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Parametric determination of conditions for bioproduction of citric acid

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Abstract : The optimization of parameters, viz. molasses concentration, pH, temperature and incubation period have been studied for parametric determination of citric acid by *Aspergillus niger* NCIM 953. It has been found that production of citric acid by *Aspergillus oryzae*-368 proceeds best when molasses solution 20.5% (w/v) is allowed to ferment for 12 days of incubation period at 30^0C temperature by maintaining the pH value of fermenting medium to 2.2 along with other nutritional ingradients required by the fungus *Aspergillus oryzae*-368.

(Key words : Citric acid fermentation, molasses, *Aspergillus oryzae*-368)

Introduction

An *optimization parameter* (or a decision variable, in the terms of optimization) is a model parameter to be optimized. During the optimization process, the parameter's value is changed in accordance to its type within an interval, specified by lower and upper bounds. The goal of the optimization process is to find the parameter values that result in a maximum or minimum of a function called the objective function. Objective function is a mathematical expression describing a relationship of the optimization parameters or the result of an operation (such as simulation) that uses the optimization parameters as inputs. The optimization objective is the objective function plus optimization criterion. The latter determines whether the goal of the optimization is to minimize or maximize the value of the objective function.

Parametric determination of citric acid production by *Aspergillus oryzae* is virtually as important to the success of an industrial fermentation as is the selection of an organism to carry out the fermentation. Medium supplies nutrients for growth, energy, building of cell substances and biosynthesis of fermentation products. A poor selection of medium components can effect cellular growth and little if any yield of fermentation products.[1-8] 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 citric acid production by *Aspergillus oryzae*-368 is very critical.

It is reported that more than 90% of the citric acid produced in the world is obtained by fermentation[13-21]. The industrial citric acid production can be carried out in three different ways: by submerged fermentation, surface fermentation and solid-state fermentation or "Koji" process. It is estimated that about 80% of the world citric acid production is obtained by submerged fermentation in stirred tanks of 40-200 m^3 or larger airlift fermentors of 200-900 m^3 capacity[22]. Submerged fermentation can be carried out in batch, fed batch or continuous systems, although the batch mode is more frequently used. It is reported that citric acid production rates and yields are highly dependent on the type of microorganism, the type of substrate and the culture conditions. Factors that have been shown to exert an effect on citric acid production are the type and concentration of carbon source of the fermentation medium, nitrogen and phosphate limitations, aeration, trace elements, initial pH, and temperature[23-25].

In the present communication author has confined her study to optimize the parameters for maximum production of citric acid by fermentation using the fungal strain of *Aspergillus oryzae*-368

Experimental

Medium :

The composition of the production medium for each fermentor flask containing 100 mL production medium is as below :

Molasses: 20.5% (w/v) NH_4NO_3:0.65% , KH_2PO_4 : 0.65%, $MgSO_4.7H_2O$: 0.65%, pH : 2.2

Preparation of the culture medium :

In order to retain metabolic activity and to maintain the growth of the selected strain of fungus, i.e; *Aspergillus oryzae*-368 it was fresh cultured periodically. Different micro- oganisms require different nutrient materials. Thus, culture medium vary in form and composition, depending on the specific species to be cultivated. The culture media for the maintenance of *Aspergillus oryzae*-368 is as follows :

Sucrose : 0.200 g, Malt-extract : 0.50 g, Yeast-extract : 0.50 g, Peptone : 0.50 g, Agar-Agar: 0.80 g, Distilled water : 100 ml, pH : 2.0

The pH of the culture medium was adjusted to 2.0 by adding requisite amount of KCl-HCl buffer solution.

The above contents was transferred in a 250 ml flat bottom conical flask and was made upto 100 ml by adding requisite amount of distilled water. The flask was then tightly plugged with non-absorbent cotton wool plugs.

Sterilizations :

The growth and production media were sterilized in an autoclave maintained at 15 lbs steam pressure for 30 min.

Strain : *Aspergillus oryzae*-368 was used in the present study. The strain was procured from NCL, Pune,India.

Aspergillus oryzae

Assay methods: Evaluation of citric acid[27] formed was made colorimetrically[28].

Age of the inoculum: 50 hours old.

Quantum of the inoculum: 0.05 ml fungal suspension of *Aspergillus oryzae*-368.

Molasses concentration : 5.5%, 10.5%, 15.5%, 20.5%, 25.5%, 30.5%, 35.5%, 40.5%, 45.5% and 50.5%

Temperature (in^{0}C) : 15, 20, 25, 30,32, 34, 36, 38, 40 and 42

Incubation period: 5, 7, 8, 9, 10, 12, 15, 18,20 and 25 days

pH : 1.4, 1.8, 1.9, 2.0, 2.1, 2.2, 2.5, 2.6, 2.7 and 2.8

Table - 1
Study of the effect of different carbohydrates on bioproduction of citric acid by *Aspergillus oryzae*-368

S. No.	Carbohydrates substrates used	Yield of Citric acid* in g/100 ml	Sugar left unfermented g/100 ml.
1	Arabinose	1.535	-
2	Rhamnose	1.444	-
3	Xylose	2.115	-
4	Glucose	4.360	-
5	Fructose	5.410	-
6	Galactose	6.116	-
7	Sorbose	1.690	-
8	Lactose	3.112	-
9	Sucrose	6.415	-
10	Maltose	5.773	-
11	Starch	1.005	-
12	Inuline	1.112	-
13	Dextrine	1.624	-
14	Raffinose	1.318	-
15	Mannitol	3.201	-
16	Molasses**	6.920 20.5% (w/v)	-

Table -2
Effect of concentration of molasses substrate, pH, temperature and incubation period on parametric determination for citric acid by *Aspergillus oryzae* -368

% of Molasses	pH	Temp. in (^{0}C)	Incubation Period in days	Corresponding yield of citric acid* in g/100ml			
5.5	1.4	15	5	1.610	1.995	2.475	1.683
10.5	1.8	20	7	2.118	2.115	3.118	2.016
15.5	1.9	25	8	3.228	3.263	5.670	3.132
20.5	2.0	30**	9	6.810***	4.178	6.836***	4.163
25.5	2.1	32	10	6.110	5.373	5.050	6.118
30.5*	2.2**	34	12**	5.346***	6.880***	4.217	6.860***
35.5	2.5	36	15	4.275	6.085	3.001	5.311***
40.5	2.6	38	18	3.110	4.110	2.269	4.010
45.5	2.7	40	20	2.050	3.116	2.015	3.002
50.5	2.8	42	25	1.115	1.865	1.115	2.105

* Each value represents mean of three trials. ** Optimum values of molasses solution, pH, temperature and incubation period *** Optimum yield of citric acid

Results and Discussion

The data recorded in the table-1 shows that a 20.5% molasses solution serves as an optimum substrate solution for the for the production of citric acid. It is obvious that higher concentration of molasses interferes with the production of citric acid producing enzyme activity of *Aspergillus oryzae*-368 and inhibits the yield of citric acid.

The results recorded in the table-2 show that *Aspergillus oryzae*-368 attains its best activity when the pH of the medium is maintained at 2.2. In much acidic medium, *Aspergillus oryzae*-368 failed to give significant yield of citric acid. However, at pH 2.5 and onwards the yield of citric acid has been found discouraging and insignificant.

It is obvious from the table-1 that *Aspergillus oryzae*-368 shows its best activity at the temperature 30^{0}C. Lower temperature of the experiment at 15, 20 and 25^{0}C caused discourasing yield of citric acid. Higher temperatures, i.e; 32^{0}C and onwards also deactivated the enzymatic system of citric acid fermentation process and the yield of citric acid was very much insignificant.

It is also obvious from the result that an incubation period of 12 days is most favourable for citric acid production by *Aspergillus oryzae*-368. No increase in the yield of citric acid has been observed even after incubation period of 15 to 25 days.

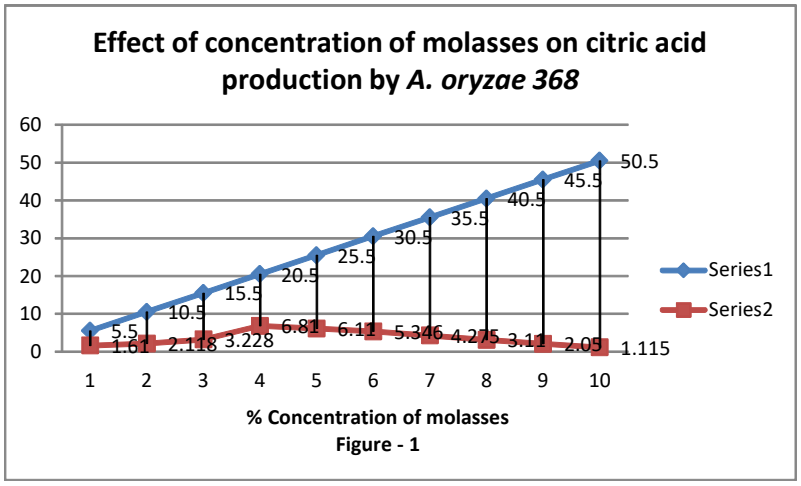

Figure - 1

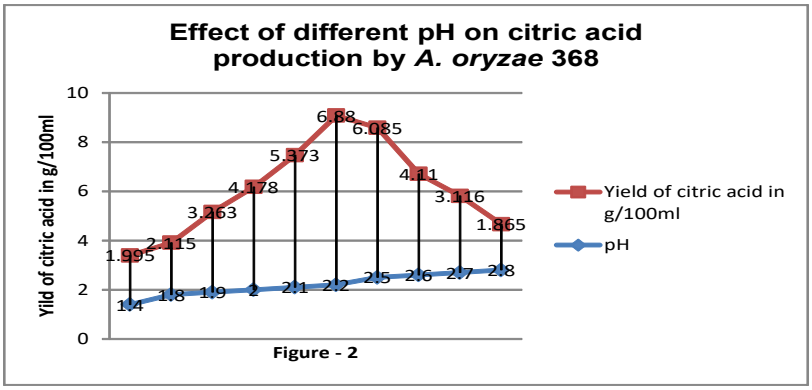

Figure - 2

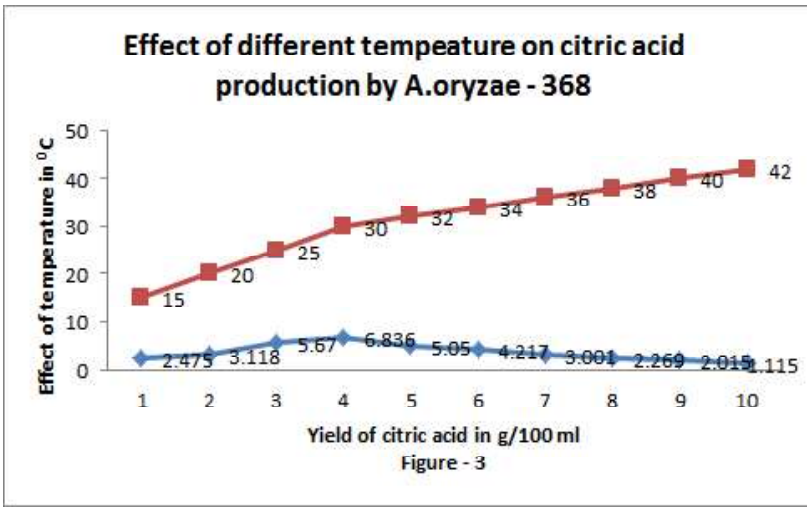

Figure - 3

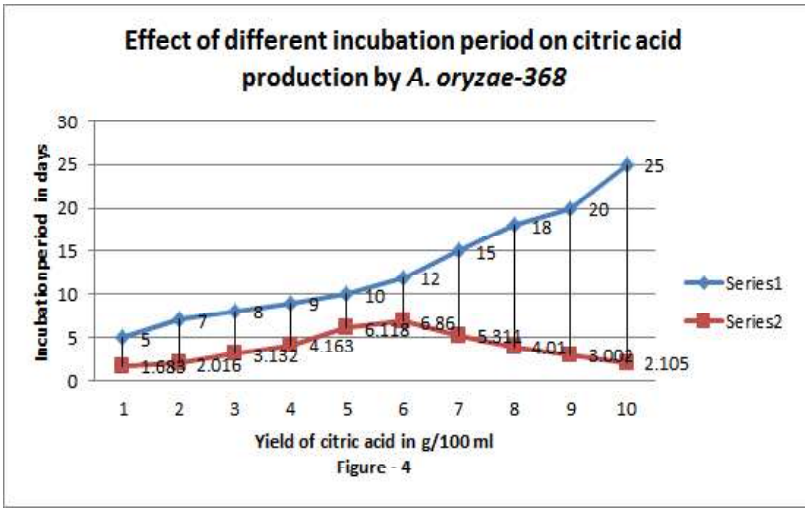

Figure - 4

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