J. Plant Biotechnology(2002) *Vol. 4(3). pp. 109~115*

Review

Journal of Plant Biotechnology

Enhanced Production of Valuable Bioactive Metabolites in Submerged Cultures of Medicinal Mushroom *Ganoderma lucidum* by Manipulation of Oxygen Supply

Jian-Jiang Zhong*, Qing-Hua Fang, Ya-Jie Tang

State Key Laboratory of Bioreactor Engineering (China), East China University of Science and Technology, 130 Meilong Road, Shanghai, 200237, China

Key words: Bioprocess engineering, Chinese medicinal mushroom, ganoderic acid, *Ganoderma lucidum*, *Ganoderma* polysaccharide, initial volumetric oxygen transfer coefficient (K_La)

Abstract

Submerged cultures of *Ganoderma lucidum*, a valuable mushroom in traditional Chinese medicine, were used for production of bioactive ganoderic acids and Ganoderma polysaccharides. The significant effects of oxygen supply were demonstrated in both shake flasks and bioreactors. By changing the medium loading volume in a shake flask, a different value of initial volumetric oxygen transfer coefficient (K_La) was obtained, and a higher K_La value led to a higher biomass density and a higher productivity of both intracellular polysaccharide and ganoderic acid. In a stirred bioreactor, at an initial K_La of 78.2 h^{-1}, a maximal cell concentration of 15.6 g/L by dry weight was obtained, as well as a maximal intracellular polysaccharide (IPS) production of 2.2 g/L and its maximal productivity of 220 mg/(L.d). An increase of initial K_La led to a higher production and productivity of GA, and the GA production and productivity at an initial K_La of 96.0 h^{-1} was 1.8-fold those at an initial K_La of 16.4 h^{-1}. The fundamental information obtained in this study may be useful for efficient large-scale production of these valuable bioactive products by the submerged cultures.

Introduction

In recent years, bioprocessing of natural products, such as production of valuable bioactive compounds by cell cultures of plants or higher fungi, has received much attention around the world. The main problems hindering the development of large-scale cultivation of these organisms include low productivity, cell line instability, and difficulty in scale-up. Medicinal mushrooms are abundant sources of a wide range of useful native products and new compounds with interesting biological activities. In contrast to various studies on fermentation of conventional filamentous microorganisms such as streptomycetes and fungi, until now there are few investigations on the development of mushroom culture processes (Tang and Zhong, 2000).

Ganoderma lucidum (Leyss.:Fr.) Karst is one of the most famous traditional Chinese medicinal mushrooms used as a health food and medicine in the Far East for more than 2000 years. Polysaccharides produced by *G. lucidum* are a type of carcinostatic agent, which have antitumor (Sone et al., 1985) and hypoglycemic activities (Hikino et al., 1985). The higher fungus can also produce many species of oxygenated triterpenes (especially ganoderic acid) with various biological functions such as cytotoxicity to hepatoma cells, inhibition of histamine release, inhibition of cholesterol synthesis and stimulation of platelet aggregation (Shiao et al., 1994; Tang and Zhong, 2000), as well as new interesting biological activities including anti-tumor and anti-HIV-1 (El-Mekkaway et al., 1998; Wu et al., 2001).

*Corresponding author, E-mail; jjzhong@ecust.edu.cn
Received Jul. 12, 2002; accepted Aug. 15, 2002

Because it usually takes several months to cultivate the mushroom and the product yield is low in soil cultivation, submerged culture of *G. lucidum* is viewed as a promising alternative for efficient production of valuable polysaccharides (Lee et al., 1999; Yang and Liau, 1998) and ganoderic acids (Tsujikura et al., 1992; Fang and Zhong, 2002a). However, there is very limited information on the simultaneous production of ganoderic acids and polysaccharides by submerged cultivation (Fang and Zhong, 2002b; Fang and Zhong, 2002c; Tang and Zhong, 2002).

Oxygen affects cell growth, cellular morphology, nutrient uptake, and metabolite biosynthesis. Ishmentskii et al. (1981) reported that a high oxygen transfer rate could reduce, enhance, or have no effect on the production of pullulan, depending on the strain ploid of *Pullaria (Aureobasidium) pullulans*. In cultivation of filamentous fungus *Schizophyllum commune*, Rau et al. (1992) reported that sufficient oxygen supply resulted in an increase in the specific growth rate and a decrease in the production rate of extracellular glucan. When oxygen partial pressure in the culture broth decreased to almost zero, the fungus responded to this oxygen limitation by reduced cell growth and increased glucan accumulation. In the submerged fermentation of *Monascus ruber*, Hajjaj et al. (1999) reported that improving the oxygen supply increased the biomass yield, consumption of nitrogen source and production of secondary metabolites (red pigment and citrinin). Yoshida et al. (1965, 1967) showed the significance of oxygen transfer in submerged cultures of a mushroom *Lentinus edodes*. Until now, there is lack of reports on effects of oxygen (O_2) supply on simultaneous production of *Ganoderma* polysaccharides and ganoderic acids.

By changing the medium loading volume in flasks, the interaction between O_2 supply, cell growth and metabolite biosynthesis can be observed. In this work, the initial volumetric oxygen transfer coefficient (K_La) in a shake flask was altered to provide some insights into the influences of O_2 supply on cultures of *G. lucidum* for simultaneous production of polysaccharides and ganoderic acids. The impact of K_La values on bioreactor cultiva-tion of *G. lucidum* was also studied. The results may be useful for efficient large-scale production of those bioactive compounds by the bioprocess.

Materials and Methods

Maintenance and preculture of *Ganoderma lucidum*

The strain *G. lucidum* was maintained on potato-agar-dextrose slants. The slant was inoculated and incubated at 28℃ for 7 days, then stored at 4℃ for about two weeks. Preculture medium and conditions were described elsewhere (Fang and Zhong, 2002a; Fang and Zhong, 2002b; Fang and Zhong, 2002c; Tang and Zhong, 2002).

O_2 supply experiments

The effects of O_2 supply were investigated by changing the medium loading volume of shake flask on a rotary shaker (30℃, 120 rpm). By setting the loading volume at 30, 50, 70 and 100 mL in 250-mL conical flasks, an initial K_La value of 32.6, 18.5, 13.2 and 6.4 h^{-1} was obtained, respectively.

For bioreactor cultures, a 3.5-L (working volume) agitated bioreactor with two six-bladed turbine impellers (6.5 cm ID) was used. Cultivation was conducted at 30℃ in the dark. The K_La value was determined using the dynamic gassing-in and gassing-out method (Wang and Zhong, 1996). The cultures were all agitated at the same speed (200 rpm), and the aeration rate was set at 220, 1050, 1750 and 3500 mL/min to obtain the desired K_La values of 16.4, 60.0, 78.2 and 96.0 h^{-1}, respectively.

Determination of cell dry weight and residual sugar

For measurement of cell dry weight, the cells from a sample were filtered through a mesh with 30 μm pore size and washed with a large amount of distilled water, then collected by centrifugation at 30,500 g (twice). The fresh cells were dried at 50℃ for sufficient time until a constant dry weight was obtained. After sampling, the culture supernatants were stored at minus 20℃, and later thawed for analyses of residual sugar (Fang and Zhong, 2002a; Tang and Zhong, 2002).

Measurements of extracellular and intracellular polysaccharides

For the determination of extracellular polysaccharides (EPS), after removal of mycelia by filtration, the culture filtrate was dialyzed, and the crude extracellular polysaccharides were precipitated with 4 volumes of 95% (v/v) ethanol, then recovered by centrifugation at 2,400 × g. The insoluble components were suspended in 1 M NaOH at 60℃ for 1 h, and reducing sugar in the supernatant were measured by phenol-sulfuric acid method (Dubois et al., 1956). Intracellular polysaccharides (IPS) were extracted from dried mycelia (100 mg) by 1N NaOH at 60℃ (for 1 h), and the supernatant was tested for reducing sugars (Dubois et al., 1956).

Assay of ganoderic acids

The dried mycelia (100 mg) were suspended in 50% (v/v) ethanol (3-mL) once for one week (twice). After removal of mycelia by centrifugation, the supernatants were dried at 50°C under vacuum. The residues were suspended by water, and later extracted with chloroform. The GA in the chloroform phase was extracted by 5% (w/v) $NaHCO_3$. After adding 2 N HCl to adjust the pH of the $NaHCO_3$ layer to be lower than 3, the GA in the $NaHCO_3$ layer was extracted with chloroform. After removal of chloroform by evaporation at 40°C, GA was dissolved in absolute ethanol, and its absorbency was detected at 245 nm by using a spectrophotometer.

Results and Discussion

Effects of O_2 supply in shake flasks

Although there are a few studies on submerged cultures of *G. lucidum* for production of extracellular polysaccharides (Lee et al., 1999; Yang and Liau, 1998), no detailed information is available regarding the effects of O_2 supply on the accumulation of the fungus biomass and culture metabolites. As shown in Figure 1A, the maximum cell density of 14.6, 14.1, 11.9 and 11.5 g DW/L was obtained at day 6, 8, 10 and 12 at K_La of 32.6, 18.5, 13.2 and 6.4 h^{-1}, respectively. The results indicate that a higher O_2 supply led to more final biomass and shorter cultivation time. By correlation (Figure 2), a linear relationship between the average growth rate and the initial K_La could be described by the following equation (r^2=0.98):

$$AGR=0.193\times K_La+1.76 \quad (1)$$

where AGR is average growth rate (d^{-1}), i.e., (Final cell density - Initial cell density)/(Initial cell density × Cultivation time). In fermentation of *Penicillium canescens* (Gaspar et al., 1997) and in cell culture of *Murine hybridomas* (Randers-Eichhorn et al., 1996), it was also reported that good O_2 supply resulted in high cell growth rate.

Figure 1B shows that glucose consumption rate was low at a low K_La. The residual sugar was almost used up on day 8 and day 10 at K_La of 32.6 and 18.5 h^{-1}, respectively. However, at low K_La values of 13.2 and 6.4 h^{-1}, there remained 2.8 and 6.7 g/L of glucose at the end of cultivation (day 14). As shown in Figure 1C, the glucose consumption rate could be linearly correlated to the cell growth rate (r^2=0.97), i.e.,

$$AGR=1.462\times GCR \quad (2)$$

where AGR is average growth rate (d^{-1}); GCR, glucose consumption rate (g/(L d)). This result suggests that the carbon flux to biomass production was quite steady under

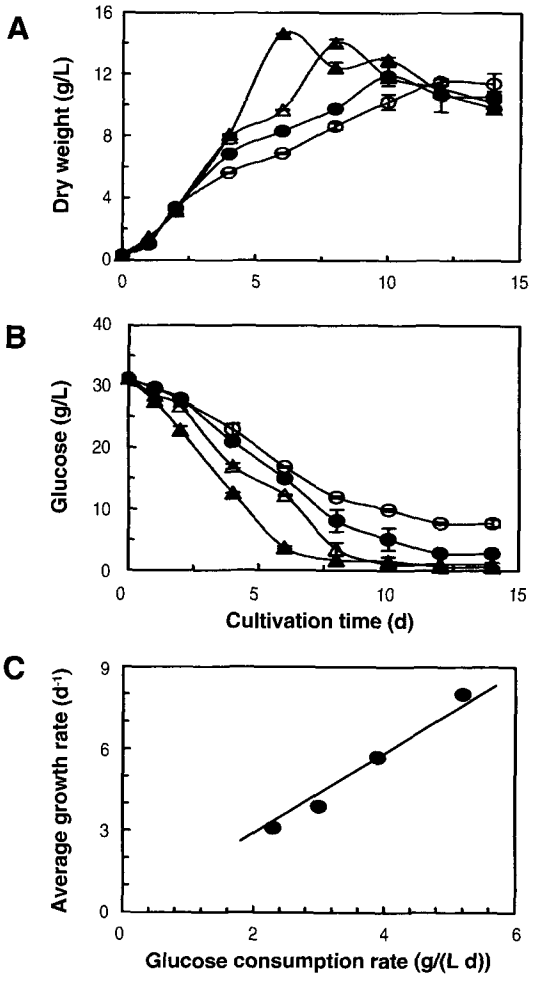

Figure 1. Effect of O_2 supply (initial K_La) on the cell growth (A) and glucose consumption (B), and the relationship between average cell growth rate and glucose consumption rate (C) in submerged cultures of *Ganoderma lucidum* in flasks. In Figs. 1A and 1B, symbols for initial K_La (h^{-1}): 32.6 (dark triangle), 18.5 (open triangle), 13.2 (dark circle), and 6.4 (open circle). The error bars in the figure indicate the standard deviations from 3 samples.

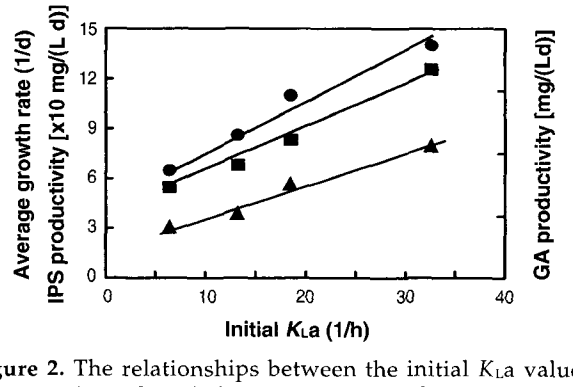

Figure 2. The relationships between the initial K_La value as investigated and the average growth rate (triangle), intracellular polysaccharide (IPS) productivity (circle), and ganoderic acid (GA) productivity (square).

Table 1. Effects of loading volume on the cell growth, yield, and production of IPS, EPS and GA[a]

Loading volume (mL)	30	50	70	100
Cell dry weight (g/L)	14.6±0.0	14.1±0.2	11.9±0.6	11.5±0.2
Y x/s (g DW/g glucose)	0.53	0.51	0.46	0.49
Average growth rate (d-1)	8.0	5.7	3.9	3.1
Maximum IPS content (mg/100 mg DW)	6.7±0.2	7.3±0.1	7.9±0.1	8.1±0.1
IPS specific productivity (mg/(g DW d))	1.7	2.1	2.6	2.7
Maximum IPS production (g/L)	0.83±0.02	0.87±0.07	0.86±0.07	0.91±0.06
IPS productivity (mg/(L d))	140	110	86	65
Maximum EPS production (g/L)	0.52±0.04	0.66±0.02	0.63±0.00	0.64±0.00
EPS productivity (mg/(L d))	62	63	61	51
Maximum GA content (mg/100 mg DW)	1.39±0.01	1.29±0.11	1.54±0.05	1.45±0.09
GA specific productivity (mg/(g DW d))	0.92	0.57	0.70	0.62
Maximum GA production (mg/L)	203±2	180±16	183±8	149±13
GA productivity (mg/(L d))	33.5	22.2	18.1	14.6

[a]Standard deviation was calculated from 3 samples

different O_2 supply conditions, and the cell yield on glucose did not change so much (Table 1).

The effect of O_2 supply on IPS formation is shown in Figure 3A. In the lag phase (for 1 day), the specific production (i.e., content) of IPS decreased from 4.3 to around 3.9 mg/100 mg DW. After that, the IPS synthesis was promoted and its content increased. At the end of cultivation (day 14), the highest IPS content at an initial K_La of 32.6, 18.5, 13.2 and 6.4 h^{-1} was 6.73, 7.25, 7.93 and 8.06 mg/100 mg DW, respectively. As shown in Figure 3B, the maximal production of IPS was obtained on day 6, 8, 10 and 14 at an initial K_La of 32.6, 18.5, 13.2 and 6.4 h^{-1}, respectively. Although the maximum IPS production was almost the same under the different O_2 supply conditions, a higher O_2 supply led to a higher IPS productivity (Figure 2). The maximal IPS production (and productivity) at an initial K_La of 32.6, 18.5, 13.2 and 6.4 h^{-1} was 0.83 (140), 0.87 (110), 0.86 (86) and 0.91 g/L (65 mg/(L d)), respectively (Table 1). The high IPS productivity under good O_2 supply was due to the high cell growth rate in the case.

Figure 3C shows the EPS production at various initial K_La values. The EPS accumulation increased rapidly after day 1 and reached a peak value on day 8, 10, 10 and 12 at an initial K_La of 32.6, 18.5, 13.2 and 6.4 h^{-1}, respectively, and its maximal production titer was 0.52, 0.66, 0.63 and 0.64 g/L for each case. Their corresponding EPS productivity was 62, 63, 61 and 51 mg/(L d) (Table 1).

The dynamic profiles of specific production (i.e., content) and total production of GA under different O_2 supply conditions were illustrated in Figure 4. At an initial K_La value of 32.6 and 18.5 h^{-1}, the GA content increased quickly after inoculation and reached a peak value at day 6 and 8, respectively (Figure 4A). At an initial K_La of 13.2 and

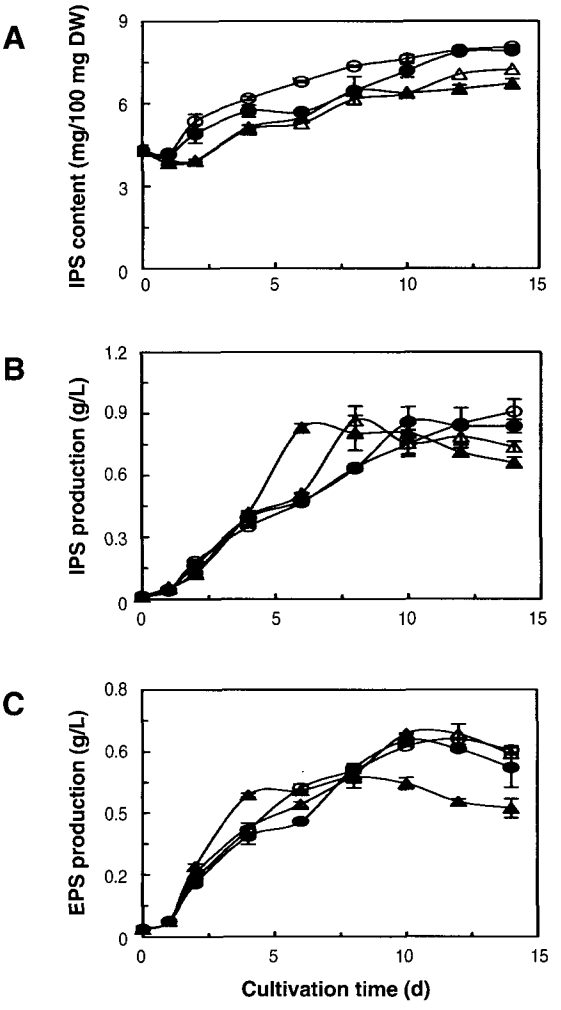

Figure 3. Effect of O_2 supply on IPS content (A), IPS production (B), and EPS accumulation (C). The symbols for initial K_La are the same as those in Figure 1.

Table 2. Effects of initial oxygen transfer coefficient (K_La) on the cell growth, yield, and production of IPS, EPS and GA[a]

Initial KLa value (per h)	16.4	60.0	78.2	96.0
Cell dry weight (g/L)	11.8 (d 8)	14.1 (d 8)	15.6 (d 8)	12.9 (d 8), 13.6 (d 13)
Average growth rate (d^{-1})	1.39	1.69	1.87	1.53
Maximum IPS content (mg/100 mg DW)	17.5	14.2	14.0	15.6
Maximum IPS production (g/L)	1.9	1.6	2.2	2.1
IPS productivity (g/(L d))	0.19	0.16	0.22	0.21
Maximum EPS production (g/L)	0.97 (d 13)	0.69 (d 13)	0.92 (d 13)	0.92 (d 13)
EPS productivity (mg/(L d))	73 (d 13)	51 (d 13)	69 (d 13)	69 (d 13)
Maximum GA content (mg/100 mg DW)	2.33 (d 13)	2.44	2.17	3.36
Maximum GA production (mg/L)	245	280	338	450
GA productivity (mg/(L d))	23.8	27.3	33.1	44.3

[a]The maximal values were obtained on day 10 during cultivation except those indicated.

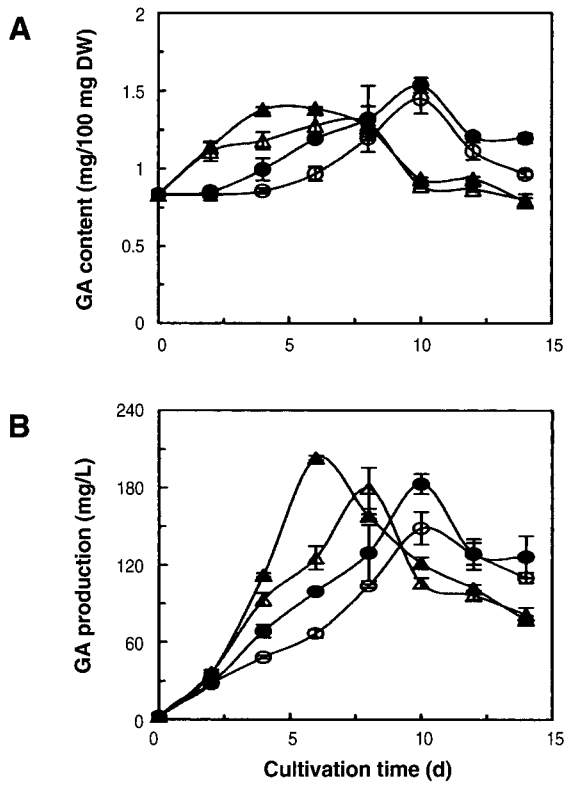

Figure 4. Effect of O_2 supply on the content (A) and total production (B) of GA. The symbols for initial K_La are the same as those in Figure 1.

6.4 h^{-1}, GA content was stable around 0.84 mg/100 mg DW for the first 2 days, increased after day 2 and reached its peak on day 10 (Figure 4A). The highest content at an initial K_La of 32.6, 18.5, 13.2 and 6.4 h^{-1} was 1.39, 1.29, 1.54 and 1.45 mg/100 mg DW, respectively. The GA content of 1.54 mg/100 mg DW obtained here was more than 3-fold in a previous work by Tsujikura et al. (1992).

The maximum production of GA (Figure 4B) was obtained on the same day as the highest GA content (Figure 4A). At an initial K_La of 32.6, 18.5, 13.2 and 6.4 h^{-1}, its highest production titer was 203, 180, 183 and 149 mg/L, respectively, and the corresponding GA productivity was 33.5, 22.2, 18.1 and 14.6 mg/(L d) for each (Figure 2 and Table 1). A higher GA productivity was obtained under higher O_2 supply because of high cell growth rate in the case. The GA productivity of 33.5 mg/(L d) reached here was also much higher than that of previous work (Tsujikura et al., 1992).

Effects of O_2 supply in bioreactors

Effect of initial K_La on *G. lucidum* cultures was also studied in bioreactors. As shown in Table 2, initial K_La affected the biomass level, and its peak value of 15.6 g DW/L was obtained at an initial K_La of 78.2 h^{-1}. The results indicate that initial K_La had a significant effect on the cell growth during cultivation and an initial K_La of 78.2 h^{-1} seemed to be best for cell growth of G. lucidum. A typical time course of residual sugar concentration (at an initial K_La of 78.2 h^{-1}) is shown in Figure 5A.

The EPS production was 0.97, 0.69, 0.92 and 0.92 g/L in the cultures at initial K_La values of 16.4, 60.0, 78.2 and 96.0 h^{-1}, respectively, and its corresponding productivity was 73, 51, 69 and 69 mg/(L.d) (Table 2). Figure 5B shows the kinetics of EPS accumulation at K_La of 78.2 h^{-1}. From day 0 to day 13, a rapid increase of EPS concentration was observed, and from day 13 to the end of culture (day 15), its accumulation level showed a slight decrease.

For the specific production (i.e., content) of IPS, as summaried in Table 2, the maximum IPS content of 17.5, 14.2, 14.0 and 15.6 mg per100 mg DW was reached on day 10 in the cultures at an initial K_La value of 16.4, 60.0, 78.2

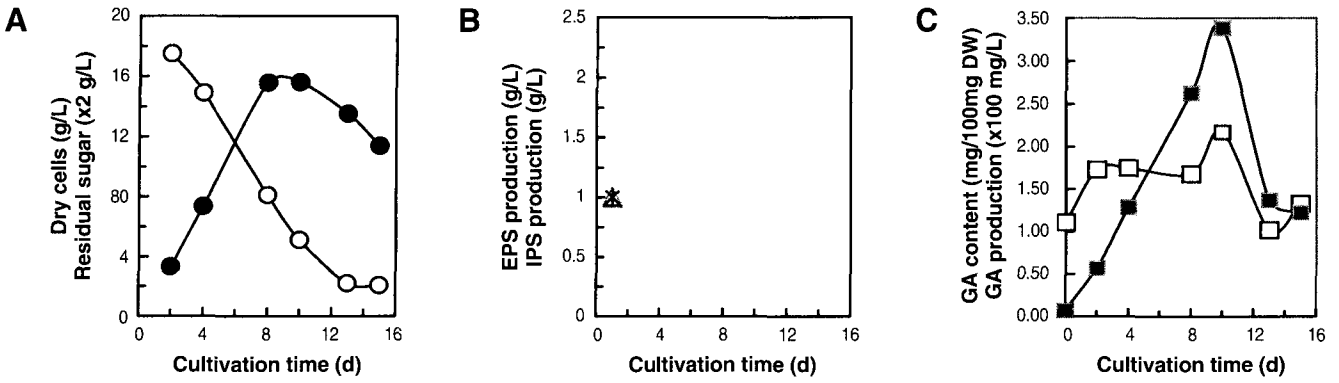

Figure 5. Time profiles of dry cell weight (A), residual sugar concentration (A), production of extracellular (EPS) and intracellular polysaccharides (IPS) (B), and specific production (i.e., content) and total production of ganoderic acids (GA) (C) in submerged cultures of *G. lucidum* at initial KLa of 78.2 h^{-1} in a bioreactor. Symbols: residual sugar (open circle), dry cell weight (dark circle), EPS (open triangle), IPS (dark triangle), GA content (open square), and total GA production (dark square).

and 96.0 h^{-1}, respectively. The maximum IPS production of 1.9, 1.6, 2.2 and 2.1 g/L was reached on day 10 in the culture grown at an initial K_La value of 16.4, 60.0, 78.2 and 96.0 h^{-1}, respectively, and its corresponding productivity was 0.19, 0.16, 0.22 and 0.21 g/(L.d). The dynamic profile of the total accumulation of IPS is shown in Figure 5B. The results indicate that a relatively higher initial K_La value was favorable for IPS production and productivity.

Figure 5C shows the kinetics of GA content and total GA production. Table 2 indicates that the maximum GA content in the culture at initial K_La values of 16.4, 60.0, 78.2 and 96.0 h^{-1} was 2.33, 2.44, 2.17 and 3.36 mg/100mg DW as obtained on day 13, 10, 10 and 10, respectively. Although the highest biomass was obtained at an initial K_La of 78.2 h^{-1}, the maximum GA production was obtained at an initial K_La of 96.0 h^{-1} because of the high GA content obtained in the latter case (Table 2). The total GA production of 245, 280, 338 and 450 mg/L was attained on day 10 in the culture grown at initial K_La values of 16.4, 60.0, 78.2 and 96.0 h^{-1}, respectively, and their corresponding productivity was 23.8, 27.3, 33.1 and 44.3 mg/(L.d). An increase in initial K_La led to an increased production and productivity of GA. The GA production and productivity at an initial K_La value of 96.0 h^{-1} was 1.8-fold those at an initial K_La value of 16.4 h^{-1}. It is clear that an initial K_La value of 96.0 h^{-1} was most suitable for both the production and productivity of GA.

Concluding Remarks

The significant effects of O_2 supply (initial K_La) on the cell growth and production of *Ganoderma* polysaccharides and ganoderic acids were demonstrated in submerged cultures of *G. lucidum*. A higher cell growth rate and a higher productivity of IPS and GA were reached under higher O_2 supply. The experiments in bioreactors were also repeated, and the same conclusions were attained. The results obtained are considered useful for the regulation and optimization of the mushroom cultures for simultaneous, highly efficient production of the cell mass, *Ganoderma* polysaccharides, and ganoderic acids on a large scale. Related work on effect of dissolved O_2 (DO) was also done in our laboratory and will be published elsewhere.

Acknowledgements

Financial support from the National Natural Science Foundation of China (NSFC project no. 20076011), the Science and Technology Commission of Shanghai Municipality (Qimingxing Jihua), and the Key Discipline Development Project of Shanghai Municipality (Shanghaishi Zhongdian Xueke Jianshe Xiangmu) is acknowledged. The Cheung Kong Scholars Program admini-stered by the Ministry of Education of China and the National Science Fund for Distinguished Young Scholars administered by NSFC are also appreciated. J.J.Z. would especially like to thank Profs. Kee-Yoeup Paek and Eun-Joo Hahn, Research Center for the Development of Advanced Horticultural Technology, Chungbuk National University, for their warmest invitation.

References

Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28: 350-356.

El-Mekkaway S, R. Meselhy M, Nakamura N, Tezuka Y, Hattori M, Kakiuchi N, Shimotohno K, Kawahata T,

Otake T (1998) Anti-HIV-1 and anti-HIV-protease substances from *Ganoderma lucidum*. Phytochemistry 49: 1651-1657.

Fang QH, Zhong JJ (2002a) Two-stage culture process for improved production of ganoderic acid by liquid fermentation of higher fungus *Ganoderma lucidum*. Biotechnol Prog 18: 51-4.

Fang QH, Zhong JJ (2002b) Effect of initial pH on production of ganoderic acid and polysaccharide by submerged fermentation of *Ganoderma lucidum*. Process Biochem 37: 769-774.

Fang QH, Zhong JJ (2002c) Submerged fermentation of higher fungus *Ganoderma lucidum* for production of valuable bioactive metabolites - ganoderic acid and polysaccharide. Biochem Eng J 10: 61-65.

Gaspar A, Cosson T, Roques C, Thonart P (1997) Study on the production of a xylanolytic complex from *Penicillium* canescens 10-10c. Appl Biochem Biotechnol 67: 45-58.

Hajjaj H, Blane PJ, Groussac E, Goma G, Uribelarrea JL, Loubiere P (1999) Improvement of red pigment/citrinin production ratio as a function of environmental conditions by *Monascus ruber*. Biotechnol Bioeng 64: 497-501.

Hikino H, Konno C, Mirin Y, Hayashi T (1985) Isolation and hypoglycemic activity of ganoderans A and B, glycans of *Ganoderma lucidum* fruit bodies. Planta Med 4: 339-340.

Ishmentskii AA, Kondrat'eva TF, Smut'ko AN (1981) Influence of the acidity of the medium, conditions of aeration and temperature on pullulan biosynthesis polyploid strains of *Pullaria* (*Aureobasidium*) *pullulans*. Mikrobiologiya 50: 471-475.

Lee KM, Lee SY, Lee HY (1999) Bistage control of pH for improving exopolysaccharide production from mycelia of *Ganoderma lucidum* in an air-lift fermentor. J Biosci Bioeng 88: 646-650.

Randers-Eichhorn L, Bartlett RA, Frey DD, Rao G (1996) Noninvasive oxygen measurements and mass transfer considerations in tissue culture flasks. Biotechnol Bioeng 51: 466-478.

Rau U, Gura E, Olszewski E, Wangner F (1992) Enhanced glucan formation of filamentous fungi by effective mixing, oxygen limitation and fed-batch processing. Ind Microbiol 9: 12-26.

Shiao MS, Lee KR, Lee JL, Cheng TW (1994) Natural products and biological activities of the Chinese medicinal fungus *Ganoderma lucidum*. In: *ACS Symp Ser 547 Food Phytochemicals for Cancer Prevention II*, Washington DC: American Chemical Society, pp. 342-354.

Sone Y, Okuda R, Wada N, Kishida E, Misaki A (1985) Structures and antitumor activities of the polysaccharides isolated from fruiting body and the growing fermentation of mycelium of *Ganoderma lucidum*. Agric Biol Chem 49: 2641-2653.

Tang YJ, Zhong JJ (2000) Submerged fermentation of higher fungi for production of valuable metabolites (a review). In: Zhong JJ, ed. Advances in Applied Biotechnology (in English). Shanghai: East China University of Science and Technology Press, pp 104-118.

Tang YJ, Zhong JJ (2002) Fed-batch fermentation of *Ganoderma lucidum* for hyperproduction of polysaccharide and ganoderic acid. Enzyme Microb Technol 31: 20-28.

Tsujikura Y, Higuchi T, Miyamoto Y, Sato S (1992) Manufacture of ganoderic acid by fermentation of *Ganoderma lucidum* (in Japanese), Jpn Kokai Tokkyo Koho JP 04304890.

Wang SJ, Zhong JJ (1996) A novel centrifugal impeller bioreactor. II. Oxygen transfer and power consumption. Biotechnol Bioeng 1996;51: 520-527.

Wu TS, Shi LS, Kuo SC (2001) Cytotoxicity of *Ganoderma lucidum* triterpenes. J Nat Prod 64: 1121-1122.

Yang FC, Liau CB (1998) The influence of environmental conditions on polysaccharide formation by *Ganoderma lucidum* in submerged fermentation. Process Biochem 33: 547-553.

Yoshida T, Shimizu T, Taguchi H, Teramoto S (1965) Studies of submerged culture of basidiomycetes (II) the effect of oxygen on the respiration of Shiitake (*Lentinus edodes*). J Ferment Technol 43: 901-908.

Yoshida T, Shimizu T, Taguchi H, Teramoto H (1967) Studies on submerged cultures of basidiomycetes (III): The oxygen transfer within the pellets of *Lentinus edodes*. J Ferment Technol 45: 1119-1129.