Effects of Dissolved Oxygen on Fungal Morphology and Process Rheology During Fed-Batch Processing of *Ganoderma lucidum*

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Controlling the dissolved oxygen (DO) in the fed-batch culture of the medicinal mushroom *Ganoderma lucidum* led to a 2-fold increase of the maximum biomass productivity compared with uncontrolled DO conditions. By contrast, extracellular polysaccharide (EPS) production was two times higher under oxygen limitation (uncontrolled DO) than under increased oxygen availability (controlled DO). Morphologically, dispersed mycelium was predominant under controlled DO conditions, with highly branched hyphae, consistent with the enhanced culture growth noted under these conditions, whereas in the uncontrolled DO process mycelial clumps were the most common morphology throughout the culture. However, in both cultures, clamp connections were found. This is an exciting new finding, which widens the applicability of this basidiomycete in submerged fermentation. In rheological terms, broths demonstrated shear-thinning behavior with a yield stress under both DO conditions. The flow curves were best described by the Herschel–Bulkley model: flow index down to 0.6 and consistency coefficient up to 0.2 and 0.6 Pa s in uncontrolled and controlled cultures DO, respectively. The pseudoplastic behavior was entirely due to the fungal biomass, and not to the presence of EPS (rheological analysis of the filtered broth showed Newtonian behavior). It is clear from this study that dissolved oxygen tension is a critical process parameter that distinctly influences *G. lucidum* morphology and rheology, affecting the overall performance of the process. This study contributes to an improved understanding of the process physiology of submerged fermentation of *G. lucidum*.

**Keywords:** Ganoderma lucidum, rheology, EPS, fed-batch, dissolved oxygen, clamp connection

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Medicinal mushrooms are viewed as a rapidly developing area of biotechnology for cancer and other therapies [27].

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*Ganoderma lucidum* (Fr.) Krast (Polyporaceae), a mushroom-like higher fungus, has been a popular folk and an oriental medicine used to treat many various human diseases such as hypertension, arthritis, bronchitis, and cancer [22]. *G. lucidum* produces several metabolites with biological activity, such as polysaccharides (EPS) and terpenoids. The major bioactive *Ganoderma* polysaccharide species are β-1-3 and β-1-6-D glucans, which have an average molecular mass of 1,050,000 Da [22]. The structure is β-1-3-D-glucopyran with 1–15 units of β-1-6-monoglycosyl side chains. These metabolites are believed to exert antitumor activities through an enhancement of host-mediated immunity, rather than a direct cytotoxic effect upon tumor cells themselves [28, 29].

Because of their perceived health benefits, there has been recent interest in developing commercial processes for the submerged culture of *G. lucidum* mycelium, prompted by the fact that wild *G. lucidum* is rarely found in nature, and traditional mushroom cultivation (solid substrate cultivation) usually takes months and presents several process control issues [1]. The optimization and control of *G. lucidum* submerged culture requires a better understanding of the macrofungi process physiology in liquid systems, which, when compared with our knowledge of bioprocessing of the lower fungi such as *Aspergillus* and *Penicillium*, is very poor [8]. Understanding the challenges in bioprocessing of these highly complex fungi is extremely important for the successful exploitation of the macrofungi at an industrial level.

Fermenter operating conditions have a critical influence on biomass concentration, mycelial morphology, and target metabolite production. The oxygen supply, which is measured as dissolved oxygen (DO) in the culture media and controlled by the mixing and aeration conditions, may affect the type of morphology of the fungi. It influences the cell wall properties, the length of hyphae, and the roughness of the clumps, and consequently the viscosity of the culture [32]. In turn, changes in mycelial morphology and the consequent changes in broth rheology affect several phenomena in
Herschel rheological models applicable to fermentation broths (adapted from [23]).

Strain and Inoculum strategies. Furthermore, the use of a systematic approach of the shear rate, giving rise to what is generally called lower viscosity [26]. In the vast majority of fermentation the pelleted form (compact discrete masses of hyphae) exhibiting non-Newtonian behavior. In complete contrast, the filamentous fungal form and its innate tendency to process performance can be optimized. In rheological terms, mixing efficiency [19]. By identifying the morphology of abbreviations:

Table 1. Comparison of the rheological models fitness for Ganoderma lucidum controlled-DO fed-batch culture (day 9), using rheological models applicable to fermentation broths (adapted from [23]).

<table>
<thead>
<tr>
<th>Model</th>
<th>Equation</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newtonian</td>
<td>( \tau = \eta \dot{\gamma} )</td>
<td>-</td>
</tr>
<tr>
<td>Bingham</td>
<td>( \tau = \tau_y + \eta \dot{\gamma} )</td>
<td>0.02 + 14.21</td>
</tr>
<tr>
<td>Power Law</td>
<td>( \tau = K_p \dot{\gamma}^n )</td>
<td>2.00</td>
</tr>
<tr>
<td>Herschel–Bulkley</td>
<td>( \tau = \tau_y + K_{mb} \dot{\gamma}^n )</td>
<td>1.13 + 3.00</td>
</tr>
<tr>
<td>Casson</td>
<td>( \tau^2 = \tau_y^2 + K_c \dot{\gamma}^2 )</td>
<td>0.01 + 7.95</td>
</tr>
</tbody>
</table>

Abbreviations: \( \tau \) = shear stress (Pa); \( \eta \) = viscosity (Pa s); \( \dot{\gamma} \) = shear rate (s\(^{-1}\)); \( \tau_y \) = yield stress (Pa); \( K_p \) and \( K_{mb} \) = consistency coefficient for Power Law and Herschel–Bulkley, respectively (Pa s\(^n\)); \( n \) = flow behavior index and \( K_c \) = Casson viscosity (Pa s\(^{n/2}\)).

bioreactors, such as mass and heat transfer, shear stress, and mixing efficiency [19]. By identifying the morphology of the organism that will give the highest process yields, the process performance can be optimized. In rheological terms, the filamentous fungal form and its innate tendency to entanglement gives rise to a highly viscous, process fluid exhibiting non-Newtonian behavior. In complete contrast, the pelleted form (compact discrete masses of hyphae) produces essentially a Newtonian system with a much lower viscosity [26]. In the vast majority of fermentation processes, the viscosity is found to decrease with an increase of the shear rate, giving rise to what is generally called shear-thinning behavior [32]. The common increase in broth viscosity with process time is usually a consequence of increased cell concentration, changes in microbial morphology, or due to the accumulation of extracellular products that alter the rheological characteristics of the fermentation broth [27]. The Power Law is the most widely used model for describing such rheological behavior; however, there are several other empirical models that describe the broth rheological behavior (Table 1) [16, 32].

Previous studies have demonstrated the impact of morphology on both cell growth and metabolite production in macrofungi, including G. lucidum [9, 25, 28]. However, the influence of dissolved oxygen on the morphology and process rheology of a macrofungus in submerged cultivation has been neglected. Therefore, the main objective of this study was to investigate the influence of dissolved oxygen, as a critical process parameter, on the growth and morphological profiles of G. lucidum in submerged culture, by using different control strategies. Furthermore, the use of a systematic approach to enhance the value of the rheological data from a G. lucidum fed-batch process is presented here for the first time.

**Materials and Methods**

**Strain and Inoculum**

The microorganism used in the present study, Ganoderma lucidum CCRC36123, was kindly obtained from Professor Fan-Chiang Yang (Tunghsi University, Taiwan). The strain was maintained on potato-agar-dextrose (PDA) slants. Plates were inoculated and incubated at 30°C for 7 days, and stored at 4°C. The inoculum preparation involved two seed culture stages, both cultivated for 3–5 days at 30°C and 200 rpm. A 20-ml mycelium suspension prepared from a newly prepared PDA plate was inoculated into a 250-ml Erlenmeyer flask containing 100 ml of medium (1st seed culture). The mycelium was then homogenized by a sterile Waring blender for 40 seconds to use as the inoculum for the second seed culture (2-l Erlenmeyer flask containing 1-l medium) and then transferred to the bioreactor.

**Culture Conditions**

The composition of the medium used in all stages was (g/l): glucose 35, yeast extract 5, peptone 5, KH₂PO₄ 1, and MgSO₄·7H₂O 0.5. All fermentations were carried out in fed-batch mode with pulse-feeding of glucose, yeast extract, and peptone every 24 h, or whenever the glucose concentration fell to 5–10 g/l. The reactor used was a 15-l (total volume) stainless steel bioreactor (BIOSTAT C.-DCU; B. Braun Biotech International, Switzerland). The pH was kept at 4.0 by automatic addition of titrants (1 M NaOH and 1 M H₂SO₄). The temperature was kept at 30°C throughout the runs and the agitation rate was set at 300 rpm. The culture conditions chosen were based on previous work [7]. 30°C and 300 rpm Uncontrolled and controlled dissolved oxygen (DO) fed-batch cultures were performed. In the DO-controlled process, the DO set-point was at 20% air saturation using a cascade control of agitation, airflow rate, and oxygen enrichment. The maximum airflow rate used was at 2.0 volume of air per volume of culture per minute (vvm) in both cultures. Real-time values of pH, dissolved oxygen, agitation speed, temperature, airflow rate, and oxygen percentage during fermentations were recorded automatically by the bioreactor software, MFCS DA (Sartorius, U.K.).

**Biochemical Assays**

Biomass was estimated by filtering a 5-ml sample through a pre-dried and weighted GF/C filter paper and washed through a pre-dried and weighted GF/C filter (Whatman Ltd., U.K.), followed by repeated washing of the mycelial biomass with distilled water. The filter cake was dried for 20 min in a microwave oven (650 W), and cooled in a desiccator before weighing. The filtrate was collected for EPS assays. The crude EPS was precipitated with addition of four volumes of 95% (v/v) ethanol and left overnight at 4°C. The precipitate was then separated by centrifugation at 10,000 rpm for 15 min, which was repeated twice. The precipitate was then filtered through a pre-dried and weighted GF/C filter paper and washed twice with 5 ml of 95% (v/v) ethanol. It was then transferred to a desiccator, left to equilibrate to constant weight, and the weight of EPS was then estimated. All assays were carried out in triplicates.
Morphological Characterization
The morphological details of the samples collected were evaluated using a light microscope (Olympus CHB) through a coupled camera (Nikon Coolpix 4500). Five ml of culture sample was resuspended in 5 ml of a fixative solution according to the method described in Packer and Thomas [20] and kept at 4°C until measurement. The fixative solution was prepared by mixing 13 ml of 40% (v/v) formaldehyde, 5 ml of glacial acetic acid, and 200 ml of 50% (v/v) ethanol. An aliquot (0.1 ml) of each fixed sample was transferred to a slide, air dried, and then stained with methylene blue.

Rheological Measurements
A rotational rheometer (Model RM180; Rheometric Scientific, Inc., NJ, U.S.A.) was used. In order to settle on a suitable measuring device for rheological analysis of G. lucidum broth, different measuring systems included in the RM180 rheometer were compared. Measuring system 22, which includes the measuring bob 1 (ϕ=30 mm, ℓ=45 mm) and the measuring tube 1 (ϕ=32.54 mm), gave consistent and constant values during the measurement period of 5 min and at each shear rate, as opposed to the other systems. Moreover, the applicability of the bob and the tube was emphasized by the observations that measured shear stress obtained at a particular shear rate for 1 min was within 5% mean error of the shear stress value obtained from a second measurement obtained 2 min later at the same shear rate. This was verified at a number of shear rates in the range of 100–1,291 s⁻¹, suggesting that there was no significant settling or separation of the broth. Shear stress, apparent viscosity, temperature, and torque were given by the software in RM180 and verified using the model fluid silicone. All rheological analyses were completed at 30°C within 10 min after sample removal from the fermenter. Apparent viscosity was estimated using the Metzner and Otto [17] approach for non-Newtonian fluids, as shown below:

\[ \ln \eta_a = \ln \frac{K \cdot \eta}{\dot{\gamma}} + (n – 1) \ln \dot{\gamma} \]

which can be rewritten as

\[ \eta_a = K \cdot \dot{\gamma}^{n-1} \]

where \( \eta_a \) is apparent viscosity (Pa s), K is the consistency coefficient (Pa s), n is the flow behavior index, and \( \dot{\gamma} \) is the shear rate (s⁻¹).

RESULTS AND DISCUSSION
Cell Growth and EPS Production
Fig. 1 shows the culture kinetics of G. lucidum fed-batch cultures under uncontrolled and controlled DO conditions. The highest biomass production (26.6 g/l) was obtained from the controlled DO culture, whereas the highest EPS production (4.55 g/l) was achieved under uncontrolled DO conditions. The onset of oxygen limitation was associated with cessation of growth and the appearance of a stationary phase in both uncontrolled and controlled DO cultures: day 4 and day 7, respectively (Fig. 1C). However, the response of EPS production to oxygen limitation was quite distinct: a dramatic increase was seen on day 4 in the uncontrolled DO culture, whereas EPS production was static under controlled DO conditions. The fermentation kinetic data of both cultures showed that controlling the oxygen above 20% saturation resulted in a 130% increase in biomass productivity relative to the uncontrolled DO culture, whereas

Table 2. Effect of DO control strategy upon growth parameters of Ganoderma lucidum fed-batch culture in a 10-l stirred tank bioreactor.

<table>
<thead>
<tr>
<th>Kinetic parameters</th>
<th>Culture conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uncontrolled</td>
</tr>
<tr>
<td>( Q_X ) (g ( \Gamma ) day⁻¹)</td>
<td>1.663</td>
</tr>
<tr>
<td>( Q_{EPS} ) (g ( \Gamma ) day⁻¹)</td>
<td>0.649</td>
</tr>
<tr>
<td>( P_{EPS/X} ) (g ( g^{-1} ) day⁻¹)</td>
<td>0.380</td>
</tr>
</tbody>
</table>

Abbreviations: \( Q_X \): maximum cell productivity; \( Q_{EPS} \): maximum EPS productivity; \( P_{EPS/X} \): specific production rate of EPS.
uncontrolled DO conditions led to more than a 2-fold increase of both maximum EPS productivity and specific production rate compared with controlled DO conditions (Table 2).

Morphological Characterization

*G. lucidum* showed different morphological characteristics throughout the different stages of the process (Fig. 2). The inoculum stage (grown in shake flasks) showed a pelleted morphology (Fig. 2A), as opposed to the dispersed hyphal elements in the bioreactor during fed-batch cultivation (Fig. 2B). These differences can be attributed to a higher hydromechanical stress present inside the bioreactor. In fact, the shear rate in the bioreactor was up to a factor of 10 higher than the lowest average limit usually seen in shake flasks cultures [24]. The associated increase on the shear stress has an effect on the morphology of *G. lucidum*, breaking the inoculum pellets into more fragmented hypha, and therefore encouraging the development of the filamentous form [3]. Clamp connections, however, were found regardless of the culture stages or conditions (Fig. 2C). Clamp connections are believed to function in ensuring that each basidiomycete “cell” or compartment has a compatible pair of nuclei, and so the dikaryotic state is maintained, as opposed to the predominant homokaryotic state seen in ascomycetes [8]. The finding of clamp connections in fed-batch cultures in the present study is both unusual and exciting, since it is believed that hyphae fail to form clamp connections in liquid culture (usually attributed to the use of intense agitation) [4]. Previous studies of submerged cultivation of ectomycorrhizal fungi (*Laccaria proxima*) revealed that clamp connections were always lost and therefore their ability to infect their plant symbiont with it. This meant that the potential commercialization of such process was immediately put aside. Although is reasonable to assume that the dikaryotic mycelial network present in the submerged cultures in the present study will exhibit reduced specific growth rates relative to a monokaryotic mycelium (less

![Fig. 2. Photomicrographs of (A) Ganoderma lucidum inoculum sample showing a pelleted morphology. Images were taken at 10-fold magnification. Bar=250 µm. B. Ganoderma lucidum sample from fed-batch cultures showing freely dispersed and clumped mycelia. Images were taken at 10-fold magnification. Bar=250 µm. C. Detail of Ganoderma lucidum hyphae showing the presence of clamp connections, septum, and hyphal tip (typical features of basidiomycetes). Images were taken at 100-fold magnification. Bar=125 µm](image)

![Fig. 3. Morphological changes in mycelia during the fed-batch fermentations of Ganoderma lucidum without (A) and with (B) DO control. Images were taken at 10-fold magnification. Bar=250 µm.](image)
“genetic burden”), the findings of the present study will widen the applicability of basidiomycetes using submerged culture techniques.

The data presented in Fig. 3 indicate that different fragmentation events took place dependent on the oxygen availability in the fed-batch cultures. In the uncontrolled DO process (Fig. 3A), although mycelial clumps were predominant, the amount of free dispersed mycelia increased considerably after day 4 (oxygen depletion occurred). When the DO was controlled (Fig. 3B), clump fragmentation was more pronounced throughout the process, until finally almost only freely dispersed hyphal elements were present. This is consistent with a culture growing more rapidly when oxygen supply was improved. These findings were also in concordance with Wongwicharn et al. [30]: two distinct micromorphologies of *Aspergillus niger* under oxygen limitation (long, sparsely branched hyphal elements) and O$_2$ enrichment (shorter hyphal elements, with more branching) were identified. These authors also concluded that oxygen-enriched cultures showed a higher percentage of “active” length (i.e., % of cytoplasm filled hyphae) and attributed the “stranded” aggregate morphology observed to a possible response to a hyperoxidant state.

In this study, *G. lucidum* adapted to the DO-control scheme by increasing the amount of freely dispersed mycelia, and switching from the mostly inactive vacuolated, long filaments with few branches (found in O$_2$-limited culture), to more branched and “active” hyphae (found in O$_2$-enriched culture). The agitation rate also contributed to the “shaving off” of the outermost hyphae in clumps in the DO controlled culture, resulting in shorter hyphal elements [13]. When a microorganism is subject to physiological stress, changes in the chemical structure of the cell wall and morphology have been shown to take place [14, 15]. For example, in a *A. oryzae* fed-batch culture, higher levels of fragmentation were dominant over tip extension and branching owing to the slow growth observed when nutrient stress was imposed [13]. The data presented here are consistent with this concept: oxygen depletion in the uncontrolled DO process (on day 4) resulted not only in a stationary phase, but also a significant increase in culture sensitivity to impeller-induced shear stress, as shown by a rise in hyphal fragmentation. Furthermore, this was also associated with an increase in EPS concentration following oxygen limitation, possibly linked to the now imperfect conditions for growth. This can be further bolstered by the mucilage of EPS observed on the outermost part of *G. lucidum* hyphae on day 5 and a number of long filaments containing highly vacuolated cell compartments observed in the end of the process.

Most published studies characterize *G. lucidum* morphology as chiefly pelleted, but it is noteworthy that most reports refer to shake flasks studies [6, 31], an essentially very low shear process. Lee et al. [11] and Park et al. [21] pointed out that “feather-like” morphology (similar to that of this work) was more favorable than pellets in the production of EPS and arachidonic acid from *Grifola frondosa* and *Mortierella alpina*, respectively. Here, the more dispersed mycelia observed under controlled DO conditions was associated with better growth (Q$_X$ = 3.82 g l$^{-1}$ d$^{-1}$), whereas the predominant clumps present in the uncontrolled DO culture led to more than two times higher EPS productivity (Q$_{EPS}$ = 0.93 g l$^{-1}$ d$^{-1}$).

**Broth Rheology**

**Flow characterization.** The culture broth demonstrated shear-thinning behavior regardless of the DO conditions (Fig. 4). By contrast, rheological analysis of filtered broth samples (i.e., with biomass removed) showed a constant apparent viscosity of 9.0 mPa.s, indicating that the shear-thinning of the broth was entirely due to the biomass and not the aqueous medium or extracellular metabolites (data not shown).

In an attempt to find the model that best described the process, the models presented in Table 1 [Eqs. (3) to (7)] were fitted to the rheological data. Measurements were taken at 1,000 s$^{-1}$ for comparability purposes (the shear rate...
Fermentation broth rheological profile.

Below, the rheology of such non-Newtonian systems, as discussed, yield stress. These parameters will help to fully characterize (K) and the flow behavior index (n) but also account for a only allows the calculation of the consistency coefficient (K) and the flow behavior index (n) but also accounts for a yield stress in the broth; that is the broth only flows when a critical value of the shear stress is exceeded. The existence of a yield stress in the broth is usually related to the formation of poorly aerated zones in the fermenter that can have a marked effect on the performance of the bioreactor as a whole [16]. The Herschel-Bulkley model was used for the remainder of this work because, being a modified form of the Power Law, it not only allows the calculation of the consistency coefficient (K) and the flow behavior index (n) but also accounts for a yield stress. These parameters will help to fully characterize the rheology of such non-Newtonian systems, as discussed below.

Fermentation broth rheological profile. Table 3 depicts the rheological parameters determined for *G. lucidum* cultures. In both uncontrolled and controlled DO processes, the shear-thinning characteristics developed slowly with a 40% decrease in the flow index (n) from unity after day 6. The consistency coefficient (K), which gives a direct indication of the increase in the apparent viscosity since, as indicated in Eq. (3), η_a is proportional to K, continued to rise to a maximum value in both cultures simultaneously to the minimal flow index value (day 7). The decrease on K at the end of both fed-batch cultures is attributed to cell lysis from nutrient limitations [23]. This observation was broadly similar to what is observed with other submerged liquid culture fermentations of macrofungi [10, 12, 25] and has also been described for other broths containing solutions of xanthan gum and pullulan [16]. Hence, a nonlinear relationship between biomass concentration and rheological parameters is not unexpected (Fig. 5). In fact, in the controlled DO culture, the relationship between K and biomass (X) was within the published range [23]: it varied to the power of approximately 5.0 (K=2×10^{-5}×X^3, R^2=0.700).

It is likely that the higher yield stress values seen on uncontrolled DO culture until day 7 are related to the presence of stagnant nonproductive zones within the bioreactor [20], since this culture was poorly mixed resulting in an early onset of oxygen limitation. As a result of EPS decrease on day 7, the broth became more Newtonian (n rose), less viscous (K and μ_a decreased), and therefore the mass and heat transport conditions of the culture improved (yield stress decreased). The improved mixing conditions (higher agitation rates and oxygen enrichment) seen on the controlled DO cultures improved the broth homogeneity and consequently the biomass productivity. A doubling in cell concentration (on day 7) caused the apparent viscosity to increase a factor of up to 40. The yield stress behavior was more accentuated towards the end of the process, reflecting the oxygen limitation conditions, whereas the broth viscosity showed a decline (despite the constant biomass concentration). It has been reported that a decrease on fungal broth density with culture age can lead to a decrease on broth viscosity, which has been attributed to an increase of fragmented mycelia [18]. It is possible that a similar phenomenon was occurring in the controlled DO culture.

Another aspect that contributed to the differences found between the controlled and uncontrolled DO cultures was the broth morphology. The broth containing chiefly mycelial clumps showed a more Newtonian flow (n closer to unity) and lower apparent viscosity. On the other hand, it is known that dispersed filamentous growth (the dominant morphology in the controlled DO culture) produces “structure” in the

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**Table 3.** The variation in flow behavior index (n), consistency coefficient (K), and yield stress of the fermentation broth of the uncontrolled and controlled-DO *Ganoderma lucidum* fed-batch processes.

<table>
<thead>
<tr>
<th>DO conditions</th>
<th>Culture days</th>
<th>Flow index (n)</th>
<th>Consistency coefficient (K, Pa)</th>
<th>Yield stress (Pa)</th>
<th>Apparent viscosity (Pa s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Uncontrolled</td>
<td>5</td>
<td>0.90</td>
<td>0.02</td>
<td>3.83</td>
<td>13</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>0.88</td>
<td>0.02</td>
<td>5.63</td>
<td>16</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.56</td>
<td>0.19</td>
<td>7.68</td>
<td>18</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.82</td>
<td>0.04</td>
<td>6.12</td>
<td>17</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.76</td>
<td>0.05</td>
<td>5.75</td>
<td>17</td>
</tr>
<tr>
<td>Controlled</td>
<td>5</td>
<td>1.08</td>
<td>0.01</td>
<td>1.04</td>
<td>10</td>
</tr>
<tr>
<td></td>
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<td>1.06</td>
<td>0.01</td>
<td>2.49</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>7</td>
<td>0.59</td>
<td>0.70</td>
<td>5.11</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>0.60</td>
<td>0.60</td>
<td>6.20</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>0.64</td>
<td>0.50</td>
<td>6.71</td>
<td>39</td>
</tr>
</tbody>
</table>

**Fig. 5.** Effect of biomass concentration on the rheological properties of *G. lucidum* DO-controlled fed-batch culture. Consistency coefficient (K, □), flow index (n, ■), and yield stress (△)
broth, resulting in higher consistency coefficients and therefore higher apparent viscosities [5]. Furthermore, the extent of branching of hyphal cells is directly proportional to the viscosity of the broth [5]; the long filaments with few branches found in the uncontrolled DO culture tend to be more flexible than the branched cells (found in controlled DO culture) and produce lower viscosities. As shown above, since cell metabolism and rheological parameters are linked, rheological data can be used for the early assessment of a fermentation trajectory. This gives the possibility to change relevant parameters or fermentation conditions (such as DO-control strategies or broth dilution), which will improve the fermentation quality and increase EPS or biomass production or both.

It may be concluded that the oxygen availability not only influenced the extracellular polysaccharide and *G. lucidum* growth, but also had an impact on the fungal morphology and broth rheology. The clump form was the most productive morphology in terms of EPS production, compared with the free filamentous mycelial form (favored under conditions of enhanced oxygen supply), which was associated with improved cell growth. The fungal morphology showed a close relationship with the broth rheology. The higher apparent viscosity in the controlled DO culture was a result of both higher biomass concentration and the highly branched hyphal forms noted, in contrast to the clumped form in the uncontrolled DO culture. However, both extremes of morphological form showed clamp connections, indicating the retention of the capability to infect plant symbionts.

**REFERENCES**


