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Full Length Research Paper

Effect of different media on production of lactic acid from whey by *Lactobacillus bulgaricus*

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Whey containing 50 g.l $^{-1}$ lactose was fermented to lactic acid in batch process by *Lactobacillus bulgaricus.* The impact of 5 different media with change in volume percent of whey and nutrient was investigated at 32 ± 0.5°C. Substrate consumption and lactic acid production were determined at 0, 12, 24, 36, 48, 60 and 72 h. In these experiments, the volume percent of whey and nutrient was changed and highest lactic acid production (20.8 g/l) and rate of productivity (0.304 g/l.h) was achieved when the volume percent of whey was 80 (volume percent of nutrient diluted with water 20). The minimum rate of process was observed at the highest nutrient percent (50%), and at highest volume percent of whey in the medium (99%), inhibition occurred.

Key words: Lactic acid, whey, *Lactobacillus bulgaricus*, batch process, productivity.

INTRODUCTION

Production of cheese whey in the world is estimated to be over 10^8 tons per year. Cheese whey is an important source of environmental pollution since 10 L cheese whey is produced from 1 kg cheese with high carbohydrate, protein and lipid contents (Grba et al., 2002). Cheese whey is usually considered as a high strength wastewater from environmental point of view because of its high chemical oxygen demand (COD) content of nearly 80 g.l^{-1}. For this reason biological treatment of cheese whey by conventional activated sludge processes is very expensive. Production of valuable chemicals from cheese whey has been considered as an attractive option because of the rich nutrient contents. Cheese whey has been used as substrate for production of organic acid, ethanol, single cell protein and methane (Siso, 1996). Typical cheese whey contains 5 - 6% lactose, 0.8 – 1% protein and 0.06% fat constituting an inexpensive and nutritionally rich raw material, high production rate and high yield for lactic acid fermentation.

Lactic acid has long history of uses for fermentation and preservation of human foodstuffs. It was first discovered in sour milk by Scheele (1780), who initially considered it a milk component. In 1789, Lavoisier named this milk component “acide lactique” which became the possible origin of the current terminology, for the lactic acid. In 1857, however Pasteur discovered that it was not a milk component, but a fermentation metabolite generated by certain microorganisms (Davison et al., 1995; Beninga, 1990; Romani et al., 2008).

Lactic acid can be produced by either fermentative processes or chemical synthesis (VickRoy, 1985; Marques et al., 2008). In early 1960 a method to synthesize lactic acid chemically was developed due to the need for heat-stable lactic acid in the baking industry (Data et al., 1995). There are two optical isomers of lactic acid; L (+) lactic acid and D (-) lactic acid. Lactic acid is classified a GRAS (generally recognize as safe) for use as a food additive by the US FDA (Food and drug Administration) but D (-) lactic acid is at times harmful to human metabolism and can result in acidosis and decalcification (Hofvendahl and Hahn-Hagerdal, 2000). Although racemic DL- lactic acid is always produced by chemical synthesis from petrochemical resources, an optically pure L(+) or D(-) lactic acid can be obtained by microbial fermentation of renewable resources when

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Table 1. Composition of different media investigated for production of lactic acid by *Lactobacillus bulgaricus*.

Composition	Number of Medium
50% volume whey + 50% volume nutrient	1
66% volume whey + 34% volume nutrient	2
75% volume whey+ 25% volume nutrient	3
80% volume whey+ 20% volume nutrient	4
99% volume whey+ 1% volume nutrient	5

the appropriate microorganism that can produce only one of the isomer is selected (Bonomo et al., 2008). Lactic acid is a versatile chemical. Besides its traditional application in food and pharmaceutical industries, lactic acid is used in manufacture of polymers (such as poly lactic acid-PLA) suitable for variety of purpose including biodegradable plastics (Onda et al., 2008; Panesar et al., 2007). One of the most important substrate for lactic acid production is whey. In this research the production of lactic acid from whey in batch process was investigated. For this the volume percent of ultra filtered whey and nutrition diluted with water was changed.

MATERIALS AND METHODS

Microorganism and culture media

Lactobacillus bulgaricus (ATCC 8001, PTCC 1332) was supplied by the research department of Science and Industry. The microorganism was grown aerobically in MRS medium which containing 5 g/l yeast extract, 20 g/l glucose, 2 g/l $K2HPO_4$, 1 g/l peptone and 0.2 g/l $MgSO_4$ agitated (100 rpm) and added supplements were sterilized at 14.7 psi g and temperature of 121°C for 20 min. The pH and temperature of solution adjusted to 6.5 and 32°C, respectively. The batch experiment conducted in 5 flasks with 500 ml total volume (200 ml working volume). The percent of whey and nutrient diluted with distilled water was changed in batch fermentation to achieve the optimized amount of whey and nutrient. All chemicals and reagents used for the experiments were analytical graded and supplied by Merck (Germany).

Analytical method

Lactose concentration was determined by 3,5-dinytrisalicylic acid (DNS) method every 12 h. By adding 1 ml of DNS solution to 1 ml of sample and heating the sample in 10 min and after this adding 8 ml of water, and reading the absorbance at 540 nm in spectrophotometer (unico 2100, USA) and using calibration curve, the lactose concentration was achieved. The pH meter, HANA 211 (Romania) model glass-electrode was employed for measuring pH values in the aqueous phase. The initial pH of the working solutions was adjusted by addition of HNO_3 or NaOH solution. Lactic acid was measured by means of HPLC (column:shim-pack CLC-ODS : SPD 6A at 210 nm).

RESULTS AND DISCUSSION

In this experiment, ultra filter whey was used for production of lactic acid. The whey contains 50 g/l lactose, 0.6 g/l phosphate, 0.02 g/l calcium and 1 g/l chloride. Analysis shows the ultra filter whey had insufficient nutrients for growth of bacteria. In these experiments the whey and nutrient volume percent was changed to achieve best medium for the production of lactic acid. Table 1 shows the different media used for the batch experiments.

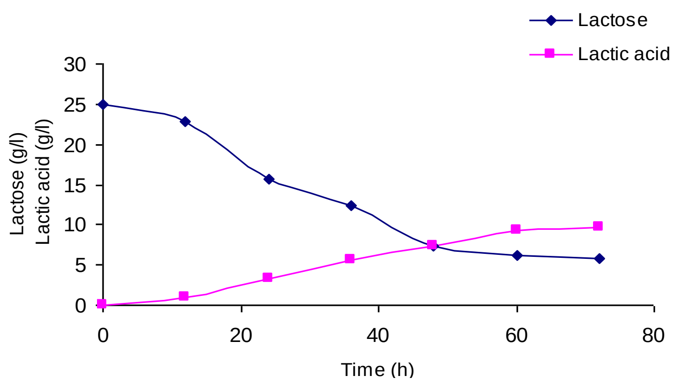

Figure 1. Lactose consumption and lactic acid production by *Lactobacillus bulgaricus* using 50% volume whey + 50% volume nutrient (medium number 1).

Figure 1 shows lactose consumption and lactic acid production with medium 1. As shown in Figure 1 when the nutrient volume percent in the medium is 50%, because of high percent of nutrient the lactose consumption was very low. At this condition after 72 h incubation, 9.7 g/l lactic acid was produced and 76.7% lactose consumed. As a result of lactic acid production the pH of the medium decreased and the process stopped. The initial lactose concentration was 25 g/l but after 72 h the medium lactose concentration was high about 5.8 g/l. This medium had the yield of 38%.

Figure 2 shows lactose consumption and lactic acid production of medium number 2. These results indicated when the whey percent in the medium increases (nutrient percent decreases) the lactose consumption increases and higher lactic acid is produced. In this medium 16.5 g/l lactic acid was produced and 91.4% lactose was consumed. The lactose remaining at this medium after 72 h incubation was 2.9 g/l. This is half of lactose remaining in medium 1. The yield of medium 2 was 43%.

The lactose consumption and lactic acid production of

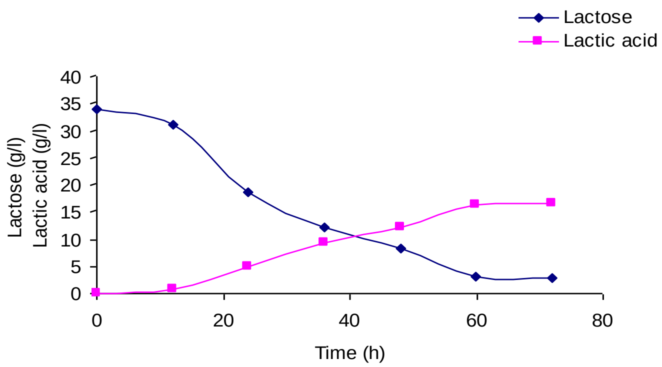

Figure 2. Lactose consumption and lactic acid production by *Lactobacillus bulgaricus* using 66% volume whey + 34% volume nutrient (medium number 2).

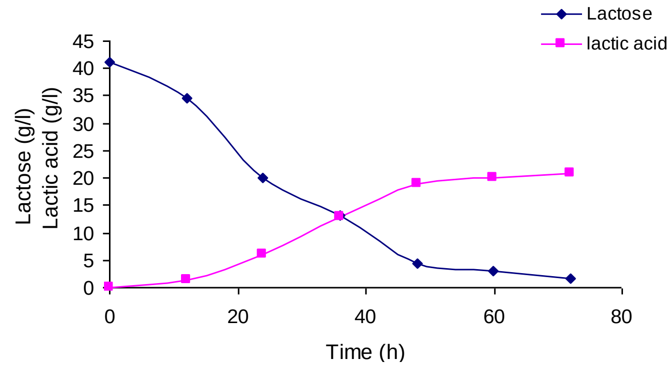

Figure 4. Lactose consumption and lactic acid production by *Lactobacillus bulgaricus* using 80% volume whey + 20% volume nutrient (medium number 4).

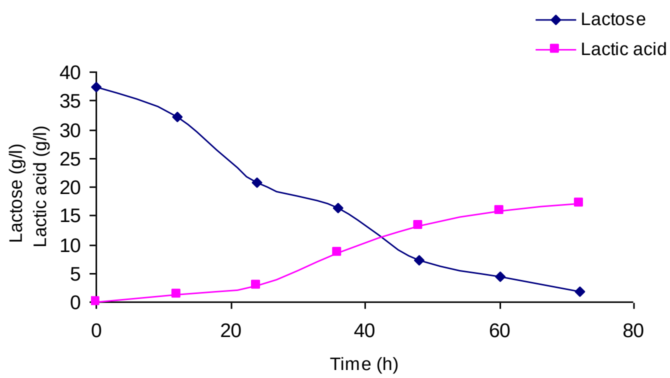

Figure 3. Lactose consumption and lactic acid production by *Lactobacillus bulgaricus* using 75% volume whey + 25% volume nutrient (medium number 3).

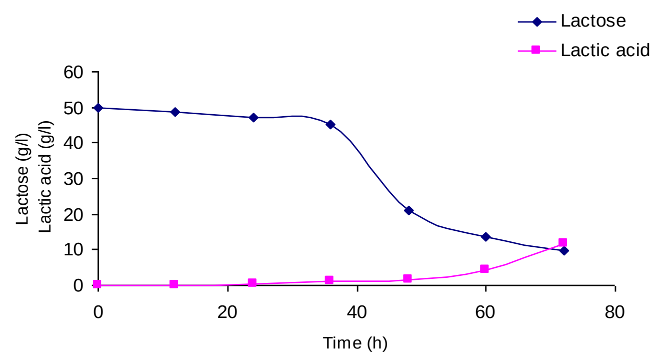

Figure 5. Lactose consumption and lactic acid production by *Lactobacillus bulgaricus* using 99% volume whey + 1% volume nutrient (medium number 5).

medium number 3, containing 75% whey and 25% nutrient is presented in Figure 3. This medium increased the lactic acid production and lactose consumption. 95.2% lactose was consumed with this medium and 17.2 g/l lactic acid was produced. The remaining lactose at this medium was 1.9 g/l. The yield of this medium was 47%, which is higher than medium number 1 and 2.

In Figure 4, the medium lactic acid production and lactose consumption of medium 4 is shown. With this medium, about 96.1% lactose was consumed and 20.8 g/l lactic acid was produced. The remaining lactose in the medium was 1.6 g/l which was lower that all the previous three media examined. Furthermore, yield of this medium was higher than the others (54.7%).

The yield higher than 50% is good for biological process but as result of high capital cost of additive to whey for lactic acid production, the last medium (medium 5), the nutrient volume percent was decreased to 1% with 99% cheese whey. The lactic acid production and lactose consumption in medium number 5 condition is shown in Figure 5. As shown in the figure, after 36 h of beginning the reaction, the medium is in lag phase and the lactose consumption is negligible. Inhibition effect as a result of high lactose concentration was observed in this condition. However, this medium has more lactose than the other media but it produced 11.6 g/l.h lactic acid which was lower than media 2, 3 and 4. And after 72 h, about 80.9% of lactose was consumed and remaining lactose was 9.6 g/l. This is approximately equal to the amount in medium number 1.

The rate of productivity of the different media is shown in Figure 6. As shown in the figure the medium with whey of 80 and 20% nutrient diluted with water has the highest productivity. The productivity shows the rate of lactic acid production.

Conclusion

Batch fermentation of lactic acid with different percent of whey in the medium was successfully carried out. The medium with 80% volume whey and 20% volume nutrient had the highest results for production of lactic acid (20.8 g/l) and also this medium had the maximum lactose consumption (96.1%), maximum yield (54.7%) and maximum

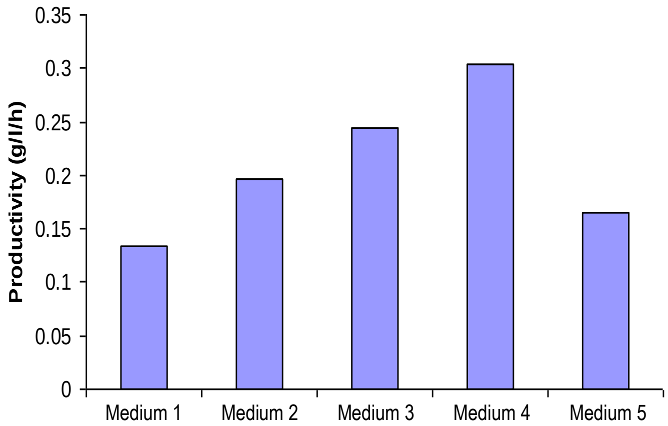

Figure 6. Comparison of lactic acid productivity by *Lactobacillus bulgaricus* using difference media (see Table 1).

rate of productivity (0.304 g/l.h). Because whey is very abundant and cheap, agro industries in dairy production should focus on how to maximize the whey percent and minimize the nutrient supplementation for lactic acid production. The inhibition effect shows that batch fermentation cannot utilize the high feed (sugar) concentration, and whey without supplementation was not a good medium for growth of bacteria and production of lactic acid. Our next study will investigate the possibility of using high feed concentration for production of lactic acid in continuous fermentation.

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