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A novel membrane bioreactor enhanced by effective microorganisms for the treatment of domestic wastewater

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Abstract The activated sludge membrane bioreactor (MBR) has been shown to have some advantages for the processing and reclamation of domestic wastewater. We hypothesized that certain microorganisms, chosen for their abilities to decompose the chemical components of raw sewage, would, when coupled with the MBR, significantly improve the stability and efficiency of this system. We selected environmental bacterial strains which oxidize ammonia and nitrites and produce protease, amylase, and cellulase for the development and testing of a novel biologically enhanced MBR (eMBR). We compared the eMBR with the activated sludge MBR. With the eMBR, the average values of effluent quality were: chemical oxygen demand (COD), 40 mg/l(average efficiency of removal 90.0%); and NH_4^+–N, 0.66 mg/l(average efficiency of removal 99.4%). Effluent qualities met the standard and were stable during the entire 90 days of this study. For the activated sludge MBR, the COD removal rate was 91.7%, and the NH_4^+–N removal (94.8%) was less than that of the eMBR. Start-up time for the eMBR was only 24–48 h, much shorter than the 7–8 days required to initiate function of the standard MBR. The biomass concentrations of total heterotrophic bacteria and autotrophic bacteria in the eMBR did not fluctuate significantly during the course of the study. Various kinds of microorganisms will establish an ecological balance in the reactor. Compared with the activated sludge MBR, the eMBR not only produced an excellent and stable quality of effluent but also resulted in a shorter time to start-up and significantly improved the efficiency of NH_4^+–N removal.

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Introduction

Rapid population growth, economic development, and the lack of effective environmental protection in many developing countries often lead to serious deterioration in the quality of water resources. Sewage discharge without sufficient treatment has become the main source of organic impurities in water. China is one country where there is a scarcity of water and where there is extensive pollution of the water sources which are available. According to the Report on the Environmental Conditions in China issued by the State Environmental Protection Administration of China (2004), the main rivers in China are severely polluted. The inland freshwater lakes, like Dianchi Lake in Yunnan Province, Taihu Lake in Jiangsu Province, and Chaohu Lake in Anhui Province, are all in eutrophication. The water quality of most of the main rivers is declining.

The membrane bioreactor (MBR) process, consisting of an activated sludge bioreactor and a microfiltration membrane, is an emerging and promising technology utilizing a biological treatment process. It takes advantage of the rapid development in membrane manufacturing and has the potential to fundamentally advance the biological treatment of wastewater. The MBR system has exhibited an excellent effluent quality, a high biomass concentration without concern for sludge settling problems, a simple flow configuration, and a small footprint demand. The MBR has been used successfully for biological treatment of wastewater and for the reclamation of treated effluents (Li and Chu 2003; Rosenberger et al. 2002; Stephenson et al. 2000; Ueda and Hata 1999; Visvanathan et al. 2000).

There are, however, several remaining disadvantages of this basic system which need to be addressed. One of these is the slow initiation of function because the beginning concentration of effective bacteria is low. Liu et al. (1999) reported that it took at least 2 weeks to get effluent quality to meet the basic standards defined by the Water Quality Standard for Urban Miscellaneous Water Consumption when using the activated sludge MBR. In addition, with an influent of low organic and high ammonia strength, a short hydraulic retention time (HRT) has to be adopted to ensure the sufficient growth and accumulation of biomass in the bioreactor. A short HRT not only requires a high transmembrane flux, which is rather difficult to achieve, but it can also jeopardize the quality of the treated effluent (Stephenson et al. 2000).

Because the membrane retains all the biomass in the reactor, producing a high internal concentration of microorganisms, the MBR is an ideal system for utilizing highly efficient chemical oxygen demand (COD) and (NH_4^+–N)-removing bacteria. We hypothesized, therefore, that the MBR augmented with such bacteria (known as "effective microorganisms") will improve the system by reducing the start-up time, increasing the stability of effluent quality, and prove to be convenient and easy to operate. The advantages of this enhancement of the MBR processing system will promote efforts to utilize and further develop the MBR technology. The purpose of this study was to develop an effective microorganism-enhancing MBR (eMBR) for the treatment of domestic wastewater. The efficiency of the eMBR at removing pollutants was analyzed and compared with that of the standard activated sludge MBR. The growth of the reactor biomass and operation characteristics such as the concentration of dissolved oxygen (DO) and HRT were also analyzed because these affect the quality of the effluent.

Nomenclature

NH_4^+–N	Ammonia nitrogen concentration (mg/l)
NO_2^-–N	Nitrite nitrogen concentration (mg/l)
NO_3^-–N	Nitrate nitrogen concentration (mg/l)
COD	Chemical oxygen demand (mg/l)
Q	Gas flux (m^3/h)
HRT	Hydraulic retention time (h)
T	Temperature (°C)
DO	Dissolved oxygen
PVDF	Polyvinylidene fluoride
MBR	Membrane bioreactor
NTU	Nephelometric turbidity unit
CFU	Colony-forming unit

Materials and methods

Effective microorganisms

The effective microorganisms used included both autotrophic and heterotrophic bacteria. The autotrophic bacteria, including the ammonia-oxidizing bacteria *Nitrosomonas europaea* (ATCC 19718) and the nitrite-oxidizing bacteria *Nitrobacteria winogradskyi* (DSMZ10237), were purchased from the Institute of Microbiology of the Chinese Academy of Sciences. The heterotrophic bacteria, including the protease-producing bacteria *Bacillus licheniformis* (CGMCC0766), the cellulase-producing bacteria *Bacillus megaterium* (CGMCC0767), and the amylase-producing bacteria *Bacillus sphaericus* (CGMCC0764), were isolated from the environment and preserved in the Chinese General Microbiological Culture Collection Center (CGMCCC).

Activated sludge

The activated sludge seeded in the MBR was taken from the secondary settling tank of the Jizhuangzi Wastewater Treatment Plant in Tianjin, China.

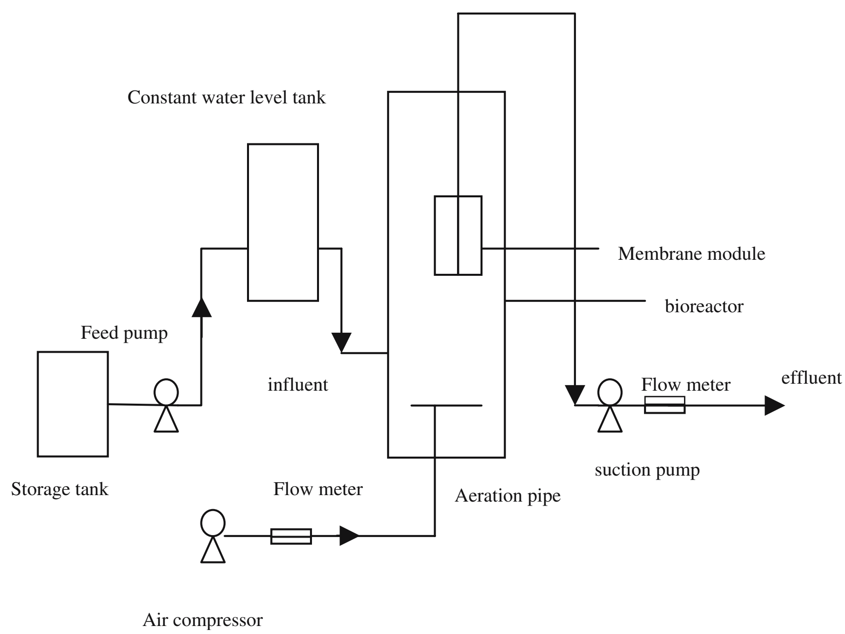


Fig. 1 Flow diagram of the experimental MBR

Table 1 Experimental operation conditions

T (°C)	HRT (h)	Flow rate (l/h)	DO (mg/l)	*Q* (m^3/h)
20–30	5	7.0	2–3	0.1

System description and operating conditions

The MBR was installed in the laboratory of the Institute of Hygiene and Environmental Medicine in Tianjin. The wastewater treated in the experiment originated from a community living near the Institute. The MBR consisted of a cylindrical bioreactor with a working volume of 36 l and a hollow fiber membrane module, as shown in Fig. 1. The membrane used was made of PVDF (Tianjin Motian Membrane Eng. Co. Ltd., China) and had an average pore diameter of 0.22 μm. The active surface area of the membrane was 0.2 m^2.

The raw wastewater was first pumped from the storage tank into the constant water level tank. This tank controlled the influent and kept the water level in the bioreactor constant as the inflow rate was set by the water level. The effluent rate of flow was controlled by a flow meter. The effluent was removed by a pump which operated periodically for 8 min, separated by 2-min intervals. Aeration pipes were placed under the membrane module to provide oxygen for the microorganisms and to generate a shear force which hindered membrane fouling.

Two MBR systems were used in the experiment, which operated in steady state. One was an eMBR without any activated sludge, in which the initial biomass concentrations (measured in colony-forming units (CFU)/ml) of the introduced bacteria were:

N. europaea 2.5×10^6, ammonia-oxidizing
N. winogradskyi 4.5×10^5, nitrite-oxidizing
B. licheniformis 8.3×10^4, protease-producing
B. megaterium 2.7×10^5, cellulase-producing
B. sphaericus 4.0×10^5, amylase-producing.

The other system was an activated sludge MBR with a seeded activated sludge concentration of 5–6 g/l. The numbers of protease-producing bacteria, cellulase-producing bacteria, amylase-producing bacteria, ammonia-oxidizing bacteria, and nitrite-oxidizing bacteria in the MBR were 1.6×10^5, 2.5×10^4, 3.6×10^5, 8.8×10^3, and 4.3×10^4 CFU/ml, respectively.

The duration of the experiment was 90 days, and no sludge was withdrawn from the MBR during its operation. The conditions of operation are summarized in Table 1.

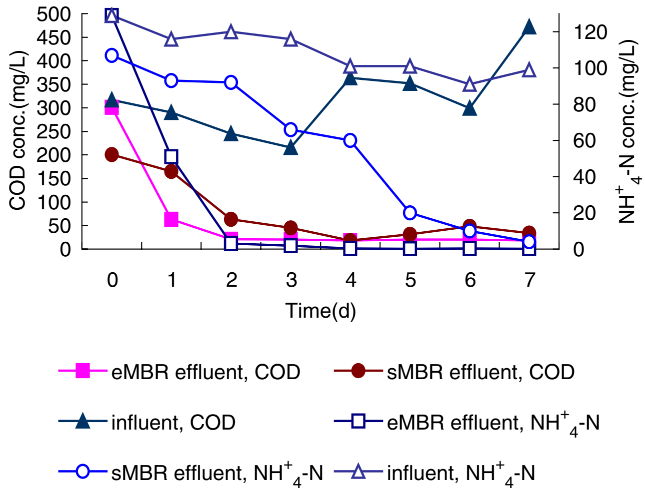


Fig. 2 Time-dependent variations of COD and NH_4^+–N in influent and effluent of the eMBR and the sMBR during the start-up

Characteristics of the domestic wastewater

The composition of domestic wastewater and effluent quality of the eMBR are presented in Table 2.

Analytical methods

Samples were collected at fixed intervals from the effluent and the storage tank. COD, NH_4^+–N, NO_2^-–N, NO_3^-–N, pH, turbidity, and temperature of the influent and effluent were measured regularly using standard methods (Clesceri et al. 1998).

Table 2 Influent and effluent characteristics of the MBRs

	Escherichia coli (CFU/l)	COD (mg/l)	NH_4^+–N (mg/l)	Turbidity (NTU)	Color (dilution times)	pH
Influent	10^7–10^8	230–580	80–130	40–80	60–80	7.1–8.5
Effluent of eMBR	0–3	29–49	0.1–1.5	<1	<30	6.5–7.5
Effluent of sMBR	0–3	18–46	2.1–7.6	<1	<30	6.5–7.5
Standard[a]	<3	<50	<10	<10	<30	6.5–9.0

[a]Water Quality Standard for Urban Miscellaneous Water Consumption (GB/T 18920-2002) (General Administration of Quality Supervision, Inspection and Quarantine of the People's Republic of China 2002)

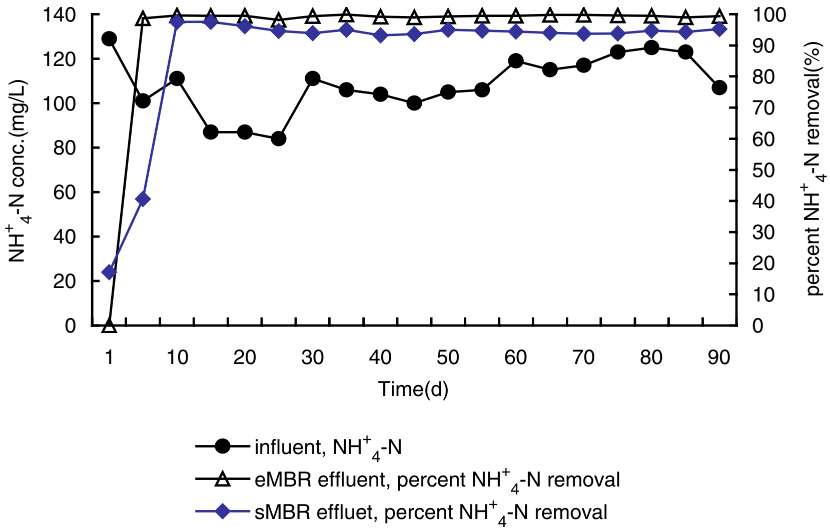


Fig. 3 Time-dependent variations of NH_4^+–N in influent and percentage of NH_4^+–N removal by the eMBR and the sMBR

The removal rates of COD and NH_4^+–N were calculated as follows:

$$\text{Removal rate of COD}(\%) = \left[(\text{CODin} - \text{CODeff})/\text{CODin}\right] \times 100$$

$$\text{Removal rate of } NH_4^+ - N(\%) = \left[(NH_4^+ - \text{Nin} - NH_4^+ - \text{Neff})/NH_4^+ - \text{N in}\right] \times 100.$$

Determination of biomass concentration in the eMBR

The chemo-autotrophic biomass concentrations of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria were determined using a selective liquid medium according to the most probable number (MPN) method (Chen and Zheng 1985). For the protease-producing bacteria, cellulase-producing bacteria, and amylase-producing bacteria, the dilution plate method and plate-screening method for the detection of responding bacteria were employed to count the CFUs of responding bacteria (Giraud et al. 1991; Hankin and Anagnostakis 1977; Teather and Wood 1982; Williams et al. 1990). The populations of heterotrophic bacteria and *Escherichia coli* were obtained by standard methods (Clesceri et al. 1998).

Results

Treatment effects of the eMBR compared with the activated sludge MBR

Start-up time of MBR

The removal efficiency of COD and NH_4^+–N by the eMBR and the activated sludge MBR during the start-up period is shown in Fig. 2.

The start-up time, i.e., the shortest time taken for the effluent quality to meet the Water Quality Standard for

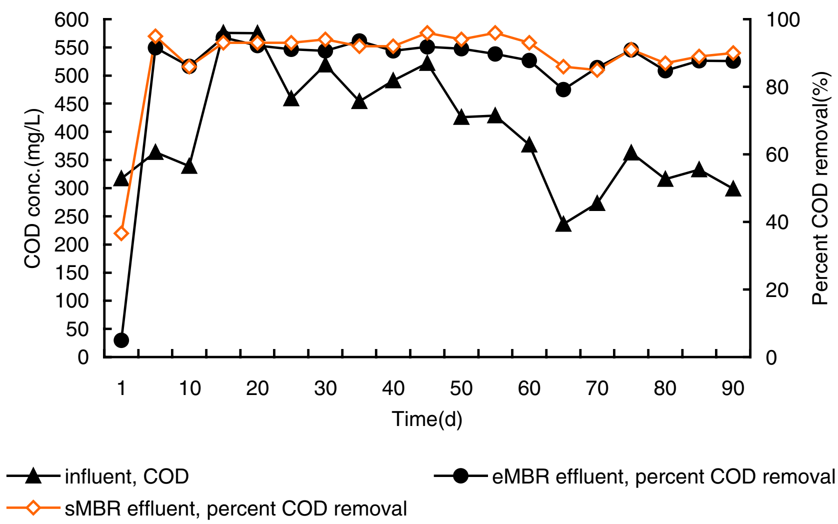


Fig. 4 Time-dependent variations of COD in influent and the percentage of COD removal by the eMBR and the sMBR

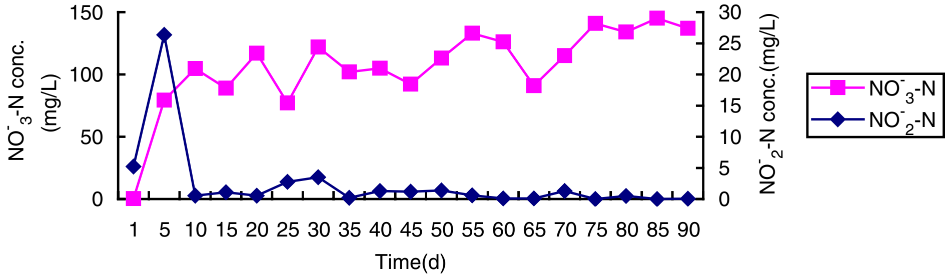


Fig. 5 Time-dependent variations of NO_2^-–N and NO_3^-–N in effluent of the eMBR

Urban Miscellaneous Water Consumption, was 24–48 h for the eMBR and 7–8 days for the activated sludge MBR.

Removal efficiency of COD and NH_4^+–N

The time-dependent variations of COD in the influent and the percentage removal of COD in the two systems during the experimental period are shown in Fig. 3. The same measurements and assessments for NH_4^+–N are shown in Fig. 4. The variations of NO_2^-–N and NO_3^-–N concentration in effluent of the eMBR are presented in Fig. 5.

The data shows that both systems can provide a consistent high efficiency of COD removal. Despite the fluctuations of influent COD concentration ranging from 230 to 580 mg/l, the effluent COD concentrations of the two systems were always lower than 50 mg/l. For the eMBR, the average effluent COD concentration was 40 mg/l, with the average efficiency of COD removal being 90.0%. For the activated sludge MBR, the average effluent COD concentration was 34 mg/l, and the average efficiency of COD removal was measured at 91.7%.

Our results also show that the eMBR had a much greater efficiency of NH_4^+–N removal compared to the activated sludge MBR. The average NH_4^+–N concentration in effluent was 0.66 mg/l, and the efficiency of NH_4^+–N removal was over 99% for the eMBR. This improved performance was observed irrespective of the variation of NH_4^+–N from 80 to 130 mg/l in the influent. For the activated sludge MBR, the average NH_4^+–N concentration in effluent was 5.7 mg/l, with the average efficiency of NH_4^+–N removal at 94.8%. With the combined function of ammonia-oxidizing bacteria, which have the metabolic ability to oxidize ammonia to nitrite aerobically, and nitrite-oxidizing bacteria, which have the metabolic ability to oxidize nitrite to nitrate aerobically, the NO_2^-–N, transformed from influent NH_4^+–N in the eMBR, was quickly oxidized to NO_3^-–N (in about 5 days), so there was no accumulation of NO_2^-–N (Fig. 5).

System effluent quality

The average concentration of pollutants in the effluent from the eMBR is shown in Table 2. The results indicate that effluent quality of the eMBR met or exceeded the requirements of the Water Quality Standard for Urban Miscellaneous Water Consumption.

Biomass concentration in sludge of the eMBR

The numbers of total heterotrophic bacteria, *E. coli*, ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, protease-producing bacteria, cellulase-producing bacteria, and amylase-producing bacteria in the eMBR were monitored at fixed intervals, as presented in Fig. 6.

In the initial stage of the experiment (days 1–7), the numbers of each of these types of bacteria in the eMBR were more than those in the activated sludge MBR in which the numbers of protease-producing bacteria, cellulase-producing bacteria, amylase-producing bacteria, ammonia-oxidizing bacteria, and nitrite-oxidizing bacteria were 10^3–10^5 CFU/ml. The numbers of each of these types of bacteria in the eMBR increased slightly due to the overall growth of bacteria populations; however, over the next 30

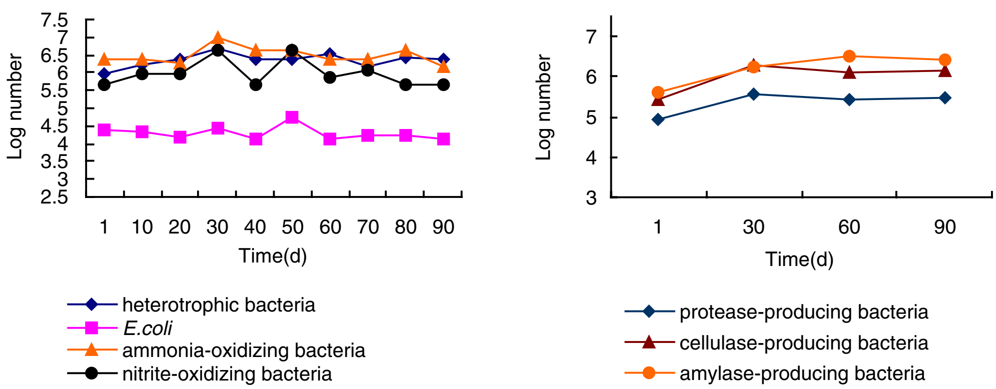
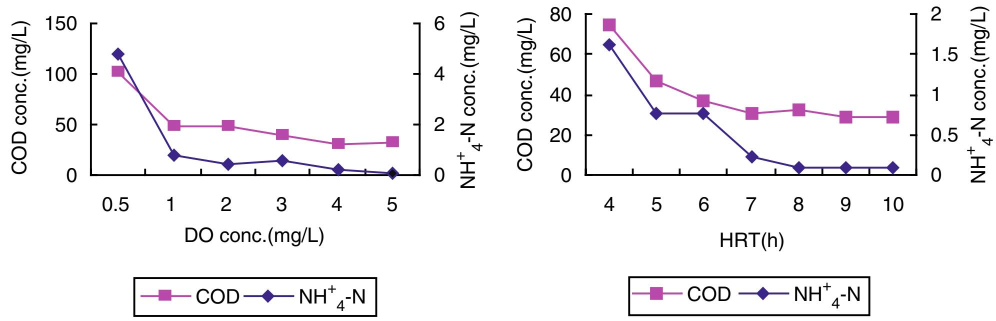


Fig. 6 The variations in the number of bacteria in the eMBR

Fig. 7 The influence of HRT and DO levels on COD and NH_4^+–N in the effluent from the eMBR



days, the number of total heterotrophic bacteria declined slightly, while the other responding bacteria populations fluctuated less. Colony characteristics of protease-producing bacteria, cellulase-producing bacteria, and amylase-producing bacteria grew to be more diverse. The total population of protease-producing bacteria, cellulase-producing bacteria, and amylase-producing bacteria was greater than the number of total heterotrophic bacteria.

These results demonstrate that the biomass concentration cannot be increased beyond a certain limit, and that different kinds of bacteria will establish an ecological balance in the reactor during operation of the system.

Effect of operation characteristics on the effluent quality from the eMBR

The influence of HRT and DO level on effluent quality of the eMBR is shown in Fig. 7.

These data indicate that both the HRT and the DO had some impact on the pollutant-degrading process, although there was more apparent influence on COD removal than NH_4^+–N removal. A DO concentration equal to or more than 1.0 mg/l or an HRT equal to or more than 5 h resulted in a COD concentration below 50 mg/l in the effluent. Under less optimal conditions of these two variables, the concentration of COD exceeded the minimum requirement of the standard. Furthermore, unfavorable conditions would lead to a quick decline in flow across the membrane because of fouling at the external membrane surface. As HRT and DO concentrations were increased, the effluent quality improved progressively. However, HRT above 7 h or DO concentration above 4 mg/l produced no further improvement in effluent quality. Therefore, to obtain the best and most consistent effluent quality, it is necessary for the bacteria to obtain adequate oxygen for growth and sufficient contact time with the pollutants to achieve optimal degradation.

Discussion

The MBR process offers several benefits over the conventional activated sludge process, including smaller space and reactor requirements, better solids removal, disinfection, increased volumetric loading, reduced sludge production, system reliability throughout hydraulic and solids load variations, potential reduction in capital expenditures, and potential reduction in energy requirements (Chiemchaisri et al. 1992; Cicek et al. 1998; Muller et al. 1995). MBR systems were initially used for municipal wastewater treatment, primarily in the area of water reuse and recycling. By the mid 1990s, the development of less expensive submerged membranes made MBRs a real alternative for high-flow, large-scale municipal wastewater applications. Over 1,000 MBRs are currently in operation around the world, largely in Japan, Europe, and North America (Van de Roest et al. 2002). In China, serious interest in this technology for treating domestic wastewater has only occurred recently because of the approval of new water reclamation projects and further advancements in membrane technology, yielding more favorable process economics.

The disadvantages associated with the MBR are mainly the slow start-up, a low efficiency of NH_4^+–N removal, unreliable effluent quality, and membrane fouling problem (Cicek 2003; Liu et al. 1999).

Lately, researchers have been noting the advantages of decentralized treatment systems over centralized ones in achieving water treatment sustainability, especially in developing countries (Bakir 2001; Sundaravadivel and Vigneswaran 2001). The perceived benefits include less need for major infrastructure development and/or maintenance, potentially lower costs, less discharge to receiving waters, and more opportunities for water reuse because the reclaimed water is locally available, and the pathogen risk is lower (Olson 2004; DiGiano et al. 2004). However, procurement of activated sludge becomes more difficult because of the absence of large wastewater treatment facilities in small towns. The preparation of effective activated sludge is another problem which limits the application of MBRs.

The concept of effective microorganisms (EM) was developed by Professor Teruo Higa of the University of the Ryukyus, Okinawa, Japan (Higa 1991; Higa and Wididana 1991). The EM consists of mixed cultures of beneficial naturally occurring microorganisms that can be applied as inoculants to increase the microbial diversity of soils and plants. The inoculation of EM cultures into the soil/plant ecosystem can improve soil quality, soil health, and the growth, yield, and quality of crops. This EM contains selected species of microorganisms, including predominant populations of lactic acid bacteria and yeasts and smaller numbers of photosynthetic bacteria, *actinomycetes*, and other types of organisms. All of these are mutually compatible with one another and can coexist in liquid culture.

We expected that the combination of effective organisms and MBR would resolve some of the problems in the application of standard MBR practices. Based on the composition of domestic sewage, we selected environmental bacterial strains which oxidize ammonia and nitrites and produce protease, amylase, and cellulase for the development of a novel biologically enhanced MBR. In fact, our results showed that not only did the eMBR yield a much shorter time to start-up, but also, effluent qualities met the standard and were stable during the entire 90 days of this study. Compared with the traditional MBR, the bacteria which degrade COD and NH_4^+–N become the predominant populations after addition to the eMBR. These bacteria are particularly advantageous for their potential to degrade organic matter in wastewater. In activated sludge, heterotrophic bacteria are considered to be more dominant organisms compared to nitrifying bacteria. Nitrifying bacteria are chemo-autotrophic bacteria which grow much slower under various environmental conditions and usually must be cultured and enriched for a long time to get adequately high concentrations. Therefore, the initial start-up for a traditional MBR is slow, and the removal rate of NH_4^+–N is also lower than in the eMBR. In the eMBR, the nitrifying bacteria were directly introduced along with a high concentration of COD-degrading bacteria, producing the rapid start-up and higher removal rate of NH_4^+–N compared to the standard MBR.

Finally, the effective organisms can be prepared as a solid product similar to EM. The solid products are convenient to use because of the ease of transport. Thus, the eMBR system, as developed in our laboratory, fulfills the need for decentralized wastewater treatment and reclamation of water for diverse purposes.

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