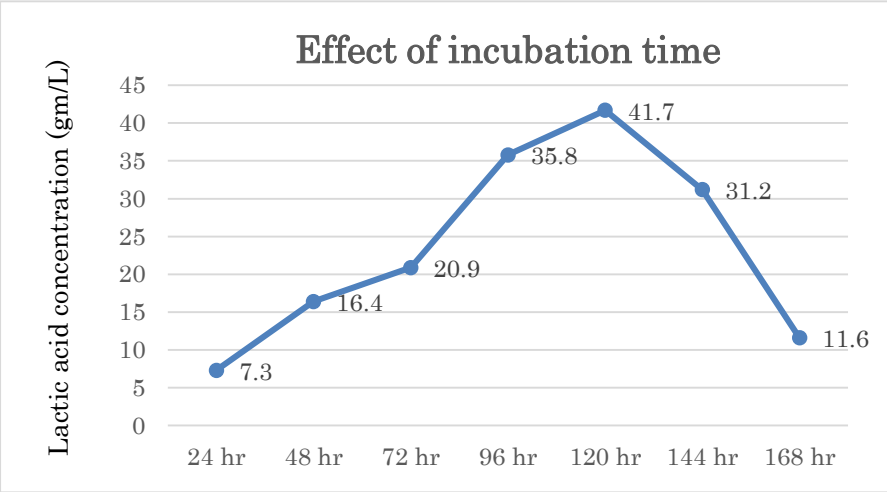

Figure 4: Effect of incubation time on lactic acid production

Effect of rotation speed

To find out optimal rotating speed in shaker incubator for the maximal lactose utilization and lactic acid production, the whey medium inoculated with bacterial culture was incubated for different rpm ranging from 50-200 (Figure 5). We found maximum production of lactic acid at 150 rpm when other parameter kept optimum. When rpm increase to 200, the quantity reduced [20,34-39]. So, it is proved that along with all other optimum parameter shaking speed also influence in lactose utilization and lactic acid production by *Lactobacillus sp.*

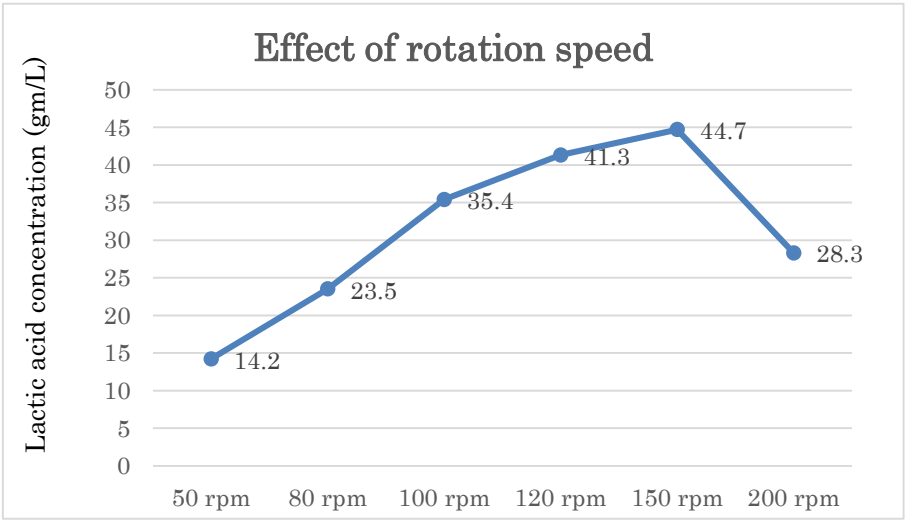

Figure 4: Effect of rotation speed on lactic acid production

4. CONCLUSION

In conclusion, Lactic acid is one of the most important chemical that can be derived from various waste products. Whey is the byproduct of milk, which can be used for lactic acid production. The data presented in this work supports the use of whey for valuable lactic acid production under presented optimized condition.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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