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Recovery of *Bacillus thuringiensis* based biopesticides from fermented sludge by cross-flow microfiltration

Ming Chang[a,b], Shungui Zhou[b,c], Qihong Sun[a], Tiexiang Li[d], Jinren Ni[b,*]

[a]*State Key laboratory of Environmental Criteria and Risk Assessment, Chinese Research Academy of Environmental Sciences, Beijing 100012, China*
[b]*Department of Environmental Engineering, The Key Laboratory of Water and Sediment Sciences, Ministry of Education, Peking University, Beijing 100871, China*
Tel. +86 10 6275 1185; Fax: +86 10 6275 6526; email: nijinren@iee.pku.edu.cn
[c]*Guangdong Key Laboratory of Agricultural Environment Pollution Integrated Control, Guangdong Institute of Eco-Environment and Soil Sciences, Guangzhou 510650, China*
[d]*The Department of Mathematics, Southeast University, Nanjing 210096, China*

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ABSTRACT

The recovery of *Bacillus thuringiensis* (Bt) based biopesticides from fermented sludge broth via cross-flow microfiltration (CFMF) was evaluated. Three types of microfiltration membranes, WX-model cellulose, cellulose acetate, and polyethersulfone, were tested for their permeate flux and restoration capacity. The cellulose acetate membrane with a pore size of 0.8 μm was selected for further process optimization and modeling of CFMF. Under optimal conditions, the permeate flux could be greater than 20 l/m^2/h if the retentate contained was less than 100 g/l. The spore and crystal proteins recoveries were found to be 99 and 95%, respectively. The standard hydraulic resistance-in-series model was used to determine the parameters of CFMF of Bt fermented sludge. The results demonstrated that CFMF was effective for Bt based biopesticide recovery from fermented sludge.

Keywords: Bacillus thuringiensis; Permeate flux; Process optimization; Membrane; Modeling

1. Introduction

Bacillus thuringiensis (Bt) is a Gram-positive, spore-forming bacteria that has been extensively used for four decades in biopesticidal formulations due to its safe environmental and human health records [1]. This microorganism is characterized by the production of crystal-shaped parasporal inclusions that contain insecticidal proteins [2]. In addition to the principal contributors of entomotoxicity (namely crystal proteins and viable spores [VS]), there are various other insecticidal factors such as proteases, chitinases, phospholipases, vegetative insecticidal proteins, and other unknown components, which all need to be recovered efficiently from the fermented broth and then formulated, packed, or dried [3]. Various methods, traditional or advanced, have been used for the recovery of the active components during downstream processing. The advantages and drawbacks of the recovery methods commonly used for Bt-based biopesticides are listed in Table 1. None of these methods can achieve high recovery efficiency with low energy and time requirements. An alternative to these approaches that has been developed over the last

*Corresponding author.

Table 1
The advantages and drawbacks of the recovery methods commonly used for Bt-based biopesticides

Methods	Advantages	Drawbacks	References
Lactose-acetone technique	• Mature method	• Highly laborious • Low recovery efficiency	[4]
Centrifugation	• Simple • Cost-effective • Versatile	• Recovery efficiency depends on the relative centrifugal force (RCF), and the RCF is difficult to increase for large amount of broth. • Loss in supernatant	[5]
Dead-end microfiltration	• High recovery efficiency	• Cost-intensive (frequent membrane replacement) • Time-consuming • Heavy membrane fouling	[6,7]

several years is cross-flow microfiltration (CFMF). In CFMF, the feed flow is tangential to the membrane surface and perpendicular to the permeation flux, and the accumulation of filtered solids can be minimized by the sharing action of the flow [8]. The advantages of CFMF over conventional separation and recovery techniques are its simplicity in operation and that it can be carried out at ambient temperature [9]. The feasibility of CFMF to separate cells and/or cell debris from fermentation broth to obtain a clear and sterile filtrate containing the targeted product has been demonstrated with bacteria [10,11], yeast [12,13], and fungi [14,15]. However, the use of CFMF for Bt cell harvesting is still limited due to the rheological characteristics of the fermentation broth, especially its impact on membrane fouling [9]. To minimize the negative effects of membrane fouling, optimization of transmembrane pressure (TMP), cross-flow feed velocity, and the characteristics (e.g. pH and temperature) of the fermentation broth are necessary.

Sewage sludge has been used successfully as a raw material for Bt-based biopesticides production because it has lower processing costs, higher fermentation entomotoxicity [16], and better stability [5] than some commercial media. However, the downstream processing efficiency of the overall Bt production process has yet to be accounted for. This study was conducted to investigate the potential applications of CFMF technologies to the recovery of Bt-based biopesticides. Three types of commercial microfiltration membranes were tested. The most suitable one was used under different operation conditions (TMP, feed velocity) and broth characteristics (pH, flocculant content) to identify the optimal process parameters. The CFMF model was subsequently established to describe the filtration process of Bt-based fermented sludge.

2. Materials and methods

2.1. Sludge samples

Sludge was obtained from the Gaobeidian Wastewater Treatment Plant in Beijing, China. The sludge, which was a mixture of primary and secondary sludge, was collected from the off-set of the concentrated tank and stored at 4°C in a plastic barrel until use. The main characteristics of the sludge used in this study were determined by the APHA standard methods [17], and the major results are summarized in Table 2. Total organic carbon and total nitrogen concentrations were determined using a Muti TOC/TN 3000 (Analytik Jena AG, Germany) after filtration through a 0.45-μm membrane. pH was measured with a pH/mV/°C-m (Hanna, Italy). The total solid concentration of the sludge was adjusted to 20 g/l with deionized water for fermentation.

2.2. Microorganism and culture media

The microorganism used in this study was *Bacillus thuringiensis* subsp. *kurstaki* 130-1 (Btk 130-1), which was provided by Dr. Hu (Sichuan Normal University, Sichuan Province, China). The strain was subcultured on beef extract-peptone (BP) agar slants [18] and stored at 4°C. A loopful of bacteria from the slant was used to inoculate 60 ml of sterilized BP medium in a 250 ml Erlenmeyer flask. The flask was incubated on a reciprocating shaker at 30 ± 1°C and 200 rpm for 10 h. After that, a 2% (v/v) inoculum of actively growing cells was transferred to the fermentor.

Table 2
Physical-chemical properties of the selected sewage sludge

Parameters	Concentration
Total solids	30.10
Volatile solids	19.34
Suspended solids	25.14
Volatile suspended solids	15.97
pH	6.88
Total organic carbon	11.33
Dissolved organic carbon	1.18
Total nitrogen	1.23
Total phosphorus	0.25
Reducing sugar	0.91

Note: All but pH measured in g/l.

2.3. Fermentation

The fermentation processes were carried out in a 5-l automatic controlled fermentor (BIOTECH-5BG, China) with a working volume of 3 l. The total solid of the sludge was adjusted to 20 g/l as described in a previous study [19]. Fermentations were conducted for about 48 h under the following conditions: temperature 30°C, air supply 1.0 v/v/m (liter of air/liter of fermentor content/min), agitation 500 rpm, and initial pH 7.0. These conditions were all controlled automatically. After fermentation, the broth was collected and stored at 4°C until membrane filtration.

2.4. Membrane and membrane system

Three common types of membranes were tested in this study: WX-model cellulose membrane (WXM), cellulose acetate membrane (CAM), and polyethersulfone membrane (PESM) with pore sizes of 1.0, 0.8, and 0.45 μm, respectively (Different membranes with different pore sizes will be expressed as "membrane-size" hereafter, for example. CAM with a pore size of 0.8 μm will be expressed as "CAM-0.8"). A flat-plate membrane module with a channel length of 25 cm, width of 0.8 cm, thickness of 0.25 cm, and a corresponding effective membrane area of 20 cm^2 was used.

Fig. 1 is a schematic diagram of the membrane filtration system. An adjustable constant flow pump (HL-6D, China) was used to pump the broth from the feed tank to circulate along the surface of the plat-plate membrane (cross-flow) in the module. The cross-flow velocity was controlled by the constant flow pump. The applied pressure was controlled by ball valves and monitored with pressure gauges. In this system, the TMP of CFMF was defined as the average pressures of the inlet and outlet pressure gauges. The gauge pressure of the permeate side was equal to zero because the permeate side of the membrane was open to the atmosphere. Permeate was collected with a narrow-neck bottle and measured using a BS223S electronic balance (Sartorius, Germany; Accuracy 0.01 g) to calculate the permeation flux.

The operation procedure was to: (1) fill the feed tank with the fermented broth; (2) turn off Ball valve 4 first and then Ball valve 5, and after that, turn on Ball valve 3; (3) start up the constant flow pump, adjust to the proper flow rate, and pump the fermented broth along A flow (recycling); (4) turn on Ball valve 5 first and then 4, after that turn off Ball valve 3, and pump the fermented broth along B and C flows; (5) turn down Ball valve 5 to the suitable TMP based on readings of Pressure gauges 6 and 7, then collect the permeate generated by CFMF along D flow.

The system was cleaned by recirculating 0.1 mol/l sodium hydroxide solution twice (each for 15 min)

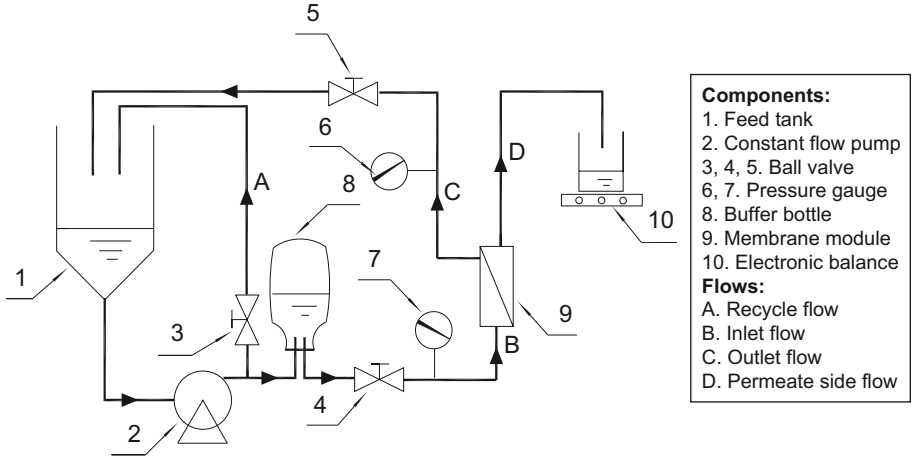

Fig. 1. Schematic diagram of the membrane filtration system.

after each batch filtration. The system was then flushed with deionized water to ensure that its original performance was restored [20].

2.5. Dead-end filtration

Dead-end filtrations were conducted using a self-made Amicon laboratory cell equipped with a separated membrane with a diameter of 80 mm. Constant pressures were applied using compressed N_2. Samples of 100 ml were filtered and the permeate weights were determined using a BS223S electronic balance. Each experiment was conducted in triplicate.

2.6. Analysis

2.6.1. Permeate flux (J) and volume concentration factor (N)

$$J = \frac{\Delta V_p}{\Delta t \times S} \left(1\ m^{-2}\ h^{-1}\right) \quad (1)$$

where ΔV_p represents the permeate volume during Δt, Δt is the minimum interval recorded by the electronic balance, and S is the effective membrane area.

$$N = \frac{V_t}{V_t - V_p} \quad (2)$$

where V_t and V_p represent the total volume of the broth at the beginning of the filtration and the permeate volume, respectively.

2.6.2. VS and spore recovery (SR)

Samples of the broth and the retentate after CFMF were analyzed for colony-forming units (CFU) of VS by serial plating on BP plates. Samples appropriately diluted with sterile saline solution (the concentration of sodium chloride was 8.5 g/l) were subject to 80°C for 15 min, after which they were plated on agar plates and incubated overnight at 30°C to form fully developed colonies. Each experiment was conducted in triplicate and plates containing 20–300 colonies were used for enumeration [21]. The standard deviation of the plate counts for VS was less than 8.5%. SR (%) was calculated as the VS in the retentate divided by the VS in the broth.

2.6.3. Evaluation of crystal proteins and protein recovery (PR)

Crystal proteins in the broth and the retentate were determined by SDS-PAGE as described by Laemmli [22], with some modifications. After dissolved in 1 M sodium hydroxide, the leavenings were added to 1% sodium dodecyl sulfate (SDS) and 1% 2-mercaptoethanol. The suspension was then heated in a boiling water bath for 6 min before centrifuged for 10 min at 3000 g in an Eppendorf centrifuge. Electrophoresis was initially conducted at 50 V until the dye front reached the separating gel. Subsequently, electrophoresis was conducted at 120 V for 3 h, after which the gel was stained with Coomassie Brilliant Blue R-250 overnight and then de-stained. The molecular weight of the crystal proteins was estimated by comparison with an Electrophoresis Calibration Kit produced by Pharmacia (High Molecular Weight). The yield of crystal proteins was determined by dividing the gel into 3 ml 25% pyridine for dissolution and then analyzing the absorbance of the samples at 605 nm spectrophotometrically. PR (%) was calculated as the yield of crystal proteins in the retentate divided by the yield of crystal proteins in the broth.

2.6.4. Membrane adsorption and deposit (A&D)

The A&D on the membrane surface and within the membrane pores could be expressed as follows.

$$A\&D = \frac{TS_o \times V_o - TS_r \times V_r}{S} \left(g\ m^{-2}\right) \quad (3)$$

where TS_o and TS_r represent the total solids in the filtration system before CFMF and in the retentate after CFMF, respectively, and V_o and V_r are the liquid volumes in the filtration system and in the retentate, respectively.

3. Results and discussion

3.1. Membrane selection

Three types of commercial membrane candidates, WXM, CAM, and PESM, were selected in this study due to their excellent performance, high permeate flux, and low protein adsorption. The most suitable one was picked according to the results of the dead-end filtration experiment. Fermented sludge broth was filtered through each membrane at a fixed TMP (0.1 MPa) and ambient temperature. The average size of the Bt 130-1 spores or crystals cultivated in the sludge was about 1.0 μm. Hence, the equivalent pore size of 1.0, 0.8, or 0.45 μm was selected to give a Bt 130-1 retentate. Fig. 2 shows the permeate flux variation within 50 min of dead-end filtration of Bt-based fermented broths with different membranes. During this period, the broth could be doubled by concentration. The highest initial permeate fluxes were observed when WXM was used. However, the corresponding reduction in permeate flux over time could also be characterized by an initial

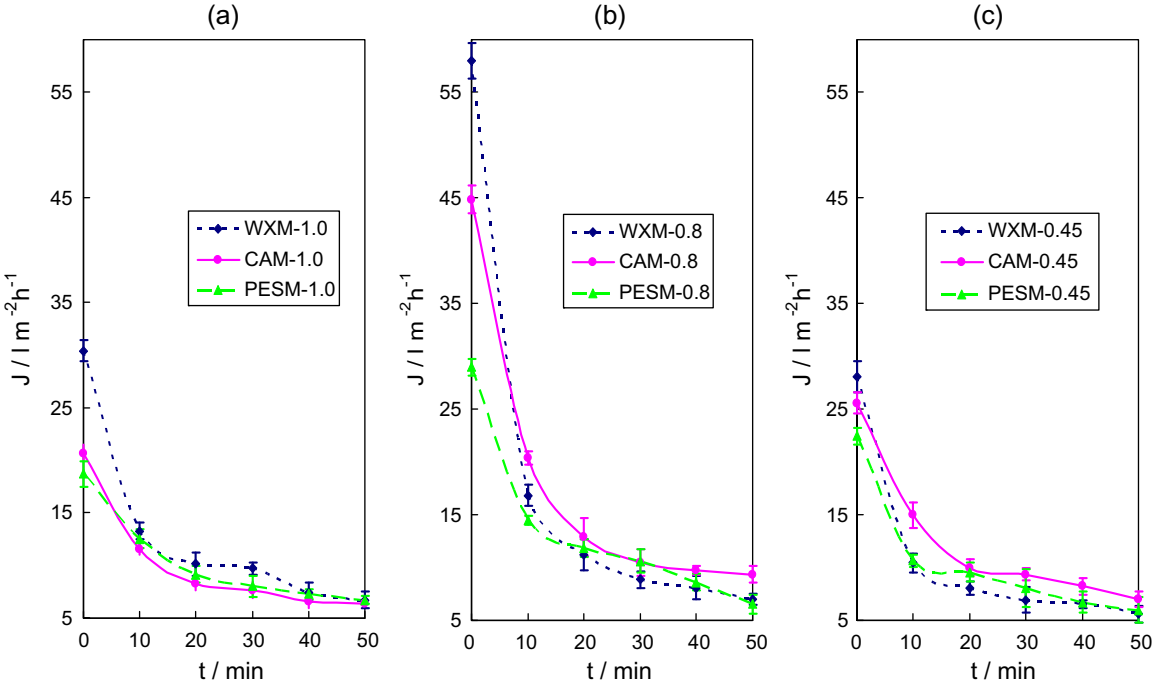

Fig. 2. Permeate flux variation of different pore size membranes: (a) 1.0, (b) 0.8, and (c) 0.45 μm.

short period of rapid permeate flux decline, which was caused by membrane fouling [9]. Apparently, WXM fouling was severer than those of CAM and PESM. By selecting three representative pore sizes for each of the three types of membranes as described above, comparisons could be made in terms of the permeate flux for different types of membranes. In general, the permeate flux of CAM declined more mildly than those of PESM and WXM. However, the initial permeate flux through the 1.0 μm membranes was lower than that of the 0.8 μm membranes, and sometimes even lower than that of the 0.45 μm membranes. This was likely because the spores and crystals of Bt 130-1 tended to get stuck in the apertures because of their sizes (around 1.0×2.0 μm). Membrane fouling became much severer when using the membranes with pore sizes similar to the diameter of the filtered materials. Overall, this process was characterized by little penetration at the beginning and subsequent serious blocking. Consequently, a lower permeate flux of membranes with a pore size of 1.0 μm was caused by the adsorption within the membrane pores, while a lower permeate flux of membranes with a pore size of 0.45 μm was caused by adsorption onto the membrane material.

The membrane restoration capacity is another important index for membrane selection. Usually, washing or back-washing can be applied to restore the permeate flux. In this study, membranes were restored by sponge erasure, water dipping (24 h), and sodium carbonate solution (pH = 8.0) dipping (24 h), successively. Sodium carbonate solution was selected for membrane restoration because Bt crystals could be dissolved in alkaline solutions. Under the same experimental conditions of 25°C and TMP = 0.1 MPa, water fluxes through new and restored membranes were obtained as shown in Fig. 3. Apparently, CAM was superior to the other membranes with respect to its capacity for restoration after dipped in water. Sodium carbonate solution can be an effective membrane restoration solvent for both CAM and PESM, although some impact was found when applying it to WXM. The high adsorption ability of WXM would restrict the restoration of its permeate capacity.

As described above, CAM gave the best performance in terms of both permeate flux and membrane restoration capacity. Therefore, the permeate flux and the crystal proteins recoveries using CAM membranes with different pore sizes were compared (Fig. 4). The advantage of CAM-0.8 was obvious in terms of permeate flux (Fig. 4(a)). Because the concentration of the broth doubled, the protein recoveries of CAM-0.8 and CAM-0.45 were twice that of the original fermented sludge (Fig. 4(b)). A lower PR of CAM-1.0 resulted from quick adsorption. According to the above analysis, CAM-0.8 was the most efficient and favorable membrane under the given conditions and was thus used as a recovery membrane for subsequent studies to evaluate CFMF of Bt fermented sludge broth.

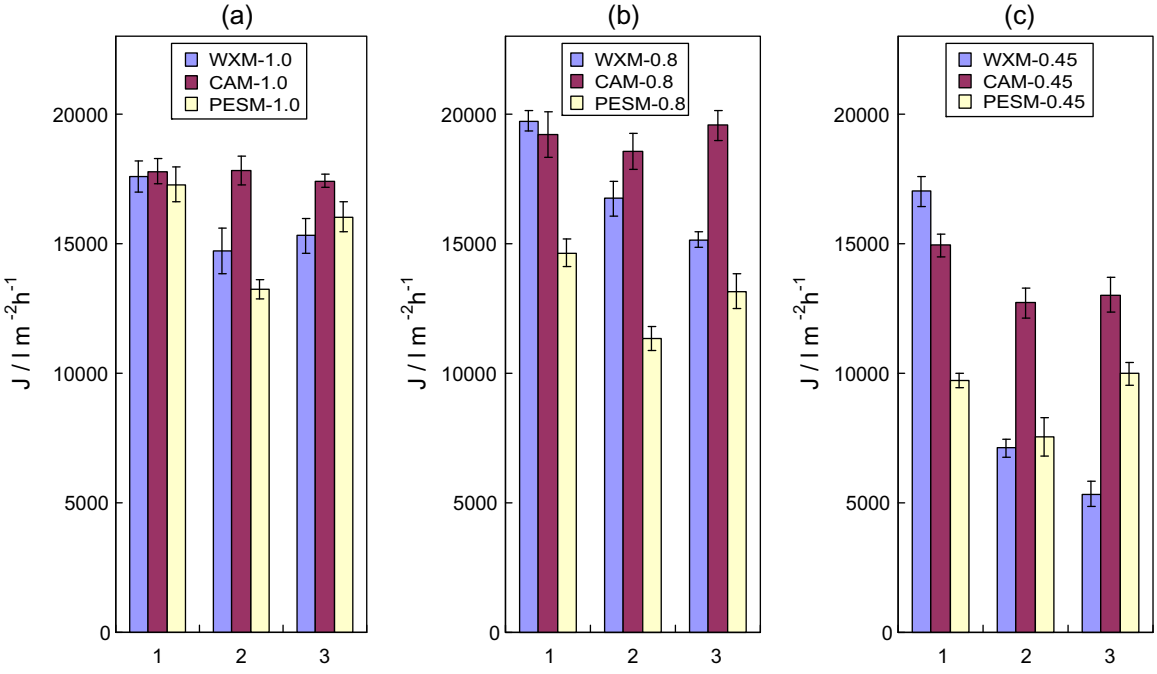

Fig. 3. Water flux of different membranes: (a) 1.0, (b) 0.8, and (c) 0.45 μm (1. new membranes; 2. water-dipped membranes; 3. sodium carbonate solution-dipped membranes).

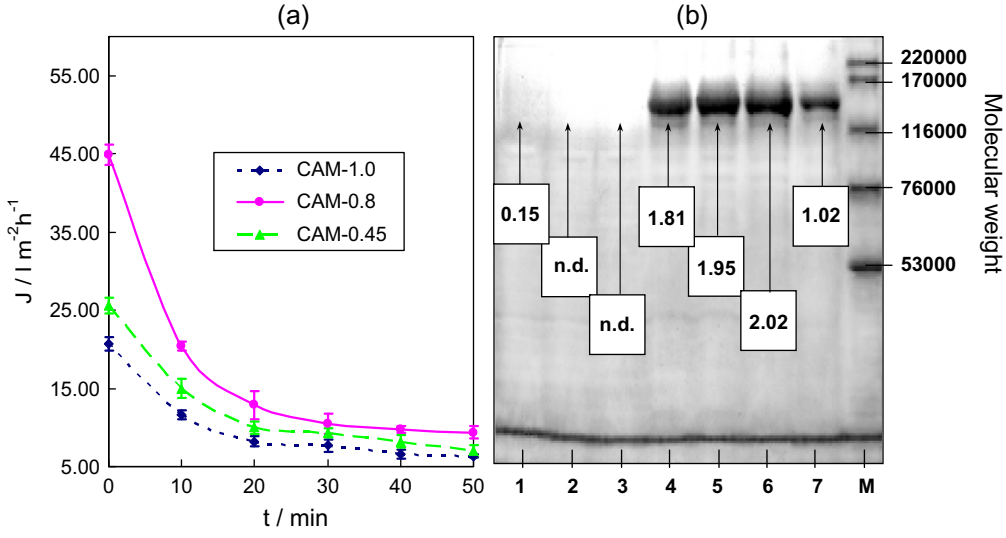

Fig. 4. Analysis of CAM for (a) permeate flux and (b) crystal proteins recovery. In Fig. 4(b), 1, 2, and 3 represent the crystal proteins penetrating the permeations after CFMF by CAM-1.0, CAM-0.8, and CAM-0.45, respectively; 4, 5, and 6 represent the crystal proteins recovered in the retentates after CFMF by CAM-1.0, CAM-0.8, and CAM-0.45, respectively; and 7 represents the crystal proteins in the original fermented broth. Herein, M is the marker, and data in the rectangle denote the concentrations of crystal proteins in mg/ml.

3.2. *Optimization of the filtration process*

Different operation conditions in terms of TMP and cross-flow velocity, and different broth characteristics in terms of pH and flocculant content were investigated using the CFMF for Bt recoveries from the fermented sludge (Fig. 5). Increasing the TMP leads to an increase in the permeate flux (Fig. 5(a)). An apparent increase in permeate flux with TMP from

0.1 to 0.15 MPa was observed, but no further increases were observed after TMP exceeded 0.15 MPa. Although a higher TMP would enhance the solvent flux through the membrane, it also increased the diffusion rate of the solute toward the membrane. The membrane A&D under different operation conditions was evaluated with Eq. (3) and is shown in Table 3. Membrane fouling caused by A&D became more severe with increasing TMP, resulting in poor mass transfer, which indicated that there might be an efficient TMP for CFMF of the fermented sludge to obtain the maximum permeate flux. Similar results were reported by Krstić et al. [9] who mentioned that the permeate fluxes at steady state showed almost no increase when the TMP was higher than 51 kPa during CFMF of *Polyporus squamosus* fermentation broth at the same cross-flow velocity. The permeate flux eventually reached equilibrium.

The permeate flux increased significantly as the cross-flow velocity increased (Fig. 5(b)). Both Fig. 5(b) and Table 3 show that less deposition on the membrane surface occurred as the velocity increased, which agreed with other studies [23–25]. As a typical non-Newtonian fluid, the filtration efficiency of fermented sludge relies on the solute retention rate and diffusion rate in the boundary layer. When compared with dead-end filtration, cross-flow filtration could greatly improve the permeate flux by intensifying the disturbance level and reducing the thickness of the boundary level with higher tangential velocity [26].

The effect of pH on permeate flux was not significant in this study, although the permeate flux at pH 7.0 and pH 5.5 was slightly lower than the control, while that at pH 4.0 was slightly higher during continuous CFMF (Fig. 5(c)). A possible explanation for these findings was that the interaction of negative charges on the protein surface promotes or restrains the particle size increments [27]. The neutralization of negative charges at pH 4.0 helped the particles agglomerate, which favored the clarification of the fermented sludge and reduced membrane fouling (Table 3). Based on this electric flocculation phenomenon, one of the chemical flocculants, polyacrylamide (PCM), was evaluated in this recovery process because it is one of the most widely used water-soluble high polymers. The results (Fig. 5(d)) showed that 1 g/l of PCM already induced an effective increase of the permeate flux. Additionally, PCM helped the broth colloid form a highly porous filtration cake and prevented deposition on the membrane during CFMF [28], which enhanced the permeate flux. However, too much PCM may have an adverse effect. All together, the optimal concentration of PCM was 2 g/l.

Based on the above analysis, transport of material away from the membrane wall and reduction of the cake formation by the tangential retained fluid flow

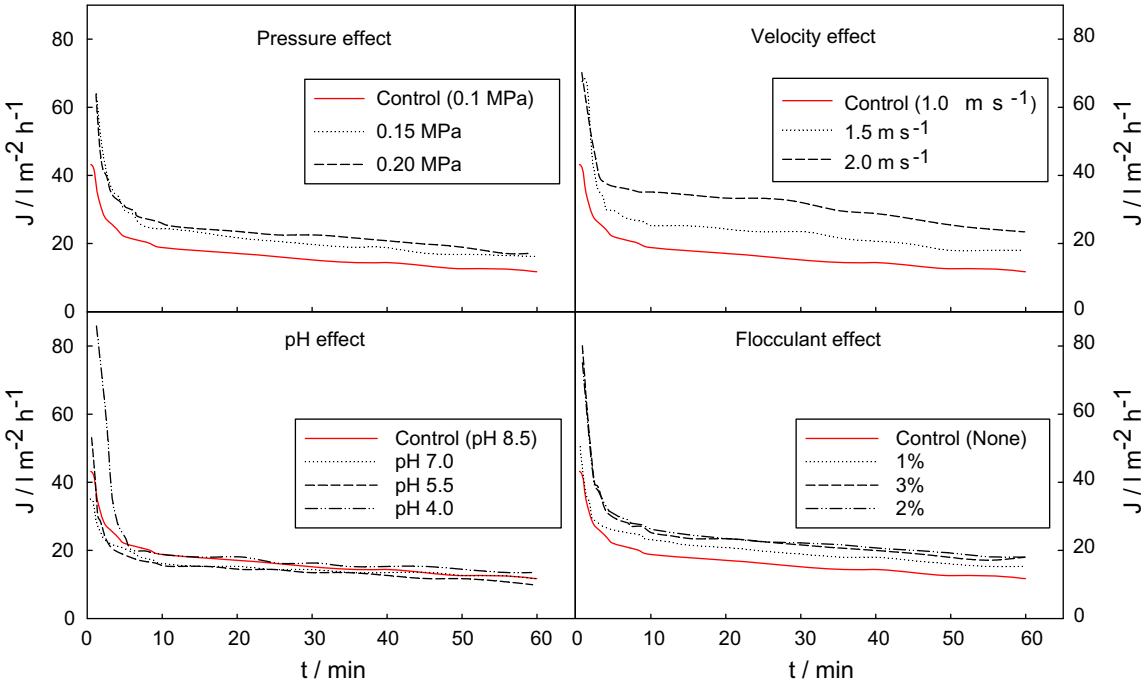

Fig. 5. Process conditions and broth optimization for (a) pressure, (b) velocity, (c) pH, and (d) flocculant effects.

were the major factors influencing the CFMF processes. Based on the SR rate and PR rate shown in Table 3 (average recoveries = 98.92 and 94.87%, respectively), the optimal CFMF operation conditions were pH: 4.0, PCM: 2 g/l, TMP: 0.15 MPa, and v: 2.0 m/s.

3.3. CFMF modeling for fermented sludge

A modified standard hydraulic resistance-in-series model described by Carrère et al. [29] was used to represent the CFMF process taking into account the membrane resistance (R_m), the adsorption resistance (R_a), the concentration polarization resistance (R_p), and the cake resistance (R_c). The permeate flux was expressed by

$$J = \frac{\mathrm{TMP}}{\mu_p(R_m + R_a + R_p + R_c)} \quad (4)$$

where TMP is the transmembrane pressure and μ_p is the permeate viscosity.

Each resistance parameter in Eq. (4) is described as follows and numerically determined using Matlab software.

R_m, which is also known as the membrane hydraulic resistance, was determined by the water flux measurements. As mentioned above, the average water flux of the selected membrane (CAM-0.8) was 19,220 l/m^2/h under a TMP of 0.1 MPa. The viscosity of the permeated solvent was around 0.4 mPa·s. Thus, R_m was calculated as 4.683×10^{10}/m.

R_a and R_p were obtained from filtration experiments conducted with a previously clarified broth, which was microfiltered in advance to remove the suspended solids. It was demonstrated that the resistances due to adsorption and concentration polarization are time dependent and tend toward constant steady-state values, $R_{a_{ss}}$ and $R_{p_{ss}}$, respectively. The resistances could be expressed as

$$R_a + R_p = (R_{a_{ss}} + R_{p_{ss}})(1 - \exp(-bt)) \quad (5)$$

When filtering the clarified broth, R_c = 0, and R_m could be considered constant. Thus,

$$R_a + R_p = \frac{\mathrm{TMP}}{J \cdot \mu_p} - R_m = (R_{a_{ss}} + R_{p_{ss}})(1 - \exp(-bt)) \quad (6)$$

Data from a series of experiments were fitted to Eq. (6). Based on the results, $(R_{a_{ss}} + R_{p_{ss}})$ and the constant b at different TMP and CFMF velocities could be calibrated (Table 4). Fig. 6 shows an example of how $(R_{a_{ss}} + R_{p_{ss}})$ and b were determined when TMP = 1×10^5 Pa and v = 1 m/s. When fitting the experimental data with Eq. (6), $(R_{a_{ss}} + R_{p_{ss}})$ and b were determined to be 1.09×10^{13}/m and b=7.4×10^{-3}/s, respectively. As shown in Table 4, velocity had little effect on $(R_{a_{ss}} + R_{p_{ss}})$. These results were similar to those reported by Carrère et al. [29], who found that $(R_{a_{ss}} + R_{p_{ss}})$ was less influenced by CFMF velocities ranging from 1 to 2 m/s. Additional procedures were taken to determine the relationship between TMP and $(R_{a_{ss}} + R_{p_{ss}})$. In the case of TMP from 0.05 to 0.20 MPa, $(R_{a_{ss}} + R_{p_{ss}})$ was determined as follows:

Table 3
Membrane adsorption, deposit, and entomotoxicity recoveries performance

		Membrane A&D (g/m^2)	VS		Crystal proteins	
			Retentate ($\times 10^8$ CFU/ml)	SR (%)	Retentate (mg/ml)	PR (%)
Control*		3.3 ± 0.6	5.0 ± 0.6	89.3	1.89 ± 0.15	92.2
TMP	0.15 MPa	5.0 ± 0.8	5.5 ± 0.3	98.2	1.87 ± 0.21	91.2
	0.20 MPa	7.1 ± 1.1	4.9 ± 0.7	87.5	1.84 ± 0.05	89.8
v	1.5 m s^{-1}	2.2 ± 0.9	5.6 ± 0.5	100.0	1.95 ± 0.32	95.1
	2.0 m s^{-1}	1.0 ± 0.3	5.7 ± 0.5	101.8	1.92 ± 0.14	93.7
pH	7.0	3.5 ± 0.2	5.1 ± 0.8	91.1	1.95 ± 0.07	95.1
	5.5	3.0 ± 0.7	5.7 ± 1.0	101.8	1.93 ± 0.26	94.1
	4.0	2.7 ± 0.3	6.0 ± 0.3	107.1	1.98 ± 0.18	96.6
PCM	0.1%	2.2 ± 0.6	5.3 ± 0.7	94.6	2.02 ± 0.23	98.5
	0.2%	2.0 ± 0.3	5.9 ± 0.3	105.3	2.01 ± 0.17	98.0
	0.3%	1.9 ± 0.1	5.7 ± 0.1	101.8	1.98 ± 0.21	96.6

Notes: *Conditions of control: TMP, 0.10 MPa, v, 1.0 m/s, pH, 8.5, PCM, none. There was only one single factor changed for others. The changed factors are listed in the first line. VS and crystal proteins concentrations of the fermented broth are (5.5 ± 0.5) × 10^8 CFU/ml and 1.02 mg/ml, respectively.

Table 4
Experiments with previously clarified broth: experimental conditions and parameters of Eq. (5) ($T = 25°C$)

TMP (MPa)	v (m/s)	$R_{a_{ss}} + R_{p_{ss}}$ (m^{-1})	b (s^{-1})
0.05	1.0	7.75×10^{12}	6.6×10^{-3}
	2.0	7.73×10^{12}	7.5×10^{-3}
0.10	1.0	1.09×10^{13}	7.4×10^{-3}
	2.0	1.10×10^{13}	8.2×10^{-3}
0.15	1.0	1.81×10^{13}	9.3×10^{-3}
	2.0	1.82×10^{13}	1.0×10^{-4}
0.20	1.0	2.46×10^{13}	9.3×10^{-3}
	2.0	2.45×10^{13}	7.8×10^{-3}

$$(R_{a_{ss}} + R_{p_{ss}}) = 9.40 \times 10^{11} + 1.15 \times 10^{8}\text{TMP} \quad (7)$$

The simulation results are shown in Fig. 6(b).

The cake resistance, R_c, could be expressed as:

$$R_c = \frac{m}{A}\alpha_0 \quad (8)$$

where m is the weight of membrane cake (kg), A is the membrane area (m^2), and α_0 is the specific cake resistance (m/kg).

m was calculated by multiplying the suspended solid concentration by the permeate volume during the dead-end filtration. The variation in m with time was expressed with the following simplized equation:

$$\frac{dm}{dt} = (JC - \frac{D}{\delta}C_m)A \quad (9)$$

where C is the suspended concentration in the retentate (kg/m^3), $\frac{D}{\delta}C_m$ is the back diffusion coefficient, C_m is the suspended solid concentration on the membrane wall (kg/m^3) which is believed to be far higher than C, D is the shear induced diffusion coefficient (m^2/s), and δ is the cake thickness (m).

Experiments of CFMF at a constant retentate concentration were conducted to determine $\frac{D}{\delta}C_m$. When steady state was reached, the retentate on the membrane face came to homeostasis, and the Eq. (9) can be rewritten as

$$J_{ss}C = \frac{D}{\delta}C_m \quad (10)$$

where J_{ss} is the steady-state flux.

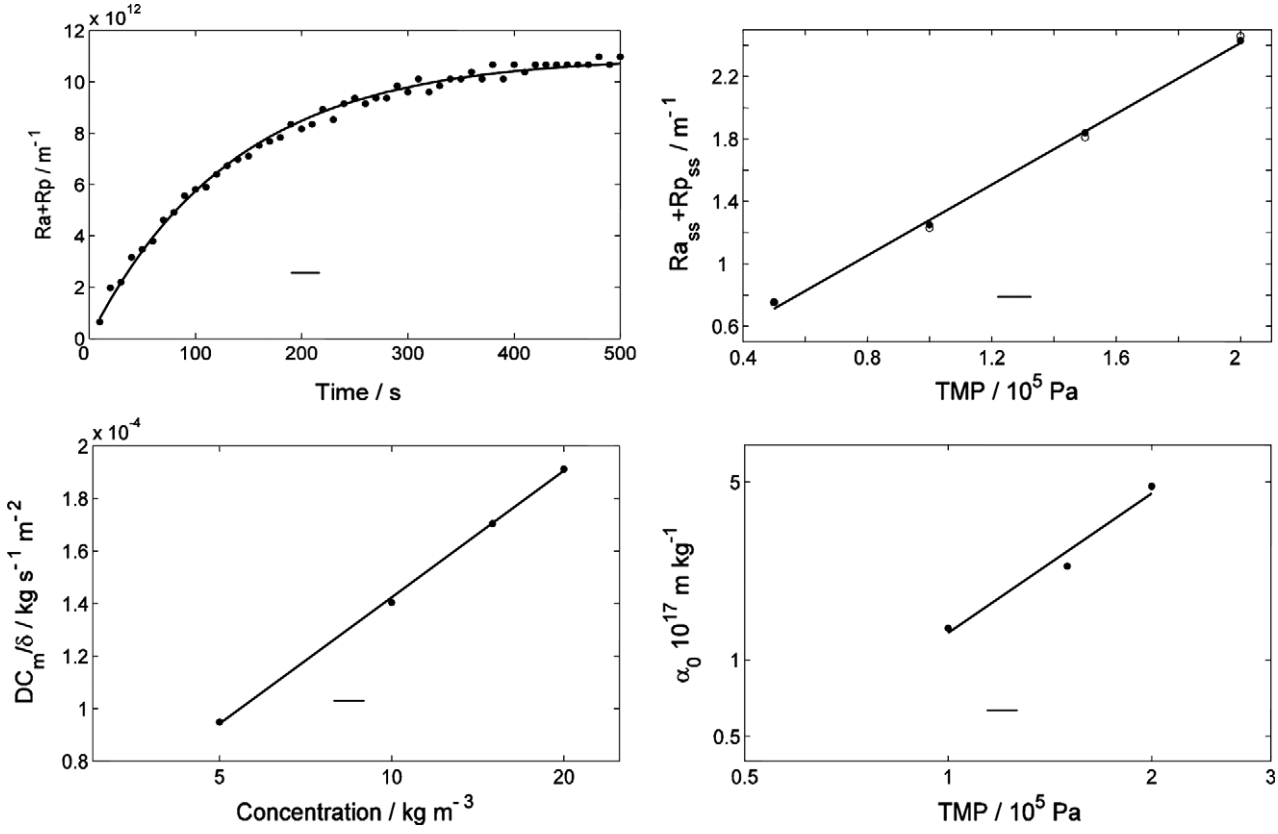

Fig. 6. Parameter determinations for (a) $(R_{a_{ss}} + R_{p_{ss}})$ and b from Eq. (5), (b) $(R_{a_{ss}} + R_{p_{ss}})$ from Eq. (7), (c) $\frac{D}{\delta}C_m$ from Eq. (11) and (d) α_0 from Eq. (13).

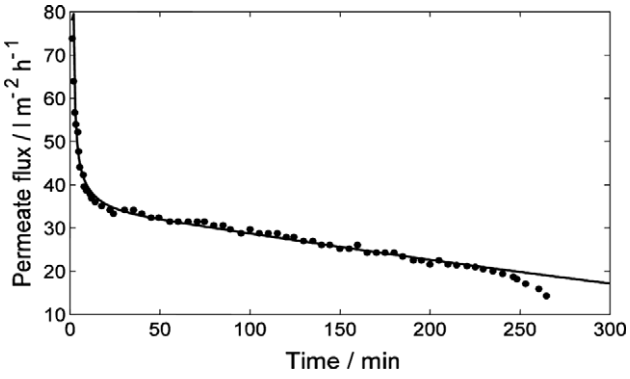

Fig. 7. Modeling the continuous CFMF of Bt-based fermented broth (TMP = 0.15 MPa, $V = 2$ m/s, $T = 25$°C).

The results of CFMF experiments at different retentate concentrations are shown in Fig. 6(c).

Under given conditions ($v = 1$ m/s, $T = 25$°C, 0.05 MPa < TMP < 0.20 MPa), $\frac{D}{\delta}C_m$ could be determined as follows:

$$\frac{D}{\delta}C_m = 6.94\times10^{-5}\ln(C) - 1.44\times10^{-5} \tag{11}$$

and C was expressed as follows:

$$C = \frac{C_0V_0}{V_0 - A\int_0^t Jdt} \tag{12}$$

The specific cake resistance α_0 was calculated as follows [30]:

$$\alpha_0 = \alpha(\mathrm{TMP})^n \tag{13}$$

where n is the cake compressibility index and α is the cake resistance coefficient.

n was determined by the dead-end filtration experiments. A series of experiments were conducted under different TMP using either the whole broth or a previously clarified one. The specific cake resistance (α_0) was calculated as follows [29]:

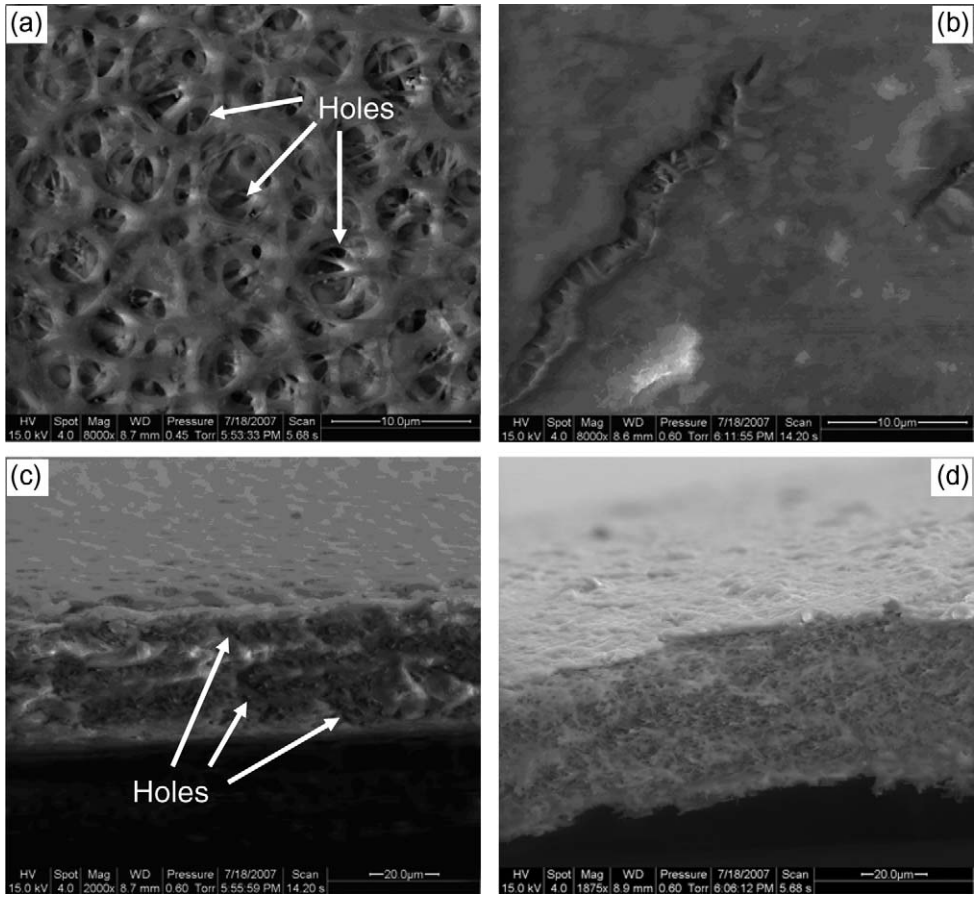

Fig. 8. SEM observations of CAM morphological changes for (a) new membrane, (b) the membrane surface after CFMF, (c) cross-section of new membrane, and (d) cross-section of the membrane after CFMF.

$$\alpha_0 = \frac{A(R_{c_w} - R_{c_c})}{C_w \times V_{P_w} - C_c \times V_{P_c}} \quad (14)$$

where the subscripts w and c represent the whole broth and the clarified broth, respectively, and V_P represents the permeate volume.

Fig. 6(d) shows the variation of α_0. n was given by the slope of the fitting line and recognized as 1.8207. This value was higher than that reported by Tanaka et al. [31], which was primarily due to differences in the suspended solids originally present in the fermented sludge broth. The bacteria cake resistance coefficient (α) was determined with the CFMF experiments at a constant TMP and was found to be equal to 1.05×10^5 Pa. Thus, α_0 could be calculated as 2.8×10^{14} m/kg under a TMP of 0.15 MPa.

Based on the mentioned parameters of the standard hydraulic resistance-in-series model obtained from corresponding experiments, continuous CFMF of Bt-based fermented sludge (initial solid concentration of 20 g/l, initial volume of 200 ml) under the predetermined optimal conditions was conducted to test the model. Good agreement was observed from the beginning to 240 min into the experiment (Fig. 7), when the broth had been concentrated by about 5-fold. Further recovery of entomotoxicity, however, became increasingly difficult due to the significant increase in the thickness of the cake on the membrane wall from the growing suspended solid concentration when a certain retentate concentration was reached. The value in this study was determined to be 10%, which implied that CFMF was suitable for Bt-based fermented sludge with a retentate solid concentration less than 10%. The continuous CFMF showed a desirable permeate flux over 20 l/m^2/h, demonstrating the applicability of CFMF for efficient recovery of Bt-based biopesticides.

3.4. Membrane morphological changes

According to SEM observations, the new CAM consisted of several porous nets stacked together. Irregular holes from net to net could be seen clearly from the membrane surface (Fig. 8), and 4–5 porous nets could be seen in the cross-section of the membrane (Fig. 8(c)). Such structures helped the CAM maintain a high water flux. After continuous CFMF, the surface of the CAM was covered with a thick cake layer. Crystal proteins and VS were recovered successfully from the membrane (Fig. 8(b)). Additionally, obvious membrane adsorption could be seen from the cross-section of the CAM before (Fig. 8(b)) and after CFMF (Fig. 8(d)). However, some inner pores were clogged by finer particles of the broth, which resulted in a thicker membrane. Despite this, the inner pores could not be blocked completely because of the structure of cellulose acetate. As a result, the permeate flux could maintain at a relatively high value in cases where the cake on the CAM was swept away in time.

4. Conclusions

The CAM with a pore size of 0.8 μm was shown to be suitable for CFMF of Bt-based fermented sludge broth. The spore and crystal proteins recovered by this membrane were up to 99 and 95%, respectively. The optimal operation conditions were determined to be TMP: 0.15 MPa, v: 2.0 m/s, pH: 4.0, PCM: 2 g/l. Under the optimal conditions, the permeate flux could be over 20 l/m^2/h upon the retentate being less than 100 g/l. The CFMF process was well described by the standard hydraulic resistance-in-series model. Overall, the CFMF was demonstrated to be very efficient for Bt-based biopesticides recovery from fermented sludge.

Acknowledgments

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