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# PRODUCTION OF OXALIC ACID BY *ASPERGILLUS NIGER*

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***ABSTRACT***

*Oxalic acid has been suggested to be essential in the metal leaching processes by Aspergillus niger. The ability of Aspergillus niger strain to produce a high amount of oxalic acid on glucose and sucrose media was investigated. The experimental results show that glucose is favorable for oxalic acid biosynthesis which can produce 14.47 g/L oxalic acid compared to 7.09 g/L oxalic acid on sucrose medium. The production pattern, however, were identical on both substrates. The main drawback of this fermentation was the low yield attained (75.47 % from theoretical yield) probably because some of glucose was oxidized to gluconic acid at the beginning of fermentation, and due to some limitation of growing the* A. niger *in shake flask condition because pH of the culture cannot be fully controlled in shake flask system. Therefore, batch culture in fully controlled fermentor can be carried out as further steps of experiment after shake flask.*

*Keywords : oxalic acid, biosynthesis,* Aspergillus niger*, glucose, sucrose*

## INTRODUCTION

Oxalic acid is known as organic acid used as precipitant extracts in the rare earth metals, cleansing agents of metal equipments (China Research and Intelligence Co., Ltd., 2009), a leaching agent for solubilizing heavy metals and purification of industrial minerals such as quartz, feldspar, bentonite, and gypsum (Styriakova and Stiriak, 2000; Gharieb, 2000). Due to its high reduction power, oxalic acid can also be used for the removal of iron present in kaolin as impurity (Cameselle et.al., 2003). Furthermore, the quality of quartz sand can be improved by means of microbial leaching of iron oxide, which is especially attributed to oxalic acid excreted by the microbes (Strasser et.al., 1993, Handayani, 2004). Therefore, our biohydrometallurgy group is interested in the possibility of producing oxalic acid in high amount through this biological process.

Oxalic acid is a common metabolic excreted by several fungi under specific condition. Among other species, Aspergillus niger is favoured as good producer of oxalic acid (Rymowics and Lenart, 2004; Podgorski and Lesniak, 2003; Musial et.al., 2006; Ruijter et.al., 1999; Handayani, 2004). This research was conducted to get an optimum condition for oxalic acid production by the strain of *Aspergillus niger,* which obtained from the quartz sample of the Karimum island. The specific aim of this part of investigation was to determine the effect of medium composition (glucose and sucrose) on the growth kinetics of Aspergillus niger.

## MATERIALS AND METHODS

### Aspergillus Niger Strain

The *Aspergillus niger* used as a tester strain in this research was isolated from the quartz sample of the Karimun island. The origin and method of maintenance of this organism has been documented (Handayani, 2004).

### Shake Flask Experiment

The submerged culture media were inoculated with a spore suspension of *Aspergillus niger* at a concentration of 5 x $10^6$ spores/ml. *A. niger* was cultivated in 500 ml Erlenmeyer flask containing 200 ml of two different carbon substrates as sole nutrient solution, that were glucose and sucrose. The media consisted of the following (g/l): glucose 35

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(or sucrose 35), $NaNO_3$ 1.5, $KH_2PO_4$ 0.5, $MgSO_4.7H_2O$ 0.025, KCl 0.025, yeast extract 1.5. The nutrient solutions were buffered with TRIS at a concentration of 1 M. The pH value was adjusted with NaOH (pH 6.0) before sterilization at 121 °C for 30 minutes.

*A niger* was cultivated at room temperature on a rotary shaker at 250 rpm for 12 days. The amount of biomass, the accumulated oxalic acid, the sugar consumption and the pH of the culture medium were measured every 24 hours.

**Analytical Method**

All samples were filtrated to get particle free solution for analysis. The production of oxalic acid was determined by Bergermann and Elliot method, and the consumption of sugar was determined by Wang method (Wang, 2002). The biomass of *A. niger* grown in the presence of different carbon substrates were measured gravimetrically. The filtrated biomass was dried at 80$^o$C until the weight was constant.

The obtained data were analyzed in term of various kinetics parameters as have been reviewed by many authors (e.g. Kubicek et.al., 1987, Ruijter et.al., 1999) as follows:

1. Specific growth rate ($\mu$)
   The growth rate was measured during the exponential growth phase of the culture. The increase in biomass during an infinitely small time interval is proportional to the amount of biomass present and time interval:
   $$dx = \mu \times dt$$
   $\mu$ is constant and can be calculated from $\mu = 0.693/dt$, where dt = doubling time

2. Overall biomass yield (Yx/s)
   The growth yield is the increase in biomass as a result of the utilization of substrate:
   $$Yx/s = (x-x_o)/(s_o-s)$$
   Where $x_o$ and $s_o$ are the initial biomass and substrate concentration and x and s are the corresponding concentration during growth of the culture.

3. Overall product yield (Yp/s)
   The overall product yield can be calculated from:
   $$Yp/s = (p-p_o)/(s_o-s)$$
   Where p and $p_o$ are respectively final and initial product concentration.

4. Maximum specific substrate utilization (uptake) rate ($q_s$)
   The utilization of substrate in a culture during an infinitely small interval time can be given by :
   $$q_s = (1/Yx/s)\ \mu$$

5. Maximum specific oxalic acid production rate ($q_p$)
   $$Q_p = (Y_p/x)\ \mu$$

**RESULTS AND DISCUSSION**

The changing culture, the biomass and accumulation of oxalic acid on different media (glucose and sucrose) was followed as a function of time (Figure 1 dan 2), and a comparison of kinetics parameter data for *Aspergillus niger* grown on those different media can be seen in Table 1.

The production of oxalic acid was clearly observed on culture media containing glucose as the carbon source. The high amount of oxalic acid started after 1 week of cultivation and reaching the maximum amount (14.67 g/L) after nine days (Fig.1.). This final concentration of oxalic acid was 1.83 times higher compared to Kubicek et.al. (1987) who reported oxalic acid accumulation up to 8 g/L in the medium. The production patern of oxalic acid on sucrose medium was similar to that of glucose, but the concentration was only about half of that on the glucose medium (7.09 g/L) (Fig.2.). The growth was estimated by measuring the dryweight of *A.niger.* The highest biomass was obtained on glucose medium (1.72 g/L) after 10 days of cultivation. The maximum biomass of *A. niger* grown on sucrose medium was also much lower compared to that of glucose, only 1.09 g/L. Those results suggest that the growth was faster on glucose, as could be expected, which may also affect the general metabolic activity and the excretion of oxalic acid. However, the precise connection of those two compounds and the activities catalyzing the production of oxalic acid remains to be studied further.

From the data shown in Fig 1, 2 and Table 1, it can be seen that *A. niger* can convert glucose into oxalic acid more efficiently and more rapidly compared to sucrose. All kinetics parameters were

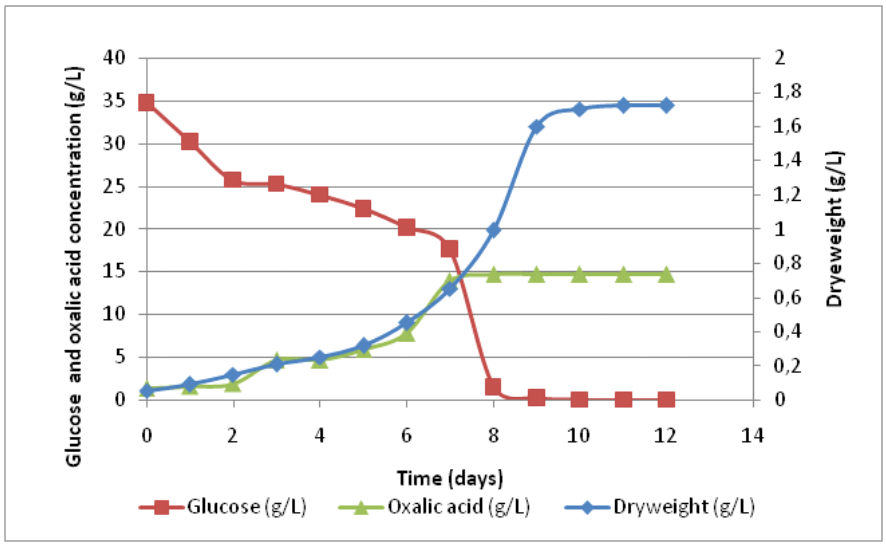


Figure 1. Batch kinetics of *Aspergillus niger* grown on glucose medium

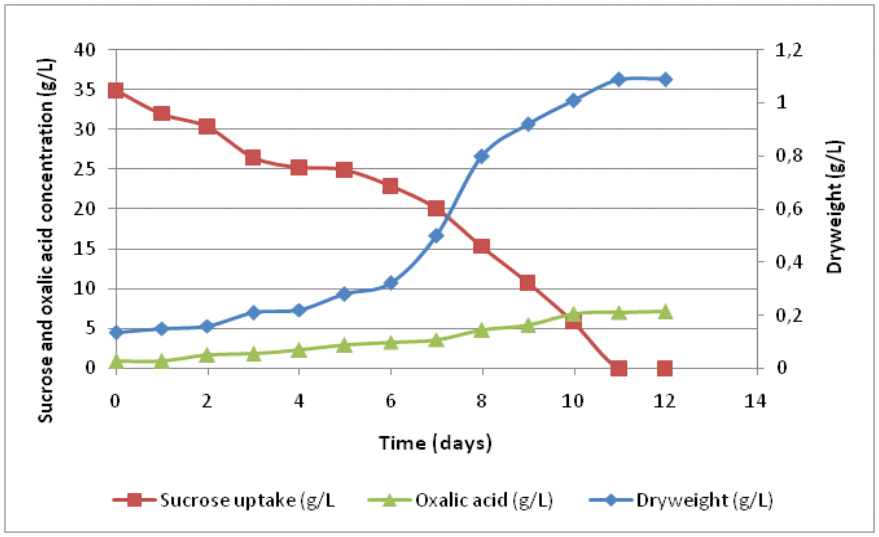


Figure 2. Batch kinetics of *Aspergillus niger* grown on sucrose medium

Table 1. Comparison of kinetics parameter data for *Aspergillus niger* grown at different media (glucose and sucrose)

| Kinetics parameters | Glucose | Sucrose |
|---|---|---|
| Maximum specific growth rate (μ) | 0.433 | 0.347 |
| Overall biomass yield (Yx/s) | 0.048 | 0.050 |
| Overal product yield (Yp/s) | 0.385 | 0.265 |
| Maximum specific substrate uptake rate (qs) | 9.039 | 6.940 |
| Maximum specific oxalic acid production rate (qp) | 3.478 | 1.833 |
| % of theoretical yield of oxalic acid | 75.49 | 51.96 |

clearly affected by the media composition. The *A. niger* had higher values of maximum specific growth rate, the overall biomass and product yield, maximum specific substrate uptake and oxalic acid production rate when they were grown in glucose medium compared to sucrose. This is probably because *A. niger* can ferment glucose as a simple sugar more readily than sucrose. A molar fermentation equation of glucose by *Aspergillus* has been reported as follows (Kubicek et.al., 1987):

1 glucose + $2CO_2$ + 2NAD → 2 oxalic acid + 2 NADH

The actual yield, however, varied depending on the strain of *Aspergillus* used and growth condition. The *Aspergillus* can also utilize sucrose by several steps; the first step is by hydrolyzing this sucrose to glucose and fructose involved invertase or with concomitant formation of levan as follows:

$nC_{12}H_{22}O_{11}$ + ROH → $R(C_6H_{10}O_5)_nOH$ + $nC_6H_{12}O_6$
sucrose acceptor levan glucose and fructose

During the exponential growth phase, the sucrose hydrolysis normally occurred rapidly to yield excess concentration of glucose. However, with growth on sucrose in this experiment, the notable differences were a reduction in oxalic acid yield (51.96%) and a lower final oxalic acid concentration (7.09 g/L) compared to glucose.

The highest oxalic acid yield and the maximum oxalic acid concentration at the end of this experiment were achieved in glucose medium, that were 0.387 (75.49 % of theoretical oxalic acid yield) and 14.67 g/L of oxalic acid. Those results were far from theoretical yield (below 90%). There were some possible reasons for it. First, perhaps there were some amount of by products produced in this process such as gluconic and citric acids that not measured in this experiment. Tkaez and Lange (2004) mentioned that *A. niger* is the source of three main organic acid: gluconic acid, citric acid and oxalic acid. Cameselle et. al. (1998) reported that glucose at pH 6 can be oxidized quickly to gluconic acid. Therefore, pH parameter should be controlled around 6 to get an optimal process condition, but this cannot be controlled in shake flask. In this experiment, the final pH was found to be varied: 4.5 (glucose) and 4.2 (sucrose). The pH decrease was mainly due to the accumulation of oxalic acid into the culture media. It is possible that changes in pH might influence the oxalic acid production as pH is known to have a major effect on the growth rate and product formation of all cultures. Kubicek et.al. (1987) reported that oxalate accumulation in *A. niger* was specifically induced by pH 6-6.5, formed by oxaloacetate hydrolase. This cytoplasmic enzyme of *A. niger* was not detectable during the growth of *A. niger* at or below pH 4. Consequently, it is essential to control the pH.

## CONCLUSION AND SUGGESTION

*Aspergillus niger* can convert glucose into oxalic acid more efficiently and more rapidly compared to sucrose. All kinetics parameters were clearly affected by the substrate composition. The best performance of this experiment was achieved in glucose medium. In this condition, the *A. niger* had higher values of maximum specific growth rate, overall biomass and product yield, maximum specific substrate uptake and oxalic acid production rate. However, the oxalic acid yield in this experiment was only 75.47 % from theoretical yield due to some limitation of growing the *A. niger* in shake flask condition because pH of the culture cannot be fully controlled in shake flask system. Therefore, batch culture in fully controlled fermentor can be carried out as further steps of experiment after shake flask.

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