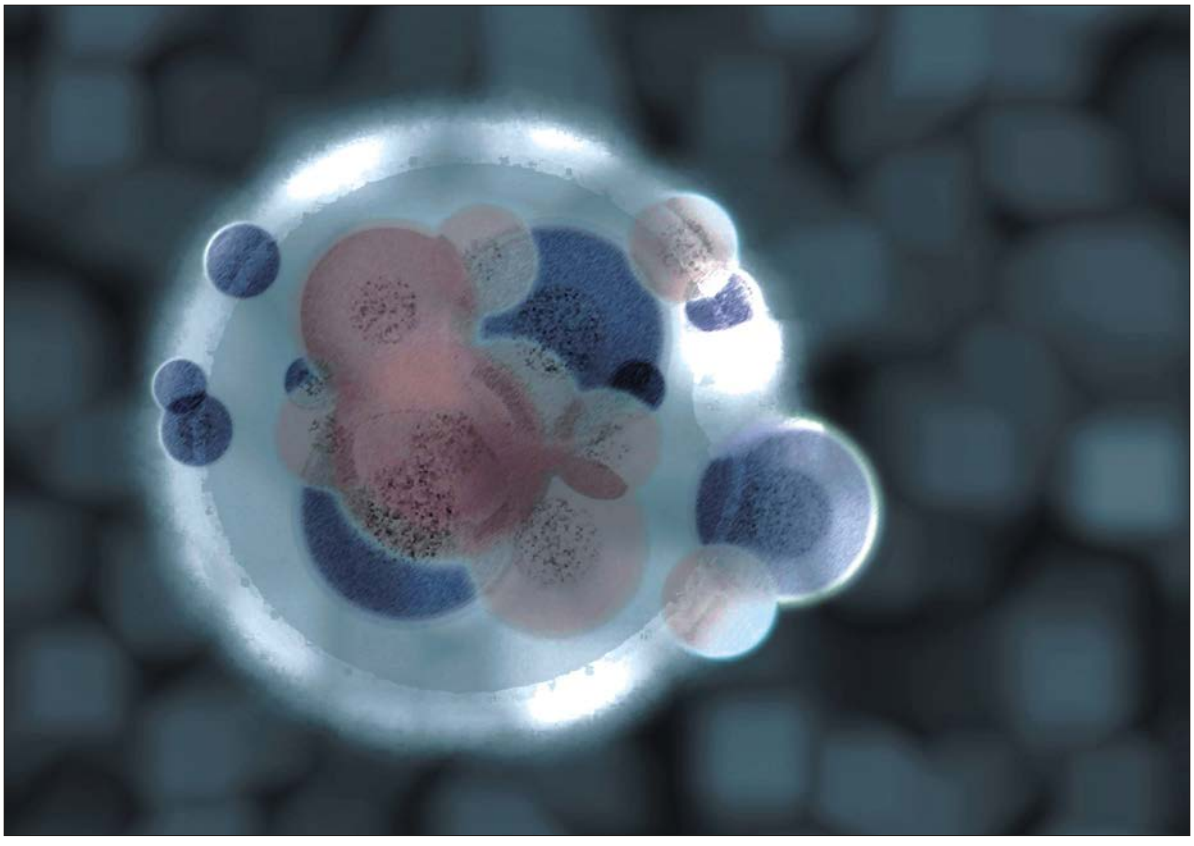

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Production of Citric Acid from Waste Bread by *Aspergillus niger*

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Abstract: *Aspergillus niger* showed maximum (7.25 mg/ml) citric acid production after 48 hours incubation in waste bread (1%) medium at pH 4.0 and 37ºC . Urea was found to be the best additional nitrogen source amongst $(NH_4)_2SO_4$, NH_4NO_3 and $(NH_4)_2HPO_4$ and it yielded optimal citric acid at 0.4% level in the optimum growth medium. Continuous shaking (120 rpm) fermentation proved to be the best fermentation mode followed by intermittent and still culture fermentation. Recovery trial yielded 6.17 mg citric acid in the form of solid, white crystals from 100 ml of culture filtrate containing 7.25 mg/100 ml citric acid.

Key words: Citric acid; waste bread; *A. niger*, Urea, continuous shaking fermentation

Introduction

Citric acid is a tribasic hydroxy acid first isolated from lemon juice and crystallized as a solid substance by Scheele (1784). It is found as a natural constituent of a variety of fruits. However, members of the citrus family are especially rich in citric acid (Reed, 1982).

Pure citric acid is colorless, odorless and easily soluble in water and alcohol. It is readily eliminated from the human body. as it is easily oxidized via Kreb's cycle and is therefore, harmless (Reed, 1982).

It is one of the most versatile industrial organic acids that are used in food preparations, cosmetics and pharmaceuticals. About 70% of citric acid is utilized in food industry, confectionary and beverages as an acidulant, flavor enhancer, preservative, chelator, buffer, emulsifier, stabilizer and antioxidant. About 10% is used in cosmetics and pharmaceuticals while remainder is employed for diverse industrial processes (Kubicek *et al*., 1985). In the present study, waste bread was used as cheaper substrate and *Aspergillus niger* as fermentative organism for the production of citric acid.

Materials and Methods

Substrate: Waste bread was obtained from "Students Mess". Tariq Hall, University of Agriculture, Faisalabad. It was dried, ground (40 mm mash) and used as a substrate for citric acid production.

Fermentative organism: Pure culture of *Aspergillus niger* obtained from Dept. of Vet. Microbiology was raised on potato starch agar slants sporulation medium (Irshad, 1999) and incubated aerobically at pH 4 and 37°C for 72 hours. For the preparation of inoculum, the spores were directly transferred into conical flask containing 100 ml pre-sterilized glucose solution (inoculum medium). Conical flask containing 100ml of waste bread medium (1.5%) and varying concentrations of urea and micronutrients were sterilized and inoculated with 5 ml of homogenous spore suspension (1.52×10^7 spores/ml). The flasks were incubated at 37°C under continuos shaking condition (120 rpm) for optimum fermentation period. The fermented biomass was filtered and filtrate was centrifuged at 10,000 rpm for 15 minutes (Nadeem *et al.,* 2000). The supernatants were analyzed for determination of citric acid content.

Optimization of Conditions: The study implicated the optimization of different culture conditions. Firstly, the growth medium of waste bread was fermented for 12, 24, 36, 48 and 60 hrs. for optimization of fermentation period with *Aspergillus niger*.

Fermentation media containing different levels of waste bread were then incubated at pH 4 and 37°C for optimum period (48 hours). In the subsequent experiment, growth media of 1% waste bread adjusted at pH values 2,3,4,5 and 6 were fermented for 48 hours). Effect of varying concentrations of nitrogen sources like urea, $(NH_4)_2SO_4$, NH_4NO_3 and $(NH_4)_2HPO_4$ on citric acid yield was studied in independent experiments. Waste bread (1%) media with different nitrogen sources were adjusted at pH 4 and fermented for 48 hours at 37°C.

The effect of mode of fermentation on citric acid yield was also investigated under optimum conditions. Optimum growth media were fermented for 48 hours, under continuous shaking, intermittent shaking and still-culture conditions.

Analytical: Citric acid in culture filtrate was determined by adopting the spectrophotometric method (430 nm) of Marier and Boulet (1958). Recovery trial of citric acid was carried out by the method of Heading and Gupta (1975).

Results and Discussion

Fermentation period: It was observed that the citric acid production increased by increasing the fermentation period upto 48 hours but decreased there after. The medium fermented for 48 hours showed maximum citric acid production (Table 1). The results are in line with those of Najam (1994) who obtained maximum citric acid in bogasse, maize and wheat bran medium after 48 hours.

Table 1: Citric acid production at different fermentation periods and substrate levels

Fermentation Period (hrs.)	Citric acid (mg/ml)	Substrate: water ratio (%)	Citric acid (mg/ml)
12	2.04	0.5	2.92
24	2.84	1.0	4.23
36	3.60	1.5	3.47
48	4.22	2.0	2.86
60	3.67	2.5	2.53

Table 2: Citric acid production with different nitrogen sources

Concentration (%)	Citric acid production (mg/ml)			
	Urea	$(NH_4)_2SO_4$	NH_4NO_3	$(NH_4)_2HPO_4$
0.1	5.06	5.25	5.18	5.24
0.2	5.76	6.75	5.62	5.72
0.3	6.41	7.10	6.17	6.09
0.4	7.25	6.82	6.92	5.78
0.5	6.76	6.57	6.45	5.16

Substrate: Water Ratio: Growth media containing different levels of substrate were fermented with *A. niger* for 48 hours. The results showed maximum citric acid production with 1% waste bread in the growth medium (Table 1). Xu *et al.* (1989) used 10% concentration of sugar for the optimum citric acid

production. Ajibad and Christine (1980) used 16% molasses for maximum citric acid production. The variation between the findings may be due to difference in substrates used, under different conditions.

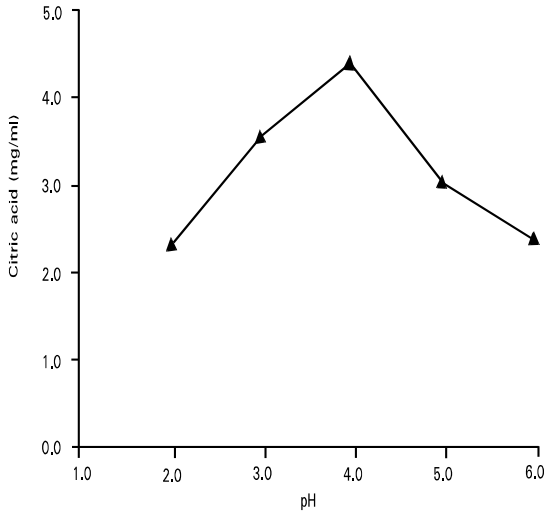

Fig. 1: Effect of pH on production of citric acid

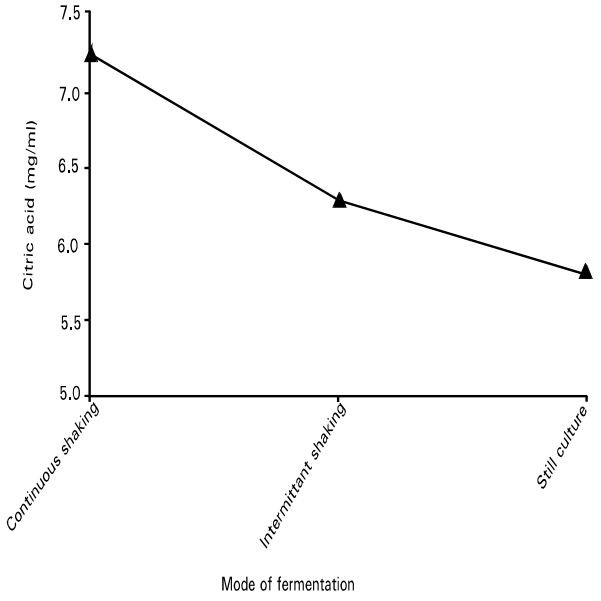

Fig. 2: Effect of fermentation mode on production of citric acid

pH of Growth Media: Growth media of 1% substrate were adjusted at different pH values viz., 2, 3, 4, 5, and 6 and harvested after 48 hours incubation at 37°C. Maximum citric acid production was observed in the growth medium adjusted at pH 4 (Fig. 1) which decreased gradually up to pH 6. These results are in accordance with Banik (1975) who reported pH 3.5 for optimum citric acid yield from sucrose. Najam (1994) reported maximum citric acid production at pH 5 using *A. niger.*

Nitrogen Sources: Growth media of waste bread containing varying concentrations of urea, $(NH_4)_2SO_4$, NH_4NO_3 and $(NH_4)_2HPO_4$ at pH 4 were fermented for 48 hours at 37°C. It was observed that 0.4% urea in the optimum growth medium gave more citric acid yield than its other concentrations as well as concentrations of other nitrogen sources. Maximum citric acid was produced in the medium containing 0.4% urea followed by 0.3% $(NH_4)_2SO_4$, 0.4% NH_4NO_3 and 0.3% $(NH_4)_2HPO_4$ (Table 2). Abou-Zeid and Muhammad (1984) observed that urea and NH_4 salts could be used as source of nitrogen for citric acid production. Garg and Sharma (1991) reported that NH_4NO_3 as better nitrogen source for citric acid production. The difference of substrates used, organism and optimum conditions may be the source of variation.

Mode of fermentation: Optimum growth media of waste bread were subjected to different modes of fermentation like continuous shaking, intermittent shaking and still culture (no shaking) for 48 hours. Growth medium subjected to continuous shaking fermentation showed (Fig. 2) more citric acid yield as compared to intermittent shaking and still-culture. Najam (1994) reported that shaking increases the citric acid production in molasses medium by *A. niger*. Results are also in line with Roukas (1991) who obtained maximum citric acid yield in shaking medium of molasses with *A. niger*.

Statistical analysis: The data obtained was subjected to statistical analysis by ANOVA under completely randomized design (CRD) (Steel and Torrie, 1984).

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