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Citric acid Production from whey by *Aspergillus niger*

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Abstract:*Aspergillusniger*is used for the production for citric acid from whey, it shows the maximum citric acid production. This work is focused on the production of citric acid from whey using different concentrations of sugars. Citric acid, biomass and pH values from whey (with 15% sucrose) fermentation media by *A.niger*during 20 days, Biomass increase continues to the 20th day. The pH value decreases from 3.0 initially to 1.5 after 15 days

Keywords: Acid, Biomass, pH, Whey, Sucrose

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I. INTRODUCTION

Citric acid is a weak, tricarboxylic, colorless, odorless, acid which is soluble in water and alcohol with a pleasant taste .It is widely used as an acidifying agent and antioxidant in food and beverages and pharmaceutical industries. The food industry is the largest consumer of citric acid, using almost 70% of total production, followed by about 12% of pharmaceutical industries and 18% for other applications. An isolate of *Aspergillusniger*was evaluated for citric acid production and enriched protein mycelium using whey and molasses for the fermentation medium. The results indicated that acid pretreated cane and beet molasses yielded 74.32 and 75.14% citric acid and 32.99 and 34.17% proteins. Ammonium nitrate (0.3, w/v) was the best additional nitrogen sources for protein and citric acid production in presence of whey and (15%; v/v) acid pretreated beet molasses. The pH 5.5 was optimum for dry cell mass (15.64 g L-1) and protein content (45.8%) while; pH 6 was the optimum for citric acid production (47.63 g L-1). Time course study during citric acid fermentation by *A. niger*showedthat 4 days was optimum for dry cell mass, economic coefficient (EC) and protein conversion coefficient (PCC), while 6 for maximum protein and citric acid production. The chemical composition of *A. niger*grown under optimal culture conditions showed that the mycelium was rich in protein (29.04%), carbohydrates (soluble 10.32% & non-soluble 34.86%), crude lipids (6.37%), nucleic acids (RNA/DNA;4.46/0.83%) and uric acid (0.43 mg g-1). Toxigenic activity of *A. niger*showed no toxin production of B1, B2, G1 and G2. Profile of *A. niger*protein showed amino acids in sufficient amounts. Therefore, whey and beet molasses were optimized as the basal fermentation medium for maximal citric acid production as well as nutritional up-grading of these wastes for single cell protein (SCP) production by *A. niger*for animal feed.(El-Aasar 2006).

Submerged citric acid fermentation by *Aspergillusniger*using blackstrap molasses as the basal fermentation media is used. A laboratory scale stirred fermenter of 15-Lcapacity having working volume of 9-L was used for cultivationCitric acid is a weak, tricarboxylic ,colorless,odorless,acid which is soluble in water and alcohol with a pleasant taste .It is widely used as an acidifying agent and antioxidant in food and beverages and pharmaceuticalindustries. The food industry is the largest consumer of citric acid, using almost 70% of total production, followed by about 12% of pharmaceutical industries and 18% forother applications and nutritional analysis. Among the10 stock cultures of *Aspergillusniger*, the strain GCBT7was found to enhance citric acid production. This strainwas subjected to parametric studies. Major effects werecaused due to oxygen tension (1.0 l/l/min), pH value (6.0)and incubation temperature (30ºC). All fermentationswere carried out following the growth on 150 g/l rawmolasses sugars for 144 hours. Ferrocyanide (200 ppm)was used to control the trace metals present in themolasses medium. Ammonium nitrate (0.2%) wasadded as nitrogen source. Maximum citric acidproduction (99.56 ± 3.5a g/l) was achieved by *Aspergillusniger*GCBT7. The dry cell mass and sugar consumptionwere 18.5 and 96.55 g/l, respectively. The mycelia were intermediate round pellets in their morphology. Thespecific productivity of GCBT7 (qp = 0.074 ± 0.02a g/gcells/h) was several folds higher than other strains. Thespecific production rate and growth coefficient revealedthehyperproducibility of citric acid using mutantGCBT7. **(**Sikander Ali and et,al 2002).

II. METHODOLOGY

Submerged Fermentation

The submerged technique is widely used for citric acid production. It is estimated that about 80% of world production is obtained by submerged fermentation. This fermentation process employed in large scale requires more sophisticated installations and rigorous control.On the other hand,it presents several advantages such as higher productivity and yield lower labor costs,lower contamination risk and labor consumption.

Submerged fermentation can be carried out in batch, fed batch or continuous systems,although the batch mode is more frequently used.Normally, citric acid fermentation is concluded in 5 to 12 days, depending on the process conditions.

Surface Fermentations

Liquid surface culture is the classic citric acid production process and was the first industrial manufacture; submerged fermentations were developed only after that.Surface fermentation is still used in industries of small and medium scale because it requires less effort in operation, installation and energy cost.

The process is carried out in fermentation chambers where a great number of trays isthe arranged in shelves. The culture solution is held in shallow trays with capacity of 0.4 to 1.2$m^3$and the fungus develops as a mycelia mat on the surface of the medium. The Culture Solution is held in shallow trays with capacity of 0.4 to 1.2m^3 and the fungus develops as a mycelial mat on the surface of the medium. The trays are made of high purity aluminium, special grade steel or polyethylene,however steel trays supply better yields of citric acid.The fermentations chambers are provided with an effective air circulation, which passes over the surface inorder to control humidity and temperature by evaporative cooling.This air is filtered through a bacteriological filter and the chambers should always be in aseptic conditions and must be conserved principally during the first two days when spores germinate.The most common contaminations are mainly caused by pencillia,other aspergilla, yeasts and lactic bacteria.

During fermentation, which is completed in 8 to 12 days, high amount of heat is generated, so high aeration rates are needed in order to control the temperature and to supply air to the microorganisms. After fermentation, the tray contents are separated into crude fermentation, fluid and mycelial mats which are washed to remove the impregnated citric acid.

Solid-State Fermentation

Solid-state fermentation, also known by Koji process,was first developed in Japan where abundant raw materials such as fruit wastes and mainly rice bran are available. It is the simplest method for citric acid production and it has been an alternative method for using agro-industrial residues solid state culture is characterized by the development of microorganism in a lower-water activity environment on an insoluble material that acts both as physical support and source of nutrients.Some similarities are observed with the surface process since the fungus also develops on material surface.The substrate is solid and it is moistened to about 70% moisture, depending on the substrate adsorption capacity.The initial pH of the material is normally adjusted to 4.5-6.0 and the temperature of the incubation is about 28º-30ºC,depending on the microorganism used.The solid culture process is completed within 96 hours under optimal conditions.

The most common organism used in solid-state fermentation is *A.niger* . However, there have also been reports with yeasts.The strains with large requirements of nitrogen and phosphorus are not ideal microorganism for solid state culture due to lower diffusion rate of nutrients and metabolites occurring at lower water activity in solid- state process.The presence of trace elements may not affect citric acid production so harmfully it does in submerged fermentation, thus, substrate pretreatment is not required.The is one of the advantages of the solid culture

III. MATERIAL AND METHODS

A.niger

A.niger ATCC 9642 stock culture was reactivated and cultivated by streaking a loop full of the culture on petri dishes containing solidified acidified (with 10% tartaric acid) potato dextrose agar(PDA) and incubated at 25ºC for 5 days. Spores formed were washed out twice with 10ml distilled sterilized water each time. Spore suspensions containing about log 8/ml were prepared and used as inoculums for the fermentation process.

Fermentation Media

Whey prepared in the laboratory was used as the basal fermentation media

. Sucrose,maltose,glucose,fructose sugar solutions of 5,10,15%(w/v) each were added to the whey in the fermentation process.Different concentrations of tricalcium phosphate(TCP),Methanol(1,2,3,4 and 5%) and riboflavin(10,20,30,40and 50mg/L) were also used to fortify the fermentation media.Surface liquid culture

fermentation process was carried out in a 500 ml Erlemeyer flask containing 100 ml media .Each flask was inoculated with the given spore suspensions and incubated at 300C for upto 20 days.

Citric acid determination

Citric acid was determined titrimetrically(AOAC,1995) by using 0.1N NAOH and phenophthalin indicator and calculated according to the formula:

$$\%CA = \frac{\text{Normality} \times \text{Volume of NAOH} \times \text{Equiv. wt of CA}}{\text{Weight of sample (g)} \times 10}$$

Biomass and pH Estimation

Biomass and pHwere determined according to AOAC,(1995). To determine biomass, the whole fungal culture growth was filtered with Whatman filter paper, washed with distilled water (250ml) and dried at 105ºC to constant weight.Results were expressed as g/l.Culture pH was adjusted to 3 using 1N of HCl and/or NAOH.

IV. PERFORMANCE EVALUATION

Table 1: Citric acid production from whey with different sucrose concentration

Media	**Citric acid (g/L) during incubation time (days)**						
Days	**4**	**6**	**8**	**10**	**12**	**14**	**16**
Whey	0.8	1.31	2.45	2.26	0.15	0.0	0.0
Whey+5% sucrose	5.57	13.41	14.51	0.95	0.0	0.0	0.0
Whey+5% +10% sucrose	4.87	9.18	15.57	27.50	37.60	34.20	19.95
Whey +15% sucrose	4.15	9.15	18.27	47.56	50.50	80.01	108.50

Table 2: Citric acid production from whey with different maltose concentration

Media	**Citric acid (g/L) during incubation time (days)**						
Days	**4**	**6**	**8**	**10**	**12**	**14**	**16**
Whey	0.8	1.31	2.45	2.26	0.15	0.0	0.0
Whey+5%Maltose	4.57	12.41	13.51	0.90	0.0	0.0	0.0
Whey+10% Maltose	4.56	8.18	12.57	23.50	35.60	32.20	15.95
Whey +15% Maltose	4.01	8.15	17.27	40.56	49.50	70.01	90.50

Table 3: Citric acid production from whey with different glucose concentration

Media	**Citric acid (g/L) during incubation time (days)**						
Days	**4**	**6**	**8**	**10**	**12**	**14**	**16**
Whey	0.8	1.31	2.45	2.26	0.15	0.0	0.0
Whey+5% glucose	2.50	7.90	2.40	0.0	0.0	0.0	0.0
Whey+10% glucose	1.95	3.70	21.10	25.64	3.79	0.0	0.0
Whey +15% glucose	2.00	4.30	14.40	30.35	54.15	46.15	29.98

Table 4: Citric acid production from whey with different fructose concentration

Media	**Citric acid (g/L) during incubation time (days)**						
Days	**4**	**6**	**8**	**10**	**12**	**14**	**16**
Whey	0.8	1.31	2.45	2.26	0.15	0.0	0.0
Whey+5% fructose	2.07	5.39	9.24	0.99	0.1	0.0	00
Whey+10% fructose	2.07	3.87	7.25	15.75	32.229	31.15	17.12
Whey +15% fructose	1.80	2.85	9.25	23.90	40.85	48.20	58.60

Table 5: Citric acid production from TCP

Media	Citric acid (g/l) during different incubation time (days)							Biomass (g/l) after 16 days
	4	6	8	10	12	14	16	
Whey	0.8	1.31	2.45	2.26	0.15	0.0	0.0	16.3
Whey + 15% sucrose	4.15	9.15	18.27	47.56	50.50	80.01	108.50	38.5
Whey + 15% sucrose + 1% methanol	0.63	1.17	3.59	10.53	36.65	69.14	92.35	39.5
Whey + 15% sucrose + 2% methanol	0.30	0.35	0.90	3.95	15.89	50.86	78.48	34.3
Whey + 15% sucrose + 3% methanol	0.97	3.85	4.20	4.12	4.76	4.89	12.50	15.5
Whey + 15% sucrose + 4% methanol	0.65	2.67	3.50	3.56	3.57	5.56	11.13	7.7
Whey + 15% sucrose + 5% methanol	0.47	2.20	2.56	2.57	3.81	5.46	5.32	6.8

Table 6: Citric acid production from methanol

Media	Citric acid (g/l) during different incubation time (days)							Biomass (g/l) after 16 days
	4	6	8	10	12	14	16	
Whey	0.8	1.31	2.45	2.26	0.15	0.0	0.0	16.3
Whey + 15% sucrose	4.15	9.15	18.27	47.56	50.50	80.01	108.50	38.5
Whey + 15% sucrose + 1% tricalcium phosphate	5.12	10.45	19.0	26.34	32.4	69.12	92.50	39.5
Whey + 15% sucrose + 2% tricalcium phosphate	4.45	8.26	15.99	17.98	27.95	32.40	31.23	35.4
Whey + 15% sucrose + 3% tricalcium phosphate	6.50	11.50	13.60	20.77	27.69	33.15	30.20	35.1
Whey + 15% sucrose + 4%tricalcium phosphate	3.30	10.53	16.8	23.15	30.70	40.56	45.58	37.6
Whey + 15% sucrose + 5% tricalcium phosphate	3.95	7.60	10.08	15.45	23.30	41.20	5.48	32.3

Table 7: Citric acid production from riboflavin

Media	Citric acid (g/l) during different incubation time (days)							Biomass (g/l) after 16 days
	4	6	8	10	12	14	16	
Whey	0.8	1.31	2.45	2.26	0.15	0.0	0.0	16.3
Whey + 15% sucrose	4.15	9.15	18.27	47.56	50.50	80.01	108.50	38.5
Whey + 15% sucrose + 10 mg/l riboflavin	1.65	3.95	20.85	43.37	62.30	50.30	17.40	43.5
Whey + 15% sucrose + 20mg/l riboflavin	1.25	3.10	7.85	22.54	43.15	45.62	30.65	44.6
Whey + 15% sucrose + 30 mg/l riboflavin	0.45	1.94	6.25	19.58	47.54	55.48	43.30	47.5
Whey + 15% sucrose + 40mg/l riboflavin	0.55	2.75	8.67	26.54	43.65	49.85	33.49	43.5
Whey + 15% sucrose + 50mg/l riboflavin	0.13	2.00	6.33	22.40	36.65	46.50	31.00	43.5

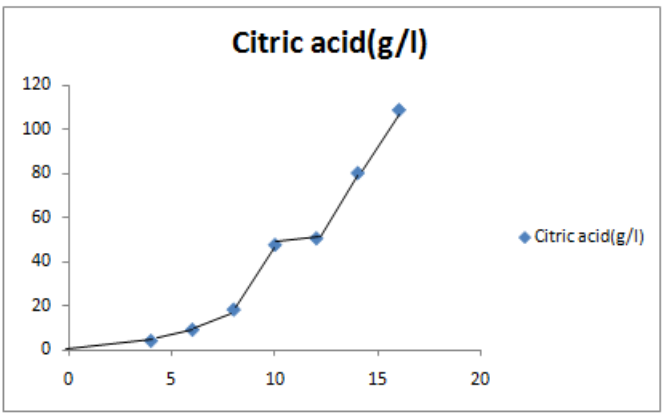

Fig 1: Citric acid production

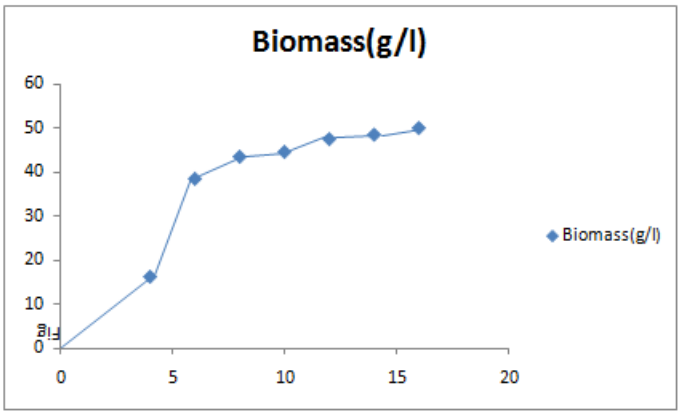

Fig.2: Biomass optimization

Table 1-4 shows citric acid production by *A. niger* from whey as a basic fermentation media, and with different concentrations of sucrose, maltose, glucose and fructose respectively. Low amount of citric acid (2.45g/l) was produced from whey alone. Adding different sugars to whey enhanced citric acid production with a maximum value of 108.50g/l with 15% sucrose. Significantly low values were obtained using same concentrations of other sugars. The poor citric acid production from whey alone is believed to be atleast partly due to presence of galactose moiety of lactose in the whey. (Hossain*et al*, 1984).

V. CONCLUSION

The result of the different concentrations of each riboflavin, tricalcium phosphate and methanol added to the whey media containing 15% sucrose on citric acid and biomass production are presented in table 5-7.The highest amount of citric acid values of 92.35-108.50g/l were produced in the whey media containing 15% sucrose with or without 1% methanol respectively. Much lower citric acid was produced with the addition of riboflavin and tricalcium phosphate throughout 16 days fermentation period. High methanol concentration caused drastic decrease in citric acid production reaching its minimum 5.32 g/l with the addition of 5% methanol. Citric acid values steadily increased with incubation time. Relatively higher biomass values (43.5-47.5g/l) were found in the cultures containing riboflavin after 16 days. Lower values (32.3-37.6g/l) were recorded in the cultures with tricalcium phosphate. Biomass in the culture containing methanol decreased from 92.35g/l with 1% methanol to 5.32g/l with 5% methanol.Citric acid, biomass and pH values from whey (with 15% sucrose) fermentation media by *A. niger*during 20 days are presented in Figure 3-6. Biomass increase continues to the 20th day. The pH value decreases from 3.0 initially to 1.5 after 15 days.

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