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Utilization of whey as a substrate for lactic acid producion by lactobacillus cells using immobilization technique

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ABSTRACT

Whey is the liquid remaining after the separation of milk fat and casein from whole milk, its disposal, is a major problem for the dairy industry, which demands simple and economical solutions. The bioconversion of lactose present in whey to valuable products has been actively explored. Production of lactic acid through lactic acid bacteria could be a processing route for whey lactose and various attempts have been made in this direction. Immobilized cell technology has also been applied to whey fermentation processes, to improve the economics of the process. A fermentative means of lactic acid production has advantages over chemical synthesis, as desirable optically pure lactic acid could be produced, and the demand for optically pure lactic acid has increased considerably because of its use in the production of poly(lactic acid), a biodegradable polymer, and other industrial applications. In our experiment lactic acid production by immobilized *Lactobacillus cells* has been studied for that the cells were immobilized in alginate beads and the effect of variations in different parameters on product formation and productivity was investigated. Repeated batch fermentations were performed with lactobacillus cells (*L. acidophilus)* cells immobilized in 2·4-2·9 mm alginate beads to investigate the possibility of reusing the gel beads. The fermentation was carried out for 48 hours in four batches in normal medium as well as whey medium . At the end of each run, the beads were washed with sterile physiological saline and transferred to fresh medium. Alginate immobilized cells were reused successfully for 3 continuous runs without marked activity loss. The highest lactic acid (31.605 g/l) was obtained using whey as substrate in the third run.

KEYWORDS

Whey;
Lactose;
Lactic acid bacteria;
Lactic acid;
Immobilization.

INTRODUCTION

Whey is a major by-product of the dairy industry which has proved to serves as an inexpensive medium for lactic acid production. It contains approximately (w/v) 5 % lactose, 1 % protein, 0.4 % fat, and some minerals. It has a high biochemical oxygen demand (BOD) content (40,000-60,000 ppm) which represents seri-

ous disposal problems. The disposal of whey, is a major problem for the dairy industry, which demands simple and economical solutions. The bioconversion of lactose present in whey to valuable products has been actively explored. Since whey and whey permeates contain significant quantities of lactose, an interesting way to upgrade this effluent could be as a substrate for fermentation. Production of lactic acid through lactic acid bacteria could be a processing route for whey lactose and various attempts have been made in this direction. Immobilised cell technology has also been applied to whey fermentation processes, to improve the economics of the process. A fermentative means of lactic acid production has advantages over chemical synthesis, as desirable optically pure lactic acid could be produced, and the demand for optically pure lactic acid has increased considerably because of its use in the production of poly (lactic acid), a biodegradable polymer, and other industrial applications[6]. Immobilization of whole cells has been widely used for lactic acid production since immobilization exhibits many advantages like relative ease of product separation, reuse of biocatalysts, high volumetric productivity, improved process control and reduced susceptibility of cells to contamination The entrapment of cells in calcium alginate gel beads is the most widely used method for viable lactic acid bacteria immobilization due to its simplicity, nontoxicity, mild gelation conditions and ease of use[3].

Immobilized cell technology has been widely applied in a variety of research and industrial applications. While numerous immobilization techniques have been described, entrapment within synthetic or natural polymers remains among the most popular due to its ease and simplicity, low cost and gentle formulation conditions ensuring high retention of cell viability.

Immobilization makes it possible to maintain the cells in a stable and viable state and also provides a means of continuous fermentation. It is natural that organisms in an immobilized state will experience different pH, product concentration in their immediate environment as compared to the bulk solution which may have some effect on the metabolism of the bacteria and hence on the overall process. Therefore, information on the physiology of the microorganism in a modified environment is an essential prerequisite to run a fermentation under optimal conditions[5], Immobilization commonly is accomplished using a high molecular hydrophilic polymeric gel such as alginate, carrageenan, agarose, etc. In these cases, the cells are immobilized by entrapment in the pertinent gel by a drop-forming procedure, When traditional fermentations are compared with the microbial conversions using immobilized cells, the productivity obtained in the latter is considerably higher, obviously partly due to high cell density and immobilization-induced cellular or genetic modifications. Nevertheless, a few critical parameters such as the cost of immobilization, mass transport limitations, applicability to a specific end-product, etc. are to be carefully examined before choosing any particular methodology[7]. Presently sugar containing substances(may it be dairy waste) are used for lactic acid production. So whey can be a low cost substrate for production of lactic acid as it contains lactose and utilizing biotechnological startegies for lactic acid production from whey can serve dual purpose, ie production of valuable product lactic acid and addressing whey disposal environmental problem. So the work was carried out to optimize the process and to find efficient way to convert whey to lactic acid. From[2].

Lactic acid fermentation

The lactic acid bacteria belong to two main groups – the homofermenters and the heterofermenters. The pathways of lactic acid production differ for the two. Homofermenters produce mainly lactic acid, via the glycolytic (Embden–Meyerhof) pathway). Heterofermenters produce lactic acid plus appreciable amounts of ethanol, acetate and carbon dioxide, via the 6-phosphoglucanate/phosphoketolase pathway. The glycolytic pathway is used by all lactic acid bacteria except leuconostocs, group III lactobacilli, oenococci and weissellas. Normal conditions required for this pathway are excess sugar and limited oxygen[1]. gives an in-depth account of the biochemical pathways for both homo- and hetero-fermenters.

Homolactic fermentation

The fermentation of 1 mole of glucose yields two moles of lactic acid;

Heterolactic fermentation

The fermentation of 1 mole of glucose yields 1 mole each of lactic acid, ethanol and carbon dioxide;

MATERIALS AND METHODS

In the study lactobacillus cells were cultured on MRS broth medium before immobilization. Then the

$C_6H_{12}O_6 \rightarrow$	2 $CH_3CHOHCOOH$
Glucose	lactic acid

TABLE 5.1 : Major lactic acid bacteria in fermented plant products.

Homofermenter	Facultative homofermenter	Obligate heterofermenter
Enterococcus faecium	*Lactobacillus bavaricus*	*Lactobacillus brevis*
Enterococcus faecalis	*Lactobacillus casei*	*Lactobacillus buchneri*
Lactobacillus acidophilus	*Lactobacillus coryniformis*	*Lactobacillus cellobiosus*
Lactobacillus lactis	*Lactobacillus curvatus*	*Lactobacillus confusus*
Lactobacillus delbrueckii	*Lactobacillus plantarum*	*Lactobacillus coprophilus*
Lactobacillusleichmannii	*Lactobacillus sake*	*Lactobacillus fermentatum*
Lactobacillus salivarius		*Lactobacillus sanfrancisco*
Streptococcus bovis		*Leuconostoc dextranicum*
Streptococcus thermophilus		*Leuconostoc mesenteroides*
Pediococcus acidilactici		*Leuconostoc paramesenteroides*
Pedicoccus damnosus		
Pediococcus pentocacus		

lactic acid production by cultured lactobacilli cells were assayed using titrimetric method (with the help of 0·5M sodium hydroxide and 0·5 M hydrochloric acid,

$C_6H_{12}O_6$ ⟶	$CH_3CHOHCOOH$+	C_2H_5OH+	CO_2
Glucose	lactic acid+	ethanol+	carbon dioxide

phenolpthelein as indicator). After the values are recorded lactobacillus cells were immobilized and cultured in whey,(whey is a waste product of dairy industry)

The immobilization procedure was carried out by using 2% sodium alginate and 50 mM calcium chloride solution. After the reaction, bead formed lactobacillus cells were then transferred to medium containing whey as substrate and after two days of culture the lactic acid production was assayed using titrimetric method. The beads were again collected by filtering the media, the filtered beads were collected and washed with saline water and transferred to fresh medium. In this way cultures were done in three batches transferring same immobilized lactobacillus cells and assayed for lactic acid production. So a comparative study was made on lactic acid production by lactobacillus cells using normal culture medium and whey as culture medium. All the culture vessels were kept in the incubator maintaining 45·c and in an anaerobic condition. Strictly aseptic and sterile conditions were maintained during the entire culture process and inoculation was performed mainly under laminar airflow hood.

Preparation of starter culture(MRS broth)

The predetermined quantity i.e. 55·15 gm MRS broth was dissolved in 500ml distilled water and volume was made upto 1000 ml with distilled water.

Method for immobilization of lactobacillus cells-

For immobilization of lactobacillus cells 10ml of 2% sodium alginate solution was made and mixed with 10ml of lactobacillus culture medium. Again 100 ml of 50mM calcium chloride solution was taken in a beaker. After that with the help of a burette, the mixture of alginate and culture media containing lactobacilli cells was poured drop wise into the calcium chloride solution carefully. The beaker was shaken for sometime untill the beads containing lactobacillus were formed**.**

Method for culture of immobilized lactobacillus cells

Immobilized lactobacilli cells were cultured in both normal medium and medium containing whey as substrate. The cultures were kept in the incubator for 2 days at 45·C. Assay for lactic acid production was carried out using 0·5M sodium hydroxide solution and 0·5 M hydrochloric acid solution, and phenolphthalein as indicator, until the pink colour discharged. The burette readings were noted and lactic acid production were calculated.

Assay of lactic acid

The cultures flasks containing lactobacillus cells were filtered and filtrate were kept for further titration procedure. assay was carried out for lactic acid production using 0·5M sodium hydroxide, 0·5 M hydrochloric acid, and phenolpthelein as indicator. 10 ml of filtrate was taken and 40 ml of 0·5M NaOH solution was mixed. Pink colour appears upon addition of phenolphthalein

FULL PAPER

indicator as the medium is still alkaline. It is then titrated with 0·5M HCl solution. Burette readings were noted carefully. Each ml of 1M NaOH is equivalent to 0·09008 gm of $C_3H_6O_3$[4].

RESULTS

The production of lactic acid by lactobacillus cells in normal culture medium

The lactic acid production by lactobacillus cells are estimated by cultivating them in medium containing lactose at optimum condition of 45.C for 48hours in four batches. (for each batch of culture, optimum culture condition i.e. temperature and p^H were maintained)The cultures were maintained by transferring a few drop of the culture to a fresh culture propagation media The results were as follows-

The production of lactic acid by immobilized lactobacillus cells in normal culture medium

The lactobacillus cells immobilized in sodium alginate beads were cultured in medium containing lactose at optimum condition of 45.C for 48 hours in four batches and their lactic acid production was estimated in each batch. This is done by filtering the beads containing lactobacillus from the old medium and transferred them into a new medium. The results were as follows-

The production of lactic acid by free lactobacillus cells in whey as culture medium-

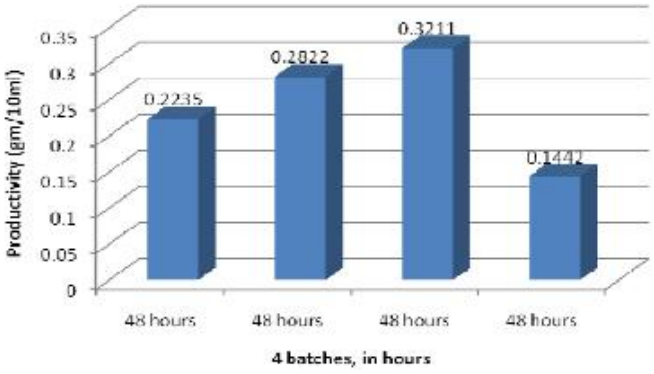

Figure 1 : Result of lactic acid production in normal culture medium

The lactic acid production by lactobacillus cells as estimated by cultivating them in whey medium at optimum condition of 45.C for 48.. hours in four batches. In each batch after 48 hours the assaying of lactic acid production is done The results were as follows.

The production of lactic acid by immobilized lactobacillus cells in whey as culture medium

The lactobacillus cells immobilized in sodium alginate beads were cultured in medium containing whey

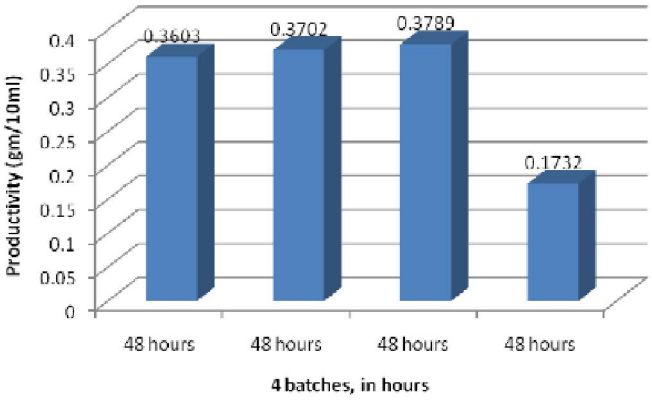

Figure 2 : Result of lactic acid production by immobilized lactobacillus cells in normal culture medium

as substrate at optimum condition of 45.C for 48 hours in four batches and their lactic acid production was estimated using titrimetric method. The results were as follows-

DISCUSSION

Repeated batch fermentations were performed with *Lactobacillus* cells immobilized in 2·4-2·9 mm alginate beads to investigate the possibility of reusing the gel beads as shown in the Table : 1 The fermentation was carried out for 48 hours in four batches in normal medium as well as whey medium. At the end of each run, the beads were washed with sterile physiological saline and transferred to fresh medium. Alginate immobilized cells were reused successfully for 3 continuous runs without marked activity loss. The highest lactic acid (31.605 g/l) was obtained using whey as

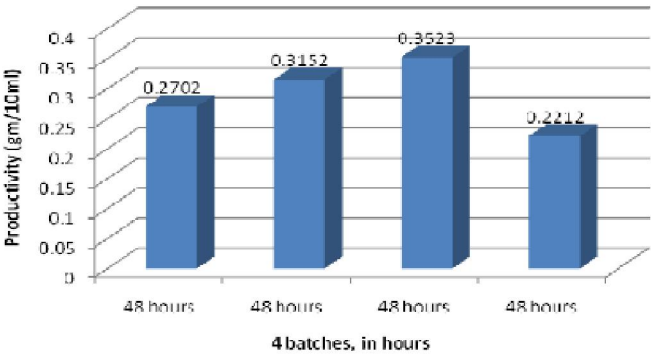

Figure 3 : Result of lactic acid production in whey culture medium

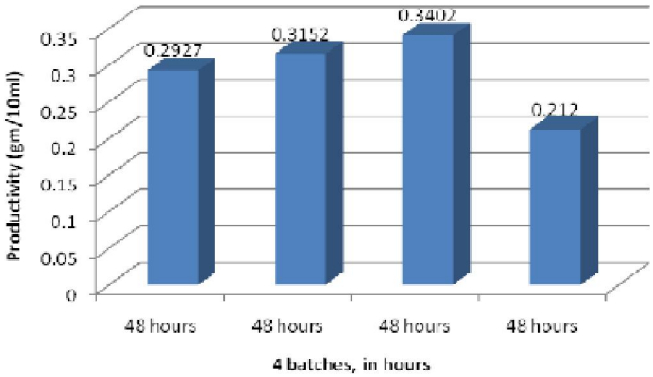

Figure 4 : Result of lactic acid production by immobilized lactobacillus cells in whey as culture medium

substrate in the third run. Shrinkage, deformation and small cracks in the surface of beads were observed in the last run and the beads lost their hardness and completely disrupted in the medium at the end of 4th run. But it was observed that the immobilization of lactobacilli cells in sodium alginate beads lead to production of lactic acid for a longer period of time using the same cells repeatedly. Though it was found that lactic acid production by immobilized cells were less compared to normal cells but it was observed for longer productivity. Although remarkable achievements in higher productivity of lactic acid have been attained since it was first recognized more than a decade ago, enormous challenge remain for researchers to improve the microbial production of lactic acid due to the increasing demand of pure lactic acid production by different industries such as pharmaceutical, chemical, food, cosmetic etc, there fore it can be envisaged that in the near future and further studies can be explored using the selected methods. As the whole project was performed in laboratory scale, to unfold the amount of lactic acid production from whey by immobilized lactobacillus cells, suitable optimization and scale up technique has to be developed. So it can be concluded that in near future lactic acid production from whey by immobilized lactobacilli cells can beused industrially for use in various industrial application.

TABLE 1: Table for comparision of lactic acid production by normal lactobacilli cells and immobilized lactobacilli cells taking normal culture medium and whey as culture medium-

Inoculation with	Incubation period (three batches)	Culture medium	Productivity in case of immobilized cells (gm/10ml)	Productivity in case of normal cells (gm/10ml)
Lactobacillus	48 hours	Normal medium	0.2235	0.3603
Lactobacillus	48 hours	Normal medium	0.2822	0.3702
Lactobacillus	48 hours	Normal medium	0.3211	0.3789
Lactobacillus	48 hours	Normal medium	0.1732	0.1442
Lactobacillus	48 hours	whey	0.2927	0.2702
Lactobacillus	48 hours	whey	0.3152	0.3152
Lactobacillus	48 hours	whey	0.3402	0.3523
Lactobacillus	48 hours	whey	0.2120	0.2212

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