# EFFECTS OF AGITATION RATE ON THE GROWTH OF *MYCENA SP* AND PRODUCTION OF ANTIFUNGAL AGENTS

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## ABSTRACT

Impeller speed or agitation rate plays a significant role in the growth of microorganism especially basidiomycetes and production of bioactive compounds via transfer of oxygen and mass. In this investigation the effects of different impeller speeds on morphology, biomass concentration and production of bioactive compounds with antifungal activity were studied using a 5-liter fermenter. It was found that use of different impeller speeds (300, 450 and 600 rpm) resulted in various growth pattern and productivity. Impeller speed of 600 rpm gave a low biomass concentration and low production of antifungal agent and the best result was obtained when impeller speed was adjusted to 450 rpm. Biomass concentration and productivity in the case of 300 rpm was less than that of 450 but higher than that of 600 rpm.

**Key words:** Mycena, Antifungal, Impeller speed, Basidiomycetes

## INTRODUCTION

Agitation rate and impeller speed play a significant role in the growth of basidiomycetes. When agitation rate is too low, oxygen and mass transfer are also low and result in a low growth and often, in the case of basidiomycetes, in the formation of large pellets. Only a few reports on basidiomycete mycelium cultivation specify the agitation conditions and therefore it is difficult to reproduce results obtained in the laboratory for the large scale operation (1). Agitation rate of 80 rpm (2 inch stroke) for growth of *Agaricus blazei* in shake flasks is reported to give low yield of mycelial pellet with average size 0.125 inch (2). Increasing the shaking frequency from 0 to 150 rpm has changed the yield and the size of mycelium of *Lentinus edodes*, and also the size of the mycelial pellets (3) and the lowest average diameter of mycelial pellets was obtained at a shaking frequency range from 150 to 200 rpm. The effect of agitation conditions on submerged culture of *Lentinus edodes* has also been reported (4). In preliminary experiments changing the agitation rate, stepwise every 10 days, the morphology of the mycelium did not change into a pulpy form during the first 30 days under the agitation condition of 350 rpm (4) but it gradually changed into a pulpy form at 400 rpm or more. The same result were also achieved by other investigations (5) where the specific growth rate of *Lentinus edodes* increased as the diameter of the mycelial pellet, which was related to the agitation condition, decreased and the specific growth rate of the pulpy form was higher than that of the pellet form. Several mushrooms e.g. *Morchella hortensis*, *Agaricus bisporus*, *Agaricus campestris* and *Agaricus blazei* develop pellets only at very low agitation rate (6). Also, agitation influences the structure and survival of pellets once they have been formed. On the basis of these reports, agitation rate has an effect on the yield of mycelial biomass and morphology of the organism and it may also change the productivity of organism. For example, loss of antibiotic activity of some streptomycete isolates in liquid culture was attributable to a fragile morphology in the isolates concerned (7). The physical damage inflicted by shake flask culture is, presumably, sufficient to cause fragmentation in these cultures and as a consequence, prevent antibiotic production. Data on the production of bioactive compounds from basidiomycetes and effect of agitation rate are not available. However, different agitation rates or impeller speeds using for production of different bioactive compounds from basidiomycetes has been reported. A 20-liter fermenter with 140 rpm has been employed for production of some antimicrobial and cytotoxic polyenes from *Mycena viridimarginata* (8). Also, for production of a new antifungal agent from culture of *Oudemansiella radicata*, a 150-liter fermenter with 100 rpm stirring has been used (9). In this investigation,

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effect of different impeller speeds on growth pattern and production of antifungal agents from a basidiomycete were studied.

## MATERIALS AND METHODS

*Culture conditions*

The fungus were collected from the north of Iran and cultivated by tissue culture method described by Mason and Jackson (10). They were identified by Professor Roy Watling from Edinbrough Botanic Garden in U.K and named *Mycena sp. Candida lypolitica* (ATCC 825). It was cultured in solid media (malt extract agar) and used as a test organism for antifungal activity. Medium used for growth and production of bioactive compound (s) was MYGP (Malt extract 10 g/l, Yeast extract 3 g/l, Mycological peptone 1 g/l and Glucose 10 g/l). The inoculum level was 10% (v/v) for each run (n=3). The fermenter used in this study was a SETRIC-set fermenter model (SETRIC Genie Industrial) with open turbine and two-pitch impeller. The pH was controlled by automatic titration with 0.1 M NaOH and 10% $H_2SO_4$. Foaming was controlled by addition of 0.5 ml polypropylene glycol 2025 (BDH Ltd.) at zero time. Antifoam was added manually when necessary, but kept in a minimum amount.

*Biomass estimation*

Five ml of fungal culture were filtered using pre dried and weighed 4.25 cm diameter Watman GF/C filters circles and a 4.25 cm Gelman filtration unit. Pellet size was determined by measurement of the diameter of 20 such pellets per culture using grid (minimum division =1mm).

*Residual carbon and nitrogen measurement*

Total carbohydrate was determined by the method of Samsel and Delap (11). The microkjeldahl method was used for determination of total nitrogen in samples by the method of Kirk and Sawyer (12).

*Gas analysis*

The percentage of $CO_2$ in the exit gas from the SETRIC fermenter was measured using infrared absorption arys analyzer. The percentage of $O_2$ in the exit gas from the SETRIC fermenter was measured using a paramagnetic oxygen analyzer (Sybron-Tylor-Servomex, Model OA 540).

*Antifungal activity*

*Candida Lipolytica* (ATCC 825) was used for growth inhibition activity of the bioactive compounds produced by selected organism as described by Warnock (13). The antifungal activity Was calculated as: (B-S)×100/0.1×D= Unit activity per ml sample where B was optical density of control= 0.4, S was optical density of sample (reducing 0.1 OD from B is equal to 100 U/ml activity) and D was dilution factor.

## RESULTS AND DISCUSSION

The lag phase at 450 rpm and 600 rpm was approximately 45 hours (Figs. 1 and 2). At 300 rpm, however the lag phase was much longer (65 hours) (Fig 3). An increase in biomass concentration was observed with increased impeller speed. Similar maximum biomass concentration were observed at 450 rpm and 600 rpm, however, the length of the growth phase was shorter at 450 rpm. One possible reason for this observation is carbon limitation, which happened after 180 hours and caused a decrease in growth of the fungus and production of the bioactive compounds. As expected, pellet size changed by increase in the impeller speed. The pellet size was reduced at 600 rpm and the fungus adopted a mycelial form rather than pellet, in the later stages of fermentation. This was similar to the result obtained at 450 rpm with the exception that during final stage of fermentation, a combination of very small pellets and mycelial form were observed. Variation in impeller speed changed growth, oxygen consumption, nutrient consumption and product formation. As shown in figures 4, 5 and table 1, increasing impeller speed from 300 rpm to 450 rpm, resulted in an increase in biomass concentration and activity of the bioactive compounds. In contrast increasing impeller speed from 450 rpm to 600 rpm caused a significant decrease in biomass concentration along with decrease in activity of the bioactive compounds compared to that obtained at 300 rpm. It can be assumed that reduction in the size of pellets may be due to the increase in impeller speed, which improves diffusion of oxygen and nutrients, thus increases the concentration of biomass and activity of the bioactive compounds. In contrast, utilization of a high impeller speed (600 rpm) caused activity of the bioactive compounds to decrease, although biomass concentration increased. This was confirmed by calculation of the yield factor, which was 0.52 using 600 rpm and 0.45 when 450 rpm was employed. At 600 rpm, most of the sugar was consumed to support biomass production rather than of antifungal agent(s), whereas at 450 rpm, sugar was consumed to support both growth and production of bioactive compounds. Another

possible reason for very low activity of the bioactive compounds using an impeller speed of 600 rpm may be the effects of mechanical stress and physical damage to the culture resulting in mycelial fragmentation. Liquid shear has been found to be a major mechanism of physical damage to filamentous fungi rather than the direct impact of the stirrer blades (14). A similar pattern has been reported during *Streptomyces* fermentations (7). In addition, increasing resistance against diffusion of nutrient components to the cells possibly caused growth and production of bioactive compounds to decrease in this study. The same observation was reported for *Lentinus edodes*. As seen with *Mycena. sp* the mycelial morphology of *L. edodes* changed gradually by increasing impeller speed, from 300 to 600 rpm. Increasing the impeller speed resulted in an increase in biomass which reached a maximum amount at 500 rpm, and then decreased at an impeller speed of 600 rpm, due to low resistance of mycelia to shear stress (4). A very dark-brownish color appeared 48 hours prior to the death phase of the fermentation process at impeller speeds of 450 and 600 rpm but not at 300 rpm. One possible reason is autolysis of the fungus due to nutrient limitation (i.e. carbon limitation).

Another possible reason is fragmentation of mycelia at high impeller speed, which results in the release of intracellular components causing the medium to become a dark-brownish color. In addition, the production of some toxic compounds at this stage is another possible explanation for this observation.

It is interesting to note that activity of the bioactive compounds declined during this period. One possible reason for this observation is the occurrence of autolysis of the fungus and possible release of intracellular components into the medium that decreases the activity of the bioactive compounds. Although autolysis was not directly apparent, there were significant changes in the morphological state of the organism by increase in branch width.

**Table 1.** Maximum biomass concentration, activity, sugar consumption, nitrogen consumption and oxygen uptake rate in different impeller speed condition

| Impeller speed (rpm) | Maximum biomass (g/l) | Maximum activity (U/ml) | carbohydrate consumption (%) | Nitrogen consumption (%) | Maximum Oxygen uptake rate (mmol $dm^{-3}$ $min^{-1}$) |
|---|---|---|---|---|---|
| 300 | 4.26 | 381 | 59.3 | 43.4 | 0.061 |
| 600 | 5.8 | 211 | 84.6 | 70 | 0.175 |
| 450 | 5.9 | 392 | 97 | 76.1 | 0.245 |

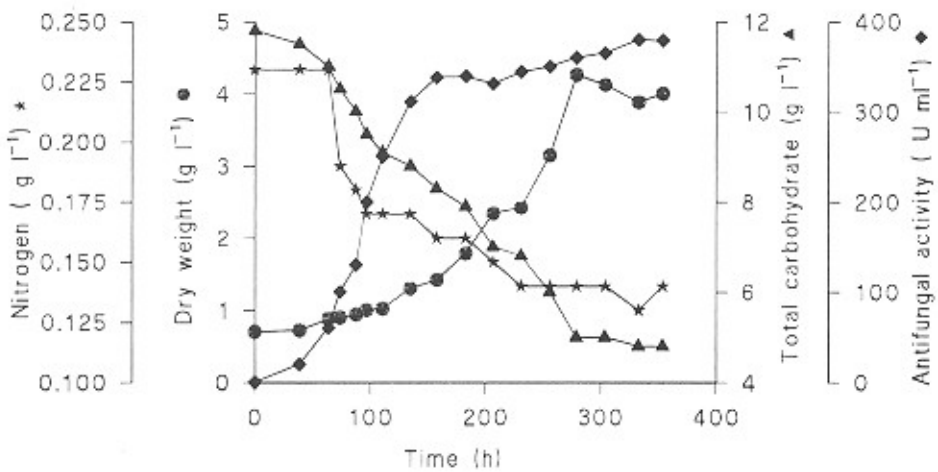


**Figure 1.** Activity of bioactive compounds, dry weight, residual carbohydrate and nitrogen V time at impellers speed of 450 rpm

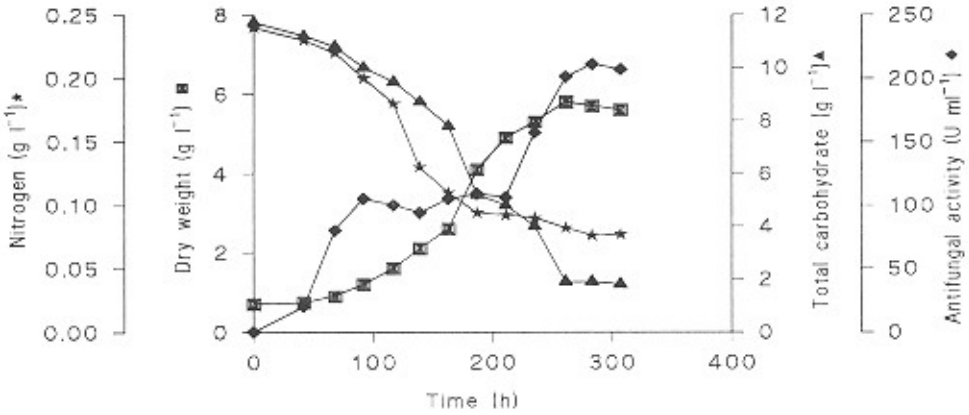


**Figure 2.** Activity of bioactive compounds, dry weight, residual sugar and nitrogen V time at impeller speed of 600 rpm

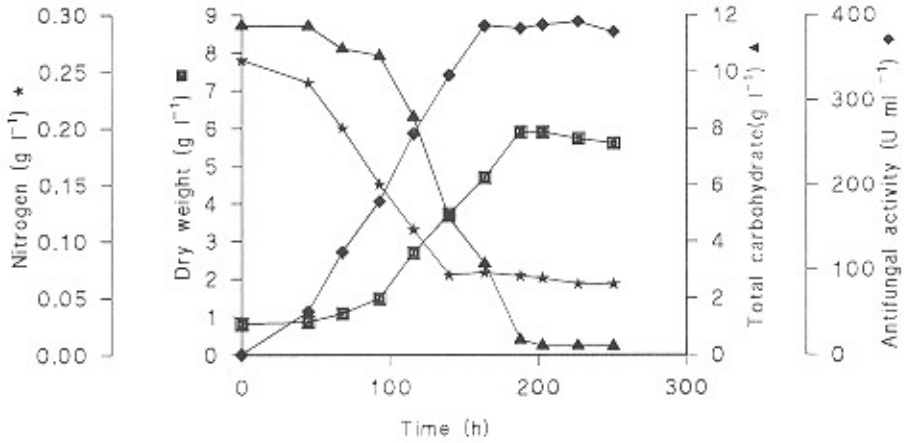


**Figure 3.** Activity of bioactive compounds, dry weight, residual sugar and nitrogen V time at impeller speed of 300 rpm

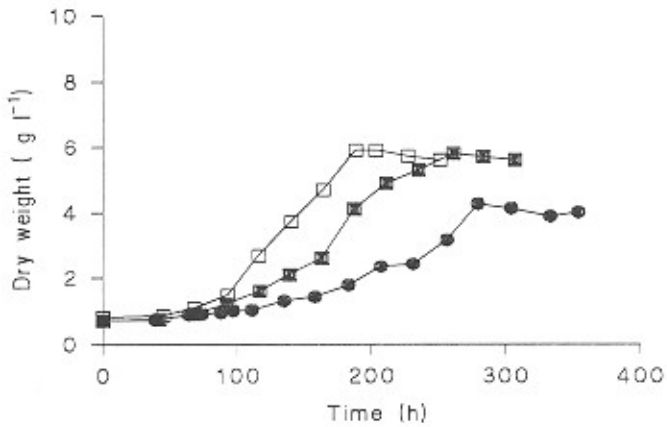


**Figure 4.** Growth of *Mycena sp* V time at different impeller speed, ● 300 rpm, ■ 600 rpm and □ 450 rpm

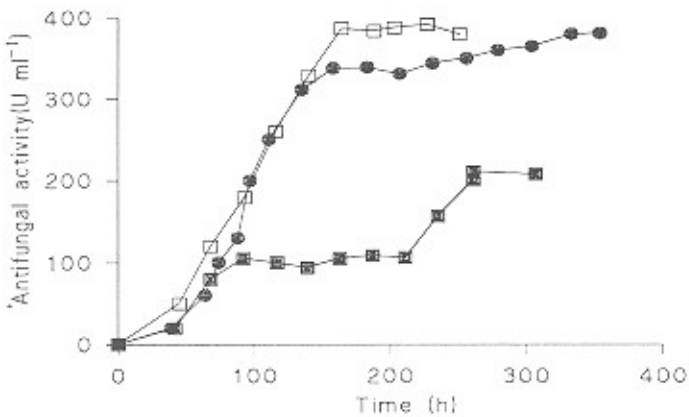


**Figure 5.** Product formation V time at different impeller speed, ● 300 rpm, ■ 600 rpm and □ 450 rpm

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