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Decolorization of Biogas Power Plant Effluent by *Phanerochaete chrysosporium* TBRC 785

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Abstract

The biogas power plant effluent has a dark brown color and exceeding Thailand's standard for effluent discharged from factories. Direct discharges into public water resources or the environment are not permitted. The aim of this study was to reduce the color and organic substances of biogas effluent from covered lagoon of distillery cane molasses wastewater using *Phanerochaete chrysosporium*. It was found that decolorization efficiency was not related to fungal growth and it could be efficiently performed in the biogas effluent concentration of 10 - 75% v/v to obtain 26.88 - 32.58% of decolorization. Organic removal during fungal cultivation was however dependent on biogas effluent concentration and the optimal value was 25% v/v. Because the biogas effluent sample contained a good balance nutrient for fungal cultivation (C:N of 10:1 measured as SCOD:TKN), nutrient supplementation especially phosphorus to maintain SCOD:N:P of 100:10:1 did not significantly improve fungal decolorization and organic removal efficiency. For efficient fungal remediation, 25% v/v (~6,912 - 7,818 mg_{SCOD}/L) biogas effluent was further used in bioreactor study to investigate the suitable aeration rate of the process. Within 7 days, the aeration rate of 1.0 $volume_{air}$/ $volume_{liquid}$/min (vvm) provided the best fungal remediation efficiency of 36.16 ± 2.93% and 43.15 ± 2.79% for decolorization and organic removal, respectively. Consequently, integration of fungal cultivation process could be a promising approach for waste remediation for the biogas power plant using distillery slop as a substrate as it requires minimal operations/ processes in existing biogas power plants. Moreover, direct fungal spore inoculation into the biogas effluent could substantially reduce cost and time during the process.

Keywords: Biogas power plant effluent; Fungal decolorization; Bioremediation; Melanoidin; White-rot fungus

1. Introduction

Cane molasses has been used as a major feedstock for ethanol production in Thailand (Sawaengsak *et al.,* 2021; Silalertruksa *et al.,* 2022). This ethanol facility generates significant amount of a liquid residue called distillery slop or vinasse which is recognized for its acidic characteristic, brownish color (from melanoidin), and high organic

load (up to ~130 g/L as chemical oxygen demand; COD) (Nitayavardhana *et al.,* 2013). Consequently, distillery wastewater discharge has to be regulated as it can potentially cause a serious environmental problem such as death of aquatic animals and plant and water bodies contamination (Nitayavardhana and Khanal, 2012; Chowdhary *et al.,* 2018).

Anaerobic digestion of the distillery wastewater is a cost-effective and environmentally friendly approach for energy generation in the form of biogas with concomitant waste remediation for ethanol facility. This technology can significantly reduce organic content in the wastewater (~40 - 50% of COD and ~60 - 65% of biochemical oxygen demand; BOD) (Arimi *et al.,* 2015). However, the biogas effluent still has a dark brown color due to the presence of melanoidin, which is highly resistant to biodegradation (Chandra and Kumar, 2017; Chandra *et al.,* 2018). Consequently, the disposal of this biogas effluent could potentially contribute to water pollution and the regulation must be applied to mitigate its environmental impact.

Decolorization of such waste has long been studied using physical-chemical approach but these are not practical for large scale application resulted from high treatment cost and toxic sludge and secondary pollution generations (Dwyer and Lant, 2008; Rai *et al.,* 2008; Liang *et al.,* 2009; Onyango *et al.,* 2011). Bioremediation, which utilizes various types of microbes working together or in succession, is a cost-effective and environmentally friendly approach for this application. White-rot fungi has been known to generate a wide variety of enzymes with specific species, including *Phanerochaete chrysosporium*, *Trametes versicolor*, and *Aspergillus niger*, can particularly effective in breaking down melanoidin (Sirianuntapiboon *et al.,* 2004; Sing and Dikshit, 2011; Kaushik and Thakur, 2013). Among the white rot fungus, *Phanerochaete chrysosporium* has become the most commonly used for wastewater remediation due to its fast growth, easy-to-handle culturing process, and presence of ligninolytic enzymes. This enzyme has potential for use in diverse industrial applications, such as food processing, cosmetics, and are also directly involved in the degradation of various environmental pollutants including polycyclic aromatic hydrocarbons, pulp paper and textiles effluent, make, them very effective for bioremediation purposes (Mielgo *et al.,* 2002; Asamudo *et al.,* 2005; Sharari *et al.,* 2013). Specifically, *Phanerochaete chrysosporium* has been successfully studied for decolorization of distillery slop but significant dilution was required (Gad and El-Sayaad, 2010; Shukla *et al.,* 2020). Moreover, the ideal inoculum for fungal growth in pellet form is an important parameter for used in submerged cultures. The technique using mycelium to produce pellet used as an inoculum for decolorization resulted in an extra 5 - 7 days for preparation in costly enrichment medium. Direct spore inoculation for fungal decolorization on biogas power plant effluent has not been examined thus far. In a previous study spore suspension was used directly for molasses decolorization, it was found the higher color removal could be achieved at 7 days cultivation (Taskin *et al.,* 2016). Successful use of fungal spore inoculation into sample for bioremediation process could potentially reduce cost and process complication and time significantly. Furthermore, a sufficient supply of oxygen through aeration will promote fungal growth, morphology and might be influences on decolorization (D'Annibale *et al.* 2006; Strong and Burgess, 2007; Dewi *et al.,* 2021). The fungus require oxygen to break down the organic matter to carbon dioxide and water and aeration also facilitating the mixing and transfer of oxygen when agitation is absent. It is essential to maintain continuous aeration. Moreover, compared to distillery slop, the biogas effluent contained less amount of biodegradable organic fraction that could have detrimental effect on the fungal decolorization process. Consequently, investigation of the feasibility of using biogas effluent for fungal decolorization is critical.

Based on the above rational, the aim of this study was to investigate the use of white-rot fungus, *Phanerochaete chrysosporium*, in color and organic reduction of biogas effluent. The specific goals were: (i) to determine suitable concentration and nutrient supplementation of the biogas effluent for fungal remediation efficiency; and (ii) to evaluate suitable aeration rate for fungal remediation in a scale up bioreactor study.

2. Materials and Methods

2.1 Biogas power plant effluent sample

The biogas effluent from an anaerobic digester using distillery slop or vinasse, liquid residue from cane-molasses ethanol production, as a starting material was provided by Millonaire Suphan Biogreen Power Co., Ltd., Suphun Buri, Thailand. The sample was analyzed for its important characteristics (APHA, 2012) and was kept at 4 ºC for later use. The biogas effluent was diluted with distillery water to obtain desired concentrations of 10%, 25%, 50%, 75% and 100% (v/v) for decolorization study.

2.2 Fungal culture and inoculum preparation

White-rot fungus, *Phanerochaete chrysosporium* (*P. chrysosporium*) TBRC 785, was obtained from Thailand Bioresource Research Center. The freeze-dried culture was reactivated using sterile water, and then grown on a potato dextrose agar plate at 30 ºC until fungal mycelium was observed. Fungal spores were harvested and kept in a solution containing 0.1% (v/v) Tween 80 and 20% (v/v) glycerol. To prepare inoculum, fungal spore stock culture was adjusted to the concentration of 10^7 spore/mL and kept at -20 °C

2.3 Bioreactor configuration

A 7-L bubble column reactor with 4-L working volume was used to determine suitable aeration rate for fungal decolorization. The bioreactor was made of clear acrylic plastic cylinder pipe with a 15 cm inner diameter, 25 cm height, and 0.5 cm thickness. Air was filtered through a polytetrafluoroethylene (PTEE) membrane filter with 0.2 µm pore size before being supplied through porous air diffusers at the bottom of the bioreactor. The pH was maintained at 5.0 using a combination of 0.5 M sulfuric acid (H_2SO_4) and 0.5 M sodium hydroxide (NaOH) solutions. The temperature was kept at 30 °C using a water bath. The schematic diagram is shown in Figure 1.

2.4 Fungal decolorization study

To determine fungal decolorization efficiency, experiments were conducted in 250-mL Erlenmeyer flasks to provide suitable biogas effluent concentration and nutrient supplementation, and subsequently in a 7-L bioreactor to give the best aeration rate for fungal decolorization process.

Fungal cultivation in flasks used 100 mL of sterile biogas effluent as a substrate with 5% v/v of fungal spore inoculum. Samples were incubated in an incubator shaker at 150 rpm and 30 °C for 10 days (cultivation time was investigated in a previous study). The pH was daily adjusted to maintain the pH value of 5.0. Fungal decolorization conditions including biogas effluent concentration (10, 25, 50, 75, and 100% v/v) and nutrient supplementation (with and without (control)) were evaluated in a sequential order. The biogas effluent concentration provided the best decolorization efficiency was used to obtain nutrient supplementation condition. Potassium dihydrogen phosphate (KH_2PO_4) was a source of phosphorus to maintain SCOD:N:P ratio of 100:10:1 in nutrient supplementation study.

Suitable aeration rate for fungal decolorization was conducted in a bubble column bioreactor with working volume of 4-L. The fungus was cultivated using conditions obtained from earlier section studies. Fungal spore suspension of 5% v/v was inoculated into biogas effluent and the growth conditions were controlled at pH 5.0 and 30 °C for 10 days. Different aeration rates of 0.5, 1.0, and 1.5 volumes of air per volume of liquid per minute (vvm) was provided in order to obtain the suitable aeration rate for fungal decolorization. A detailed experimental is summarized in Figure 2.

2.5 Analytical methods

The biogas effluent sample was analyzed for various characteristics as listed in Table 1. Biochemical oxygen demand (BOD), total and soluble chemical oxygen demand (TCOD and SCOD), total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN), and total phosphorus (TP) were determined

following procedure outlined in the Standard Methods (APHA, 2012). The biogas effluent samples before and after fungal decolorization studies were analyzed for SCOD and color to evaluate organic removal and decolorization efficiencies. Color was analyzed in ADMI (American Dye Manufacturers Institute) unit using standard method 2120F (APHA, 2012). The reported color values were based on an inherent pH of the sample and at pH 7.0. Decolorization efficiency was calculated based on color values at pH 7.0.

At the end of cultivation, fungal biomass was harvested using tea filter bag. The biomass was dried at 105 ± 2 °C until constant weight was obtained and the reported fungal biomass yield was based on dry basis.

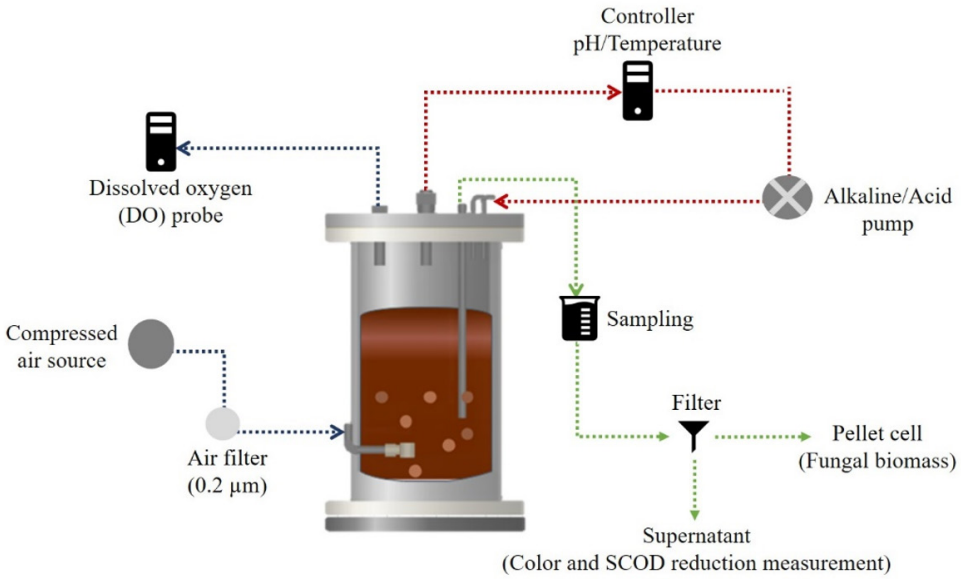


Figure 1. Schematic diagram of 7-L bubble column bioreactor

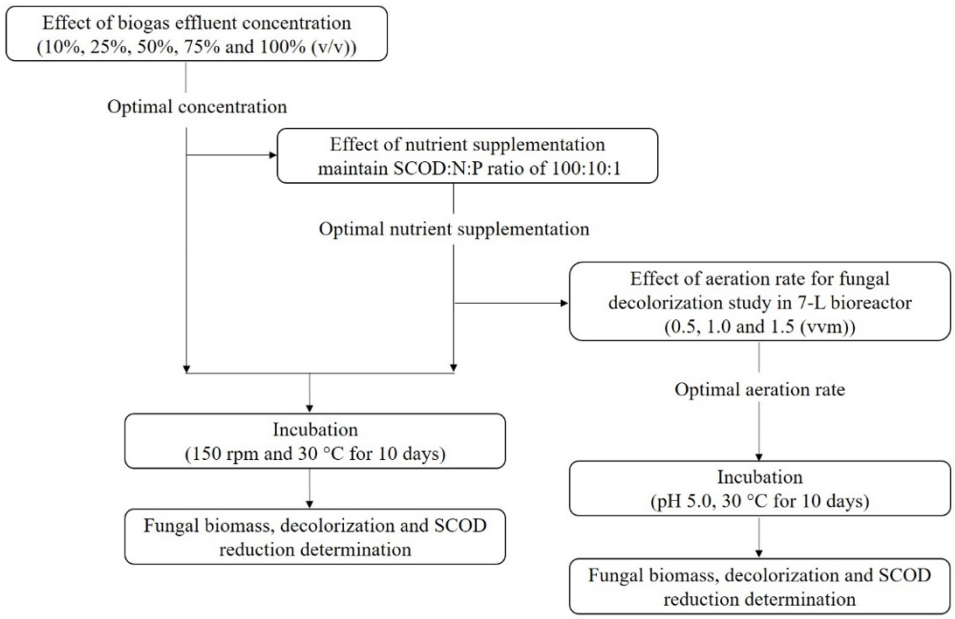


Figure 2. Summary of experimental plan for fungal decolorization and SCOD reduction of biogas effluent

Table 1. Characteristics of biogas power plant effluent sample

Parameters	Values
pH	8.04 ± 0.13
Color (ADMI)	172,733 ± 1,747 (pH 8.04) 151,200 ± 2,466 (pH 7.00)
Biochemical oxygen demand (BOD) (mg/L)	1,801 ± 89
Total chemical oxygen demand (TCOD) (mg/L)	43,946 ± 1,754
Soluble chemical oxygen demand (SCOD) (mg/L)	29,460 ± 1,812
Total solids (TS) (mg/L)	40,156 ± 445
Volatile solids (VS) (mg/L)	22,471 ± 507
Total Kjeldahl nitrogen (TKN) (mgN/L)	2,960 ± 34
Total phosphorus (TP) (mgP/L)	36 ± 3

All values are expressed as mean ± standard deviation of the mean and the sample size (n) = 3

2.6 Statistical analysis

Statistical significance was determined by a one-way analysis of variance (ANOVA) followed by a Tukey's Honest Significant Difference (HSD) at a 95% confidence level. All statistical analyses were carried out using MINITAB statistics software version 16 (Minitab Pty Ltd, Martin place, Sydney NSW, Australia). All experiments were conducted in triplicates. The reported values were mean values with standard deviation and the resulting statistical analyses with significantly difference/same were presented with superscript letters.

3. Results and Discussion

3.1 Effect of biogas effluent concentration and nutrient supplementation on fungal remediation process

Although *P. chrysosporium* was successfully used to break down melanoidin-containing distillery waste, a major concern was a high level of organic and non-biodegradable materials present in the biogas effluent. Compared to distillery slop from sugarcane molasses ethanol facility, BOD:COD ratio of the biogas effluent was approximately 10 times lower. Moreover, high solids content of the biogas effluent could also hinder the growth and spread of fungal mycelia in the cultivation process. To address this, the study first investigated the use of biogas effluent as a sole carbon source for fungal growth by cultivating the fungus on various concentrations of the biogas effluent.

The fungal biomass yields obtained from fungal cultivation at different biogas effluent concentrations are summarized in Table 2. Fungal biomass yields in all experiments were insignificant different at 95% confidential level suggesting that *P. chrysosporium* can metabolize biogas effluent to support its growth and no growth inhibition was observed. Although BOD value of the biogas effluent was low, the white-rot fungus could potentially degrade non-readily digested organic material (which would not be measured as BOD). Moreover, the carbon to nitrogen [C:N] ratio of biogas effluent sample was about 9.95:1 (as SCOD:TKN) which was a close number to C:N ratio of a balance fungal medium (10:1) (Carlile and Watkinson, 1994). Consequently, nutrient supplementation did not significantly improve the fungal biomass yield (discussed later). No nutrient supplementation requirement could be excellent for cost-effective and environmentally friendly bioremediation approach for commercial application.

Fungal decolorization and organic reduction are summarized in Figure 3. Although fungal growth was not inhibited in all biogas effluent concentrations, decolorization efficiency of the sample without dilution (100% v/v) was significantly low. This finding is consistent with other researches indicating that the fungal decolorization was related to the secondary metabolic reaction and was not related to the fungal growth (Kumar *et al.,* 1998; Dahiya *et al.,* 2001). Except for non-diluted biogas effluent sample, fungal decolorization efficiency was independent on biogas effluent concentration and the highest

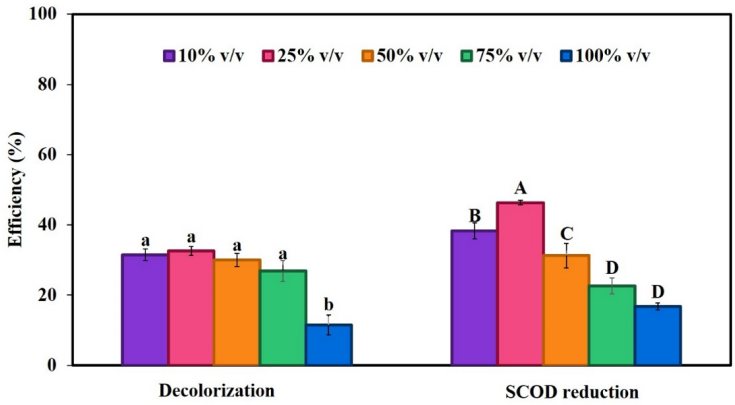
value was derived from fungal cultivation on 25% v/v of biogas effluent which resulted in color reduction of 32.58 ± 1.33%. Significant drop in fungal decolorization efficiency obtained from 100% biogas effluent sample (11.52 ± 2.90%) could be due to antimicrobial property from high melanoidin content in the sample, which the conventional biological wastewater treatment could potentially remove only approximately 6 - 7% of the melanoidin (Satyawali and Balakrishnan, 2008; Kaushik *et al.,* 2017; Johnson *et al.,* 2019).

The organic removal in fungal bioremediation process was however significantly dependent on biogas effluent concentration. As shown in Figure 3, biogas effluent concentration of 25% v/v resulted in the highest SCOD removal efficiency of 46.33 ± 0.65%. Dilution was needed as the biogas effluent sample contained significantly high amounts of organic matter, solids, and chemical constituents especially potassium and heavy metals which could negatively impact the organic removal by fungus (Fahy *et al.,* 1997; Nitayavardhana and Khanal, 2010; Kumar and Chandra, 2020).

In addition, nutrient supplementation to obtain SCOD:N:P ratio of 100:10:1 could not improve the fungal remediation efficiency (Figure 4). As previously mentioned, the biogas effluent sample had balance nutrient for fungal cultivation measured as C:N ratio where the addition of phosphorus would not significantly help to enhance the degradation of color and organic matter. The insignificant different of fungal biomass yields for both samples with (2.45 ± 0.31 g/L) and without nutrient supplementation (2.60 ± 0.93 g/L) were achieved. Consequently, the fungus, *P. chrysosporium* (TBRC 785), could potentially degrade color and organic matter in the biogas effluent from anaerobically digested of cane-molasses vinasse. Although it has been found that addition of easily digestible carbon source such as glucose could improve decolorization efficiency, this would not practical for commercial application (Kumar *et al.,* 1998; Dahiya *et al.,* 2001). The fungal cultivation condition for efficient bioremediation process was 25% v/v of biogas effluent concentration without nutrient supplementation which was used for further study in bioreactor.

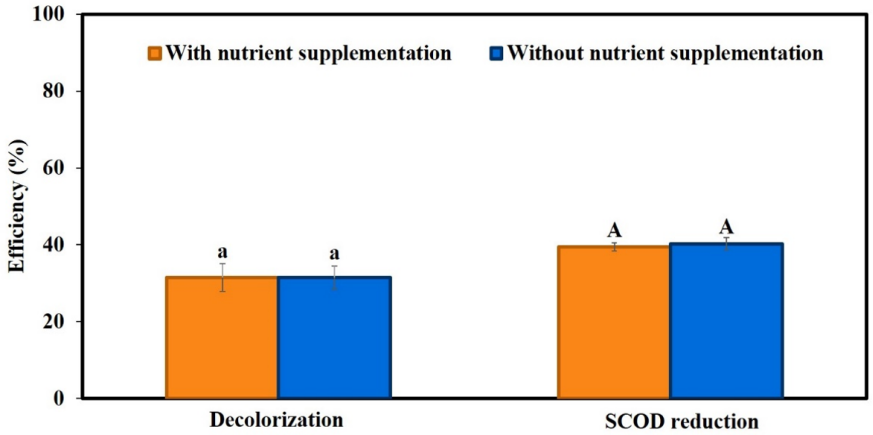
Table 2. Fungal biomass yields from fungal cultivation on various biogas effluent concentrations

Biogas effluent concentrations (% v/v)	Fungal biomass yield (g/L)
10	2.68 ± 0.78[a]
25	2.88 ± 0.33[a]
50	2.68 ± 0.24[a]
75	2.77 ± 0.12[a]
100	2.51 ± 0.52[a]



Note: The letters above the bar graphs correlate statistical analyses. All analyses are based on n (sample size) = 3.

Figure 3. Decolorization and soluble chemical oxygen demand (SCOD) reduction efficiencies during fungal cultivation on biogas power plant effluent samples at various concentrations (10, 25, 50, 75, and 100% v/v)



Note: The letters above the bar graphs correlate statistical analyses. All analyses are based on n (sample size) = 3.

Figure 4. Effect of nutrient supplementation on fungal decolorization and SCOD reduction

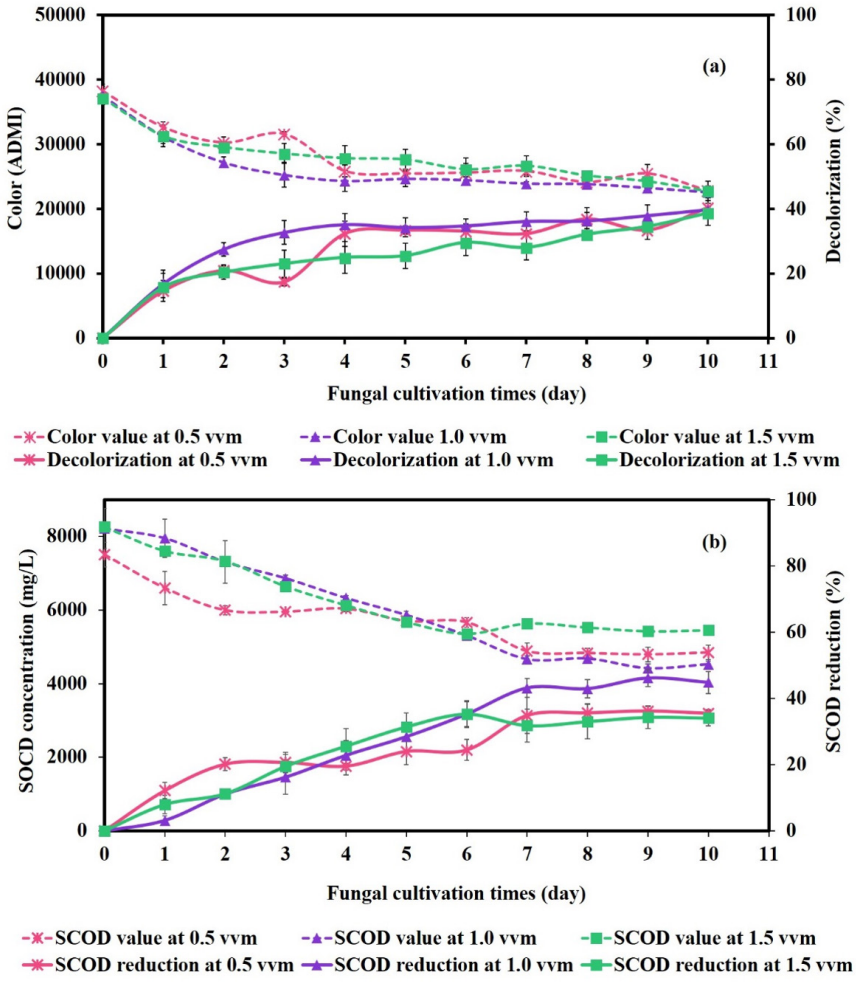


Figure 5. Color and SCOD removal efficiencies during fungal cultivation at various aeration rates of 0.5, 1.0, and 1.5 vvm (a) Color and decolorization, (b) SCOD concentration and reduction

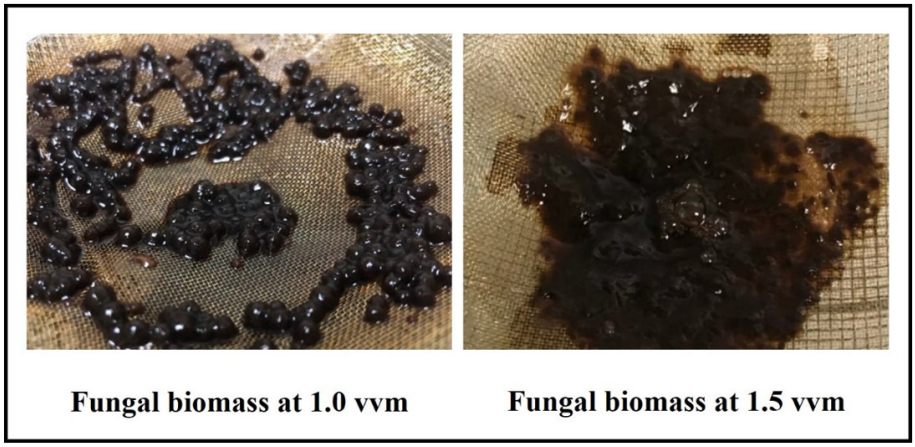


Figure 6. Fungal biomass under the cultivation aeration rates of 1.0 and 1.5 vvm

3.2 Effect of aeration rate on fungal decolorization and organic removal

Fungal remediation process was further investigated in a bubble column bioreactor under different aeration rates of 0.5, 1.0, and 1.5 vvm for 10 days. The fungal biomass yields obtained after 10 days of cultivation under 0.5, 1.0, and 1.5 vvm were 3.05 ± 0.54^{a}, 4.41 ± 0.63^{b}, and 3.02 ± 0.89^{a}, respectively. Rapid reductions of both color and SCOD were observed at the beginning of fungal cultivation and the reductions of both color and SCOD remained constant afterward. This resulted from more available in easily digestible organic material at the beginning of fungal cultivation (Kumar *et al.,* 1998). However, although fungal cultivation time for decolorization varied depending on aeration rate applied, fungal decolorization efficiency at the end of cultivation (10 days) was not dependent on aeration rate (Figure 5a). To achieve desired decolorization efficiency, fungal decolorization under 0.5 and 1.0 vvm required less time of only 4 days, while 10 days was necessary for fungal decolorization under high aeration rate (1.5 vvm). This could be due to high air velocity resulted in interference of spherical fungal pellet formation and more dispersed fungal mycelia was observed under fungal cultivation at 1.5 vvm (Figure 6), which reduced mass and oxygen transfer during the process (Wang *et al.,* 2005; Liu *et al.,* 2008; Nitayavardhana *et al.,* 2013). Moreover, fungal pellet has been reported to have a bioaccumulation effect, where the pollutant was adsorbed and degraded more efficient (Yesilada *et al.,* 2003; Ravikumar, 2015; Wang *et al.,* 2019). The pellet formation could also facilitate efficient settling and recovery of fungal biomass from the biogas effluent sample after remediation process. On the other hand, SCOD reduction was related to aeration rate and fungal cultivation at 1.0 vvm provided the best SCOD reduction during fungal remediation process (Figure 5b). Under the optimal aeration rate (1.0 vvm), efficient organic removal of biogas effluent sample using fungal remediation process required 7 days of fungal cultivation. Consequently, the bioremediation of biogas effluent by *P. chrysosporium* is a feasible approach to apply in wastewater treatment where nutrient supplementation is not necessary in the process.

4. Conclusion

Fungal remediation process for color and organic removal of the biogas effluent is promising. This research showed dramatically time reduction during the process as no fungal mycelia preparation in enrichment medium was required. Direct fungal spore inoculation into biogas effluent sample could reduce 5 - 7 days for the entire process. Fungal decolorization could be performed on biogas effluent concentration ranging from 10 - 75% v/v but the organic removal during fungal cultivation should be done on the biogas effluent concentration of 25% v/v (~6,912 - 7,818 mg_{SCOD}/L).

Moreover, due to the balance C:N content of the biogas effluent sample, nutrient supplementation to maintain the value of SCOD:N:P of 100:10:1 was unnecessary. Fungal remediation process in a bioreactor revealed that the suitable aeration rate for fungal cultivation on 25% v/v biogas effluent was 1.0 vvm providing good condition for fungal biomass pellet formation with 36.16 ± 2.93% and 43.15 ± 2.79% of color and organic removal efficiencies, respectively, within 7 days. However, further investigation to achieve desired color value to meet environmental standard would help for industrial application. This could be possible by integration of fungal remediation process with other physical processes for cost reduction.

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