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A comparative study between fungal pellet- and spore-assisted microalgae harvesting methods for algae bioflocculation

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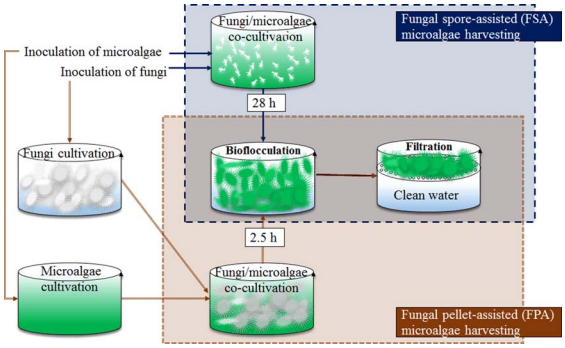
GRAPHICAL ABSTRACT



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ABSTRACT

Fungi assisted microalgae bioflocculation is an emerging, efficient and cost-effective microalgal harvesting method, but no study has systematically evaluated and compared fungal spore-assisted (FSA) and fungal pellet-assisted (FPA) microalgal harvesting methods. In this study, harvesting *Chlorella* sp. cells by co-culture with *Penicillium* sp. spores or pellets was compared. Temperature, glucose concentration, pH and fungi:algae ratio were the critical parameters for harvesting efficiency. The highest flocculation efficiency (99%) of FSA method was achieved in 28 h at 40 °C, 160 rpm, 5 g glucose/L and 1.1×10^4 cells/mL (spore). FPA method can harvest 98.26% algae cells in 2.5 h at 34 °C, 160 rpm, pH 4.0 with the fungi:algae ratio of 1:2. The carbon input for FPA is only half of that for FSA. FPA takes less time and needs less glucose input compared with FSA and may be more promising to be further developed as an effective microalgae bioflocculation method.

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1. Introduction

Microalgae have found potential applications in pharmaceutical industry, food and animal feed production, environmental engineering, wastewater treatment, renewable energy and other fields (Chen et al., 2011; Drexler and Yeh, 2014; Ge and Champagne, 2017; Makareviciene and Skorupskaite, 2013; Shanthakumar, 2016; Shen et al., 2017; Zhou et al., 2011). Microalgae are one of the most promising feedstocks for commercial products because of their fast growth, high photosynthetic efficiency, and high lipid content (up to 70%) (Posten, 2009). However, microalgae production is not economically viable yet due to technical and economic constrains. Recent research showed that three strategies can improve the economics of the microalgae production (Barros et al., 2015). (i) Cultivating microalgae in wastewater to reduce the cost associated with both nutrients and freshwater requirements (Ramsundar et al., 2016). (ii) Using microalgae to produce the third-generation biofuels by integrating carbon dioxide fixation (Zhou et al., 2017). (iii) Using efficient harvesting methods to reduce the harvesting cost which contributes to 20–30% of the total cost of biomass production. Regarding the third approach, various microalgae harvesting methods have been reported (Prajapati and Kumar, 2014; Wan et al., 2015). The ideal harvesting process should be effective and flexible to biomass concentration fluctuations (typically in the range of 0.3–5 g/L), and require low cost of operation, energy and maintenance (Olaizola, 2003). It is common to harvest microalgae in a two-step separation thickening and dewatering procedures. For instance, thickening methods include coagulation/flocculation, gravity sedimentation, flotation, electrical based method; dewatering methods include centrifugal sedimentation and filtration. Although these methods are rapid and efficient, they are either energy intensive or liable to contamination of microalgal biomass with introduced detrimental chemicals (Chen et al., 2013; Grima et al., 2003; Schlesinger et al., 2012). Bioflocculation is a chemical-free flocculation method due to the use of biological agents. The use of bio-flocculants is non-toxic to microalgae biomass, and allows culture medium recycling, which can further reduce the overall cost (Beevi et al., 2017). Moreover, bioflocculation can increase the total biomass production, lipid yield, and wastewater bioremediation efficiency (Muradov et al., 2015).

Bioflocculation of algae has been previously performed by employing suitable microbial partner, such as algae-algae, algae-bacteria and algae-fungi (Alam et al., 2016). Fungal spore-assisted (FSA) microalgae harvesting method could harvest microalgae by co-culture of microalgae with the filamentous fungal spores. It has been recently reported as an efficient algal harvesting method. (Prajapati and Kumar, 2014; Wan et al., 2015; Zhang and Hu, 2012; Zhou et al., 2013) Filamentous fungi, such as *Aspergillus* sp., *Mucor* sp., and *Penicillium* sp. can serve as bioflocculating agents because of their self-pelletization and high efficiency on harvesting microalgae (Gultom and Hu, 2013; Xie et al., 2013). Zhou et al. (2013) harvested 100% *Chlorella vulgaris* biomass after co-cultivating it with pellet-forming fungal strain (*Aspergillus oryzae*) isolated from municipal wastewater sludge; *Chlorella sorokiniana* was co-cultured with the filamentous fungus *Isaria fumosorosea*, and the fungal-algae pellet was used as sustainable feedstock for hydrothermal gasification(Mackay et al., 2015); Although the efficiency of harvesting by FSA method seems to be high, and biomass of fungal-algae pellets could be used as feedstock for production of biogas and liquid petrochemicals (Prajapati et al., 2016), the process of harvesting takes a long time (about 24–48 h). On the other hand, fungal pellet-assisted (FPA) microalgae harvesting method, which includes two steps: culturing fungal pellets and adding active pellets into the microalgae liquid, takes a much shorter time. For example, Wrede et al. (2014) reported that a mass of morphologically and physiologically different microalgal cells were harvested by co-cultivation with *A. fumigatus* pellets. Bhattacharya et al. (2017) harvested microalgae by FPA and obtained 99% harvest efficiency within 2.5 h using pre-cultivated pellets of *Aspergillus lentulus*. However, the comparative study between FSA and FPA harvesting methods for the same pair of fungi and microalgae were not yet reported.

In this study, a systematic technical evaluation and an economic analysis were conducted to compare FSA and FPA microalgae harvesting methods. *Penicillium* sp. (isolated from a local sewage treatment plant) spores and pellets were compared for the flocculation of *Chlorella* sp. (isolated from the same source) under various culture conditions. Response surface methodology (RSM, Box-Behnken design) was employed to study interactions of different factors for co-culture of fungal with microalgae. The harvesting cost of FSA and FPA was calculated separately, and the industrial application prospect of the technology is discussed.

Table 1
Corresponding independent factors and response variables code.

Run No.	Temperature (°C)	Agitation (r/min)	pH	harvest efficiency % (28 h)
1	40(0)	160(0)	7(0)	96.58
2	40(0)	120(−1)	8.2(+1)	98.32
3	35(−1)	160(0)	8.2(+1)	64.67
4	40(0)	160(0)	7(0)	97.56
5	45(+1)	160(0)	8.2(+1)	85.61
6	40(0)	160(0)	7(0)	98.42
7	45(+1)	160(0)	6(−1)	100.00
8	40(0)	160(0)	7(0)	97.63
9	45(+1)	120(−1)	7(0)	97.82
10	40(0)	160(0)	7(0)	97.86
11	40(0)	120(−1)	6(−1)	94.82
12	35(−1)	160(0)	6(−1)	41.19
13	45(+1)	200(+1)	7(0)	100.00
14	35(−1)	120(−1)	7(0)	56.16
15	35(−1)	200(+1)	7(0)	65.50
16	40(0)	200(+1)	6(−1)	98.43
17	40(0)	200(+1)	8.2(+1)	98.19

Table 2
Effect of inoculums size on the fungi–algae pellet formation.

Parameter	Inoculums size (spores/mL)				
	1.1×10^2	1.1×10^3	1.1×10^4	1.1×10^5	1.1×10^6
Pellet formation	o*	o	o	×*	×
Culture time (h)	36	24	24	48	48
Initial pH	4.1	4.1	4.1	4.1	4.1
Final pH	6.5	5.5	6	6.5	7.0

* "×" means no pellets; "o" means pellets.

2. Materials and methods

2.1. Isolation and screening of fungal strains from municipal wastewater sludge

Fungal isolates were sourced from a sewage treatment plant of Wangcheng town (Nanchang, China, GPS position: 28° 10′ N, 115° 27′ E). These samples were stored at −4 °C for further investigation. Samples were directly streaked on potato dextrose agar (PDA) plates with inoculums loop after serial dilution using previous methods (Bala et al., 2006). The isolated fungal strains were tested for pellets formation ability by cultivating spores of candidate strains on potato dextrose broth (PDB) in preliminary experiments. The mycelia morphological of ideal candidate on the PDA agar plates were characterized by optical microscope (CX31RTSF, Olympus Inc., Philippines).

It was observed in preliminary experiments that stable pellets (3–7 mm in diameter) formed rapidly at pH 5, 30 °C, 120 rpm, and 1×10^3 spores/mL (inoculation size) on PDB broth after 2-day cultivation. The stable pellet was also formed rapidly on algae medium, BG-11 (supplemented with 10 g/L glucose).