Effect of aeration on the growth and sporulation of *Aspergillus niger* in cassava stalks bioconversion

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Abstract. *Aspergillus niger* is a mould which can be used in bioconversion of cassava stalks through solid-state fermentation. Mould is aerobic microorganism and require oxygen for their growth. Sufficient supply of oxygen by aeration will give better growth of mold, but excess of oxygen will act as a bad condition and encourage production of spores. Moulds at sporulation stage will not produced enzymes required for bioconversion of lignocellulosic material, for example cassava stalks. The aim of this study was to know effect of aeration on the growth and sporulation of *Aspergillus niger* in bioconversion of cassava stalks. *Aspergillus niger* FNCC 6114 were grown on media of cassava stalks powder moistened with water in 1:1 ratio, incubated inside of two kinds of incubators. The first, incubator of plastic box at 20 L capacities without aeration. The second, incubator equipped with aquarium aerator for force-aeration and IV needles for controlling the speed of aeration at 40 mL/min. Based on the result, it was shown that fermented media from aerated incubator were: higher glucosamine content, lower reducing sugar content, and higher spores quantity. These indicated that aeration at the speed of 40 mL/min will encourage growth and increase sporulation of *Aspergillus niger* FNCC 6114.

1. Introduction

Oxygen is a major component of environmental conditions for aerobic fungal growth. *Aspergillus niger* is one of the aerobic fungi. *Aspergillus niger* need oxygen during their growth. Oxygen used in aerobic respiration of fungus as the last electron acceptor in glucose oxidation for obtaining energy.

Oxygen can be provided through aeration. Aeration in solid media fermentation will also play a roles to: 1). absorb back CO\textsubscript{2}, 2). remove metabolic heat that produced during fermentation, 3). maintain temperature of media, and 4). ensure water content on the surface and inside the media [1].

The mechanism used by aeration to fulfill four functions apart from providing aerobic conditions is by aeration in the form of airflow containing oxygen. Oxygen will replace the position of CO\textsubscript{2} in the air so the air composition reaches equilibrium. The air blowing will also push the metabolic heat accumulated in the media so the temperature of the media decreases. This temperature drop will encourage condensation of steam to become water, and it will maintain the water content of the media. Aeration will also keep the level of low carbon dioxide (CO\textsubscript{2}) remain low in the inter-particle solid substrates. High level of CO\textsubscript{2} on solid media can inhibit the growth of fungus, for example, *Rhizopus oligosporus*, another kind of aerobic fungus as same as *Aspergillus niger* [2]. Low level of oxygen will lead to reduce respiration activity of *Aspergillus niger* and alter the metabolism from biomass
formation into citric acid synthesis [3]. Unfortunately, excess oxygen (at a level of 80%) will give negative effect to the growth of some moulds [4].

Sporulation or asexual spore production is the vegetative stage in moulds’ life cycle. Conidia produced by *Aspergillus niger* in abundant numbers, especially for distribution into wider areas. Spores still undergo respiration, even though at a low level [5]. In fermentation process with high number of spores, oxygen will be used for aerobic respiration inside the spores. Solid-state fermentation with low free water will prevent germination of spores into mycelium. Excess oxygen level will also lead to spores overproduction, and give negative impact on sustainability of fermentation process.

The type of aeration applied can be in the form of additional aeration and natural aeration. That is intentionally flown into the incubator or by relying on the availability of natural oxygen in the air around the media expanse, respectively [6]. The choice of aeration type depends on the ease and cost of operation. Type of incubator can be selected for the fulfilment of aeration in accordance with mould requirements but it is easy to operate at low cost.

In the fermentation process, the incubator/fermenter/bioreactor is a very important aspect of the process and becomes the core of the fermentation process. The use of an improper fermenter can hinder the progress of the fermentation process. Therefore, the fermenter model used must fulfill the need as a container to accommodate the substrate, for the microorganisms involved in the process, to protect the microorganisms used from contaminant attack, and maintain the conditions of the fermentation environment at optimal levels for the growth and product synthesis [1].

The aim of this study was found out the effect of aeration provided by additional aeration on the growth and sporulation of *Aspergillus niger* during bioconversion of cassava stalks. The effect had been evaluated through grown the fungus inside incubators had been designed in previous study, which was closed-chamber incubators. Closed-chamber incubator made of plastic container with 20 L capacity and sealed with tight plastic cover. Model B.1 was natural (no aeration), while model B.2 was additional incubator equipped with aquarium aerator and intravenous (IV) needles for controlling the speed of aeration at 40 mL/min [7].

2. Methods

2.1. Inoculum preparation

Inoculum used in this study was inoculum of *Aspergillus niger* FNCC 6114 obtained from Laboratory of Biotechnology, Faculty of Agricultural Technology, Universitas Gadjah Mada, Indonesia which served in powdery form.

Pure culture of *Aspergillus niger* FNCC 6114 in agar slant were harvested using sterilized 0.1 % Tween 80, and inoculated in sterilized solid media mixture, consisted of steamed rice, rice bran, and cassava stalks powder in a ratio of 1:1:2 (w/w). Incubation conducted for 6 days at ambient temperature. Fermented media rich of spores dried in oven at 50 °C for 2 days. Grounded, counted for spores number, and used as powdered inoculum.

2.2. Media preparation

Cassava stalks were chopped, grounded, crushed, sieved with fine size sieve of 50-100 mesh, and weighed into 160 gr and placed in perforated plastic tray of 3 L capacity, moistened with distilled water in a ratio of 1:1.5 (w/w), covered by perforated plastic cover, put in heat-resistant plastic bags, and sterilized at 121 °C for 15 minutes. Stayed overnight, and sterilized once more in same condition. Cassava stalks media used furthermore in bioconversion without any nutrients supplementation.

2.3. Solid substrate fermentation

Media prepared from previous step inoculated with powder inoculum, covered with perforated plastic lid, and incubated inside incubator model B.1 and B.2 during a process of evaluation. Samples of fermented media were taken every day until 7 days.

2.4. Evaluation of environmental condition
Environmental condition evaluated over the condition inside the incubators, and inside the fermented media. Environmental conditions inside the incubators were Relative humidity (RH) and temperature, while environmental conditions inside the fermented media were relative to water activity and pH.

2.5. Evaluation of fungal growth and metabolism

Fungus growth was evaluated through glucosamine level, while fungus metabolism was evaluated by detecting reducing sugar produced. Glucosamine was dominant in mycelium structure of fungi, while reducing glucose was dominant metabolite product from degradation of cellulose and hemicellulose of cassava stalks media.

Glucosamine detected by spectrophotometer using standard solution of N glucosamine-hydrochloride according to the method of Souza et al. [8]. Reducing sugar detected by spectrophotometer using DNS (Dinitro Salisilyc acid Solution) reagent according to the method of Miller et al. [9].

2.6. Evaluation of spores quantity

The sample preparation for spores calculation analysis was maceration (immersion) of the sample in 0.1% Tween 80 solution. The sample was then diluted in series to a dilution level of $10^{-1}$ or $10^{-2}$ depending on the density of the spores. Soaking in a Tween solution was to release off the spores. The spores calculation was carried out under a microscope on Neubauer Haemacytometer.

3. Result and Discussion

3.1. Environmental factors

Environmental factors which were evaluated were conditions inside two kinds of closed-chamber incubators namely incubator model B.1 and B.2, and inside fermented media (cassava stalks). Environmental conditions inside incubators were: 1). Relative Humidity (RH) in %, and 2). Temperature (°C), whereas conditions inside fermented media were water activity ($a_w$) and level of acidity (pH). The result of environmental evaluation shown in Figure 1.

**Figure 1.** Relative humidity (RH) and temperature inside closed-chamber incubators. (a). Incubator model B.1 and (b). Incubator model B.2, using media of cassava stalks, in a size of 50-100 mesh.

Figure 1 informs that RH and temperature inside incubator model B.2 were slightly higher than inside incubator model B.1. The incubator model B.2 was equipped with an apparatus to provide air flow passed through heated distilled water and the flow rate was adjusted using a valve from the series of infusion (intravenous) needles used. Incubator model B.2 also acted as aerated and humidified incubator.

According to Figure 1, it can be seen that maximum RH inside incubators were 82 % and 86%, respectively for incubators model B.1 and B.2. These RH levels were slightly under a level for *Aspergillus niger* optimal growth, which is 92% [10]. Temperatures inside both incubators were nearly equal, and had reached a value for optimal growth of *Aspergillus niger* [11].
Environmental conditions inside fermented media had been evaluated for the level of water activity ($a_w$) and acidity (pH). The results served in Figure 2. It is shown that $a_w$ from both two kinds of incubators gave nearly equal in a range between 0.97 until 0.99. This value are in an optimal level of $a_w$ for the growth of *Aspergillus niger*, which is higher than 0.9 [12].

![Figure 2](image)

**Figure 2.** Environmental condition inside fermented media incubated in incubator model B.1 and B.2. (a) water activity ($a_w$) level, and (b) pH level.

pH value obtained in fermented media from both incubators was not significantly different, except for fermented media from incubator B.2 at 30 hours of incubation, which was very low. This pH results can be explained through the fact that there was a problem in aerator at that time. This also prove that aeration in a form of warm and humid air gave a positive effects on controlling environmental condition inside the media.

3.2. Growth and metabolism of *Aspergillus niger*

Evaluation of growth and metabolism conducted by detection of glucosamine and reducing sugar content were shown in Figure 3 (a) and (b) respectively.

![Figure 3](image)

**Figure 3.** Growth and metabolism activities of *Aspergillus niger* inside incubator model B.1 and B.2. (a) glucosamine content, and (b) reducing sugar content.

Maximum values of glucosamine and reducing sugar content from fermented media after incubated in incubator B.2 relatively higher than B.1. These results indicate that additional aeration give better growth and metabolism, because of important roles of oxygen for aerobic fungus [13].

3.3. Effect of additional aeration on spores production

Spores produced in fermented media from two kinds of incubator served in Figure 4, which can be seen that spores quantity in incubator B.2 were higher than B.1.
Figure 4. Spore production of *Aspergillus niger* FNCC 6114 from fermented media incubated in incubator model B.1 and B.2

Oxygen will induce spores production for many reasons depending on strain of fungi used [14, 15]. In this study, addition aeration at a rate of 40 mL/min induced sporulation of *Aspergillus niger* FNCC 6114.

4. Conclusion
The results of this study can be concluded that aeration increased the growth and metabolism of *Aspergillus niger* FNCC 6114, but can not suppress sporulation.

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