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RESEARCH ARTICLE

Harvesting of *Chlorella* sp. using hollow fiber ultrafiltration

Cui Huang · Xiaolin Chen · Tianzhong Liu · Zhaohui Yang · Yong Xiao · Guangming Zeng · Xiuxuan Sun

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Abstract
Introduction The suitability of the application of ultrafiltration (UF) to harvest *Chlorella* sp. from the culture medium was examined. We investigated the effects of two improved UF system, forward air–water flushing and backwash with permeate, on the concentration process.
Materials and methods Backwash with permeate was selected as an optimization of the improved UF system, which was more effective for permeate flux recovery. Moreover, the hollow fiber UF system by adding periodical backwash with permeate was examined for *Chlorella* sp. harvesting.
Results and discussion It was found that *Chlorella* sp. could be concentrated with high recovery in a lab-scale experiment. An overall algal biomass recovery of above 90% was achieved when the volume concentration factor was 10. For an original biomass of 1.3±0.05 g/L, 1 min backwash followed by 20 min forward concentrating was more effective, which resulted in a recovery of 94% and a high average flux of 30.3 L/m^2/h. In addition, the algal recovery was highly correlated to the volume concentration factor and the initial biomass. A high concentration factor or a high initial biomass resulted in a low biomass recovery.

Keywords *Chlorella* sp. · Harvest · Ultrafiltration · Backwash

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C. Huang · X. Chen (✉) · T. Liu · X. Sun
Key Laboratory of Biofuels, Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences,
Qingdao 266101, People's Republic of China
e-mail: chenxl@qibebt.ac.cn

C. Huang
China Water Resources Pearl River Planning, Designing & Surveying Co., Ltd.,
Guangzhou 510610, People's Republic of China

C. Huang · Z. Yang · G. Zeng
College of Environmental Science and Engineering, Hunan University,
Changsha 410082, People's Republic of China

Y. Xiao
Institute of Urban Environment, Chinese Academy of Sciences,
Xiamen, Fujian Province 361021, People's Republic of China

1 Introduction

Marine microalgae are primary producers and play an important role in marine ecosystems. Over the last century, there has been increasing interest in the production of photosynthetic microalgae for commercial use in many fields such as depollution, therapeutics, dermocosmetics bioenergy, and, in a large part, food and feed industries (Rossi et al. 2008). Such microorganisms are produced in photobioreactors generally coupled with harvesting devices for spatial (Morist et al. 2001; Rossi et al. 2004), biotechnological (Rossignol et al. 2000a, b), and aquacultural (Rossignol et al. 1999) applications. Among these microorganisms, *Chlorella* sp. is one of the main species being exploited (Wang et al. 2010; Chiua et al. 2008; Petruševski et al. 1995). Marine *Chlorella* sp. is a significant potential source of eicosapentaenoic acid and is widely used in aquaculture in China (Feng et al. 2005). The biomass of *Chlorella* sp. from large-scale culture facilities is at a rather low concentration, which is one of the major barriers to the implementation of *Chlorella* sp. A possible solution to the problem is, therefore, to concentrate the algae in the culture medium. An appropriate concentration can enhance the performance of extracting algae oil or some useful sources from *Chlorella* sp.

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Separation of *Chlorella* sp. from their culture medium is a critical process in the biotechnology. Membrane techniques seem to be effective, reliable, and safe, despite some limitations such as the progressive fouling and the permeate flux decline. The cross-flow filtration has many advantages over conventional filtration, centrifugation, and flocculation–floatation sedimentation processes. Cross-flow filtration performs better than dead-end filtration, exhibiting higher permeate flux and less damage on cell integrity. So cross-flow filtration often appears as a suitable process for the purpose of *Chlorella* sp. harvest. Nevertheless, a drawback of membrane filtration is the permeate flux decline. The irreversible fouling of the membrane, mainly due to adsorption, concentration polarization, and eventually pore clogging, is a well-known phenomenon. There are different techniques to limit the fouling phenomenon, such as the utilization of cross-flow rather than frontal filtration, working with high fluid velocity (Morineau-Thomas et al. 2001), physical or chemical cleaning (Smith et al. 2006; Lee et al. 2001), pretreating filters with chemical dispersant (Hill et al. 2005), and so on. But there are few reports about the techniques to limit the fouling phenomenon in *Chlorella* sp. harvesting by ultrafiltration (Zhang et al. 2010).

In this study, backwash followed by forward concentration was selected as an optimization for flux recovery from two kinds of cleaning methods (forward air–water flushing and periodical backwash with permeate). Then periodical backwash was adopted in the following experiment. With the aim of concentrating the *Chlorella* sp. suspension (initial concentration around 1.3 g/L), hollow fiber ultrafiltration technology has been carried out in the experiment. In order to reduce the membrane fouling, the present work was focused on the method of periodical backwash with permeate seawater. Based on different backwash frequency, the permeate flux and biomass recovery were estimated to examine the harvesting effect with volume concentration factor up to 10. Finally, the effect of initial biomass and volume concentration factor on the flux and the biomass recovery were discussed.

2 Materials and methods

2.1 Microalgal suspensions

The *Chlorella* sp. was obtained from Qingdao Institute of Biomass Energy and Bioprocess (China), which was isolated from Jiaozhou bay in Qingdao and cultured in artificial sea water enriched with BG11 nutrients (Kuhl and Lorenzen 1964). The medium filled in a panel photobioreactor (50×50×5 cm) was aerated for 24 h and then inoculated with precultured *Chlorella* sp. at 25°C. The light was provided by eight solar light tubes on one side of the photobioreactor for continuous illumination. Light intensity was 100 μmol/m^2/s as measured by a digital light meter (LI-250A, USA) at the light surface of the cultivator. The culture was continuously aerated with 2% (*v*/*v*) CO_2 at 0.25 volume of gas per volume of media per min (Ong et al. 2010). In order to compare the performance of the backwash frequency, all comparative experiments have been conducted with the same biomass level of 1.3±0.05 g/L.

2.2 Analytical techniques

Biomass concentration was evaluated by dry weight measurements carried out at 105°C (cellulose acetate filters) for 16 h (Takagi and Karseno 2006). The criteria assessments of the UF system's performance were algal recovery rate and average penetrate flux.

Hollow fiber ultrafiltration is typically run in a tangential mode where the retentate is recirculated until the desired volume concentration factor is achieved. In this study the effect of volume concentration factor on algal recovery was investigated with different factors: 10, 15, and 20. Generally the volume concentration factor (C) is expressed by

$$C = V_{\mathrm{in}}/V_{\mathrm{fi}} \tag{1}$$

where V_{in} is the initial volume of medium before the concentration process and V_{fi} is the final volume of algal liquid after the concentration process.

Biomass recovery rate (R) in different conditions is calculated as the following equation:

$$R = V_{\mathrm{fi}} \cdot B_{\mathrm{fi}} \cdot 100/V_{\mathrm{in}} \cdot B_{\mathrm{in}} \tag{2}$$

where R is the defined volume concentration factor (in percent), B_{in} is the initial biomass (in grams per liter) in the tank before concentration, and B_{fi} is the final biomass (in grams per liter) in the tank after the concentration.

Average flux (F) in a process is defined as:

$$F = (V_{\mathrm{in}} - V_{\mathrm{fi}})/S/T \tag{3}$$

where F is the average flux in a process (in liters per square meter per hour), S is the effective membrane area (in square meters), and T is the time for concentration process including the backwash time (in hours).

2.3 Filtration equipment

Chlorella sp. was harvested by the ultrafiltration at 25°C (temperature for laboratory) with a hollow fiber membrane module (Motian Membrane ENG&TECH, Co., Ltd., Tianjin, China). The effective membrane area was 0.4 m^2, while the mean pore diameter of the membrane was 0.2 μm. The membrane material is polyvinylidene difluoride (PVDF).

Figure 1 shows the experimental setup. In a usual concentration process, the culture medium was continuously recycled to the tank through the filtration module; the permeate seawater was collected to another tank. To maintain the setup's security and stabilization, the retentate operating pressure was adjusted at 0.05 MPa and controlled by proportion integration differentiation (PID) press controller. The filtration unit was equipped with transmembrane pressure (TMP) PID controller connected with the digital pressure gauge, which kept the TMP at the desired level automatically. A 10-L algal culture medium was used for every concentration process; samples of the retentate were taken when the desired condition was attained.

In order to concentrate *Chlorella* sp. normally, energizing the power and the "Direction" switch must be in the "positive" position; the system must be ready to run in "concentrate" mode. When backwash is needed, the "Direction" switch must be in the "reverse" position; the system must be ready to run in "backwash" mode. After each experiment, a cleaning procedure was required. An alkaline solution of soda (1% NaOH) was used for PVDF membrane cleaning until a flux of 140±5 L/m^2/h was achieved.

2.4 Method

We applied preliminary design for an optimization of the permeate flux recovery. Two cleaning methods in recycled process were investigated: forward air–water flushing and backwash with permeate. Effects of two cleaning methods on flux behavior were observed when the permeate seawater and retentate were continuously recycled to the culture intake tank (different from Fig. 1). Duration of every hydraulic cleaning was fixed as 1 min. When the filtration system was in hydraulic cleaning mode, the culture medium and air were used for forward air–water flushing manually and UF permeate was used for backwashing by the peristaltic pump. The membrane cleaning efficiencies were determined by the flux recovery percent (flux at a time/the initial flux).

In the study backwash was confirmed as the optimization of cleaning method between forward air–water flushing and backwash with permeate during harvesting. After the optimization was confirmed, periodical backwash frequencies were introduced to the filtration system. In the study, the UF permeate was adopted in backwash procedure to save water resource. Backwash with permeate seawater was followed by forward concentration in every concentration experiment. Generally the research contents were as follows:

1. Effect of backwash frequency on flux and biomass recovery

   The effects of backwash frequency (10, 20, and 30 min for once) on flux behavior and biomass recovery were observed at the same pressure, fluid velocity, and initial biomass.
2. Effect of initial biomass on flux and biomass recovery

   The effects of different initial biomass (0.40±0.04, 1.67±0.05, and 1.92±0.04 g/L) on flux behavior and biomass recovery were observed when the backwash frequency was 20 min for once.
3. Effect of volume concentration factor on biomass recovery

   The recovery of different volume concentration factors (C=10, 20, 30) was observed when the backwash frequency was 20 min for once and the initial concentration was 1.3±0.05 g/L.

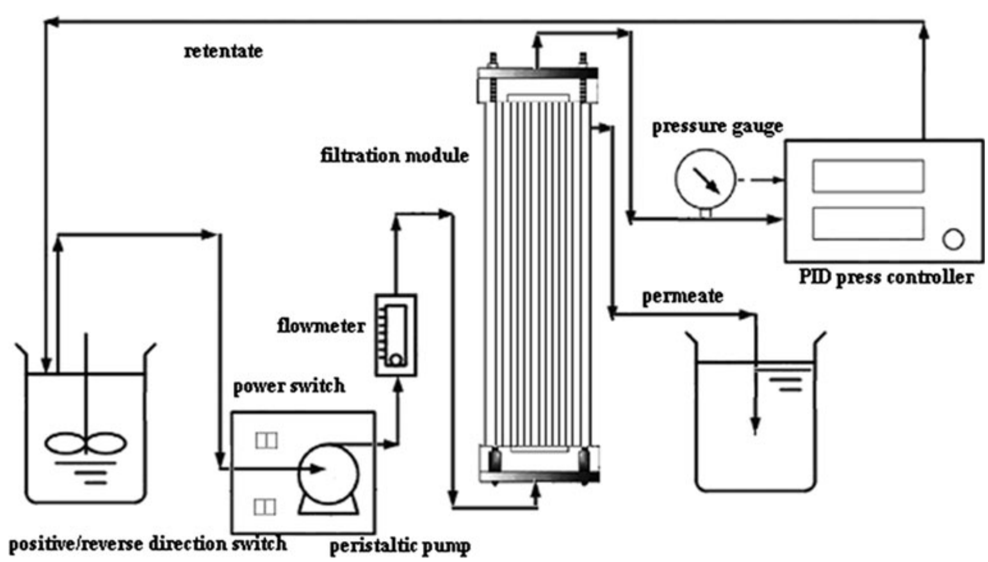

Fig. 1 The scheme of ultrafiltration apparatus

Table 1 Results of flux recovery percent in hydraulic cleaning

Time (min)	Forward air–water flushing flux recovery (%)		Backwashing by UF permeate flux recovery (%)	
	Flush per 10 min	Flush per 20 min	Backwash per 10 min	Backwash per 20 min
11.00	65.89		100.00	
21.00	43.29	40.73	100.00	100.00
31.00	38.49		100.00	
41.00	38.00	32.18	97.70	97.10
51.00	37.78		94.60	
61.00	35.48	29.56	91.90	90.60

3 Results and discussion

3.1 Optimization of flux recovery

The optimization of flux recovery test was performed, and the results were shown in Table 1. The flux recovery of 29% to 66% was achieved by adding forward air–water flushing, while the flux recovery of 90% to 100% was achieved by adding periodical backwash in the concentration process. Certain fouling problems were solved by hydraulic flushing, which coincided with Heng's former report (Liang et al. 2008). It was shown that backwashing was more effective than forward air–water flushing, which coincided with Chen's former report (Chen et al. 2003).

As seen from Table 1, forward air–water flushing could not satisfy with a relatively high flux recovery. To confirm the result, additional experiments were performed.

Two forward air–water flushing frequencies on flux behavior were observed (showed in Fig. 2), while the flux without air–water flushing was also observed in every group. Whether the frequency was 10 or 20 min for once, the results showed that flux increased small after the 1-min air–water flushing. However, flux recovery was better when air–water flushed per 10 min. Since the flux recovery was tiny, we focused on backwash to reduce the membrane fouling in the following experiment.

3.2 Effect of backwash frequency on permeate flux and biomass recovery

In this part, we focused on determination of the optimized backwash frequency by utilizing hollow fiber UF technique. Whatever the frequency was, the results (Fig. 3) showed that flux increased after the 1-min backwash, but drastic flux decline was observed when no backwash was operated. It was because gradual fouling was forming linked to the adsorption phenomenon (Metsämuuronen et al. 2002). Generally speaking, backwash improved performance of ultrafiltration by reducing cake compressibility, increasing the turbulence and decreasing algal layer on membrane surface. However, only certain fouling problems could be solved by backwash; consequently, fouling resistance of the membrane became higher, so a lower average flux in a next interval was observed.

In addition, as shown in Fig. 3, raising backwash frequency could increase the average flux. However, if the backwash frequency was too high, the concentration time would be long, which resulted in lower average flux. While the backwash frequency was too low, fouling resistance became higher, which also resulted in a lower average flux. According to our results, the backwash frequency of 20 min for once exhibited the best performance in terms of permeate flux. The average permeate fluxes of different backwash

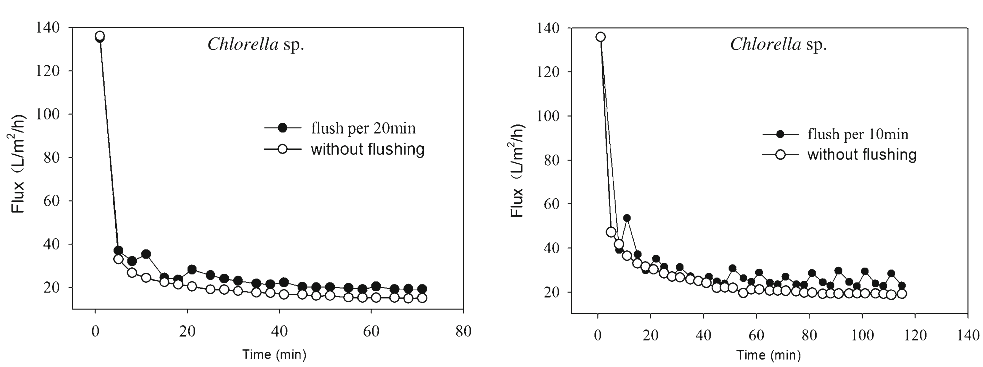

Fig. 2 Flux with different forward air–water flushing frequency

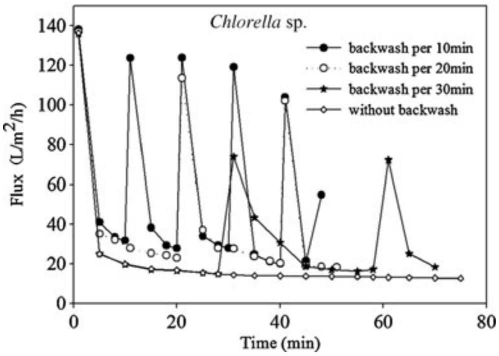

Fig. 3 Flux with different backwash frequency

frequency (10, 20, and 30 min for once) were 28.75, 30.3, and 19.28 L/m^2/h, respectively. But when no backwash was operated, the average permeate flux was only 8.00 L/m^2/h.

Additional experiments were performed to check the effect of backwash frequency on biomass recovery, while the algal biomass was 1.3±0.05 g/L. The 10-L culture medium was concentrated to 1 L, depending on the experimental condition. Consequently, when the backwash frequencies were 10, 20, and 30 min for once, respectively, the algal biomass recoveries were 95.5%, 94%, and 87.2%, respectively. It was concluded that a high backwash frequency was helpful to a high algal biomass recovery, so the algal biomass recovery of the 10-min backwash frequency was slightly higher than that of the 20-min backwash frequency. Therefore, according to the results of permeate flux and algal biomass recovery, we chose the frequency of 20 min for once in the next experiments.

In all, the hydraulic cleaning method of backwash followed by forward flushing with medium was recommended. A hypothesis to explain such a biomass recovery phenomenon with varied backwash frequency is that backwashing may disturb foulants depositing on the membrane surface and the following forward flushing may bring about a beneficial effect on flushing out the debris, which remain in the module during backwash (Liang et al. 2008).

3.3 Effect of initial biomass on the flux and biomass recovery

The effect of initial biomass (0.40±0.04, 1.67±0.05, and 1.92±0.04 g/L) on flux behavior was observed when the backwash frequency was 20 min for once and the concentration factor was 10. As shown in Fig. 4, when the biomass was 0.40 g/L, the recovery of flux was higher than the two others, and it showed a higher average flux. For a higher biomass (1.92 g/L), the recovery of flux was lower after the backwash. As the biomass fluctuated from 0.40, 1.67, and to 1.92 g/L, the corresponding average fluxes were 54.0, 30.7, and 13.5 L/m^2/h, respectively; consequently, the algal

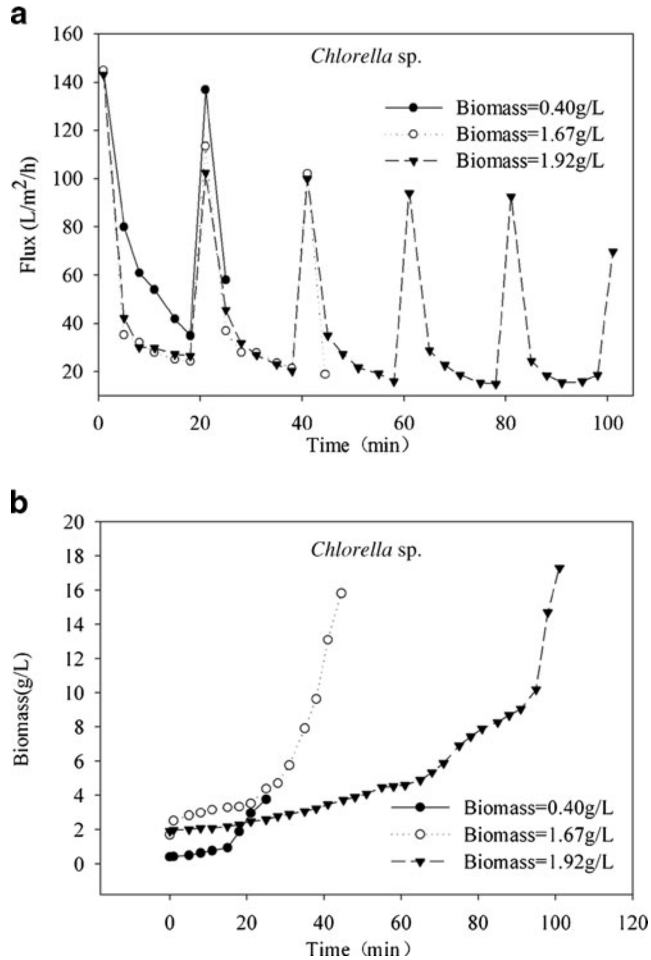

Fig. 4 Flux and biomass with different initial biomass. **a** Flux with different initial biomass. **b** Recovered biomass with different initial biomass density

biomass recoveries were 94.3%, 93%, and 90.2%, respectively. It was because a higher biomass resulted in a severe membrane fouling, while the flux recovery after backwash was not notable compared with the lower biomass experiment.

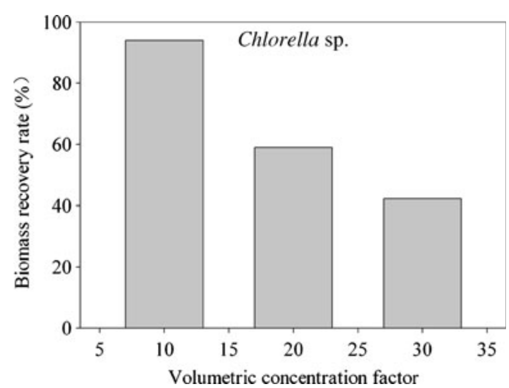

Fig. 5 Effect of volume concentration factor on biomass recovery

3.4 Effect of volume concentration factor on biomass recovery

To assess the relationship between the recovery of algal biomass and volume concentration factor, the recovery of three concentration factors (C=10, 20, 30) was determined when the backwash frequency was 20 min for once and the initial biomass was 1.3±0.05 g/L. In Fig. 5, the results showed that the biomass recovery for the lowest examined (42.2%) was achieved at a high concentration factor (C=30) because the fouling phenomenon aggravated in the filtration module. However, recovery ratios for C=10 and C=20 were found to be 94% and 59%, respectively. Compared with the group (C=10), an increase to C=20 did cause additional biomass loss (about 35%). Further accumulation of culture medium corresponding to C=30 increased biomass losses to 57.8%. Hence, it should be pointed out that the volume concentration factor was a significant influencing factor on the *Chlorella* sp. concentration, and the C=10 was necessary to guarantee a biomass recovery higher than 90%.

4 Conclusions

Backwash with permeate was more effective than forward air–water flushing in the concentration process, which was selected as an optimization of flux recovery. The hollow fiber membrane module has been proved to be a useful laboratory tool for the concentration of *Chlorella* sp. by adding periodical backwash. In general, periodical backwash was efficient to control membrane fouling, the recovery was found to be high (87.2–95.5%) when the initial biomass was 1.3±0.05 g/L. For a biomass of 1.3±0.05 g/L, a frequency of 20 min for once exhibited the best performance in terms of permeate flux and a relatively high biomass recovery. Algal recovery was highly correlated to the initial biomass and the volume concentration factor. A high volume concentration factor or a high initial biomass resulted in a low biomass recovery.

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