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Preparation and water purification applications of microbial-induced porous calcium carbonate microfiltration membranes

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Abstract: Filtration is a prevalent treatment modality in the domain of wastewater management. Depending on the materials and properties of the filtration media, filtration can be classified into four main categories: microfiltration, ultrafiltration, nanofiltration, and reverse osmosis. The present study focuses on the preparation of a novel porous $CaCO_3$ microfiltration membrane, which is based on the microbial-induced calcium carbonate precipitation (MICP) biomineralization process. Initially, $CaCO_3$ crystal particles with urease activity are prepared by controlling the MICP mineralization process. Secondary microbial mineralization is used to cement the loose calcium carbonate particles, forming a continuous porous solid $CaCO_3$ membrane with certain mechanical strength. Filtration tests on bacterial cells, extracellular proteins, and polysaccharides show that the MICP-driven porous $CaCO_3$ membrane effectively removes *Escherichia coli*, *Brachybacterium* sp., and activated sludge, with removal rates of 99.998%, 99.983%, and 99.996%, respectively. Compared to conventional filter paper, this porous $CaCO_3$ membrane demonstrates superior capability in removing extracellular polymers (EPS). Furthermore, the $CaCO_3$ microfiltration membrane prepared using the MICP process also exhibits ideal pore space, non-blocking characteristics, and high permeability.

Keywords: wastewater treatment; microfiltration; filter; microbial-induced calcium carbonate precipitation (MICP); water purification

基于微生物矿化诱导的多孔碳酸钙微滤膜制备及水净化应用

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摘　要:过滤是废水处理中常用的方法,根据过滤介质材料和性质的不同,可以分为4大类:微滤、超滤、纳滤和反渗透。基于微生物诱导碳酸钙沉淀(MICP)生物矿化过程制备一种新型多孔$CaCO_3$微滤膜。首先,通过控制MICP矿化过程制备具有脲酶活性的$CaCO_3$晶体颗粒;随后,利用二次微

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生物矿化将松散的碳酸钙颗粒进行胶结，从而形成具有一定机械强度的连续多孔固体$CaCO_3$微滤膜。对细菌体、胞外蛋白质和多糖的过滤试验表明，MICP驱动的多孔$CaCO_3$膜能高效去除大肠杆菌、布氏杆菌和活性污泥，去除率分别达到99.998%、99.983%和99.996%。与普通滤纸相比，该多孔$CaCO_3$膜在去除胞外聚合物(EPS)方面表现出优越的性能。此外，基于MICP工艺制备的$CaCO_3$微滤膜还展示了理想的孔隙空间、非堵塞特性和高渗透性。

关键词：污水处理；微滤；过滤器；微生物诱导碳酸钙沉淀(MICP)；水质净化
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1 Introduction

Rapid urbanization and population growth have resulted in the widespread exploitation of water resources to meet increasing demand. Both developed and developing countries are increasingly turning to the reuse of treated wastewater for potable purposes, and new and innovative methods are being proposed and implemented in many wastewater treatment plants. Filtration is the most widely used process for removing particles from wastewater, and the most commonly used filtration technique is membrane filtration, which involves a semi-permeable membrane that selectively allows certain submicron-sized particles to pass through while retaining others. There are four major categories of membrane filtration: microfiltration (0.1 μm pores), ultrafiltration (0.01 μm pores), nanofiltration (0.001 μm pores), and reverse osmosis (nonporous and impermeable to monovalent ions). The mechanism of particle removal in microfiltration and ultrafiltration is based on size exclusion, whereas nanofiltration and reverse osmosis involve mass transfer through diffusion that depends on the concentration and pressure rate of flow through the membrane flux.

Microfiltration is the process of removing particles with pore sizes of approximately 0.3 to 10 microns, which includes sand, silt, clay, cysts of parasites (e.g., *Giridia lambia*, *Cryptosporidium*), algae, and some bacterial species. Its primary role is to control microorganisms, thereby reducing the need for chlorination in water treatment. Microfiltration also removes natural and synthetic organic matter and can therefore be used as a pretreatment process for reverse osmosis and nanofiltration to reduce fouling potential. Several studies have proposed innovative approaches to improve microfiltration, such as using low-cost ceramic membrane scaffolds made from cheap clay, developing an electrochemical microfiltration granular activated carbon adsorption (e-MF-GAC) hybrid pretreatment process to control reverse osmosis pollution, and combining ceramic membranes with ozone for reverse osmosis pretreatment [1-3].

In the context of materials used for manufacturing microfiltration membranes, cost-effective polymeric substances have gained popularity due to their scalability, good separation characteristics, and suitability for industrial liquid phase separation, including wastewater treatment [4]. In water treatment, filters are commonly used to capture most of the particles present, and several effective technologies are available for the purification of potable water, including physical processes such as slow sand filters and activated carbon, and chemical processes such as ultraviolet radiation, ozonation, chlorination, flocculation, and membrane technology using polymeric, ceramic, or composite membranes [5]. Ceramic membranes have shown more promise than polymeric membranes due to their lower fouling rates and mechanical stability, whereas polymeric membranes are prone to fouling due to biological activity and pH variation [6].

However, there is a paucity of literature on the performance of ceramic filters for the separation of microbes. A study by Srivastava et al. [7] reported the effectiveness of carbon nanotube filters (CNT) in separating *E. coli* (2-5 μm), *Staphylococcus aureus* (~1 μm), and *poliovirus* (~0.025 μm) from drinking water, and Kaniganti et al. [5] investigated the log reduction value (LRV) of *E. coli*, *S. fecalis*, and *B. cereus* cultures using ceramic filters, which were 5.5, 4.2, and 3.6, respectively, for feed concentrations of 6, 0.02, and 1.1×10^4. Likewise, Mwabi et al. [8] studied various filters, including the silver-impregnated porous pot, ceramic candle filter, and sand filter, as alternate household drinking water systems and found that the silver-impregnated porous

pot demonstrated the best performance in terms of flux (0.05-2.49 L/h) and separation efficiency (LRV>5) for feed concentrations ranging from 3×10^2 to 3.68×10^6 cfu/mL. Furthermore, Liu et al. [9] found that the percentage of saturated passage was comparable at different cell densities and that saturated filtration status was achieved earlier at high cell densities as well. The filter materials, pore size, and filtering flux also had significant effects on the passage percentage of filterable bacteria. Specifically, mixed cellulose esters (MCE) membranes were more efficient than polyvinylidene fluoride (PVDF), PES, and PC membranes in retaining bacteria under the same pore conditions. The effect of filtering flux on filterable bacteria was reversed among different pore size filters (0.45/0.22 μm MCE/PVDF filters vs. 0.1 μm PVDF/PES filters), however.

All previously mentioned microfilters necessitate advanced manufacturing methods and processes for their production, which can occasionally prove challenging for developing and remote regions. In contrast, microbially-induced carbonate precipitation (MICP) has been shown to be an effective approach for reducing the porosity of porous soils by producing insoluble calcium carbonate ($CaCO_3$) precipitates that fill the soil matrix's pores, consequently decreasing porosity and permeability. For example, Yang et al. [10] devised a novel technique for seepage control in sandy environments through the formation of a precipitated $CaCO_3$ layer generated from bioslurry, a suspension of $CaCO_3$ crystals created using a microbial calcium carbonate precipitation (MICP) process. Furthermore, permeability can be regulated by applying varying degrees of cementation due to alterations in the pore size of the precipitated $CaCO_3$ layer, which ranges from a few hundred nanometers to several micrometers. Consequently, the current study focuses on the implementation of an innovative bioslurry-based filter fabricated utilizing calcium carbonate and microbial precipitation with the enzyme urease. This membrane-based filter was experimentally employed for the filtration of microorganisms, such as *Escherichia coli* (*E. coli*). Additionally, the bioslurry-based filter was evaluated in terms of its ability to remove exopolysaccharides from bacterial cultures and enriched activated sludge.

2 Materials and methods

2.1 Microorganisms and culture condition

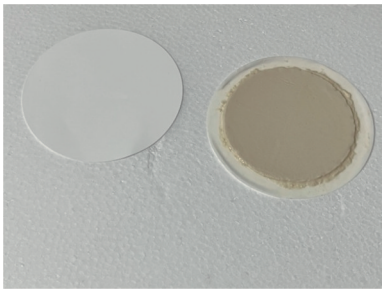
Testing of the efficacy of bacterial filtration using the bioslurry-based filter was conducted using a gram-negative strain, gram-positive strain, and a mixed consortium of bacteria. *E. coli* BL21 and *Brachybacterium* sp. were used as the model gram-negative and gram-positive strains respectively. *E. coli* BL21 was grown in lysogeny broth (LB) under shaking conditions at 37 ℃. Halotolerant *Brachybacterium* sp. was grown in minimal media composed of 8.2 g/L sodium acetate, 2 g/L yeast extract, and 10 g/L NaCl under shaking conditions at 30 ℃. Acetate utilizing the mixed consortium was enriched from activated sludge (Zhenjiang municipal wastewater treatment plant, Jingkou District, Zhenjiang, China) after several batches of subculturing under shaking conditions at room temperature. The enrichment was carried out using the minimal media made up of 16.4 g/L sodium acetate, 2 g/L yeast extract, and 10 g/L NaCl.

2.2 Bioslurry preparation and the bioslurry-based filter

Bioslurry was prepared according to the process by Cheng et al. [11] by adding 44.4 g of calcium chloride and 24 g of urea into 1 L of the ureolytic bacteria culture (DSM 33, $OD_{600}=3.5$, urease activity=20 U/mL), followed by stirring at a speed of 200 r/min for 24h. Calcium carbonate crystals with embedded highly active ureolytic bacteria were thusly produced. After complete settlement, the clean supernatant was disposed, and the settled crystals were collected and stored in a refrigerator prior to use. The solid content of the collected bioslurry was about 25%±3% (w/w). It should be noted that the urease activity of the urease-active bioslurry often correlates with the urease activity of the ureolytic bacterial culture and the amount of bioslurry formed. With higher urease activity in the ureolytic bacterial culture and fewer bioslurry crystals formed, there would be more urease activity present in the bioslurry[11].

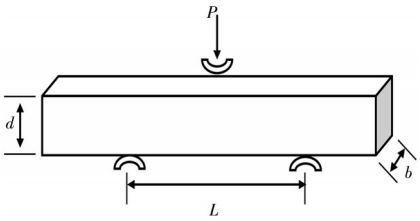
The bioslurry-based filter was fabricated using the following procedure: (1) A urease-active slurry was deposited onto the surface of filter paper, forming a bioslurry layer with an average thickness of 1 mm and a diameter of approximately 4 cm; (2) 10 mL of cementation solution, composed of 1 mol/L urea and

1 mol/L calcium chloride ($CaCl_2$), was sprayed onto the bioslurry layer to solidify it into a porous membrane; (3) Step 2 was repeated 2-6 times at 24-hour intervals until a robust and solid porous calcium carbonate ($CaCO_3$) membrane was obtained after removing the filter paper (Fig. 1(a), and 1(b)). The bioslurry-based filter was then subjected to various tests, including filtration, permeability, and flexural tests (Fig. 1(c)).



(a) bioslurry deposit layer

(b) solidified bioslurry-based filter for filtration test

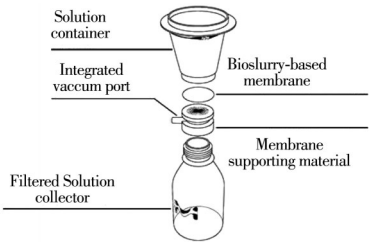


(c) three-point loading test

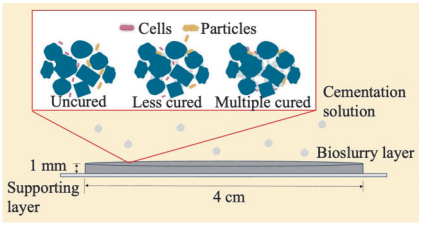
Fig. 1　Photos of the bioslurry-based filter

2.3　Bacterial filtration using the bioslurry-based filter

All filtration tests were conducted using vacuum filtration (500 mL Millipore filter unit) at an applied vacuum pressure of 15～30 kPa. The bioslurry-based filter was placed in the vacuum filtration unit (Fig. 2(a)), which was initially washed with autoclaved water to eliminate any leachable materials. The unit was then sterilized by washing it multiple times with 70% ethanol, followed by washing with autoclaved water to remove residual ethanol. Approximately 200 mL of well-mixed bacterial influent was poured onto the filter unit. The application of the vacuum ensured that filtration of biomass by the bioslurry-based filter actually occurred (Fig. 2(b)), and the permeate was collected in the bottom clean flask. The cell density of all bacterial influents was maintained relatively constant (approximately 10^6～10^7 colony-forming units per milliliter (CFU/mL)). After filtration, enumeration of biomass in the influent and effluent was carried out. Enumeration of *E. coli* BL21 colonies was performed on LB agar plates, and *Brachybacterium* sp. and activated sludge enrichment were enumerated on minimal media agar supplemented with 16.4 g/L sodium acetate, 2 g/L yeast extract, and 10 g/L NaCl. The biomass filtration capacity of the bioslurry-based filter was then determined by measuring the difference in CFU/mL between the influent and effluent. For comparison, a sterile Whatman filter paper (pore size 6 μm, Whatman filter paper grade 3) was employed as a control.



(a) Laboratory scale vacuum filtration setup



(b) bioslurry-based filter structure

Fig. 2　Schematic diagram of the laboratory scale vacuum filtration setup and bioslurry-based filter structure

2.4　Filtration of extracellular polymeric substances

The bioslurry-based filter was additionally evaluated in terms of filtration of extracellular polymeric substances (EPSs) from *E. coli* BL21, *Brachybacterium* sp., and activated sludge enrichment. EPSs were extracted from cells during their exponential phase at an OD_{600} of 2.0 (approximately 10^8 cells/mL), with all strains grown under the operating conditions described above. Specifically, the EPSs were extracted by centrifuging the cells at 6 000*g* for 20 minutes at 4 °C. The cell pellets were discarded, and the supernatant containing the total EPSs was collected. The

cell-free supernatant (approximately 100 mL) was subsequently poured onto the filter column, and all filtration tests for EPSs were conducted using the same vacuum filtration method described above. Filtration efficiency was expressed in terms of total EPSs retained (dry weight, g) in the filter per total EPSs tested. As a negative control, a sterile Whatman cellulose filter paper was also employed for EPS filtration.

2.5 Quantification of polysaccharides and proteins in EPSs

The total polysaccharides extracted from the EPSs were quantified using Dubois' assay [12-13]. About 50 μL of the extracted EPS (100 times diluted) was mixed with 50 μL of phenol (4% w/v) and 100 μL of H_2SO_4 (98%), which was then incubated at room temperature for 10~15 min. The OD_{490} of the reaction mixture was then measured [13]. Glucose was used as the standard sugar for the assay, and the total polysaccharides in the EPSs were quantified in terms of g/L. Furthermore, the total extracellular proteins from the EPSs were quantified by the bicinchoninic acid (BCA) assay. For this assay, 20 μL of the extracted EPSs were mixed with BCA reagent composed of $CuSO_4 \cdot 5H_2O$ (4% w/v) and bicinchoninic acid. The total proteins in the EPSs were then quantified by measuring OD_{562} in terms of g/L. The efficiency of EPS filtration was further expressed in terms of the protein (PN) and polysaccharide (PS) rejection.

2.6 Bioslurry-based biofilter permeability and strength

The permeability of the bioslurry-based biofilter was tested according to the ASTM D2434-68 method with a rigid side-wall device set-up while the samples remained within the test columns. Prior to the permeability tests, tap water (2 L) was pre-flushed through the filter samples under 15 kPa of back pressure (hydraulic head of approximately 150 cm) to release trapped pore air and saturate the samples before measuring permeability. Following the initial saturation step, permeability tests were conducted until steady hydraulic conductivity values (k, cm/s) were attained.

Three-point loading tests were performed to determine the flexural strength of the bioslurry filter. After the filtration test, the solidified bioslurry layer was cut into small specimens of 4 cm×2 cm×1 mm each for the determination of flexural strength, which was calculated as follows (Fig 1(c)).

$$\sigma = \frac{3PL}{2bd^2}$$

where σ is flexural strength (Pa) at the midpoint, P is load (N) at a given point on the load-deflection curve, L is the length of the support span (m), b is the width of the specimen (m), and d is the thickness (m).

2.7 Microstructure analysis

Scanning electron microscopy (SEM) analysis was also carried out to characterize the structure and pore size of the bioslurry-based filter. Prior to the SEM analysis, the filter was dried at 105 ℃ for 24 hours. The microscopy examination was then conducted using a scanning electron microscope (JSM-7001F, Jiangsu University).

3 Results and Discussion

3.1 Bacterial cell filtration efficiency

Bacterial cultures with cell density of approximately 10^6-10^7 cfu/mL were selected to test the efficacy of the bioslurry-based filter in removing biomass from the solution by recording reductions in cell concentrations. Fig. 3 shows that the bioslurry-based filter had a removal efficiency of 99.998%, 99.983%, and 99.996% for pure strains of *E. coli*, *Brachybacterium* sp., and enriched activated sludge, respectively. In comparison, the filter paper only removed about 16%-32% of the biomass. This suggests that the bioslurry-based filter has high solid retention capacity, which is probably due to the small pore size (less than 1 μm) of the $CaCO_3$ filter. Furthermore, the CFU assay (Fig. 4) clearly indicated that after filtration by the bioslurry-based filter, more than 99.98% of the biomass was removed from the culture, which means that this filter could be potentially used for water purification. It was noted that the contamination from ureolytic bacteria can be avoided by standard sterilization procedures such as autoclaving or high-concentration alcohol.

3.2 EPS filtration efficiency

Fig. 5 shows the extracted EPSs and their removal efficiency for both the normal filter paper and the bioslurry-based filter. The removal efficiency of the EPSs extracted from the *E. coli* culture by the bioslurry-based biofilter was about 57.5%, which

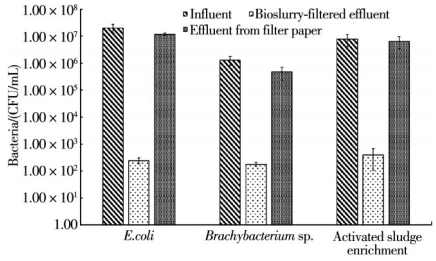
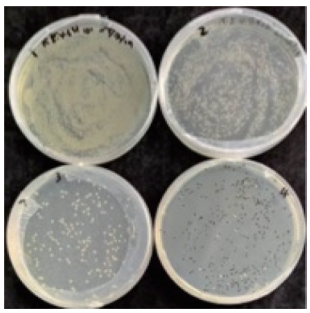
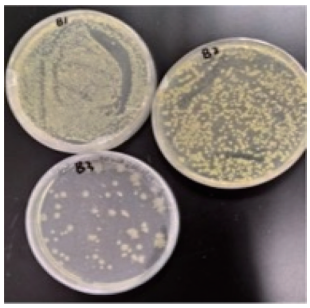


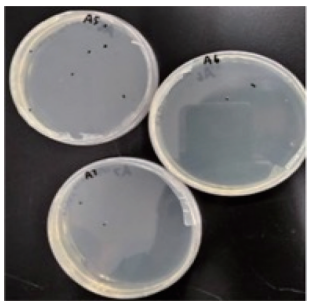
Fig. 3　The removal efficiency of *E. coli*, *Brachybacterium* sp., and enriched activated sludge biomass by the bioslurry-based filter (6 treatments) and normal filter paper



(a) unfiltered *E. coli* culture



(b) filtered *E. coli* culture using filter paper



(c) filtered *E. coli* culture using the bioslurry-based filter

Fig. 4　The CFU of unfiltered *E. coli* culture, filtered *E. coli* culture using filter paper, and filtered *E. coli* culture using the bioslurry-based filter (6 treatments)

was about 7 times larger than that achieved by the normal filter paper. Similar higher EPS removal efficacy was also observed from the bioslurry-based filter for *Brachybacterium* sp. and activated sludge. The higher EPS removal efficiency of the bioslurry-based filter was probably due to the adsorption of the EPS molecules onto the porous $CaCO_3$ filter. Due to their high surface area, porous $CaCO_3$ crystals exhibit adsorption capacity for both heavy metals and organic polymers [14].

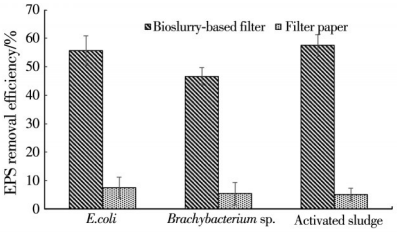


Fig. 5　EPS removal by normal filter paper and bioslurry-based filter (6 treatments)

3.3　Quantification of polysaccharide and protein rejection

Table 1 shows the rejection rates of PN and PS for the bioslurry-based and normal filters. For both filters PS rejection was much higher than for PN. Additionally, the bioslurry-based filter had much higher PN and PS rejection rates compared to the normal filter paper. Researchers have previously reported that this rejection behavior can be attributed to two possible mechanisms. One is the interaction between proteins and polysaccharides, and the other is size exclusion by membrane pore size[15]. By having a smaller pore size, the contribution of the sieving effect by membrane pore size to the overall solute rejection is higher in the bioslurry-based filter than in the paper filter. Maximous et al. [16] further revealed that the solute removal rate depends on the pollutants contributing to cake formation or pore plugging. In the current study, the high PS and PN rejection rates of the bioslurry-based filter could also be due to the porous calcium carbonate crystals that provide a large surface area for PN and PS absorption. However, direct evidence is needed to identify the possible combined effects of absorption and filtration for PS and PN removal, which we intend to investigate in a future study.

3.4　The effect of the number of treatments on filtration efficiency

The above test results clearly show that after 6 times of treatment, the loose bioslurry deposit layer turned into a solid filter, enabling efficient biomass removal. This is because the $CaCO_3$ crystals formed during the MICP treatment combine the original

Table 1 PN and PS rejection by the bioslurry-based filter (6 treatments) and normal filter paper

Culture	PN concentration/(g/L)	PS concentration/(g/L)	PN/PS ratio	Bioslurry-basedfilter/%		Filter paper/%	
				PN	PS	PN	PS
E. coli	1.81	9.69	0.19	55	76	1	12
Brachybacterium sp.	1.98	15.77	0.13	50	79	2	11
Activated sludge	3.45	42	0.08	70	91	0	20

Note: These values represent the average of tests performed in triplicate.

$CaCO_3$ crystals together to form a strong and solid $CaCO_3$ layer. Thus as more treatments were carried out, more crystals were formed within the pore space of the bioslurry deposit layer, thus reducing pore size. An investigation of the evolution of the filtration efficiency during treatment revealed that only poor biomass removal efficiency was achieved after treatments but that this rapidly improved after both 4 and 6 treatments (Fig. 6). Further treatment up to 8 times significantly reduced the permeability and flux, however, which was probably due to the high extent of pore space filling and reduced porosity.

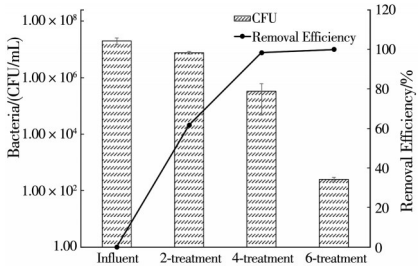


Fig. 6 The effect of the number of treatments on the biomass removal efficacy of the bioslurry-based filter

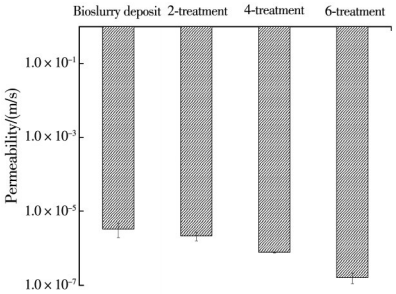
3.5 The effect of the number of treatments on permeability and flexural strength

The permeability reduction of the bioslurry-based filter changed based on the number of treatments applied: it decreased from 3.30×10^{-6} to 2.14×10^{-6}, 7.72×10^{-7}, and 1.62×10^{-7} m/s with 2, 4, and 6 treatments, respectively (Fig. 7(a)). This is in line with a previous study that presented an increase in the number of treatments decreased the permeability of a MICP-treated sand column [17]. A higher number of treatments enhances the mechanical properties of the filter. However, it also leads to complete pore blocking after excessive amounts of $CaCO_3$ form, which reduces its total flux and makes it less effective. A well-designed filter should always present good filtration efficiency and high flux. Therefore, for practical applications, it is worthwhile to investigate the optimal number of treatments so that both high removal efficiency (small pore size) and high flux (large porosity) can be achieved.

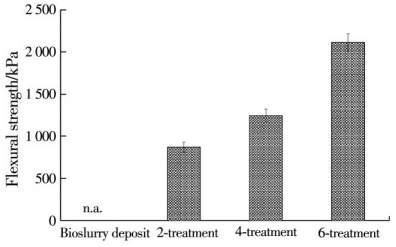
As shown in Fig. 7(b), the flexural strength of the solidified bioslurry filter was found to range from 0.87 to 2.1 MPa after two to four treatments. This indicates that the strength of the bioslurry filter was directly proportional to the number of treatments. The enhanced flexural strength significantly exceeded the negative pressure generated by the vacuum pump as well. Moreover, this increase in strength was attributable to the MICP process, which precipitated the $CaCO_3$ crystals. These crystals act as a bonding agent that coheres to the loose bioslurry $CaCO_3$ crystals and thereby enhances their mechanical properties of the loose bioslurry particles.

3.6 The microstructure of the bioslurry-based biofilter

The microfeatures of the raw bioslurry and bioslurry-based filter were examined using SEM (Fig. 8 (a)-(b)). This analysis revealed that the raw bioslurry



(a) permeability



(b) flexural strength of the bioslurry-based filter

Fig. 7 The effect of the number of treatments on the permeability and flexural strength of the bioslurry-based filter

was dominated by spherical-shaped crystals with various particle sizes ranging 2-5 μm. Imprints of the bacterial cell shapes at the surface of the spherical crystals were observed, which is in line with previous studies [11, 18-19]. Additionally, the bioslurry had high residual urease activity and was able to induce crystals precipitation [11]. Therefore, after the deposited loose bioslurry layer was 2 times with cementation solution, the crystals induced by the bioslurry were able to adhere to each other and connect together to form a solid filter membrane. However, the pore size of the filter membrane was around 10-50 μm due to insufficient $CaCO_3$ production (Fig. 8(c)-(d)), and such large pore size resulted in poor biomass filtration efficiency as indicated above (Fig. 6). Furthermore, after 6 treatments more crystals were produced within the pores, and a much denser filter membrane with smaller pore size ranging from 50 to 150 nm was achieved (Fig. 8(e)-(f)), enabling higher biomass removal efficiency as well as PS and PN rejection compared to the normal filter paper.

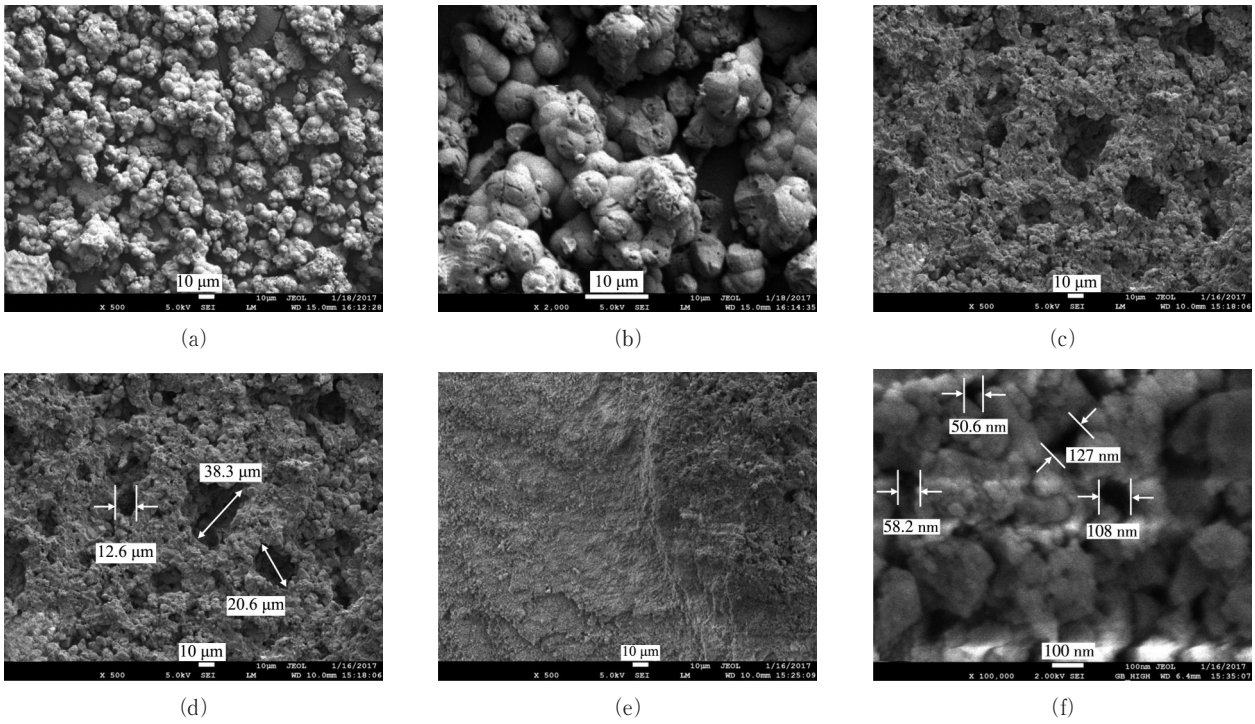


Fig. 8　Scanning electron microscopy (SEM) images of (a, b) raw bioslurry crystals, (c, d) the bioslurry-based filter treated 2 times, and (e, f) the bioslurry-based filter treated 6 times

The newly developed porous filter made from secondary biocementation $CaCO_3$ crystal particles represents an innovative and straightforward approach to manufacturing pore-size controllable porous filters. In comparison to traditional ceramic filters that often require firing [20-22], this study presents a cost-effective and facile production method for a porous filter that operates at room temperature and requires minimal technological input. This advancement is particularly advantageous for rural or low-resource areas as it offers an economical and accessible solution for household water purification, industrial applications, and emergency scenarios.

4　Conclusion

In this study, a novel bioslurry-based filter membrane derived from microbially induced carbonate precipitation was proposed to have a high removal efficiency for *Escherichia coli*, *Brachybacterium* sp., and activated sludge, with removal rates of approximately 99.998%, 99.983%, and 99.996%, respectively. The study also showed that the pore size of the bioslurry-based filter had a direct impact on treatment times. After six times of treatments, a much denser bioslurry-based filter with a smaller pore size ranging from 50 to 150 nm was achieved, enabling a significantly greater PN and PS rejection rates compared to normal filter paper.

The newly developed porous filter, composed of secondary biocementation $CaCO_3$ crystal particles, offers an innovative and straightforward method for manufacturing pore-size controllable porous filters.

Unlike traditional ceramic filters that often require high-temperature firing, this study introduces a cost-effective and easy-to-produce filter that operates at room temperature with minimal technological input. This advancement is particularly beneficial for rural or low-resource areas, providing an economical and accessible solution for household water purification, industrial applications, and emergencies.

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