Effect of aeration and agitation on the production of mycelial biomass and exopolysaccharides in an enthomopathogenic fungus *Paecilomyces sinclairii*

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ABSTRACT

S. W. KIM, H. J. HWA NG, C. P. X U, J.W. CHOI AND J. W. YU N. 2003. Aims: The objective of the present study was to investigate the influence of aeration rate and agitation intensity on the production of mycelial biomass and exopolysaccharide (EPS) in *Paecilomyces sinclairii*. Methods and Results: The *P. sinclairii* was cultivated under various aeration and agitation conditions in a 5 l stirred-tank bioreactor. The highest mycelial biomass (30.5 g l⁻¹) and EPS production (11.5 g l⁻¹) were obtained at a high aeration rate (3.5 v.v.m.) and at a high agitation speed (250 rev min⁻¹). The apparent viscosities (6000–8000 cP) of fermentation broth increased rapidly towards the end of fermentations at high aeration and agitation conditions. Conclusions: The high level of dissolved oxygen achieved at a high aeration rate (3.5 v.v.m.) associated with higher hyphal density eventually resulted in enhanced EPS production. Agitation intensity was also proved to be a critical factor influencing on both the mycelial biomass and EPS production: high agitation speeds up to 250 rev min⁻¹ were preferred to the yields of biomass and EPS production. Significance and Impact of the Study: The critical effects of aeration and agitation in the culture process of *P. sinclairii* were found, which is widely applicable to other kinds of basidiomycetes or ascomycetes in their submerged culture processes. Keywords: Aeration rate, agitation intensity, exopolysaccharide, morphology, *P. sinclairii*, rheology.

INTRODUCTION

Much interest in biotechnological methods for the production of microbial polysaccharides has been generated for applications in the food, pharmaceutical, cosmetic and other industries (Mansell 1994; Kuo *et al.* 1996; Liu *et al.* 1997). Most of the polysaccharides with various physiological activities frequently originated from fungi, especially mushrooms. A number of reports on biological activities of microbial polysaccharides from higher fungi like *Cordyceps* sp., *Ganoderma lucidum* and *Phellinus* sp. are available (Sone *et al.* 1985; Song *et al.* 1998; Yang *et al.* 2000; Koh *et al.* 2001). *Paecilomyces* species are parasitic on the larvae of various lepidopteran insects and form characteristic fruiting bodies or synnemata. The production of exopolysaccharides (EPS) has been investigated within a wide range of environmental parameters. A great number of reports have been documented, concerning factors influencing the fungal morphology, rheology and product formation from many kinds of fungi (Kim *et al.* 1983; Packer and Thomas 1990; Cox and Thomas 1992; Paul and Thomas 1998; Gibbs *et al.* 2000; Pazouki and Panda 2000; Sinha *et al.* 2001). The effects of aeration rate and agitation speed on microbial EPS production are important factors affecting successful progress of fermentation. Aeration could be beneficial to the growth and performance of microbial cells by improving the mass transfer characteristics with respect to substrate, product and oxygen (Martin and Bailey 1985; Olsvik and Kristiansen 1992; Rau *et al.* 1992; Cui *et al.* 1998; Sinha *et al.* 2001; Mantzourioudou *et al.* 2002). Agitation is also an important parameter for adequate mixing, mass and heat transfer inside the fermentation broth.
transfer. Agitation creates shear forces, causing morphological changes, variation in their growth and product formation, and also damage to the cell structure (Taguchi et al. 1968; Martin and Bailey 1985; McNeil and Kristiansen 1987; Smith and Lilly 1990; Pfefferle et al. 2000; Mantzouridou et al. 2002; Park et al. 2002b). In our previous study (Kim et al. 2002), optimal culture conditions of \textit{P. sinclairii} for the production of the mycelial biomass and EPS were investigated in shake flask culture. The purpose of the present study was to examine the effects of aeration rate and agitation speed on mycelial biomass and EPS production by \textit{P. sinclairii} in a stirred-tank reactor. The morphological and rheological features between the different culture conditions of aeration and agitation were studied and fermentation kinetics were also described.

**MATERIALS AND METHODS**

**Microorganism and culture media**

\textit{Paecilomyces sinclairii} was from a culture collection of our laboratory, isolated from a mountainous district in Korea. The stock culture was maintained on a potato dextrose agar (PDA) slant. Slants were inoculated and incubated at 25°C for 7 days and then stored at 4°C.

**Inoculum preparation**

\textit{Paecilomyces sinclairii} was initially grown on PDA medium in a Petri dish and then transferred to the seed culture medium by punching out 5 mm of the agar plate culture with a sterilized self-designed cutter (Bae et al. 2000). The seed culture was grown in a 250 ml flask containing 50 ml yeast medium (YM) (3 g yeast extract, 3 g malt extract, 5 g peptone, 10 g glucose in 1 l of distilled water) at 25°C on a rotary shaker at 150 rev min\(^{-1}\) for 4 days.

**Fermentations**

The fermentation was carried out in a 5 l stirred-tank reactor (KoBioTech Co., Seoul, Korea) with a six-blade Rushton turbine impeller (working volume of 3 l). The culture medium was inoculated with 4% (v/v) (approximately 0.22 g l\(^{-1}\) dry equivalent of cells) of the seed culture. The influences of aeration rate and agitation speed were examined within the ranges from 0.5 to 3.5 v.v.m. and from 50 to 250 rev min\(^{-1}\), respectively.

**Analytical methods**

The fermentation broth was collected at various intervals from the bioreactor and centrifuged at 10 000 \(\times\)g for 20 min. The supernatant was the filtered through a Whatman filter paper No. 2 (Whatman International Ltd, Maidstone, UK). The resulting culture broth was mixed with four times the volume of absolute ethanol, stirred vigorously and kept overnight at 4°C. The precipitated EPS was centrifuged at 10 000 \(\times\)g for 20 min discarding the supernatant (Bae et al. 2000). The concentration of residual sugar was analysed by high-performance liquid chromatography, using an Aminex HPX-42C column (0.78 \(\times\) 30 cm; BioRad Laboratories, Hercules, CA, USA) equipped with a refractive index detector (Shimadzu Co., Kyoto, Japan) (Bae et al. 2000; Park et al. 2002a).

**Measurements of rheology and morphology**

The rheological measurements were performed on samples collected from the bioreactor at regular intervals using a Brookfield programmable LVDVII + digital viscometer (Brookfield Engineering Laboratories Inc., Stoughton, MA, USA) fitted with a small sample adapter. During fermentation, photographs of morphological changes in mycelium were obtained using an Axiolab microscope (ZEISS, Jena, Germany), and the morphological properties of the samples collected were evaluated using an image analyser (Matrox Electronic System Ltd, Dorval, Quebec, Canada) with software coupled to a light microscope (Olympus Optical Co., Ltd, Tokyo, Japan) through a CCD camera (Matsushita Communication Industrial Co., Ltd, Yokohama, Japan). The samples were fixed with an equal volume of fixative (13 ml of 40% formaldehyde, 5 ml glacial acetic acid with 200 ml of 50% ethanol). A 0.1 ml portion of each fixed sample was transferred to a slide, air-dried and stained with methylene blue (0.3 g methylene blue, 30 ml 95% ethanol in 100 ml water) (Packer and Thomas 1990). Normally, a magnification of \(\times\)30 was used.

**Estimation procedures in fermentation kinetics**

The specific growth rate, \(\mu (h^{-1})\) was calculated from the equation:

\[
\mu = (1/X)(dX/dt)
\]

where \(X\) is the cell concentration (g l\(^{-1}\)) at time \(t (h)\). The specific consumption rate of substrate, \(Q_{S/X} (g g^{-1} day^{-1})\) was estimated by the equation:

\[
Q_{S/X} = (dS/dt)/(1/X)
\]

where \(S\) is the concentration of sucrose (g l\(^{-1}\)) at time \(t (day)\). The specific production rate of EPS, \(P_{P/X} (g g^{-1} day^{-1})\) was estimated by the equation:

\[
P_{P/X} = (dP/dt)/(1/X)
\]
where \( P \) is the concentration of EPS (g l\(^{-1}\)) at time \( t \) (day). The yield of EPS on substrate, \( Y_{P/S} \) (g g\(^{-1}\)) was estimated by the equation:

\[
Y_{P/S} = \frac{(dP/dt)}{(dS/dt)}.
\]

**RESULTS AND DISCUSSION**

**Effect of aeration rate on the mycelial biomass and EPS production**

The critical effect of aeration rate for mycelial biomass and EPS production is shown in Fig. 1. Unexpectedly, unusually high level of mycelial biomass (44·90 g l\(^{-1}\)) and EPS production (10·89 g l\(^{-1}\)) were observed at the highest aeration rate (3·5 v.v.m.) examined (Fig. 1a,b). Almost complete sugar depletion was observed at day 14 irrespective of culture conditions of different aeration rates (Fig. 1c). The dissolved oxygen (DO) levels at all aeration rates were reduced from 100% at the beginning of fermentation to around 0–10% at days 2–5. The DO level at aeration rates of 2·0 v.v.m. and 3·5 v.v.m. increased slowly as the growth shifted towards stationary, thereafter a low DO level (30%) was maintained throughout the fermentation (Fig. 1d). Several investigators have reported similar results during secondary metabolite production in batch fermentations (Martin and Bailey 1985; Pfefferle et al. 2000). Martin and Bailey (1985) observed that higher aeration rates enhanced the final dry mycelium concentration in submerged culture of the medicinal mushroom *Agaricus campestris*. Park et al. (2002a) reported an adverse result where an inhibitory effect of high aeration rates on EPS production was observed in *Cordyceps militaris* fermentation. Olsvik and Kristiansen (1992) observed that the effect of the specific growth rate was strongly influenced by the DO in filamentous fermentation broth. The growth kinetic data of *P. sinclairii* with different aeration rates are illustrated in Table 1. The yield of EPS formation from substrate \( Y_{P/S} \) and the specific growth rate of the cells \( \mu \) at 3·5 v.v.m. were 0·153 g g\(^{-1}\) day\(^{-1}\) and 0·154 h\(^{-1}\), respectively.

**Effect of agitation speed on the mycelial biomass and EPS production**

In submerged cultures under the different agitation speeds, the maximum mycelial biomass (30·5 g l\(^{-1}\)) and EPS (11·5 g l\(^{-1}\)) production were observed at 250 rev min\(^{-1}\) (Fig. 2a,b). However, the extent of increment by elevating agitation intensity was not so significant compared with the case of aeration effect. The DO level at an agitation speed of 50 rev min\(^{-1}\) was reduced from 100% at the beginning of fermentation to around 5–10% at days 3–4. The DO profiles at different agitation conditions were quite similar to those observed in varied aeration conditions as described earlier (Fig. 2d). Table 2 shows the growth kinetic data of *P. sinclairii* under different agitation speeds. The yield of EPS formation from substrate \( Y_{P/S} \) and the specific growth rate of the cells \( \mu \) at 250 rev min\(^{-1}\) were 0·189 g g\(^{-1}\) day\(^{-1}\) and 0·168 h\(^{-1}\), respectively. Martin and Bailey (1985) observed that higher agitation speed caused an increase in mycelial biomass concentration associated with

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**Table 1** Fermentation kinetics of *Paecilomyces sinclairii* on the different aeration conditions

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>0·5 v.v.m.</th>
<th>1·5 v.v.m.</th>
<th>3·5 v.v.m.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum biomass concentration ( X ) (g l(^{-1}))</td>
<td>14·15</td>
<td>17·15</td>
<td>44·9</td>
</tr>
<tr>
<td>Maximum EPS concentration ( P ) (g l(^{-1}))</td>
<td>4·46</td>
<td>8·00</td>
<td>10·89</td>
</tr>
<tr>
<td>Specific growth rate ( \mu ) (h(^{-1}))</td>
<td>0·048</td>
<td>0·060</td>
<td>0·154</td>
</tr>
<tr>
<td>Specific consumption rate of substrate ( Q_{S/X} ) (g g(^{-1}) day(^{-1}))</td>
<td>0·447</td>
<td>0·327</td>
<td>0·108</td>
</tr>
<tr>
<td>Specific production rate of EPS ( P_{P/X} ) (g g(^{-1}) day(^{-1}))</td>
<td>0·012</td>
<td>0·041</td>
<td>0·017</td>
</tr>
<tr>
<td>Yield of EPS on substrate ( Y_{P/S} ) (g g(^{-1}))</td>
<td>0·024</td>
<td>0·124</td>
<td>0·153</td>
</tr>
</tbody>
</table>

EPS, exopolysaccharide.
high frequency of filamentous mycelia but a decrease in the pellet size of *Agaricus* mycelium.

**Effect of morphology and rheology**

Many investigators have stressed that the morphology of individual mycelia is significantly affected by aeration rates and agitation speeds in submerged cultures (Taguchi et al. 1968; Smith and Lilly 1990; Park et al. 2002b) because vivid mixing is closely linked to modification of morphology and transport phenomena within the bioreactors. Cui et al. (1998) reported that pellet size, the hairy length of pellets, and the free filamentous mycelial fraction in the total biomass were found to be independent of the DO tension provided that the dissolved oxygen tension was neither too low nor too high. McNeil and Kristiansen (1987) observed that the rheological properties of the broth are dependent on pullulan concentration as well as culture morphology in the fermentations under different agitation conditions. In the present work, denser and smaller pellets were observed (Figures not shown) at a high agitation speed (250 rev min\(^{-1}\)) resulting in increased production of mycelial biomass and EPS (see Fig. 2). Figure 3 shows the typical morphological changes in *P. sinclairii* at the final fermentation period under different aeration conditions. The cells were mainly observed to form pellets during the entire culture period irrespective of aeration conditions. It should be mentioned here that the mycelial pellets were too big to capture the whole images, which unfortunately did not allow the analysis of the detailed morphological parameters. At the later stages of fermentation, the pellets rapidly increased in hyphal length and density. The highest hyphal length was found at a low aeration rate (0-5 v.v.m.) whereas the highest hyphal density was observed at an extremely high aeration condition (3-5 v.v.m.) with increased mycelial biomass and EPS production.

### Table 2  
Fermentation kinetics of *Paecilomyces sinclairii* on the different agitation conditions

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>50 rev min(^{-1})</th>
<th>150 rev min(^{-1})</th>
<th>250 rev min(^{-1})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum biomass concentration (X) (g l(^{-1}))</td>
<td>23-35</td>
<td>30-4</td>
<td>30-5</td>
</tr>
<tr>
<td>Maximum EPS concentration (P) (g l(^{-1}))</td>
<td>7-75</td>
<td>10-75</td>
<td>11-50</td>
</tr>
<tr>
<td>Specific growth rate (\mu) (h(^{-1}))</td>
<td>0-142</td>
<td>0-149</td>
<td>0-168</td>
</tr>
<tr>
<td>Specific consumption rate of substrate (Q_{s/X}) (g g(^{-1}) day(^{-1}))</td>
<td>0-401</td>
<td>0-280</td>
<td>0-285</td>
</tr>
<tr>
<td>Specific production rate of EPS (P_{P/X}) (g g(^{-1}) day(^{-1}))</td>
<td>0-038</td>
<td>0-046</td>
<td>0-054</td>
</tr>
<tr>
<td>Yield of EPS on substrate (Y_{P/S}) (g g(^{-1}))</td>
<td>0-095</td>
<td>0-162</td>
<td>0-189</td>
</tr>
</tbody>
</table>

EPS, exopolysaccharide.
The broth rheology in fungal fermentations is frequently related to the morphology and the yield of mycelial biomass and EPS production. Figure 4 clearly shows that the rheological behaviour of the broth is obviously pseudoplastic behaviour, which is frequently found in other fungal fermentations. Figure 5 shows the apparent viscosity of the whole broth for the entire fermentation period at different aeration rates and agitation intensities. The apparent viscosities of fermentation broth increased rapidly towards the end of fermentations at high aeration and agitation conditions, indicating extremely high levels (6000–8000 cP) because of high amount of EPS accumulated at the later stages of fermentation. It is noteworthy to mention that the combination of DO concentration with desirable morphology played an important role in higher EPS production.

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REFERENCES


