Effects of glucose and yeast cream content on the Pelletization Behavior of Fungi-Chlorella Sp. Symbiosis System

Zou Yuchun[1, a] , Luo Shanshan[1,b] and Li Wenkui[2,c *]

1Department of Pharmacy, Jiangxi Science & Technology Normal University, Nanchang, 330038, CHINA

2Key Lab of Surface Engineering, Jiangxi Science & Technology Normal University, Nanchang, 330038, CHINA

[a]18770053987, [b]luoss82@163.com, [c]liwenkui1976@163.com

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Abstract. High cost of microalgae harvesting impeded the development of algae biofuel technology and wastewater treatment to commercial practicality. Filamentous fungi and microalgae could form pellets under certain culture condition and the symbiosis system is a novel method to reduce the cost of microalgae harvesting. In this paper, effects of glucose and yeast cream content on the pelletization behaviors of fungi and fungi-*Chlorella* sp. symbiosis system are investigated. The results show that the fungi are easier to form pellets with introduction of glucose or yeast cream.

Introduction

Due to the declining supplies of fossil energy resources and the contribution of these fuels to the accumulation of CO_2 in the environment, much attention on the biomass-based biofuel production has been triggered[1]. Microalgae, with merits of environmental adaptability, high photosynthetic efficiency and high lipid content, have become one of the most promising raw materials for bio-fuels production. Since microalgae are also widely applied in waste water treatment, one promising way to make algae biofuel production more cost-effective is to couple wastewater treatment[2].

However, high cost of microalgae harvesting is a great challenge for algae biofuel technology and wastewater treatment to commercial practicality. The costs of harvesting process is generally accounting for 20-30% of the total costs of production[3]. Traditional algae harvesting methods, including centrifugation[4], filtration[5, 6] and flocculation[7], can only be applied in case of high value products accumulation in algae cells due to its high capital, energy and operational cost[8]. For ordinary microalgae culture solutions, such as *Chlorella* sp., low cell densities (typically 0.3-5g/L) and small cell size (typically 1-30μm) make the recovery of biomass very difficult. Some efforts, including using environmental flow diagram and computer modeling, has performed to reduce the costs of microalgae harvesting[9-11], however, the cost is still very high. Therefore, to reduce the energy consumption of harvesting microalgae, novel harvesting technology is needed.

Fungal have various morphology, including pellets, mycelia, clumps and the morphology could be controlled by adjusting culture parameters, including temperature, medium composition, viscosity, pH, inoculums and additives *etc*[12]. There is great deal of valuable information concerning effect of these parameters on the pelletization behavior of fungi[13, 14]. Recently, Zhou and Zhang *et al* developed an effective fungal pelletization-assisted bioflocculation method for effective algae biomass harvesting and to apply the pelletized fungi –algae symbiosis system as immobilized cells to treat wastewater for improved nutrient removal and wastewater recycling[15-17]. Since the size

of fungal pellets could reach more than several millimeters, the pelletized fungi –algae could be harvest feasibly by traditional filter screen.

The pelletization behavior of fungi-algae system is much more complicated than fungi alone, so further investigation on the effect of culture condition on that is needed. Glucose and yeast cream are commonly used as carbon and nitrogen resources in culture media. In this paper, effects of glucose and yeast cream content on the pelletization behavior of Fungal *Chlorella* sp. symbiosis system are investigated.

Material and Methods

Algal and Fungal Strain Preparation

Algal strain NCU C01 is isolated from local wastewater treatment plants and identified as *Chlorella* sp. The NCU C01 strain was able to grow on both the classic BG-11 medium under light and the modified BG-11 medium (in which 10g/L glucose was added) in the dark condition, suggesting that NCU 01 has both the autotrophic and the heterotrophic pathways and thus could be considered a facultative heterotrophic strain.

Aspergillus sp. NCU F01 is isolated from local environment and identified as *Aspergillus* sp. by morphological analysis. NCU F01 shows white color when grows on solid slant. The fungal species were stored in slant medium (24g/L potato dextrose broth with 20 g/L agar). The spore suspension was obtained by rinsing the slant with distilled water and the number of spores in the suspension was counted by using an optical microscope (B203LED, Chongqing Ao'te Optical and Scientific Instruments Inc., Chongqing, China).

Experiment design:

BG-11 medium, used in this study, contains following chemicals: $K_2HPO_4 \cdot 3H_2O$ (0.04g/L), $MgSO_4 \cdot 7H_2O$ (0.075g/L), $CaCl_2 \cdot 2H_2O$ (0.036g/L), citric acid (0.006g/L), ferric ammonium citrate (0.006g/L), EDTA (0.001g/L), $NaNO_3$ (1.5g/L), Na_2CO_3 (0.02g/L) and trace metal mix A5 (1.0ml). Trace metal mix A5 solution consisted of H_3BO_3 (2.86g/L), $MnCl_2 \cdot 4H_2O$ (1.81g/L), $ZnSO_4 \cdot 7H_2O$ (0.222g/L), $NaMoO_4 \cdot 2H_2O$ (0.39g/L), $CuSO_4 \cdot 5H_2O$ (0.079g/L) and $CoCl_2 \cdot 6H_2O$ (0.05g/L). pH was adjusted to 7.0 and 0-10g/L glucose, 0-9g/L yeast cream was added when needed.

The enriched *Chlorella* sp seed cultures were inoculated at 10 % on 100 mL liquid medium in 250-mL Erlenmeyer flask, placed on a horizontal shaker (150 rpm). The culture condition was kept at 26 ± 2 ℃ with illumination by white incandescent lights (100μmol/m^2/s). The initial inoculums sizes of fungal spores from 7E4/L to 1.2E9/L were added to algae culture medium for fungi –*Chlorella* sp. complex pellets formation.

Results and Discussion

As shown in Fig. 1, fungi pellets could not be formed without the introduction of glucose as while 0.5-2 mm pellets were formed after 72h culture time with addition of glucose in range from 2 to 10g/L. With glucose increasing from 2 to 6g/L, more pellets formed, but no remarkable difference was observed when the glucose exceed 6g/L. The results indicate that C resource is helpful for the formation of fungi pellets in present experiment condition.

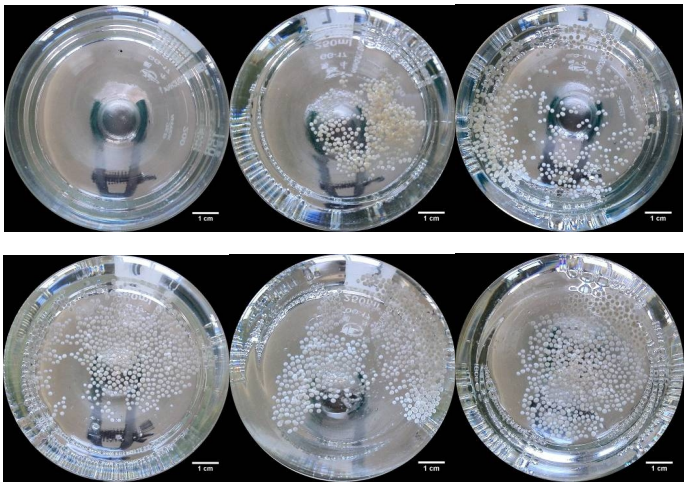

Fig. 1 Fungi pellets formed after 72h cultivation (glucose = 0, 2, 4, 6, 8,10g/L)

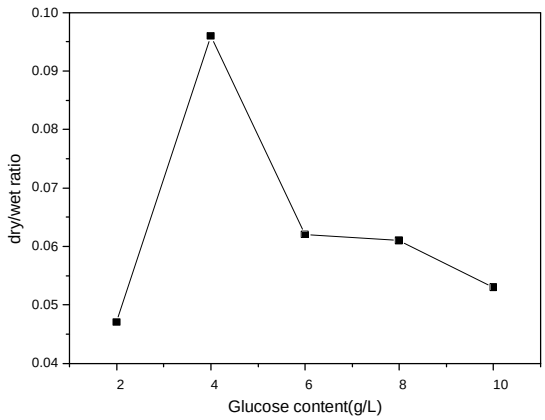

Fig. 2 Dry/wet weight ratios of pellets as function of glucose

Dry/wet weight ratios of pellets are shown in Fig. 2. When the glucose content is 4g/L, pellets have the largest density.

In order to investigate the effect of yeast cream content on the pelletization behavior of fungi, yeast cream, ranging various from 1 to 9g/L were added into the culture media. Fig. 3 is the results of pelletization, where it can be seen that with increasing yeast cream, more pellets were formed. The pellets are looser larger than those of samples with only glucose added. Compared with the samples with only glucose, the samples with yeast cream tend to form clumps.

In the case of fungi and Chlorella sp. co-cultured with 2g/L, and yeast cream ranged from 0 to 9g/L, green pellets could be formed after 24h. After 72 culture period, the pellets turn into gray, which indicates the Chlorella sp. have died, as shown in Fig. 4. The size of pellets is similar to that in Fig. 3, which implies that the growth of Chlorella make little impact in fungi pellets. However, the Chlorella sp. inside the pellets has a shorter live period than others in the culture media. Possible reason is that the Chlorella sp. inside the pellets could not gain enough nutrient substances because the pellets impede the transport of nutrient from media to the centre of the pellets.

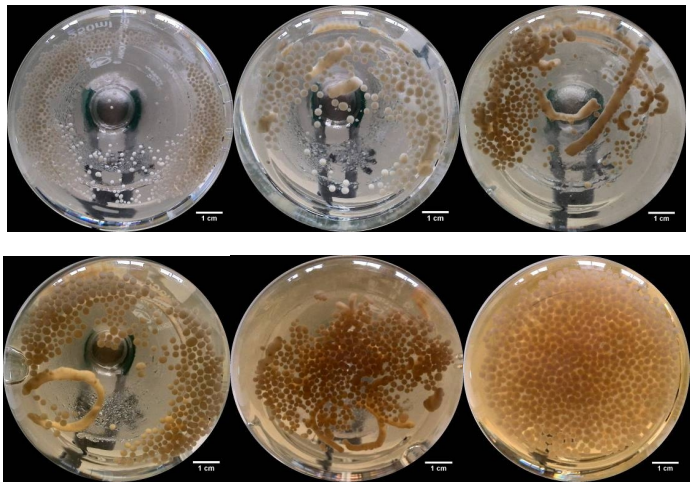

Fig. 3 Fungi pellets formed after 72h with different yeast cream (glucose =2g/L, yeast cream =0, 1, 3, 5, 7, 9g/L)

The growth of *Chlorella* sp. will influence the pH value of the culture media. The start and final pH value of the culture media is shown in Fig. 5. Although the start pH value is decreased with increasing yeast cream content, the final pH value reaches 8.1-8.2 for all samples after 72h, which indicates that the *Chlorella* sp. has different grow rate with different yeast cream addition. The growth of *Chlorella* sp is sensitive to the pH value of the culture media. However more data need collected for thorough investigation and discussion on the relation between growth of fungi-*Chlorella* sp. pellets and the pH value.

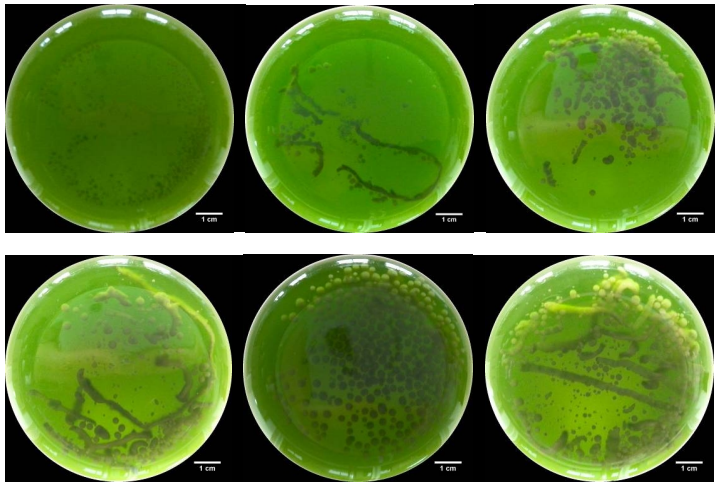

Fig. 4 Fungi-*Chlorella* sp pellets formed after 72h culture. (Glucose=2g/L, yeast cream=0,1,3,5,7,9g/L)

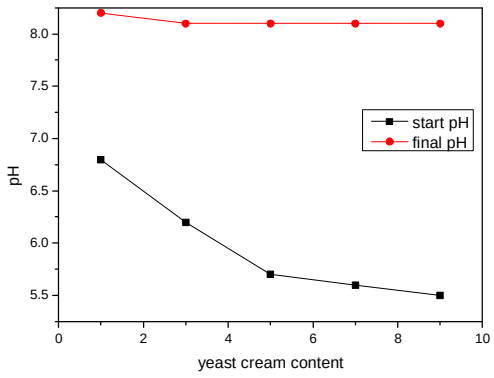

Fig. 5 Start and final pH value of fungi-*Chlorella* sp symbiosis system

Conclusion

Effects of glucose and yeast cream content on the pelletization behaviors of fungi *Chlorella* sp. are investigated. The results show that the introduction of either glucose or yeast cream is helpful to the formation of fungi pellets. The number of fungi pellets reach maximum at 6g/L glucose content. With increasing yeast cream, more fungi pellets could be formed. When fungi and *Chlorella* co-cultured, the growth of *Chlorella* sp make few impact on the pellets formation, but the pellets impede the transportation of nutrient form media to *Chlorella* sp inside pellets.

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