Mycelia Growth and Production of Total Flavonoids and 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- by *Schizophyllum commune* Using a Bubble Column Bioreactor Considering Aeration Effect and Mass Transfer Study

doi: 10.15255/CABEQ.2014.2024

Y. P. Teoh and M. Mat Don*
School of Chemical Engineering, University Sains Malaysia,
14300 Nibong Tebal, Seberang Perai South, Penang, Malaysia

Original scientific paper
Received: April 8, 2014
Accepted: December 15, 2014

Higher fungi are a major source of bioactive secondary metabolites, and *Schizophyllum commune* secreted Schizophyllan that could possess antifungal activity. 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP), a compound with flavonoid fraction, is an important bioactive chemical which exhibited antifungal activity to inhibit growth or spore germination. In view of the potential of DDMP, bench scale production of total flavonoid content (TFC) and DDMP of *Schizophyllum commune* was carried out in laboratory fermenter at 1.5 L scale. The growth of *S. commune* was affected by the supply of oxygen during the fermentation. The optimum condition of aeration rate for TFC and DDMP production from *S. commune* was found to be 4 L min^{-1}. The mass transfer coefficient (k_La) of *S. commune* was also studied to investigate the oxygen transfer capabilities in the bioreactor by considering the effect of aeration rate. On the other hand, the k_La value increased with increasing the aeration rate and the maximum k_La value (0.0814 s^{-1}) was obtained with aeration rate of 8 L min^{-1}.

Key words:
total flavonoid content (TFC), 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP), aeration, bubble column bioreactor, volumetric oxygen transfer coefficient (k_La)

Introduction

Higher fungi are a major source of bioactive secondary metabolites, and researchers have established the existence of biochemical pathways solely for the purpose of producing mycotoxins and other natural products in fungi through the study of ecological chemical interactions.[1] Recently, wood-degrading fungi received great attention for its possible use as antimicrobial agent. *Schizophyllum commune*, a species of basidiomycetes belonging to the Schizophyllaceae of Agaricales, played an important ecological role in degrading woody forest litter. This filamentously growing fungus secreted a neutral homoglucan that consists of a backbone chain of 1,3-*β*-D-glucopyranoses units linked with single 1,6-bonded *β*-D-glucopyranoses at about every third glucose molecule in the basic chain, called "Schizophyllan".[2] This polysaccharide has attracted attention in recent years in the pharmaceutical industry as an immunomodulatory, antineoplastic, and anti-viral agent with activities higher than other glucans. In addition, it is also applied in enhanced oil recovery, and cosmetics.[3]

Based on literature review, flavonoids are ubiquitous in photosynthesizing cells and could exert antifungal activity to inhibit the growth or spore germination.[4] For example, Quercetin (3,3',4',5,7-pentahydroxy flavone) is one of the most common native flavonoids occurring mainly in glycoside forms such as rutin (5,7,3',4'-OH, 3-rutinose).[5] Previous study done by Teoh *et al.*[6] revealed that 4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl- (DDMP) belonged to the flavonoid fraction, and it was found to provide antifungal activities towards selected wood-degrading fungi of rubberwood. This unique sugar residue allowed saponins to scavenge superoxides by forming hydroperoxide intermediates, thus preventing biomolecular damage by free radicals.[7]

Bubble column bioreactor is a promising device for gas-liquid mass transfer and was being considered in biochemical application, especially for fermentation process.[8] It also provides a number of advantages in both design and operation, such as high heat and mass transfer rates, compactness and low operating and maintenance costs. Furthermore, there is no mechanical agitation, and the air bubbles forced through the air compressor were responsible for the induced turbulent liquid mixing and the ac-

*Corresponding author: tel: 604-5996468; fax: 604-5941013
Email: chmashitah@usm.my

companying mass transfer.[9] Bubble column bioreactor with a bubble-free aeration through membrane provided a suitable alternative for transferring gas without inducing cell damage through shear stress. It has been reported that batch bioreactor study was convenient for determination of the suitable conditions for maximum productivity.[10]

High oxygen concentration may be toxic to the cells metabolite activities and may strip nutrients from the culture broth. Therefore, the volumetric oxygen transfer coefficient (k_La) was considered as one of the most important scale-up factors in fermentations. For example, high aeration would lead to severe foaming, which could considerably influence the growth and secondary metabolite production.[10] Kantarci *et al.*[8] concluded that the volumetric mass transfer coefficient, k_La increased with increasing gas velocity, gas density, and pressure, whereas it decreased with increasing solid concentration and liquid viscosity.

The aim of this present work was to investigate the effect of aeration on the growth and antifungal agent production by *Schizophyllum commune* under a bubble column bioreactor. Furthermore, the role of oxygen transfer rate was also determined.

Materials and methods

Fungal strain used

A locally isolated wild species of *Schizophyllum commune* was obtained from the Biocomposite and Protection of Timber Forest Products Laboratory, Forest Research Institute Malaysia (FRIM), Kepong, Malaysia. Each stock culture was grown on malt extract agar (MEA) at 30 ± 2 °C and maintained on agar slants prior to subsequent studies.

Mycelia suspension preparation

Mycelia suspension was prepared by suspending mycelia discs from 7-day-old culture plates in sampling bottles containing sterilized distilled water, and 0.1 % (v/v) Tween 80. The disc of 5 mm diameter was cut on the mycelia mats of the agar plate using a sterilized cork borer. A total of 10 discs for every 100 mL sterilized distilled water were vortexed for 5 minutes in order to homogenize the mycelia suspensions.

Medium preparation for bench scale fermentation

The optimum conditions for production and effect of oxygen transfer rate on fungus mycelia and bioactive compounds at a scale of 1.5 L were investigated for *S. commune*. The medium components and different physical parameters were optimized in shake flask level and the optimized parameters were used for bench scale fermentation (data not shown). The fermentation medium (pH 6.78) contained 18.74 g L^{-1} yeast extract, 10 g L^{-1} malt extract, 38.65 g L^{-1} glucose, 1 g L^{-1} KH_2PO_4, 1 g L^{-1} K_2HPO_4, and 0.59 g L^{-1} $MgSO_4 \cdot 7H_2O$.

Bubble column bioreactor study

After several trial and error experiments, it was found that a larger amount of pelleted mycelium was obtained in a bubble column bioreactor than in a stirred tank bioreactor (data not shown). In fact, the tested macro-fungus, *S. commune*, grew very slowly and the pellets were not shear damaged which was often found in the mechanically stirred tank bioreactor. Instead, it formed clean, clear and non-viscous like water which facilitated the control and operation of the bubble column bioreactor during the fermentation process. In addition, the pelleted mycelia also produced a greater amount of anti-fungal agent as compared to that in filamentous form. Therefore, this bioreactor was chosen in subsequent studies.

The bioreactor used in this study was fabricated by Fermentec (Malaysia). Its dimensions were 500 mm length, and 50 mm internal diameter, with 1.5 L working volume. The top of the column was closed with a rubber stopper. For dispersion of the air bubble, a porous membrane with 50 mm diameter and 3 mm thickness incorporated with pore size 16–40 μm was used in the bottom part of the system. This column is connected to the Biostat-B control panel board supplied by Sartorius (Malaysia). The bioreactor is equipped with digitally-controlled pH electrode, temperature probe, and polarographic dissolved oxygen (pO_2) electrode. The air flow rate was measured using a rotameter, and the temperature was maintained through the design of double jacket on the column. Silicone-based antifoam (Witeg GmbH, Germany) was used to control the formation of foam, while the pH of the culture broth during fermentation was controlled by automatic addition of 2 mol L^{-1} NaOH and 2 mol L^{-1} HCl.

Effect of aeration rate on growth and production of antifungal agent by *Schizophyllum commune*

In this study, mixing was done by bubbling air up in the column operated by controlling the air flow rate. The effect of aeration rate on the growth and production of antifungal agent by *S. commune* and change in dissolved oxygen (DO) and glucose concentration profile of the fermentation broth was investigated under varying aeration rate ranging from 1 to 8 L min^{-1} at an initial glucose concentration 38.64 g L^{-1} with controlled pH 6.96, incubated at temperature 31 °C.

Extraction of biomass

The culture broth obtained after fermentation process was then harvested and centrifuged at 4,000 *g* for 15 minutes. The biomass was then dried and homogenized before the extraction process. Dried biomass (100 g) was boiled in 80 % (v/v) methanol-water mixture in a ratio of 1 g : 20 mL for 48 hours. Then, the extraction solvent was separated via filtration and the filtered extract was evaporated using rotary evaporator. The extract obtained (called biomass extract) was then dried and kept at 4 °C for further analysis.

Analytical method

Determination of biomass

The procedure was carried out using the method of Branco *et al.*,[11] with slight modification. The biomass harvested at different time intervals was homogenized at 50 Hz for 6 minutes. According to Banerjee *et al.*,[12] the mycelia biomass can be analyzed using a spectrophotometer after fragmentation of the mycelia by homogenization. And sensitivity of this method depends on the extent of fungal hyphal fragmentation achieved during the homogenization procedure; short homogenization periods (≤ 6 min) are sufficient for the required sensitivity. The biomass concentration was determined by optical density (OD) measurements at 600 nm, in a spectrophotometer (XMA 1200V).

Determination of glucose concentration

The glucose concentration was estimated based on DNS method as described by Ghose.[13]

Determination of total flavonoid content (TFC)

Total flavonoid content (TFC) was measured spectrophotometrically by the aluminium chloride colorimetric assay, as presented by Ordonez *et al.*[14] with slight modification. A sample of 100 mg (biomass extract) was diluted with 85 % ethanol, and 0.5 mL of the diluted sample was then pipetted into 0.5 mL of 2 % (v/v) $AlCl_3$ ethanol solution (2 g $AlCl_3$ in 100 mL ethanol). Ethanol was used as blank in this study. The absorbance was then measured at 420 nm after 30 minutes at room temperature. The yellow color indicated the presence of flavonoids. The flavonoid content was expressed as micrograms of quercetin/milligram of sample (μg QE mg^{-1} sample).

Analysis using UV-visible spectrophotometer for determination of DDMP

In this analysis, the DDMP was determined using the method of Cechovska *et al.*[15] Since the commercial DDMP was not available in the market, the concentration of this compound was determined using a spectrophotometer with a UV-Vis (Evolution 201). Norfuraneol, a pentose-derived analogue of DDMP, which had similar electrochemical properties-half-wave potential at range 0.30 – 0.33 V, was used as a calibration standard for the quantitation of DDMP.

Determination of volumetric mass transfer coefficient

The determination of volumetric oxygen transfer coefficient, k_La was conducted using the dynamic gassing out method as described by Chauhan *et al.*[16] In this study, the fermentation process was in active respiration, aeration was stopped temporarily, and the decrease in dissolved oxygen concentration (γ_L) was measured as a function of time in order to determine the oxygen uptake rate (OUR). Aeration was then re-established and the increase in γ_L was also measured as a function of time. Assuming re-oxygenation of the broth was fast relative to fungal growth, the dissolved oxygen (DO) level would soon reach a steady state called γ_{LSS}, in which it reflected a balance between oxygen supply and oxygen consumption in the system. Thus, the k_La determination using the dynamic gassing out method was based on the dynamic oxygen balance equation as in Eq. (1).

$$\frac{d\gamma_L}{dt} = k_L a\left(\gamma_{LSS} - \gamma_L\right) \quad (1)$$

Rearranging Eq. (1) into Eq. (2):

$$ln\left(\frac{\gamma_{LSS} - \gamma_{L1}}{\gamma_{LSS} - \gamma_{L2}}\right) = k_L a\left(t_1 - t_2\right) \quad (2)$$

Results and discussion

Effect of aeration rate on the growth and antifungal agent production by *Schizophyllum commune*

Mixing is one of the most important and necessary operations used in bioprocessing to achieve uniform concentration, temperature and other properties of the environment for fermentation in a bioreactor.

Figure 1 summarizes the results obtained and it can be clearly seen that the highest biomass (32.38 g L^{-1}), total flavonoid content (TFC) (1.329 μg QE mg^{-1} sample), and DDMP (1.279 μg mg^{-1} sample) were attained in a culture grown at an aeration rate of 4 L min^{-1}. It could be observed that the biomass produced, TFC and DDMP, was lower at lower aeration rate (e.g. 1 and 2 L min^{-1}) compared to aeration rate of 4 L min^{-1}. According to Pavko *et al.*[17]

and Shohael and Paek,[18] the growth and metabolite production were reduced due to inadequate mixing and limited oxygen supply. However, beyond the aeration rate of 4 L min^{-1}, a reverse trend was observed (Figure 1). Many researchers had reviewed that oxygen was necessary for the growth of fungal in aerobic culture, yet a higher aeration rate could damage the cells.[19] In a bubble column bioreactor, the extreme oxygen flow which is provided by larger and faster air bubbles could disrupt the growth of fungal mycelia/pellet, and also the activity of microorganisms by blocking the nutrient transfer and consumption of glucose during growth,[20,21] and hence reduce the biomass and product formation ability. From Figure 1(a), it was found that the biomass produced was reduced to 15.75 % and 22.71 % at higher aeration rate (6 and 8 L min^{-1}), respectively, as compared to the biomass produced at 4 L min^{-1}. This condition was similar to the statement of Shuler and Kargi,[22] who mentioned that in a bubble column reactor, growth of any microorganism was often limited by the presence of bubble coalescence. Similar trend was observed for the production of TFC and DDMP as shown in Figures 1(b) and 1(c). During higher aeration rate of 6 and 8 L min^{-1}, foaming was formed vigorously which limited the ultimate productivity and metabolite activity during the fermentation. Thus indicating that excessive oxygen supply (6 and 8 L min^{-1}) decreased fungal growth as well as the TFC and DDMP production.

The result shown in Figure 1 suggests that the aeration rate had a direct and obvious impact on the growth of *S. commune* in a bubble column bioreactor. At an aeration rate 1 L min^{-1}, the growth of *S. commune* exhibited a longer lag phase (36 h), and then approached a hardly logarithmic phase. After 108 h of culture, it reached a stationary phase with the maximum biomass reached 22.68 g L^{-1}. On the other hand, at aeration rate of 8 L min^{-1}, the growth of *S. commune* exhibited a shorter lag phase (16 h), and approached a faster growth phase to attain maximum biomass (25.55 g L^{-1}) during 56 h. As in this study, the TFC and DDMP profiles exhibited a longer lag phase for each level of aeration rate compared to that in growth profile. As reported by Guo *et al.*,[23] flavonoid compound was a diverse class of secondary metabolites. Figures 1(b) and 1(c) show that TFC and DDMP provided a faster exponential phase to reach maximal production, and then it failed gradually. This might be due to aggressive aeration rate that caused reduction in pellet formation,[24] and thus hindered the fungal secondary metabolites. With this, the optimal aeration condition for *S. commune* growth and antifungal agent production were closely linked to each other.

Typical patterns of glucose consumption and partial dissolved oxygen (pO_2) profiles may be observed in Figure 2. Figure 2(a) shows that the glucose consumption by the tested fungus, *S. com-*

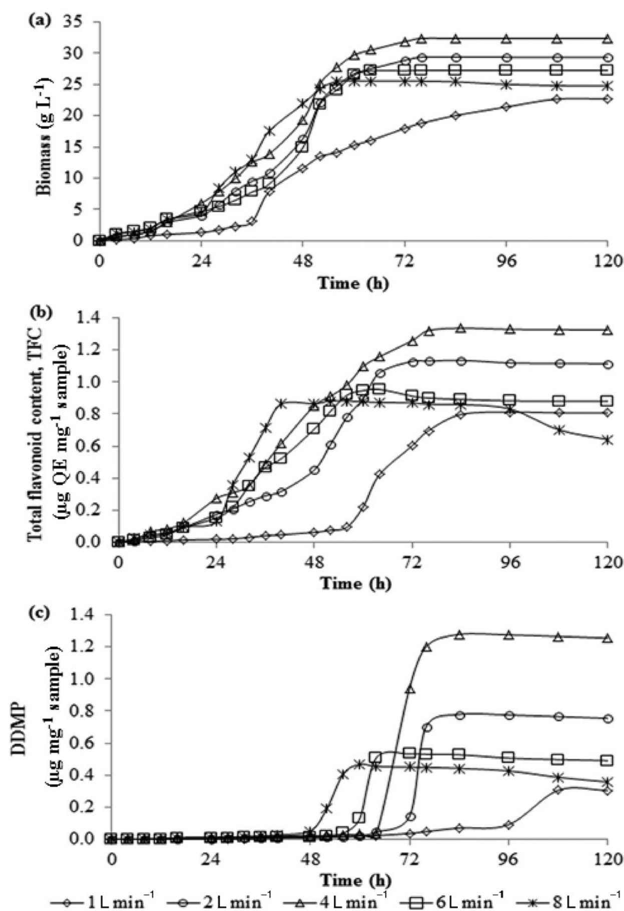

Fig. 1 – *Effect of aeration rate on (a) growth, (b) TFC, and (c) DDMP production by Schizophyllum commune in a bubble column bioreactor*

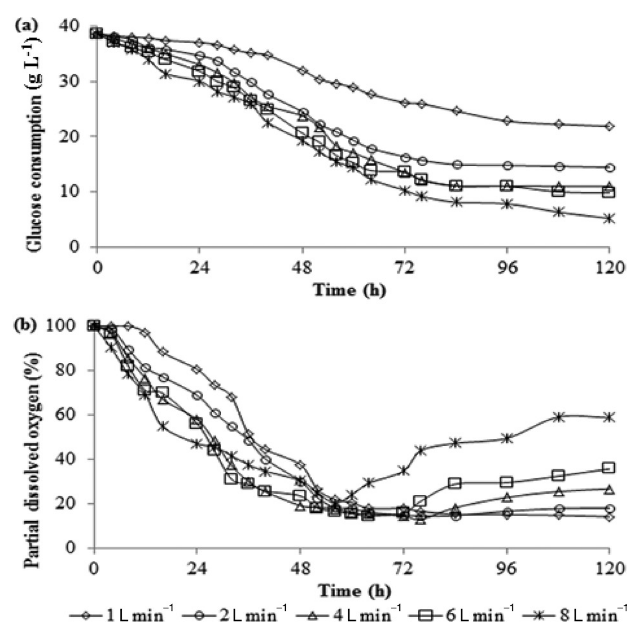

Fig. 2 – *Effect of aeration rate on (a) glucose consumption and (b) partial dissolved oxygen by Schizophyllum commune in a bubble column bioreactor*

mune, increased with increase in aeration rates. In other words, more glucose was utilized for the synthesis of mycelia when higher amount of oxygen was supplied to it. The dissolved oxygen (DO) levels at all aeration rates tested were reduced from 100 % saturation at the beginning of the fermentation to around 15 – 22 % saturation for period of 56 – 84 h (Figure 2(b)). Then, the DO level at aeration rate of 8 L min^{-1} increased gradually as the growth shifted to stationary phase, and therefore a high DO level (about 50 %) was maintained towards the end of the fermentation period. As mentioned by Calik *et al.*,[25] the transfer of oxygen into microbial cells in an aerobic fermentation process showed diverse effect on product formation by influencing the metabolic pathways and changing the metabolic fluxes. In fact, it played an important role since it was often the limiting factor in order to obtain an appropriate volumetric mass transfer coefficient (k_La) that correlated with the productivity in any specific culture media.[19]

Volumetric mass transfer coefficient (k_La) of *Schizophyllum commune* in a bubble column bioreactor

In many aerobic fermentation systems, the rate of oxygen transfer to the cells is the limiting factor which determines the rate of biological conversion.

As shown in Table 1, the maximum k_La (0.0814 s^{-1}) was obtained for the batch fermentation of *S. commune* with aeration rate of 8 l/min. In the present study, the OTR values were proportional to the k_La values as the aeration rates increased. Hikita *et al.*[26] stated that the lower value of k_La decreased the dispersion of the carrier gas as the gas rose up to the column, and hence reduced the gas uptake and transfer capability. The C_L values depended on the relative rate of oxygen transfer and utilization. It must be greater than the critical value of DO in order to avoid oxygen limitation.[19] Chauhan *et al.*[16] suggested that the critical γ_L value was set within

Table 1 – *Effect of aeration rate on the oxygen uptake rate (OUR), volumetric oxygen transfer coefficient (k_La), and oxygen transfer rate (OTR) during cultivation of Schizophyllum commune in a bubble column bioreactor*

Aeration rate (L min^{-1})	Aeration rate (vvm)	OUR (mg L^{-1} s^{-1})	OTR (mg L^{-1} s^{-1})	k_La (s^{-1})	γ_L (mg L^{-1})
1	0.5	0.5039	0.0148	0.0098	2.62
2	1	0.5053	0.0256	0.0188	2.92
4	2	0.5333	0.0845	0.0382	3.58
6	3	0.4491	0.1069	0.0612	4.26
8	4	0.3613	0.1721	0.0814	6.02

*OTR at steady state with γ^* = 7.64 mg L^{-1} at 31 °C

5–10 % (0.382–0.764 mg L^{-1}) of the oxygen solubility in a fermenter. It was found that, although the γ_L value was shown to be higher than the critical γ_L value of the present study for all aeration level tested, the OTR values were relatively lower. This might be due to the pellet formation and the oxygen transfer becoming limited by the increase in pellet size, thus making the bulk γ_L value higher than that inside of the pellet.

Besides, the k_La value is also one of the important scale-up factors in any fermentation process. As shown in Figure 3(a), the growth of *S. commune* increased with increase in k_La from 0.0098 to 0.0382 s^{-1} and thereafter decreased suddenly with further increase in k_La. Maximum biomass (32.38 g L^{-1}) was attained at 0.0382 s^{-1} k_La. Similar pattern was observed in the case of TFC and DDMP production by *S. commune* (Figure 3(b)). Initially, the antifungal agent production increased with increase in k_La up to a certain limit and then decreased. The maximum TFC (1.336 µg QE mg^{-1} sample) and DDMP (1.279 µg/mg sample) were also obtained at 0.0382 s^{-1} k_La. These results exactly coincide with the profile obtained for the growth of *S. commune*. In fact, this result is in good agreement with the research of Chauhan *et al.*[16] who found that the growth of *Acetobacter tropicalis* and dextran sucrase production increased with increase in k_La value to an optimum limit, and then started decreasing with increase in k_La value. Hikita *et al.*[26] explained that the larger k_La value might cause the decreased product in fermentation process due to the bubble coalescence hindering phenomenon. This might be due to high k_La value during

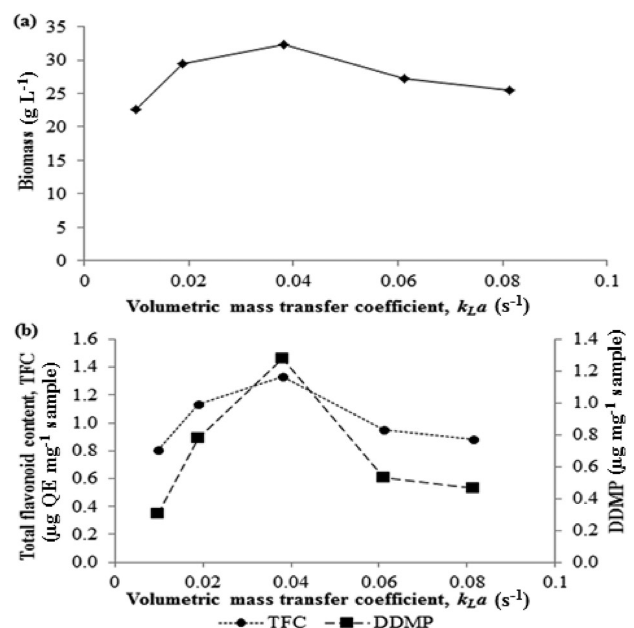

Fig. 3 – *Effect of k_La on (a) growth and (b) TFC and DDMP production by Schizophyllum commune in a bubble column bioreactor*

high aeration rate in which fragmentation or break up of the mycelia frequently occurred, thus reducing the fungal growth as well as product formation. According to Karen,[27] when the growth rate of *Streptomyces* species decreased, the break-up of mycelia resulted in no antibiotic production.

Conclusion

A 1.5 L bubble column bioreactor was chosen in this work. During the study of the effect of aeration, the highest biomass, TFC, and DDMP production were at 32.38 g L^{-1}, 1.329 µg QE mg^{-1} sample, and 1.279 µg mg^{-1} sample, respectively. It could be concluded that oxygen transfer rate plays an important role in the production of antifungal agent from *Schizophyllum commune*. It was found that the k_La values increased with increase in the aeration rate, and hence the maximal k_La value (0.08 s^{-1}) was obtained during aeration rate 8 L min^{-1}. However, it was found that the maximal biomass and antifungal agent production was achieved at 4 L min^{-1} with k_La value of 0.04 s^{-1}.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the National Science Fellowship (NSF) from the Ministry of Science, Technology and Innovation (MOSTI) of Malaysia and the Research University (RU) grant (Grant Account No.: 814057) provided by University Sains Malaysia to support this research work.

List of symbols

γ_L – Dissolved oxygen concentration, mg L^{-1}
γ_{LSS} – Dissolved oxygen concentration at steady state, mg L^{-1}
k_La – Volumetric oxygen transfer coefficient, s^{-1}
t – Time, h

References

1. *Frisvad, J. C., Andersen, B., Thrane, U.,* The use of secondary metabolite profiling in chemotaxonomy of filamentous fungi. Mycol. Res. **112** (2008) 231. http://dx.doi.org/10.1016/j.mycres.2007.08.018
2. *Hao, L. M., Xing, X. H., Li, Z., Zhang, J. C., Sun, J. X., Jia, S. R., Qiao, C. S., Wu, T. Y.,* Optimization of effect factors for mycelia growth and exopolysaccharide production by *Schizophyllum commune*. Appl. Biochem. Biotechnol. **160** (2010) 621. http://dx.doi.org/10.1007/s12010-008-8507-6
3. *Kumari, M., Survase, S. A., Singhal, R. S.,* Production of schizophylan using *Schizophyllum commune* NRCM. Bioresource Technol. **99** (2008) 1036. http://dx.doi.org/10.1016/j.biortech.2007.02.029
4. *Cushnie, T. P. T., Lamb, A. J.,* Antimicrobial activity of flavonoids. Int. J. Antimicrob. Agents **26** (2005) 343. http://dx.doi.org/10.1016/j.ijantimicag.2005.09.002
5. *Kamalakkannan, N., Prince, P. S. M.,* Antihyperglycaemic and antioxidant effect of rutin, a polyphenol flavonoid, in streptozotocin-induced diabetic wistar rats. Basic Clin. Pharmacol. Toxicol. **98** (2006) 97. http://dx.doi.org/10.1111/j.1742-7843.2006.pto_241.x
6. *Teoh, Y. P., Mashitah, M. D., Ujang, S.,* Media selection for mycelia growth, antifungal activity against wood-degrading fungi, and GC-MS study by *Pycnoporus sanguineus*. Bioresources **6** (2011) 2719.
7. *Francis, G., Kerem, Z., Makkar, H. P. S., Becker, K.,* The biological action of saponins in animal systems: A review. Br. J. Nutr. **88** (2002) 587. http://dx.doi.org/10.1079/BJN2002725
8. *Kantarci, N., Borak, F., Ulgen, K. O.,* Bubble column reactors. Process Biochem. **40** (2005) 2263. http://dx.doi.org/10.1016/j.procbio.2004.10.004
9. *Degaleesan, S., Dudukovic, M., Pan, Y.,* Experimental study of gas-induced liquid-flow structures in bubble columns. AIChE J. **47** (2001) 1913. http://dx.doi.org/10.1002/aic.690470904
10. *Chattopadhyay, S., Farkya, S., Srivastava, A. K., Bisaria, V. S.,* Bioprocess considerations for production of secondary metabolites by plant cell suspension cultures. Biotechnol. Bioprocess Eng. **7** (2002) 138. http://dx.doi.org/10.1007/BF02932911
11. *Branco, R. F., Santos, J. C., Murakami, L. Y., Mussatto, S. I., Dragone, G., Silva, S. S.,* Xylitol production in a bubble column bioreactor: Influence of the aeration rate and immobilized system concentration. Process Biochem. **42** (2007) 258. http://dx.doi.org/10.1016/j.procbio.2006.07.010
12. *Banerjee, U. C., Chisti, Y., Moo-Young, M.,* (1993). Spectrophotometric determination of mycelial biomass. Biotechnol. Tech. **7** (1993) 313. http://dx.doi.org/10.1007/BF00150905
13. *Ghose, T. K.,* Measurement of cellulase activities. IUPAC **59** (1987) 257.
14. *Ordonez, A. A. L., Gomez, J. D., Vattuone, M. A., Isla, M. I.,* Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. Food Chem. **97** (2006) 452. http://dx.doi.org/10.1016/j.foodchem.2005.05.024
15. *Cechovska, L., Cejpek, K., Konecny, M., Velisek, J.,* On the role of 2,3-dihydro-3,5-dihydroxy-6-methyl-(4H)-pyran-4-one in antioxidant capacity of prunes. Eur. Food Res. Technol. **233** (2011) 367. http://dx.doi.org/10.1007/s00217-011-1527-4
16. *Chauhan, S., Nisha, Azmi, W.,* J. Oxygen transfer rate modulates the dextransucrase production by *Acetobacter tropicalis*. Biochem. Microb. Technol. **1** (2013) 1. http://dx.doi.org/10.14312/2053-2482.2013-1
17. *Pavko, A., Perdih, A., Lipovsek, M.,* Production of lignin peroxidases with *Phanerochaete chrysosporium* in laboratory bubble column bioreactor. Acta Chimica Slovenica **42** (1995) 249.
18. *Shohael, A. M., Paek, K. Y.,* Production of eleutherosides, total phenolics and total flavonoids from somatic embryos of Siberian ginseng affected by different aeration volume in bioreactor. Int. J. Biosci. **3** (2013) 213. http://dx.doi.org/10.12692/ijb/3.4.213-221
19. *Yazid, N. A.,* Production of Fructosyltransferase and Fructooligosacchaides by *Penicillium simplicissimum*. MSc Thesis, University Sains Malaysia, Penang, Malaysia, 2010.

20. *Bandaiphet, C., Prasertsan, P.,* Effect of aeration and agitation rates and scale-up on oxygen transfer coefficient, k_La in an exopolysaccharide production from *Enterobacter cloacae* WD7. Carbohydr. Polym. **66** (2006) 216.
http://dx.doi.org/10.1016/j.carbpol.2006.03.004
21. *Abd-Aziz, S. A., Fernandez, C. C., Salleh, M. M., Illias, R. M., Hassan, M. A.,* Effect of agitation and aeration rates on chitinase production using *Trichoderma virens* UKM1 in 2l stirred tank reactor. Appl. Biochem. Biotechnol. **150** (2008) 193.
http://dx.doi.org/10.1007/s12010-008-8140-4
22. *Shuler, M. L., Kargi, F.,* Bioprocess Engineering: Basic Concepts, 2nd Edn., Prentice Hall Inc., New Jersey, 2002.
23. *Guo, C. Y., Wang, J., Hou, Y., Zhao, Y. M., Shen, L. X., Zhang, D. S.,* Orthogonal test design for optimizing the extraction of total flavonoids from *Inula helenium*. Pharmacogn. Mag. **9** (2013) 192.
http://dx.doi.org/10.4103/0973-1296.113260
24. *Kim, H. M., Kim, S. W., Hwang, H. J., Park, M. K., Mahmoud, Y. A., Choi, J. W., Yun, J. W.,* Influence of agitation intensity and aeration rate on production of antioxidant exopolysaccharides from submerged mycelial culture of *Ganoderma resinaceum*. J. Microbiol. Biotechnol. **16** (2006) 1240.
25. *Calik, P., Calik, G., Ozdamar, T. H.,* Oxygen transfer effects in serine alkaline protease fermentation by *Bacillus licheniformis*: Use of citric acid as the carbon source. Enzyme Microb. Technol. **23** (1998) 451.
http://dx.doi.org/10.1016/S0141-0229(98)00069-6
26. *Hikita, H., Asai, S., Tanigawa, K., Segawa, K., Kitao, M.,* The volumetric liquid-phase mass transfer coefficient in bubble columns. The Chem. Eng. J. **22** (1981) 61.
http://dx.doi.org/10.1016/0300-9467(81)85006-X
27. *Karen, M. P.,* The Influence of Mycelial Morphology on the Ability of "*Streptomyces*" Species to Produce Antibiotics in Liquid Culture, PhD Thesis, University of Surrey, Guilford, 1990.