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Study the optimum values of concentration of sugar, incubation period, temperature of incubation and pH of medium for fermentation of molasses by L. bulgaricus MG.

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Abstract

During my research work I was found that biosynthesis of lactic acid in SMF system by facultative aerobe proceeds the optimum values of concentration of sugar, incubation period, temperature of incubation and pH of medium for fermentation of molasses by L. bulgaricus MG. Growth and activity of bacteria has been found to be maximum when a 20% (w/v) solution of molasses (4.4% Sugar) in allowed to ferment for 6 days at 47^0C temperature by maintaining the pH value of the fermentation medium between 5.8-6.0.

Keyword :- Temperature, pH, Sugar, Molasses, L. bulgaricus MG.

Introduction

Fermentation was one of the first biological phenomena to stimulate the curiosity of natural philosophers and inquisitive observes and thus to become object of intensive investigation. Like all living organisms the life activities of microorganisms are conditioned by their environment. Environmental (Cultural) condition is an important for Fermentation are the hydrogen ion concentration p^H of the medium, temperature of reaction, incubation period and concentration of the substrate. Temperature has a universal influence on microorganism in that the rates of enzyme reactions increase with the increasing temperature and since microbial activity and growth are manifestations of enzymetic action, their rates are temperature dependent. This explains the existance of certain thermophilic microorganisms in the northern region of the globe. Knowing the favourable environment for growth and activity of certain microorganisms it is possible either to muitiply it better, as in the case of industrially important microorganisms, or to kill them, as in the case of harmful organisms and pathogenic bacteria.

Experimental

Study of the effect of concentration of substrate

8 sets of flasks (each set of 3) were prepared as described in experimental chapter with the only difference that the concentration nelesses in flasks of Ist to VIIlth set was 10, 15, 20, 25, 30, 35, 40, and 45% respectively. The flasks were sterilized, cooled and inoculated with L.bulgaricus. All and incubated at 37°.The contents of flasks were analysed colorimetrically for lactic acid (produced) and sugar (left unfermented) after 6 days of incubation.

Study of the effect of incubation period

The time period during which microorganisms inoculated into a medium are allowed to grow is called incubation period. The food requirements of fungus differ profoundly from species to species the composition of the media may also effect the growth and activity of Tungus thereby influence incubation period too.

Incubation period also controls economic position of a fermentation product tied to the costs associated with its production and distribution. Short incubation period fermentations usually are less costly, and this applies both to inoculum buildup and to production. Thus short incubation period fermentations allow a greater turmover of fermentation equipment; that is, more fermentations can be run in the same equipment during the same period of time. In this regard, it is assumed that at the termination of fermentation; accessory storage and work-up fermentor tanks will be available so that the fermentation tank itself can be drained, since the holding of completed fermentation both is a fermentor ties up equipment needed for further fermentation runs. It is established that fermentations requiring long incubation periods provide additional.

8 sets, each of three Erlenmeyer flasks, were prepared as described in experimental chapter. The flasks were sterilized, cooled and after inoculation with L. bulgaricus Au, incubated at 370. The contents of flasks were analysed after every 24 hrs (first set of flasks were analysed after every

24 hrs of incubation and the eighth set after 192 hrs of incubation) produced and sugar (left unfermented).

Study of the effect of temperature of incubation

8 sets, each consisting of three flasks, were prepared as described in experimental chapter. These were sterilized, cooled and inoculated with L bulgaricus MG 1^{st} to 8^{th} set of flasks were incubated at 30,35, 40, 45,47, 49, 51 and 55^0 C respectively for 6 days. The contents of fiasks were analysed colorimetrically for lactic acid 22 (produced) and sugar (left unfermentd)

Study of the Effect of pH of Medium

Microorganisms have a wide range of pH optima depending upon the group or species. Many yeasts and molds and certain bacteria grow well in acidic media (pH 5.0 or lower) while most bacteria require a pH near neutrality (6.5 to 8.0) for optimal growth. The pH of the medium profoundly affects the growth of microorganisms. Since enzymes are sensitive to alterations in pH. Each organism therefore has a minimum, optimum and maximum limit of normal growth. The growth rate is maximum at the optimum pH and becomes slower at lower or higher pH values. Thus, the erect of pH on multiplication rate is very marked but varies with different organism.13 sets, each comprising 3 flasks, were prepared as described experimental chapter. The pH of 1^{st} to 8^{th} set was maintained 5.0 5.2, 5.4, 5.6, 5.8, 6.0, 6.2 and 6.4 respectively by adding requisite amount of phosphate citrate buffer solution (Table - 6). All the flask was maintained between 5.5-5.6, 5.6-5.8, 5.8-6.0, 6.0-6.2 and 6.2-6.4 respectively by gradual addition of $CaCO_3$ in fermentation medium during course of fermentation. The contents of flasks were analysed colorimetrically for lactic acid (produced) and sugar (left unfermented)

Results and Discusison

The results of colorimetiric analysis are given in Tables 1-5. The values reported are mean values of the three observaitons in each case.

Table - 1

S. No.	Conc. Of molasses	Respective conc. Of Sugar (in g)	Yield of lactic acid g/100ml*	Sugar left unfermented g/100 ml*
1.	10	2.2	0.95375	1.14535
2.	15	3.3	1.44263	1.70285
3.	20	4.4	1.94735	2.27345
4.	25	5.5	2.31810	2.93483
5.	30	6.6	2.77613	3.52532
6.	35	7.7	3.15285	4.16413
7.	40	8.8	3.51176	4.70654
8.	45	9.9	3.96321	5.24721

Effect of concentration of substrate on lactic acid fermentation

***Each value represents the mean of three trails

Experimental deviation ± 1.5 to 3.5%

Table – 2

Lactic acid fermentation With Varied incubation period

S. No.	Incubation period (in Days)	Yield of lactic acid g/100ml*	Sugar left unfermented g/100 ml*
1.	1	0.80820	3.51235
2.	2	1.02786	3.27326
3.	3	1.85332	3.10587
4.	4	1.52420	2.70639
5.	5	1.80351	2.44778
6.	6	1.95224	2.26921
7.	7	1.95186	2.25456
8.	8	1.95435	2.25289

*Each value represent mean of three trails.

Experimental deviation ± 1.5 to 3.5%

Table - 3

Lactic acid fermentation with varied temperature

S. No.	Temp. (in ^{0}C)	Yield of lactic acid g/100ml*	Sugar left unfermented g/100 ml*
1.	30	0.62525	3.71336
2.	35	0.83786	3.48950
3.	40	1.54395	2.69721
4.	45	1.88235	2.33665
5.	47	1.94235	2.72210
6.	49	1.93225	2.29305
7.	51	1.92265	2.25635
8.	55	1.47235	2.78884

*Each value represent mean of three trails.

Experimental deviation ± 1.5 to 3.5%

Table – 4

Lactic acid fermentation with varied p^H

S. No.	Hydrogen ion concentration (p^H)**	Yield of lactic acid g/100ml*	Sugar left unfermented g/100 ml*
1.	5.0	1.11235	3.15256
2.	5.2	1.37358	2.85556
3.	5.4	1.42554	2.81658
4.	5.6	1.82657	2.37368
5.	5.8	1.95998	2.25648
6.	6.0	1.95325	2.26213
7.	6.2	1.68256	2.51113
8.	6.4	1.60954	2.58913

*Each value represent mean of three trails.

**p^H Maintained via Table – 6. Experimental deviation ± 1.5 to 3.5%

Table – 5

Lactic acid fermentation with varied p^H

S. No	Range of pH**	Yield of lactic acid g/100ml*	Sugar left unfermented g/100 ml*
1.	5.4 – 5.6	1.71305	2.46735
2.	5.6 – 5.8	1.84658	2.34587
3.	5.8 – 6.0	1.95265	2.26667
4.	6.0 – 6.2	1.70354	2.51156
5.	6.2 – 6.4	1.63588	2.59265

*Each value represent mean of three trails.

Experimental deviation ± 1.5 to 3.5%

Table – 6

p^H values of phosphate-citrate buffer solution

S. No.	Range of p^H	0.2M Na_2SO_4*** (in ml)	0.1M citric acid**** (in ml)
1.	5.0	10.30	9.70
2.	5.2	10.72	9.28
3.	5.4	11.15	8.85
4.	5.6	11.60	8.40
5.	5.8	12.09	7.91
6.	6.0	12.63	7.37
7.	6.2	13.22	6.78
8.	6.4	13.85	6.15

*Each value represents the mean of three trail

**P^H maintained by Ca CO_{3*}

*** 0.2M sodium phosphate prepared by dissolving 28.42 g in 1000 ml of distilled water.

Discussion

The results obtained show the optimum values of concentration of sugar, incubation period, temperature of incubation and pH of medium tor fermentation of molasses by L. bulgaricus MG. Growth and activity of bacteria has been found to be maximum when a 20% solution of molasses (4.4% Sugar) in allowed to ferment for 6 days at 47^0 temperature by maintaining the pH value of the fermentation medium between 5.8-6.0.

The results of the Table-1 show the influence of varied concentration of substrate (sugar present in molasses) on lactic acid fermentation. The best activity of bacteria has been observed at 4.4% Initial sugar concentration. It is observed that higher concentrations of Sugar (7.75 and onwards) interfere with the bacterial activity.

Lactic acid fermentation completes within6 days of incubation period (Table 2). After six days of incubation, maximum yield of lactic acid (1.96 g/100 ml) has been obtained. No increased in the yield of lactic acid has been observed with the increase in incubation period.

The data recorded in Table - 3 show the influence of temperature on the acid producing activity L. bulgaricus MG. Optimum temperature on the acid producing a acid (44% of total Sugar) has been obtained at 47°C yield of lactic acid (440 o total sugar) has temperature of incubation.

The factors that limit bacterial growth and activity at low temperature are not clearly known. It is know, however, that at temperature below the minimum tor growth, cells continue to take up oxygen and transport nutrients, but they do not synthesize macromolecule. There is, therefore, either a cold sensitive fundamental reaction such as oxidative phosphorylation, which prevents the synthesis of macromolecules. Death at higher temperature is thought to be caused by the denaturation of cellular proteins. Increased temperature results in increased rates of sysnthesis of macromolecules but also increased rates of protein denaturation.

The results of pH studies have been recorded in tables 6 and 7. The data of table - 6 show the results of pH studies in which pH has been maintained by buffer action while in table 7, pH as been maintained by gradual addition of $CaCO_3$ (Neutralishing agent) in the fermentation medium during the course of fermentation. It is evident the L. Bulgaricus MG attains its best activity of

producing acid at the pH 5.8-6.0 in both e cases of maintaining the pH. It also appears that the bacteria failed to tolerate high acidity present in the medium (at 5.0 pH only 27.9% of total sugar has been converted into lactic acid). At higher pH values of the experiment, there is again decrease in the yield of lactic acid.

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