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Effect of *o*-Cresol, Resorcinol and Hydroquinone on Citric Acid Productivity by *Aspergillus niger* in Stirred Fermentor

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Effects of *o*-cresol, resorcinol and hydroquinone, which are toxic chemicals, on citric acid biosynthesis and biomass in the artificial culture setting of *Aspergillus niger* using batch fermenter are examined in the most favourable fermentation conditions and a model is proposed. According to this model, maximum citric acid concentration is 48.3 g L^{-1} in a culture that does not contain any toxic chemicals, whereas the maximum concentrations obtained in cultures containing 15 mg L^{-1} *o*-cresol, 45 mg L^{-1} resorcinol and 35 mg L^{-1} hydroquinone are 86.7, 82.8 and 86.8 g L^{-1}, respectively. Moreover, addition of toxic chemicals to the culture reduces fermentation time by 24 h.

Key Words: Fermenter, Citric acid, *Aspergillus niger*, Toxic chemicals.

INTRODUCTION

Citric acid is a primary product of *Aspergillus niger* metabolism and widely used in the food, beverage, chemical, pharmaceutical and other industries[1]. Although conventional citric acid production by submerged culture of high-yielding strains of *Aspergillus niger* has been optimized, there is still interest in redesigning the traditional manufacturing process to increase yield and subsequently minimize overall operating cost[2,3].

It is well known that citric acid fermentation is strongly influenced by a number of culture parameters including initial pH, aeration rate, temperature and trace metals in toxic concentrations. The trace elements such as iron, zinc, copper and manganese present a critical problem during submerged fermentation of crude substrates. Copper and cadmium inhibited the growth, as well as citric acid production (depending on the heavy metal concentrations) of citric acid producing *Aspergillus niger*[4]. The concentration of these heavy metals, therefore, should be decreased well below that required for optimal growth, as well as maximum citric acid production[5,6]. It has been reported that[7] citric acid production by *A. niger* under surface culture fermentation conditions using synthetic glucose as the substrate was affected by addition of toxic chemicals.

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As yet, few attempts have been made to examine the production of citric acid in erlen cultures with toxic chemicals. The present study deals with the effect of addition of phenols on citric acid synthesis by *A. niger* in a stirred fermenter.

EXPERIMENTAL

Organism and culture maintenance: The original culture of *Aspergillus niger* isolated from soy bean was used throughout this study. The cultures of *A. niger* was maintained on potato dextrose agar (PDA, Difco) slants at 4 °C and sub-cultured at intervals ranging between 15-30 d. The cultures were incubated on potato dextrose agar petri dishes at 30 °C for 7 d. The sporulated culture was scraped off and suspended in 5.0 mL of sterile-distilled water to prepare the inoculum. All culture media were sterilized by autoclaving at 121 ºC for 20 min.

Fermentation media and culture conditions: Fermentation procedure carried out in the present study was developed according to the method of Haq *et al.*[5]. The fermentation was carried out in a 5 L glass bioreactor (Unises 1 Model) with a working volume of 4 L. The medium having initial pH 3.0 was autoclaved at 121 ºC for 20 min. After cooling, the fermentation medium composed of (g L^{-1}) saccharose, 140; $(NH_4)_2SO_4$, 3.0; KH_2PO_4, 3.0; $MgSO_4.7H_2O$, 0.5; $(NH_4)_2SO_4 \cdot FeSO_4 \cdot 12H_2O$, 0.86; $ZnSO_4.7H_2O$, 0.44 and $CuSO_4.5H_2O$, 0.24 was added to the fermenter. The medium was inoculated with 5 mL of vegetative inoculum under aseptic conditions. The incubation temperature was kept at 30 ± 1 °C throughout the fermentation period of 168 h (7 d). After the aeration rate in the fermenter medium was adjusted to maximum, the experiments were carried out with a Maria Model 9071 type probe so that DO_2 values would be 8.2 mg/L. Agitation speed of the stirrer was 200 rpm. Sterilized silicone oil was used to control foaming during fermentation. At appropriate time intervals, fermentation broth (25 mL) was removed from the reactor and transferred to a weighed Whatman filter paper (no. 541) to remove mycelium. The filtrate was washed three times with distilled water, dried at 105 ºC to a constant mass and weighed as the biomass.

After the fermenter was induced to produce citric acid, *o*-cresol was added to fermenter medium so that its concentration would be 5 mg L^{-1} and the experiment which was to continue for 7 d was initiated. These procedures were repeated for each concentration, after *o*-cresol concentration in the batch fermenter medium was set to 15, 25, 35, 45, 55 and 65 mg L^{-1}. All experiments conducted for *o*-cresol were then carried out in the same way for resorcinol and hydroquinone.

Analytical methods: The citric acid and sucrose concentration were assayed by the method of Marier and Boulet[8] and Dubois *et al.*[9], respectively. Ammonium was determined by the method of Weichselbaum *et al.*[10].

RESULTS AND DISCUSSION

Results of citric acid production in batch culture are presented in Fig. 1. The process is consistent with the literature[10]. Following a 30 to 35 h period of biomass

formation, citric acid production started and reached the maximum level of 48.3 g L^{-1} in 7 d. Saccharose was consumed depending on the amount of synthesized citric acid and biomass amount and 50.0 g L^{-1} of saccharose was left in the medium after fermentation.

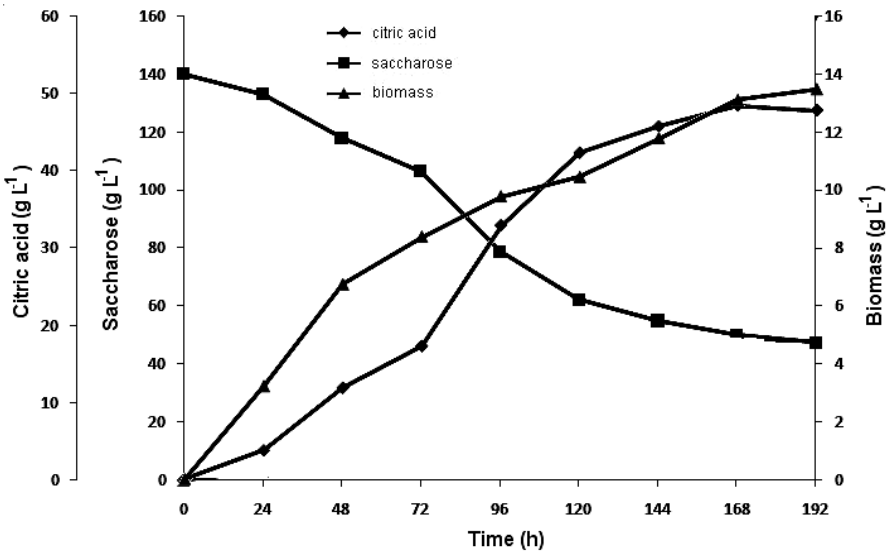

Fig. 1. Citric acid production without toxic chemicals in batch culture

An overall evaluation of results shows that maximum concentrations of citric acid obtained from a fermenter medium containing toxic chemicals are higher than those obtained from fermenter media without toxic chemicals. Furthermore, it was observed that the time that lapsed for maximum citric acid concentrations to form in the fermenter medium with toxic chemicals was 24 h shorter than that passed in the fermenter medium without toxic chemicals.

Fig. 2 demonstrates the effect of *o*-cresol concentration on citric acid, saccharose and biomass amounts. Addition of 15 mg L^{-1} *o*-cresol to the culture elevated citric acid production to 86.7 g L^{-1}. Addition of more than 15 mg L^{-1} of *o*-cresol to the fermentation medium reduce the citric acid production. The value obtained (86.7 g L^{-1}) is higher than 48.3 g L^{-1}, which is the maximum citric acid concentration of a fermenter medium that does not contain *o*-cresol.

The highest citric acid concentration of 82.8 g L^{-1} obtained in the experiments where resorcinol was used as the toxic chemical was reached with 45 mg L^{-1} resorcinol. This value is higher than the maximum citric acid concentration of 48.3 g L^{-1} obtained in the fermenter medium without resorcinol. Effects of resorcinol concentration on citric acid, saccharose and biomass concentrations are presented in Fig. 3. Addition of more than 45 mg L^{-1} of resorcinol was observed to reduce citric acid production.

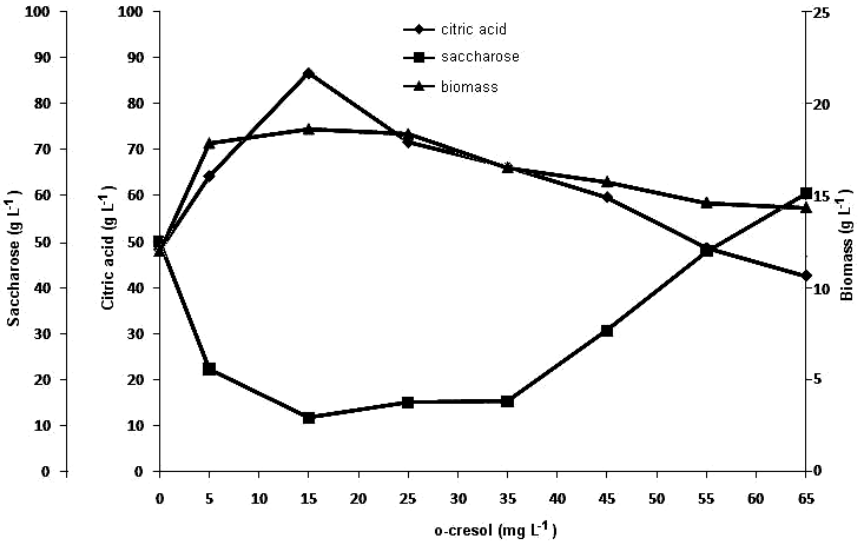

Fig. 2. Effect of *o*-cresol concentration on citric acid, saccharose and biomass concentrations

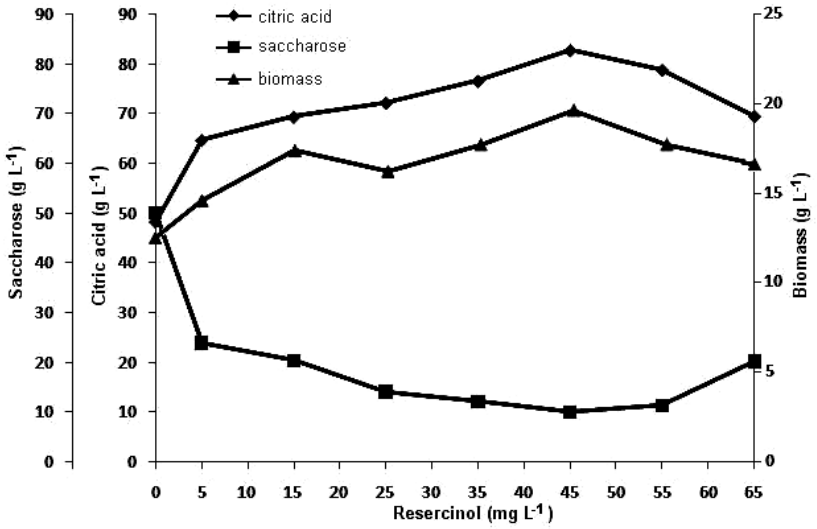

Fig. 3. Effect of resorcinol concentration on citric acid, saccharose and biomass concentrations

Fig. 4 presents the effects of hydroquinone concentration on amounts of citric acid, saccharose and biomass. Addition of 35 mg L^{-1} of hydroquinone to the culture elevated citric acid production to 86.8 g L^{-1}. Addition of a mean of 55 mg L^{-1} hydroquinone reduced citric acid production to the level of 35.0 g L^{-1}.

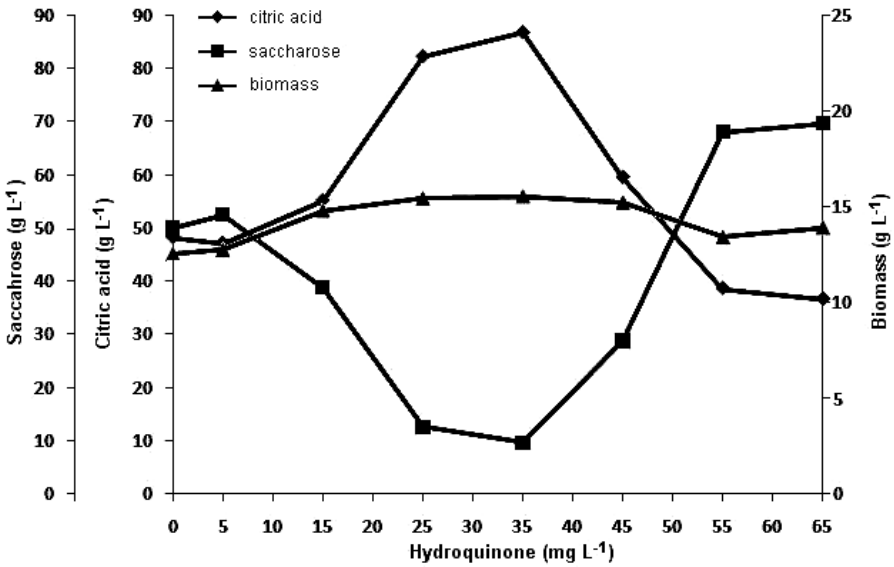

Fig. 4. Effect of hydroquinone concentration on citric acid, saccharose and biomass concentrations

All concentrations of *o*-cresol, resorcinol and hydroquinone in the studied interval added to the fermentation media led to an increase in biomass amount. It is obvious that newly formed cells will synthesize more citric acid. Previous studies showed that addition of ammonium[11] and saccharose[12] to the fermentation media caused synthesis of new cells, thus increasing citric acid production.

Similarly, acid production was increased 3 folds in another study with the addition of methyl alcohol to the culture[13]. Although the mechanism by which addition of alcohol affects citric acid production in this manner is unknown, it was assumed that methanol addition caused the microorganism tolerate[14] Fe^{2+}, Zn^{2+} and Mn^{2+}. *o*-Cresol, resorcinol and hydroquinone used in this study may form weak covalent bonds with Fe^{2+}, Zn^{2+} and Mn^{2+} found in the solution media. Unshared electrons found on the oxygen of these molecules may interact with Fe^{2+}, Zn^{2+} and Mn^{2+} ions. Acting as a sort of adsorbent, these molecules captured these elements, which are superfluous than the microorganism needs. Thus, the maximum level in acid production is reached. However, as a considerable amount of trace elements needed by the microorganism is captured when the amount of toxic chemicals increase too much, citric acid production declines markedly, dropping below the amount of citric acid produced in a culture that does not contain toxic chemicals.

In the present study, *Aspergillus niger* used in a batch fermenter medium as the microorganism, effect of toxic chemicals like *o*-cresol, resorcinol and hydroquinone on citric acid production was tested and was established that the values obtained were higher than those obtained in similar studies carried out according to erlen culture method. It is observed that the results obtained are consistent with those of

other studies[7,15]. Another reason why citric acid yield is higher than that in other studies is that DO_2 saturation in the fermenter medium was not allowed to drop below 80 % throughout the experiment[16,17]. Nitrogen concentration's [as $(NH_4)_2SO_4$] being kept at 3 g L^{-1}, instead of 2 g L^{-1}, increased citric acid yield. The results of this study are similar to the observations of Yigitoglu and McNeil[11]. Lastly, by the use of fermenter, instead of erlen culture method, it is assumed that mutation of toxic chemicals increases citric acid concentration and shortens the duration of fermentation, thereby leading to acceleration in substrate consumption[15,18,19].

In conclusion, it is suggested that addition of certain concentrations of *o*-cresol, resorcinol and hydroquinone to the culture positively affects the citric acid process.

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