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Parametric determination for facile bioproduction of 2-hydroxypropanoic acid

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Abstract:The optimization of parametric conditions, viz. molasses concentration, pH, temperature and incubation period have been studied for bioproduction of lactic acid by *Lactobacillus acidophilus -2930* . It has been found that lactic acid fermentation by *Lactobacillus acidophilus -2930* proceeds best when a molasses solution of 25% (W/V) is allowed to ferment for 7 days of incubation period at 35°C temperature by maintaining the pH values of fermenting medium at 5.8 along with other bioingredients supplements required by the *Lactobacillus acidophilus -2930*.

(Key words : Lactic acid fermentation, molasses, *Lactobacillus acidophilus -2930*)

Introduction

Parametric determination for microbial search for production of lactic acid by *Lactobacillus acidophilus* -2930 is virtually as important to the success of a lactic acid fermentation as is the selection of an organism to carry out the fermentation[1-4]. Medium supplies nutrients for growth, energy, building of cell substances and biosynthesis of fermentation bioprocess products. A poor selection of medium components can effect cellular growth and little if any yield of fermentation products.[5-8] The optimization of parameters like concentration of selected raw material, hydrogen ion concentration, temperature and incubation period of the fermentation medium can partially or fully influence the types and ratios of products from among those for which a microorganism has biosynthetic capability[9-12]. Thus, parametric determination of lactic acid fermentation by *Lactobacillus acidophilus -2930* is very critical. The optimizaton of culture for various lactic acid fermentations have been studied by different workers[13-17], Liu and Chen[18] reported significant yield of lactic acid from 11-23% sucrose at 32-33^{0}C temperature and 6.1 pH. Tiwari and Pandey[19] reported maximum production of lactic acid by *L. bugaricus* when 20% solution of molasses was allowed to ferment for 7 days at 35^{0}C and 5.8 - 6.0 pH. Singh *el al.*[20] found that *L. delbrueckii* attains its best activity of producing lactic acid when 5% solution of sugar at 6.2 pH is allowed to ferment at 35^{0}C for 7 days.

Singh *et al.*[21-27] and some others[28-50] studied lactic acid fermentation by different strains of lactic acid bacteria and found their best activity when sugar substrate concentration was taken in between 10-15%; pH 5.8-6.2, temperature 42-48^{0}C and incubation period 4-6 days.

In the present communication the author has confined his study to optimize the parameters for maximum production of lactic acid by fermentation using the bacterial strain of *Lactobacillus acidophilus* -2930.

Experimental

Medium :

Study of the impact of different carbohydrates :

15 - sets of flat bottam conical flasks (each set having three flasks) were well prepared as mentioned in general technique with the only difference that in the place of sugar molasses dissimlar carbohydrates under trial were taken in flat bottom fermentor flasks of the 1st to 15th set

to give 10.00 g of carbon substrate source to each of the 15 sets of the flat bottom fermentor flasks . Then the total volume in the flat bottom fermentor flasks were made up to 100 ml by adding requisite amount of distilled water. The flasks were then plugged with non-absorbent cotton and steam sterilized, cooled and then inoculated with 0.05 ml of *Lactobacillus acidophilus* -2930 and incubated at 35^{0}C. The contents of the flat bottom fermentor flasks were analysed colorimetrically for lactic acid produced after 7 days of optimum incubation period.

Table - 1

Effect of different carbohydrates on facile bioproduction of 2-hydroxypropanoic acid by Lactobacillus acidophilus -2930 in 7 days of optimum incubation period

S. No.	Carbohydrates substrates used	Yield of lactic acid* in g/100 ml	Sugar left Unfermented g/100 ml.
1	Arabinose	1.475	-
2	Rhamnose	0.429	-
3	Xylose	1.110	-
4	Glucose	1.115	-
5	Fructose	7.280	-
6	Galactose	5.875	-
7	Sorbose	0.996	-
8	Lactose	5.369	-
9	Sucrose	8.660	-
10	Maltose	2.545	-
11	Starch	0.785	-
12	Inuline	0.819	-
13	Dextrine	0.913	-
14	Raffinose	4.235	-
15	Mannitol	2.002	-
16	Molasses**	9.862	-

* Each value represents mean of three observation; (1-7) monosaccharides; (8-10)-disaccharides; (11-14) poly saccarides; (15) - Polyalcohol; S. No. (16) Molasses contains approximately 40% fermentable sugars.

** Molasses was employed as raw material due to economical cost.

Study of the effect of substrate concentrations:

10-sets, each of 3-flat bottom conical flasks were prepared with the only difference that the concentrations of sugar (molasses) in flat bottom fermentor flasks of 1st to 10th sets was 2%, 5%, 10%, 15%, 18%, 21%, 25%, 30%, 35% and 40% respectively. The above flasks were then steam sterilized, cooled, and incubated at 35^{0}C for 7 days. The contents of the flasks were analysed colorimetrically for lactic acid produced and sugar left unused.

Study of the effect of hydrogen ion concentration :

10 - sets, each consisting of 3-flat bottom conical flasks were prepared and requisite amount of phosphate-buffer solution were added to first 5 sets, to adjust the pH at 5.0, 5.1, 5.2, 5.3 and 5.6 In the remaining 5 sets, the pH values 5.7, 5.8, 5.9, 6.0 and 6.1 were maintained respectively the flat bottom fermentor flasks were then steam sterilized, cooled and inoculated with 0.05 ml of *Lactobacillus acidophilus-2930* and incubated at 35^{0}C for 7 days.

The contents of the flat bottom fermentor flasks were analysed colorimetrically for lactic acid produced and sugar left unused.

Study of the temperature :

10 sets, each consisting of 3-flat bottom fermentor flasks were prepared. These flat bottom fermentor flasks were steam sterilized, cooled and inoculated with 0.05 ml of *Lactobacillus acidophilus* -2930. 1st to 10th sets of flat bottom fermentor flasks were incubated at 16^{0}C, 20^{0}C, 25^{0}C, 30^{0}C, 35^{0}C, 38^{0}C, 40^{0}C, 42^{0}C, 43^{0}C and 45^{0}C respectively for 7 days. The contents of the flat bottom fermentor flasks were analysed colorimentrically for lactic acid formed and sugar left unused.

Study of the effect of Incubation period of fermentation medium :

10 sets,each of 3- flat bottom fermentor flasks were prepared. The flat bottom fermentor flasks were sterilized, cooled, and inoculated with

0.05 ml of *Lactobacillus acidophilus* -2930 and were incubated at 35^{0}C in an incubator. The contents of the flat bottom fermentor flasks were analysed carefully colorimentrically after 2, 3, 4, 5, 7, 9, 10, 11, 12 and 14 days of incubation period for lactic acid produced and sugar left unused

Results and Discussion

The results of lactic acid (LA) fermentation bioprocess by *Lactobac*illus *acidophilus* -2930 obtained in the present research investigation support the facts that in general lactic acid (LA) fermentation bioprocess by lacic culture becomes very easier as the size and colecular weight of substrate molecule decreases. It is obvious from the result that monosaccharide simple sugars glucose as well as fructose both are most easily fermentable most probably due to the presence of aldehydic and ketonic gorups which actively participates in the bioprocess of phosphorelation. The result also shows that the fermentability of galactose sugar is like sugars fructose and glucose. The lactic acid (LA) bioproducing capacity of sugars arabinose, rhamnose and sorbose was noted to be negligible. In the case of disaccharides, sucrose sugar has been found most suitable for the lactic acid fermentation lactose sugar was found to be unsuitable while maltose was fermented to lesser extent, i.e; 2.545g/100ml production of lactic acid (LA).

In the case of sugars polysaccharides, starch was found to be very less fermentable and therefore, unsuitable for lactic acid fermentation bioprocess. In the case of inuline, dextrin and raffinose insignificant amount of lactic acid was produced. In the case of polyalcohols: mannitol gave insignificant yield of lactic acid, i. e., 2.002g/ 100 ml lactic acid (LA) only. These observation therefore, suggests that glucose amongst monosaccharides and sucrose amongst disaccharides are most useful for lactic acid fermentation bioprocess by *Lactobac*illus acidophilus -2930

In the case of molasses 9.862g/100 ml lactic acid (LA) has been found from 25% (W/V) molasses solution. The fermentable sugar in molasses solution ranges about 50% thus, gives 12.00gm of fermentable sugar. Thus molasses solution has been employed as a cheapest and richest source of carbohydrate substrate during the course of present investigation for the facile bioproduction of 2-hydroxypropanoic acid by *Lactobacillus acidophilus* -2930.

The results recorded reveals the influence of different concentrations of selected substrate, i.e., molasses solution. The best functioning and activity of *Lactobac*illus acidophilus -2930 has been observed when 25% molasses solution (W/V) concentration were employed. It has been found that higher concentrations of molasses solution interferes with the potentialities and activity of enzymes of *Lactobac*illus acidophilus -2930 and therefore, retards the bioproduction of lactic acid.

The results of the influence of hydrogen ion concentrations, i.e. pH are recorded . The data shows that the lactic acid bioproduction increases with the increase of pH towards acidic side of neutrality. The bioproduction of lactic acid is much retarded in strong acidic medium. It was found that on increasing the pH values from 5.0 to 5.8 lactic acid yield also increase i. e. 3.111 g/100 ml to 9.843 g/100 ml and further increase of pH from 5.9 to 6.1 and onwards decreases the bioproduction of lactic acid. It was therefore, concluded that production of lactic acid does not proceed smoothly in strong acidic and neutral medium. The optimum hydrogen ion concentrations of the fermentation medium was recorded to best 5.8 which was most suitable for the maximum bioproduction of lactic acid and thus, all the experiments conducted in the present investigation was maintained at optimum pH value of 5.8.

The results recorded shows that table - 1 bioproduction of lactic acid increases with the increase of temperature from 16^{0}C to 35^{0}C. At the lower temperature the yield of lactic acid (LA) was minimum, i.e., 4.818 g/100ml at 35^0C while the maximum yield of lactic acid 9.852 g/100 ml was found at temperature 35^0C. The bioproduction of lactic acid (LA) gradually falls with the rise in temperature from 38^0C to 45^0C. At higher temperature enzymes of *Lactobacillus acidophilus* -2930 are deactivated and on other hand low temperature requires more energy for the activation of enzymes associated to LABs under trial.

It was, thus, sum up that 35^0C temperature was most favourable and effective for maximum bioproduction of lactic acid by *Lactobac*illus *acidophilus*-2930 and therefore, this temperature, i.e., 35^0C was maintained throughout the experiments described in the present thesis.

The results recorded reveals that bioproduction of lactic acid (LA) by *Lactobac*illus *acidophilus* -2930 proceeds in several phases. The data indicates that the bioproduction of lactic acid increases with the increase in incubation period from 2 days to 7 days and then gradually falls. It was also found that usually consumption of molasses solution corresponded with the yield of lactic acid. After completion of 7 days (optimum incubation period) maximum yield of lactic acid, i.e., 9.678 g/100 ml has ben obtained.

It was, therefore, concluded that bioproduction of lactic acid (LA) by *Lactobac*illus *acidophilus* -2930 goes best when molasses solution 25% (w/v) is admitted to ferment for 7 days of optimum incubation period at 35^0C by maintaining the hydrogen ion concentrations value of fermentation medium 5.8, along with other nutritional ingradients required by the lactic acid bacteria *Lactobacillus acidophilus* -2930.

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