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Production of lactic acid from whey using *Lactobacillus plantarum* 2621

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ABSTRACT

The dairy industry throughout the world is facing the problem of disposal and reutilization of surplus whey. The present work is done to produce a valuable industrial product like lactic acid from the whey with the use of *Lactobacillus plantarum* MTCC 2621. The effect of various environmental conditions was studied to maximize the lactic acid content. The pH 6.0 and 6.5 and temperature of 37°C showed the maximum production of lactic acid i.e. 1,40,000 mg/l. 60gm/l D-glucose concentration shows the maximum growth of *L. plantarum* i.e. 1,72,000 cells/m^3. The best working sample size, inoculum size and the incubation temperature for *L. plantarum* to produce lactic acid were found to be 50ml, 10% (v/v) and 48h. The immobilized cells worked best upto 3rd cycle and the results are compared to those of free cells and the values found out to be 8,00,000 mg/l and 1,08,000 mg/l respectively.

KEYWORDS

Lactic acid;
Lactose;
Whey;
Lactobacillus plantarum;
Continous fermentation.

INTRODUCTION

The dairy industry represents a major part of food industry and contributes significant liquid waste, whose disposal requires large amount of capital investment. Whey is the watery portion or serum that separates from curd during conventional cheese making or casein manufacturing. The disposal of whey is major problem for the diary industry which demands simple and economical solution. Since lactose is the major component of whey solids, numerous biotechnological processes have been developed to utilize whey to make useful products of industrial importance such as lactic acid. Lactic acid (2-hydroxypropanoic acid), also known as milk acid is a versatile organic acid, which finds major application in food, cosmetics, pharmaceuticals and chemical industries and receive attention for use as monomer for the production of biodegradable poly (lactic acid). Due to disadvantages of fungal fermentation like pathogenic, tedious to use, low aeration, productivity rate and mass transfer limitations we have opted for Lactic Acid Bacteria which are Gram positive, acid tolerant, non-sporulating, non-respiring rod or cocci. *Lactobacillus plantarum p*roduces both the isomers of lactic acid, high lactic acid yield, the complete conversion rate, tolerance of low pH conditions and high cell growth rate. *Lactobacillus helveticus* strain was used for the continous fermentation of lactic acid in cheese whey yeast extract permeate medium. The best lactic acid productivity of 9.7g/l/h at a dilution rate of 0.352/h was

TABLE 1 : Increase in lactic acid content with decreasing sample size of whey

Sr. no.	Sample Size (ml)	Lactic acid content		%age increased
		Initial	Final	
1	50	4,000	1,45,000	3.625
2	100	4,000	1,39,000	0.98
3	150	4,000	98,000	0.58
4	200	4,000	60,000	0.20
5	250	4,000	50,000	0.10

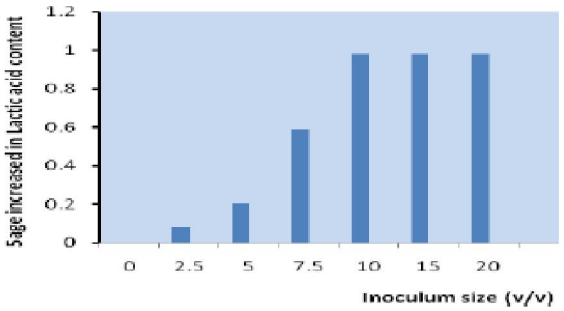

Figure 1 : Increased lactic acid content of whey with increase in inoculum size

observed[1].The effect of various concentrations of yeast extract and lactomine on the growth of *Lactobacillus helviticus* and enhancing lactic acid production from cheese whey under optimized pH and temperature was studied[2]. Lactic acid was produced from cheese whey and corn steep liquor using *Lactobacillus sp*[3]. The results have shown that cell recycle repeated batch fermentation, lactic acid productivity was maximized to 6.34g/l/h.

MATERIALS AND METHODS

Procurement and Maintenance of Microorganism

Lactobacillus plantarum MTCC 2621 was procured from IMTECH, Chandigarh. It was maintained on MRS (de Mann Rogosa Sharpe) Slants and Incubated at 37°C for 48 hr and subcultured aseptically at regular intervals.

Physico-chemical characterisation of whey sample from Verka Milk Plant (Patiala) was done (APHA, 1995). The results of characterization are as follows: color (pale yellow), odour (unpleasant), pH (3.27), Temperature (30°C), moisture content (93.5%) and Chemical characteristics include BOD (350000mg/l), COD (1,05000mg/l), Total solids (72000mg/l), Total dissolved solids (56000mg/l), TSS (16000mg/l), Total Sugars (21000mg/l), Lactose content (45000mg/l) was estimated by reduction of picric acid to the mahogany red picramic acid in an alkaline medium[4]. Lactic acid content (4000mg/l) was determined[5]. Lactic acid in the presence of sulphuric acid gets converted into acetaldehyde, react with p-hydroxydiphenyl solution forms a violet colored complex.

Preparation of whey using different sources 100ml of procured milk sample was being processed with lemon juice, curd, tomato juice ascorbic acid and citric acid for the production of whey. The results revealed that more production of whey was produced by ascorbic acid i.e. 84ml at the rate of 1.6gm/100ml of milk. Next being the citric acid, which produce 78ml of whey at rate of 2.0gm/100ml of milk. Lemon juice, curd & tomato juice produced 78ml, 47.70ml & 26.53ml of whey at rate of 2ml/100ml, 15.0ml/100ml & 37.7ml/100ml of milk respectively.

RESULTS AND DISCUSSION

Optimization of carbon source supplementation

The effect of D-glucose at different concentrations (60g/l, 80g/l and 110gm/l) on growth of *L. plantarum* was studied. In the observations, the cell number counted for different concentrations of D-Glucose were 172000 cells/m^3, 16500 cells/m^3 and 15000 cells/m^3 respectively. A uniform decreased in the cell density was recorded with increase in D-Glucose concentration. It was inferred from the results that highest cell number was observed at low glucose concentration due to maximum glucose conversion[6]. Supplementation of whey with lower concentrations of nutrients greatly enhances fermentation of *L.helveticus* but higher concentrations diminish the cell concentration as a result of toxicity[7].

Effect of sample size on lactic acid production

Different sample volumes (50ml, 100ml, 150ml, 200ml, and 250ml) were inoculated with 10ml inoculum. Samples were incubated and lactic acid content was estimated. As seen from the Table 1 the initial lactic acid content of whey was 4000mg/l and after processing with *L.plantarum* the maximum lactic acid content of 145000mg/l was observed for 50ml sample and goes on decreasing with increase in sample size.

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TABLE 2 : Increased lactic acid production in whey with varying incubation time

Sr. No.	Incubation time (hours)	Lactic acid content		% Increase
		Initial	Final	
1	0	3,200	4,000	0.08
2	12	3,200	6,400	0.32
3	24	3,200	8,900	0.57
4	36	3,200	1,00,000	0.68
5	48	3,200	1,38,000	1.06
6	60	3,200	1,24,000	0.92

TABLE 3 : Comparative lactic acid production with free & immobilized *L. plantarum*.

Cell system	Lactic acid		% Increase
	Initial	Final	
Free Cells	3,000	1,38,000	1.08
Immobilized cells 1st cycle	3,000	8,00,000	7.7
2nd	3,000	8,00,000	7.7
3rd	3,000	8,00,000	7.7
4th	3,000	7,80,000	7.0

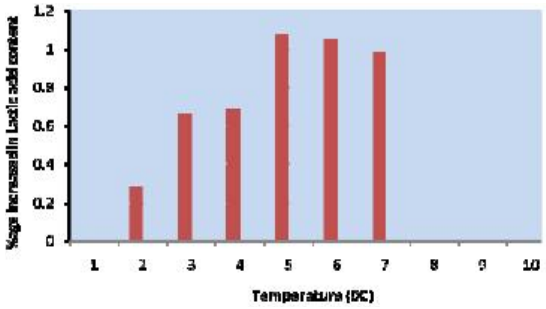

Figure 2 : Increased lactic acid content of whey with temperature as function

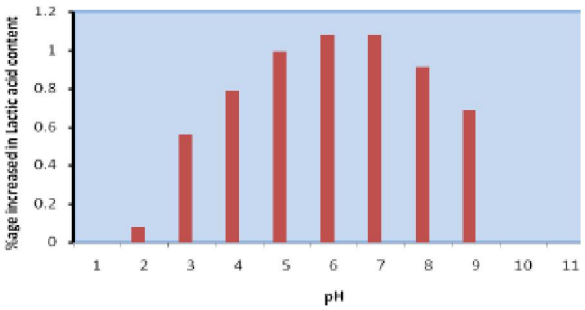

Figure 3 : Effect of pH on production of lactic acid content

Effect of inoculum size on lactic acid production

The effect of varying inoculum size (2.5-2.0%) on lactic acid production in whey sample is presented in figure 1. The increase in lactic acid content of whey effluent with increase in starter culture was recorded upto an inoculum concentration of 10% (v/v) i.e. 138000mg/l whereas with 15% (v/v) and 20% (v/v), no marked change was observed.

Effect of incubation time for lactic acid production

From the TABLE 2 it was found that the lactic acid content of whey increased with an increase in incubation time upto 48 hour and showed maximum i.e. 138000mg/l. A slight decrease in lactic acid content was observed at 60 hours incubation time. This might be due to cell lyses, consequent release of toxins and depletion of nutrients into the medium[8]. Maximum lactic acid production of 13.5g/15.2g starch utilized at 48 hour incubation time was observed[9].

Effect of temperature on *L. plantarum* for lactic acid formation

The effect of temperature was monitored between 20°C-45°C for the potential of bacterial cells to increase the lactic acid concentration and the results are presented in figure 2. The maximum efficiency of *L. plantarum* was achieved at 37°C. The optimal lactic acid production obtained was 1,40,000 mg/l, similar results were given by some researchers at 37°C for *L. casei* & *L. rhamnosus*[10]. However some workers have reported 15°C & 45°C for maximum activity of Lactic acid bacteria[11]. The higher efficiencies at higher temperature than the optimal could be due to their inhibitory effect on RNA and protein synthesis resulting in poor growth. Maximum lactose conversion (95.62%) to lactic acid (33.73g/l) at pH 6.5, temperature 37°C and inoculum size 2%(v/v) of 20 hour old culture (*Lactobacillus casei*) under stationery conditions with an incubation of 36 hours was achieved[12].

Effect of pH on the Lactic acid production

The pH of the whey sample was adjusted between 3.0 -7.5 to evaluate the effect of hydrogen ion concentration on the production of lactic acid. The maximum efficiency had been reported at pH at 6 and 6.5, which was accordance with a study conducted by researchers to show the effect of pH on the activity of lactic acid bacteria[11]. The other pH values showed lower lactic acid production. In another study 60% of the lactic acid is produced from 25 to 45% of sugar fermented at pH

6.5. After this pH, the lactic acid content falls, as shown in figure 3, which may be attributed to the production of other acids like formic, lactic acid and ethyl alcohol[13]. Similarly, pH 6.5 was found to be optimal for maximum lactic acid production of 32.93g/l[14]. Lactic acid concentrations up to 35g/l was obtained at pH of 6.0 using a *Lactococcus lactis* strain[6]. The maximum lactic acid production obtained by bacterial cells at pH 6.0 & 6.5 was 1,40,000 mg/l (Figure 3).

Comparative lactic acid production by free & immobilized cells of *L. plantarum*

The results in TABLE 3 revealed that immobilization increases the efficiency *L. plantarum* to produce lactic acid. The immobilized cells show 8% lactic acid production as compared to 1.38% lactic acid production by free cells. Four cycles of use of immobilization had been implored. The beads show same lactic acid production efficiency up to three cycles. After 3rd cycle there was drop in lactic acid production which can be due to the initiation of degeneration of cells with time, deformation or loss of hardness of beads[14]. A high lactose conversion (94.37%) to lactic acid (32.95g/l) was achieved with the developed immobilized system up to 16th cycle. *L. helveticus* cells immobilized in calcium alginate beads showed higher lactic acid production rates than free cells[15].

CONCLUSIONS

To do the present research work, one sugar utilizing bacteria, *Lactobacillus plantarum* MTCC 2621, was procured from IMTECH, Chandigarh. The pH of whey sample was 3.27 while the temperature was slightly higher than atmospheric temperature i.e. 30°C. It had very high BOD and COD values (35,000 and 1,05,000 mg/l). The total sugar, lactose and lactic acid content obtained were 21,000, 45,000 and 4.000 mg/l, respectively. The whey was produced at laboratory scale using different sources like lemon juice, curd, tomato juice, ascorbic acid and citric acid at different concentrations. Among these ascorbic acid @ 0.2gm/100ml milk was considered to be most suitable one. The optimized pH and temperature of 6.0-6.5and 37°C showed the maximum production of lactic acid i.e. 1,40,000 mg/l. D-glucose concentration (60gm/l)shows the maximum growth of *L. plantarum* i.e. 1,72,000 cells/$m^{3.}$ The best working sample size, inoculum size and the incubation time for *L. plantarum* to produce lactic acid were found to be 50ml, 10% (v/v) and 48hours. The beads inoculated worked best upto 3rd cycle and the results are compared to those of free cells which was found to be 7,70,000 mg/l and 1,08,000 mg/l respectively.

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