Investigation of Anti-Alga Properties and Anti-Bacteria Effects of Composite Nanofiltration Membranes Based on Chitosan Derivatives

J. Miao, H. C. Lin, and L. C. Zhang

***Abstract*—The anti-alga properties and anti-bacteria effects of composite nanofiltration (NF) membranes prepared from sulfated chitosan (SCS) and N, O-carboxymethyl chitosan (NOCC) were investigated in this study. The base membranes, polyacrylonitrile (PAN) and polysulfone (PS) ultrafiltration (UF) membranes, were used to be as the controls. Compared with the controls, the adsorptions of the alga on the composite NF membranes were less severe. It suggested that the SCS and NOCC composite NF membranes have anti-alga and antifouling abilities. The chosen bacteria were escherichia coli, bacillus subtilis, staureus, penicillium chrysogenum, and streptomyces jinyangensis. By comparing the colony diameters of different bacteria on various membranes and the growth of bacteria after different time periods, the qualitative conclusions of the anti-bacterial effects of the membranes were drawn. It suggested that all the investigated membranes have some anti-bacterial effects on the five kinds of bacteria and the anti-bacterial effects are related to the active layer material of the composite NF membrane and the cross-linking agent.**

***Index Terms*—Composite NF membrane, sulfated chitosan (SCS), N, O-carboxymethyl chitosan (NOCC), anti-alga and anti-bacterial abilities.**

I. Introduction

Among the polysaccharides, chitosan has been investigated extensively in the industries due to its excellent film-forming, antimicrobial, physical and mechanical properties [1]-[3]. Chitosan could inhibit the growth of numerous species of bacteria, pathogens, and fungi [4]-[9]. Chitosan derivatives also have the inhibition effects on bacteria. For example, carboxymethyl chitosan have different inhibition effects on Staphylococcus aureus, pseudomonas aeruginosa, salmonella typhi, escherichia coli (E. coli), isospora belli, streptococcus mutans, oral lactobacilli [10]-[13]. J. W. Song *et al*. [14] synthesized anti-microbial soldium alginate (SA) with chito-oligosaccharid (COS): SA–COS showed excellent anti-microbial activity with the growth of microorganisms completely suppressed by a small amount of COS (1.8 wt%). The crosslinked chitosan also has excellent bactericidal action [15]-[17].

In this work, the anti-alga and anti-bacteria effects of the crosslinked thin-film composite (TFC) nanofiltration (NF) membranes using sulfated chitosan (SCS) and N, O-carboxymethyl chitosan (NOCC) as the active layer materials were investigated preliminarily. The base membranes as the support for the active layer, PAN and PS UF membranes were employed as the control membranes for the anti-alga and anti-bacteria tests. The anti-alga effects of the SCS and NOCC composite membranes were analyzed qualitatively after comparing the adsorptions of the alga on the base membranes and the composite NF membranes.

The chosen bacteria were E. coli, bacillus subtilis, staureus, penicillium chrysogenum, and streptomyces jinyangensis. The qualitative conclusions of the anti-bacteria effects of the membranes were drawn after comparing the colony diameter of different bacteria on various composite NF membranes and the bacteria growth after different time periods.

II. Experimental Section

A. Materials

SCS and NOCC composite NF membranes were fabricated according to the preparation technologies in the references, surface crosslinked with hexamethylene diisocyanate (HDI), glutaraldhyde (GA), and epichlorohydrin (ECH), respectively. Table I shows the characteristics of these two series of composite NF membranes.

PAN UF membrane was self-cast and PS UF membranes were provided by Hangzhou Water Treatment Technology Development Center, China. The water samples containing freshwater alga were taken from a pond. Beef extract, peptone, sodium chloride, agar, and other reagents were of analytical grade. E. coli, bacillus subtilis, staureus, penicillium chrysogenum, and streptomyces jinyangensis, were provided by a Microbiology Laboratory. All the bacteria were activated and purified before using.

B. Experimental Methods

1) Anti-alga tests

The composite NF membranes and their base membranes (PAN, PS UF membranes) were immersed in freshwater containing alga, under the irradiation of sunlight. 26 days later, the adsorptions of freshwater alga on the membrane surfaces were observed with OLYMPUS BX51 Microscope (OLYMPUS Optical Co., Ltd., Japan): magnification: 40×10

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for more severe adsorption; 10 × 10 for no or light adsorption.

TABLE I: CHARACTERISTICS OF SCS & NOCC COMPOSITE NF MEMBRANES

		GA-SCS/PAN [18]		ECH-SCS/PS [19]		HDI-SCS/PAN [20]		GA-NOCC/PS [21]		ECH-NOCC/PS [22]	
Gradient β of Zeta Potential (E) vs. operating pressure (ΔP)		–10.3		–8.78		–10.5		–8.00		–6.00	
Molecular Weight cut-off (MWCO)/Da		780		925		720		625		760	
Electrical characteristic		Amphoteric		Amphoteric		Amphoteric		Amphoteric		Amphoteric	
Charged groups in the active layer		$-NH_3^+$, $-OSO_3^-$		$-NH_3^+$, $-OSO_3^-$		$-NH_3^+$, $-OSO_3^-$		$-NH_3^+$, $-CH_2COO^-$		$-NH_3^+$, $-CH_2COO^-$	
Operating conditions	Solute	F/ kg m^{-2} h^{-1}	R/ %	F/ kg m^{-2} h^{-1}	R/ %	F/ kg m^{-2} h^{-1}	R/ %	F/ kg m^{-2} h^{-1}	R/ %	F/ kg m^{-2} h^{-1}	R/ %
1000 mg L^{-1}, 0.40MPa	Na_2SO_4	7.1	92.1	22.9	90.8	7.1	89.9	3.0	92.7	7.9	90.4
	NaCl	10.4	50.3	58.4	32.5	9.0	37.1	5.1	30.2	10.8	27.4
Rejection order to inorganic electrolytes		Na_2SO_4 > NaCl > $MgSO_4$ > $MgCl_2$				Na_2SO_4> $MgSO_4$ > NaCl > $MgCl_2$		Na_2SO_4 > NaCl > $MgSO_4$ > $MgCl_2$			

TABLE II: THE BACTERIA CONCENTRATIONS

Bactria	Bacillus subtilis	E.coli	Staureus	Penicillium chrysogenum	Streptomyces jinyangensis (5406)
Concentration (×10^8 cell/mL)	2.2	3.0	1.5	1.8	1.8

2) Antibacterial test

The strains were cultured under different conditions: bacillus subtilis, E. coli, and staureus cultured on beef extract peptone medium at 37 ℃ for 24 h; Penicillium chrysogenum cultured on Czapek medium slant at 28 ℃ for 72 h or more; Streptomyces jinyangensis (5406) cultured on Gauserime synthetic medium slant for 4 to 5 days. The living bacteria were counted with direct microscopic method. Table II shows the bacteria concentrations.

3) Determination of anti-bacterial properties of the composite NF membranes

All membranes were rinsed with distilled water, placed in an empty Petri dish for moist heat sterilization. The sterilized membranes were placed on the incubated plates for the parallel experiments. 20 uL bacteria inoculums were transferred to the centers of the membrane surfaces with microinjection gun, and then the inoculated membranes were incubated for a period of time under different conditions according to the bacteria. PAN and PS UF membrane were employed as the controls. All specimens were prepared with the diameter of 15 mm for antibacterial tests. After incubation, the diameters of the colonies on the membrane surfaces were measured. The qualitative conclusions of the antibacterial effects of the membranes could be drawn after comparing the colony diameters of different bacteria on various membranes and the bacteria growth after different time periods.

III. RESULTS AND DISCUSSIONS

A. Anti-Alga Properties of Composite NF Membranes

The adsorptions of freshwater alga on the surfaces of the base membranes and the composite NF membranes were shown in Fig. 1, where the alga were mainly blue alga and green algae, a small amount of diatoms.

As could be seen clearly from Fig. 1, the alga adsorptions on SCS and NOCC composite NF membrane surfaces were not so sever as those on PS and PAN UF membranes. It might be due to charge repulsion between the alga and the surfaces of SCS and NOCC composite NF membranes. The alga surfaces are usually negatively charged, while there are negatively-charged functional groups in the active layers of both SCS and NOCC composite NF membranes, $-OSO_3^-$ and $-CH_2COO^-$. Hence, SCS and NOCC composite NF membrane surfaces could have some anti-alga and antifouling abilities.

B. Anti-Bacteria Properties of SCS and NOCC Composite NF Membranes

Table III shows the experimental results of the growth diameters of bacteria on the membranes after different time periods, including the controls (PAN and PS UF membranes), and SCS and NOCC composite NF membranes.

By comparing the growth of different strains on the membranes, and the colony growth in 24 h, the inhibition abilities of the membranes on different bacteria could be qualitatively analyzed. It could be known from Table II, SCS and NOCC composite NF membrane had certain inhibition effects on the growth of all five kinds of bacteria; HDI-SCS/PAN and ECH-SCS/PS had better antibacterial abilities on these bacteria. The following conclusions were drawn:

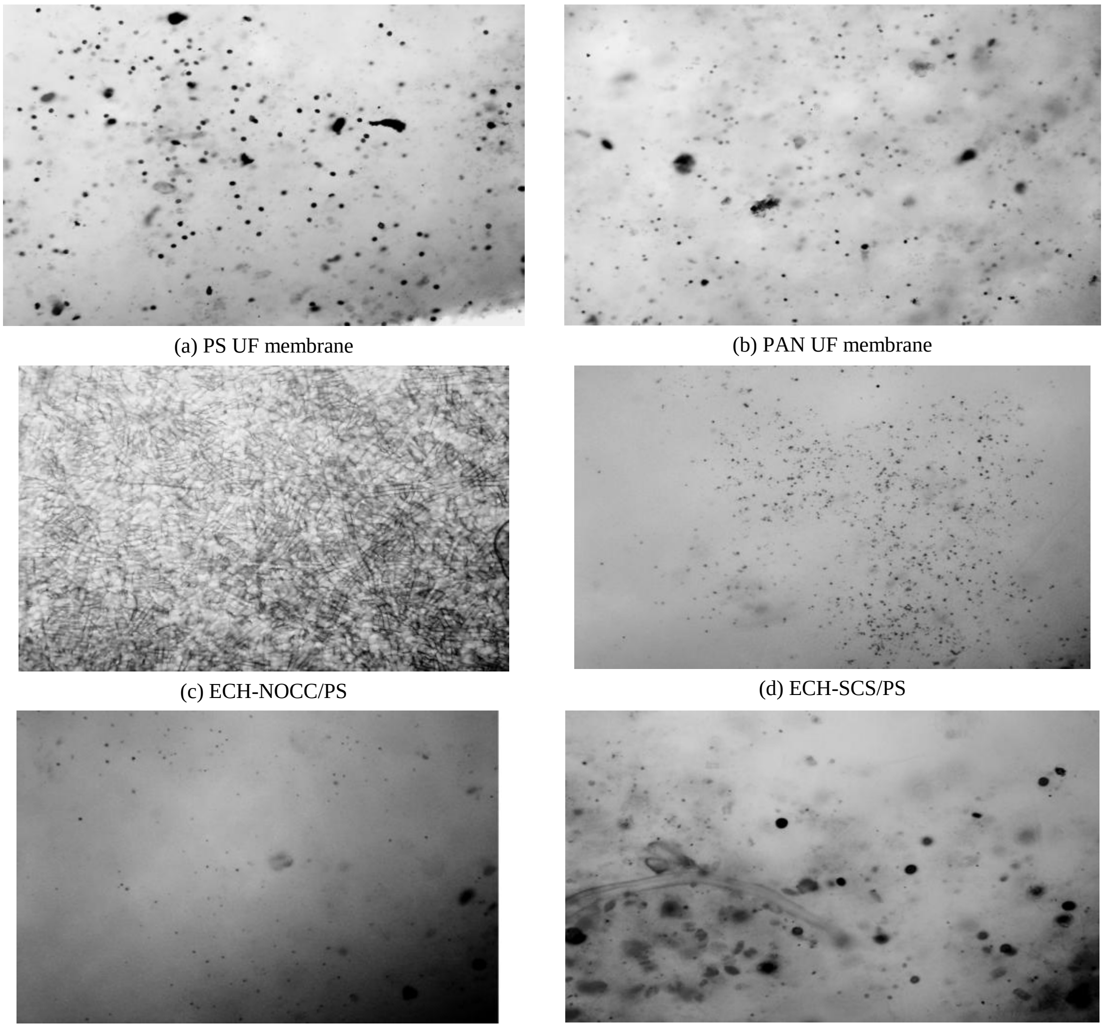
1) SCS and NOCC composite NF membranes showed different inhibition abilities on different types of Bacteria

The colonies of Bacillus subtilis on HDI-SCS/PAN and ECH-SCS/PS were smaller than those on the controls.

As for E. Coli, three kinds of composite NF membranes, ECH-SCS/PS, GA-SCS/PAN, and HDI-SCS/PAN, showed better inhibition effects. Staureus did not grow on the surface of ECH-NOCC/PS composite NF membrane, suggesting that ECH-NOCC/PS have excellent inhibition ability on it. The others showed almost the same inhibition effects on Staureus.

Considering both the growth of Penicillium chrysogenum in 48 h and 72 h, the inhibition ability decreased in the order of HDI-SCS/PAN, ECH-NOCC/PS, and GA-SCS/PAN. However, GA-NOCC/PS, ECH-SCS/PS, and HDI-SCS/PAN, showed better inhibition effects in 24 h.

Compared with the other composite NF membranes, HDI-SCS/PAN and ECH-SCS/PS showed better inhibition effects on Streptomyces jinyangensis (5406).



(a) PS UF membrane (b) PAN UF membrane

(c) ECH-NOCC/PS (d) ECH-SCS/PS

(e) GA-NOCC/PS (f) HDI-SCS/PAN

Fig. 1. The adsorption of alga on the base membranes and the composite NF membranes.

TABLE III: THE GROWTH DIAMETERS OF BACTERIA ON THE MEMBRANES AFTER DIFFERENT TIME PERIODS

	PAN	PS	ECH-NOCC/PS	ECH-SCS/PS	GA-NOCC/PS	HDI-SCS/PAN	GA-SCS/PAN
Bacillus subtilis	10.5	7.7	8.5	5.5	7.5	5.3	7.7
E. Coli	9.3	8.0	8.0	5.1	6.0	5.8	5.3
Staureus	9.0	8.3	—	5.6	6.8	6.0	6.0
	PAN	PS	ECH-NOCC/PS	ECH-SCS/PS	GA-NOCC/PS	HDI-SCS/PAN	GA-SCS/PAN
Penicillium chrysogenum in 48 h	8.0	9.5	6.0	8.0	9.0	4.7	7.3
Penicillium chrysogenum72 h	8.5	10.5	7.0	8.3	9.0	5.5	11.0
Streptomyces jinyangensis (5406) 72 h	7.1	9.1	9.5	5.9	7.9	4.5	7.1
Streptomyces jinyangensis (5406) 96 h	9.0	9.5	9.5	6.5	8.5	6.0	9.0

2) Different composite NF membranes shows different inhibition effects on bacteria

ECH-NOCC/PS composite NF membrane could inhibit the growth of staureus completely, streptomyces jinyangensis (5406) in 24 h, also inhibit penicillium chrysogenum well, but showed bad inhibition effects on bacillus subtilis and E. Coli.

ECH-SCS/PS composite NF membrane could inhibit bacillus subtilis, E. Coli., staureus, and streptomyces jinyangensis (5406) well, but showed bad effect on penicillium chrysogenum in 48 h.

GA-NOCC/PS composite NF membrane inhibited E. Coli., staureus, and streptomyces jinyangensis (5406) well. The colony diameter of penicillium chrysogenum did not change after 24 h, suggesting it also could inhibit penicillium chrysogenum well. Its inhibition effect on bacillus subtilis was not so good as on the other four bacteria.

HDI-SCS/PAN inhibited all five kinds of bacteria well.

GA-SCS/PA showed not good inhibition effects on the other 4 bacteria except staureus. It suggested that the colony diameters of penicillium chrysogenum and streptomyces jinyangensis (5406) increased significantly in 24 h, suggesting the colonies grow continually.

3) The composite NF membranes surface crosslinked with different crosslinking agent showed different inhibition effects on the bacteria

The composite NF membranes crosslinked with HDI showed better inhibition effects than those crosslinked by GA and ECH. It might be related to the occurring positions of the crosslinking reactions. GA mainly reacts with the amine groups of SCS and NOCC, while HDI react with both amine and hydroxyl groups. The anti-bacterial abilities of chitosan and its derivatives are originated from the density of $-NH_3^+$ [23].

IV. Conclusions

The anti-alga and anti-bacterial effects of SCS and NOCC composite NF membranes were investigated preliminarily. The adsorptions of freshwater alga on the composite NF membranes were less severe than those on the support membranes, PAN and PS UF membranes. It suggested that SCS and NOCC composite NF membranes have anti-alga and antifouling properties in certain degrees.

The anti-bacterial tests suggested that all the investigated composite NF membranes have some inhibition effects on the five kinds of bacteria. The anti-bacterial effects are related to the active layer material of the composite NF membrane and the cross-linking agent, which might be due to the occurring positions of the crosslinking reactions.

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