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Coliform Removal in Membrane Bioreactor and Disinfection during Hospital Wastewater Treatment

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Highlights:

- MBR treated hospital wastewater effectively at an HRT of 6 hours.
- High coliform and *E. coli* removals (6 log) were observed in MBR.
- Effective disinfection of solid-free MBR effluent was attained.
- Complete removal of *E. coli* was achieved after chlorination of MBR effluent at 2 mg/L and 15 min contact time.
- Ozonation of MBR effluent required only 1 mg/L for completion of *E. coli* removal.

Abstract. This study investigated coliform removal from hospital wastewater in a membrane bioreactor (MBR) and disinfection using either chlorine or ozone. A laboratory-scale MBR equipped with a hollow-fiber membrane module was operated with hydraulic retention times varied at 3 and 6 hours. The disinfection of MBR effluent was carried out using either chlorine or ozone with concentrations varied between 1 and 10 mg/l and contact time varied between 1 and 30 min. During 150 days of MBR operation, organic removal efficiencies averaged 96.5% and 97.7% for BOD and 73.6% and 84.1% during its operation at an HRT of 3 and 6 hours respectively. Simultaneously, 6.7 and 6.4 log reduction of total coliforms and 6.2 and 6.1 log reduction of *E. coli* were achieved under these respective HRT conditions. The use of chlorine for disinfection of MBR effluent could eliminate total coliforms and *E. coli* completely at >6 and 3 mg/L at 30 min contact time, whereas only >3 and 1 mg/L was required for ozone at the same contact time. There was a significant improvement of disinfection efficacy of solid-free MBR effluent compared to that of activated sludge effluent.

Keywords: *chlorination; coliform; E. coli; hospital wastewater; MBR; ozonation.*

1 Introduction

Hospital wastewater is a potential source of hazard contamination of urban water environments. According to Khan, *et al.* [1], hospital effluent should be considered as a breeding ground for pathogenic bacteria, but many countries do not have strict regulations regarding the disposal of hospital effluent. Over the past few decades, health care services have been improving significantly, in

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Asian countries like China, yielding a huge increase in hospital wastewater generation and related treatment requirements, as described in Lui, *et al.* [2]. Generally, hospital wastewater is handled by conventional wastewater treatment processes, which are designed for the removal of organic pollutants and suspended solids but not for pathogens. Hendricks and Pool [3] found that the processes used in sewage treatment could not effectively prevent the discharge of bacteria and antibiotic residues into the environment. In order to overcome these problems, integration of membrane filtration with biological wastewater treatment processes has been successfully demonstrated in enhancing organic, suspended solids and indicator bacteria removals, as described in Arrojo, *et al.* [4]. The development of direct solid-liquid separation using a hollow-fiber membrane inside an activated sludge reactor, as reported in Chiemchaisri, *et al.* [5], allowed simultaneous organic, nitrogen and microorganism removals in a membrane bioreactor (MBR). A beneficial feature of using an MBR for hospital wastewater treatment is its superior performance in retaining most suspended solids, including pathogenic microorganisms contaminated in hospital wastewater, thus significantly reducing the microbial risk of pathogens in the treated water. The use of membrane separation instead of gravity sedimentation also enables the MBR to operate under long sludge age condition regardless of sludge settleability. Thus, excess sludge removal was not performed in this study to eliminate the effect of coliform bacteria reduction with excess sludge. This unique operation would also help to minimize health risks arising from exposure to pathogens during sludge handling and its disposal. The application of MBRs in hospital wastewater treatment has increased recently in China [2], however, their use is still limited in other countries. Hai, *et al.* [6] explained that the biomass processes in an MBR, including spontaneous decay, aggregation/biosorption and biodegradation, are mainly responsible for pathogen removal, but membrane rejection is an essential supplement of the biomass processes.

High removals of indicator organisms were achieved in an MBR equipped with a microfiltration membrane (MF), yielding almost bacterial-free effluent qualities for reuse in urban and agriculture settings [7,8]. Nevertheless, regardless of the high-quality effluent achieved from an MBR, a post-disinfection process is normally required to ensure the safety of its reuse [6]. For disinfection of hospital effluent, several disinfectants can be used, such as chlorine, ozone, chlorine dioxide and ultraviolet irradiation [9]. Among them, chlorine is the most cost-effective option [10], whereas ozone is considered a more powerful disinfectant for pathogen inactivation [11]. Based on the aforementioned information, application of an MBR and disinfection using chlorine or ozone would be highly effective for pathogen control in hospital wastewater treatment. Several previous studies to evaluate pathogen removal in an MBR and disinfection treatments were performed using simulated wastewater [4,12,13] to ensure that conclusive results were obtained. However, information regarding the efficacy and appropriate operating conditions for microbial control when applied to real hospital wastewater is still very limited. Therefore, the present study was conducted for the removal of indicator bacteria, i.e., total coliforms and *E. coli*, during treatment of real hospital wastewater using an MBR and disinfection units.

2 Materials and Methods

2.1 MBR Experiment

A laboratory-scale MBR (Figure 1) made of acrylic with a working volume of 5.25 L was equipped with hollow-fiber membrane modules (Mitsubishi, PVDF material, 0.4 μm nominal pore size, 0.315 m^2 total surface area) for solid-liquid separation. The membrane filtration in the MBR was operated under a constant permeate suction rate using a peristaltic pump (Masterflex, USA), while the transmembrane pressure was measured using a vacuum gauge (TASCO, Thailand) and allowed to increase to a maximum limit of 40 kPa, at which membrane cleaning would be required. Aeration was continuously supplied using an air blower at 10 L/min flow rate to maintain dissolved oxygen (DO) of 4 mg/L or more in the MBR.

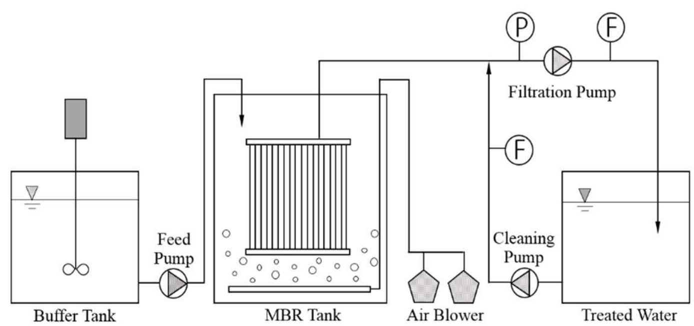

Figure 1 Schematic of the laboratory-scale MBR.

Real wastewater collected from all activities, including healthcare facility, restrooms, laundry and food services at a public general hospital with a capacity of 480 beds, located in the eastern part of Thailand, was fed to the reactor at the same rate as the permeate flow through a membrane of 42 and 21 L/d, yielding a hydraulic retention time (HRT) of 3 and 6 hours in the MBR from day 1 to day 77 (Run 1) and from day 78 to day 147 (Run 2) of the operation. Under these operating conditions, the average membrane permeate fluxes in the MBR were 5.6 and 2.8 $L/m^2/h$, respectively. During the whole operation period, membrane backwashing was not performed to avoid its impact on the microbial sludge in the MBR. The initial seed sludge was obtained from an activated sludge process at the wastewater source, with mixed liquor suspended solids (MLSS) in the MBR initially set at 10 g/L at the beginning of each run. The average mixed liquor volatile suspended solids (MLVSS) to MLSS ratios of the MBR sludge were 0.82 and 0.84 during Run 1 and Run 2, respectively. Along the operation period, no sludge wastage was performed except for the purpose of sampling (100 mL on a weekly basis). Biomass (MLSS, MLVSS) concentrations were analyzed weekly.

2.2 Analytical Methods

The treatment performance of the MBR was evaluated by analyzing the influent and effluent characteristics on a weekly basis. The analytical parameters were: pH, electrical conductivity (EC), suspended solids (SS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), total kjeldahl nitrogen (TKN), nitrite (NO_2^-), and nitrate (NO_3^-). All analyses, including those measured in mixed liquor (DO, MLSS, MLVSS), were performed following the procedures described in the Standard Methods for the Examination of Water and Wastewater [14] using pH/EC and DO portable meters (Hanna Instruments, USA). To determine total coliform and *E. coli* removal efficacy, their concentrations in the influent and effluent samples were analyzed weekly using membrane filtration and Chromocult Coliform Agar (CCA) following the standard procedures described in the Standard Methods [14,15] with some modifications.

The bacteria in water sample of known volume were initially retained on a 0.45 μm membrane filter, after which the filter was placed on a CCA agar plate. The plate was then incubated at 36 ± 0.5 °C for 24 h. The total and blue color bacterial colonies grown on the plate were counted and determined as total coliforms and *E. coli* respectively. Quality control and assurance of sampling was performed using blank (pure water) samples as control under the same sampling procedure; no total coliforms and *E. coli* were detected in the blank samples.

The pollutant removal efficiencies under two experimental runs were compared for statistical significance of the mean difference using the t-test. The test results showed that the difference was significant at $p < 0.05$. Microsoft Excel version 2010 was used for the statistical analysis of experimental data.

2.3 Disinfection Experiment

The disinfection efficacy in terms of total coliform and *E. coli* reduction was examined using chlorine and ozone as disinfectants for the MBR effluent. The effluent samples from the MBR experiment were kept at room temperature (25 to 30 °C) for less than 24 h, during which natural enumeration of total coliforms and *E. coli* took place prior to their use in the disinfection experiment. Sodium hypochlorite (NaOCl) stock solution was used for the chlorination experiment, while ozone was supplied from an ozone generator (Cocoozone, CCba15, 0.18kW) through a diffuser into 1000 mL flasks containing the effluent samples. To enable direct comparison of disinfection efficacy with that currently employed at the hospital, i.e., activated sludge (AS) processing with post-chlorination (2 mg/L dosage, 30 min contact time), both effluent samples from the MBR and AS processes were tested in the disinfection experiments.

In the first set of experiments, either chlorine or ozone was supplied at various doses (C) of 0, 1, 2, 3, 4, 6 and 10 mg/L and their disinfection efficacy was examined at a fixed contact time of 30 min. Subsequent experiments were done at different contact times (T) of 0, 1, 2, 3, 5, 10, 20, 30, 40, 60 min using a fixed dose of 2 mg/L of either chlorine or ozone. The CT indicators for chlorine and ozone, similarly used to evaluate disinfectant efficacy in Pak, *et al.* [12], yielding complete removal of *E. coli* in MBR and AS effluent samples, were compared to evaluate the disinfection efficacy in the respective samples.

3 Results and Discussion

3.1 Treatment Performance of MBR

Table 1 shows the water qualities and pollutant removal efficiencies in the MBR operated under different HRTs. At an HRT of 3 hours (Run 1), organic removal in terms of BOD and COD averaged at 96.5% and 73.6%, respectively, whereas relatively low nitrogen removals were achieved at only 34.2% due to a limited nitrification reaction, as the majority of residual nitrogen in the effluent was found in TKN form. It was anticipated that oxygen transfer for nitrifying microorganisms in the mixed liquor sludge might be limited during an increase of biomass concentration from 10 g/L to 18 g/L during operation (Figure 2).

Prasertkulsak, *et al.* [16] also reported inhibition of nitrification in an MBR treating hospital wastewater when the MLSS concentrations increased beyond 13 g/L due to limited oxygen supply, as the DO concentration available for microbial activities was reduced to 1 mg/L. Nevertheless, the removal of BOD and COD remained relatively stable, producing good effluent qualities regardless of the oxygen supply conditions in the MBR.

When HRT was adjusted to 6 hours (Run 2), the biomass concentrations in the MBR could be kept relatively constant at 10 g/L while achieving high organic removals of 97.7% for BOD and 84.1% for COD. There was also significant improvement of TKN removal, up to 93.5%, from better oxygen transfer facilitation at this operating condition. The residual nitrogen in the effluent was mainly in nitrate form, as denitrification was limited in the MBR operated under continuous aeration mode.

Table 1 Water qualities and pollutant removal efficiencies in the MBR.

Parameter	Run 1 (HRT 3 h, day 1-77)			Run 2 (HRT 6 h, day 78-147)		
	Influent	Effluent	Removal (%)	Influent	Effluent	Removal (%)
pH (-)	6.8±0.2	7.0±0.1	-	7.1±0.3	7.0±0.2	-
EC (mS/cm)	1.9±0.5	1.8±0.6	-	1.9±0.5	1.4±0.4	-
SS (mg/L)	81±31	ND	100	90±41	ND	100
BOD (mg/L)	83±14	3.1±3.9	96.5±3.6	78±24	1.7±1.3	97.7±2.1
COD (mg/L)	148±32	38±10	73.6±6.3*	133±17	20±6	84.1±5.8 *
TKN (mg/L)	36±4	24±11	34.2±24.5*	30±3	2.0±0.9	93.5±3.0 *
NO_2^- (mg/L)	0.4±0.3	0.1±0.4	-	0.5±0.1	2.0±1.4	-
NO_3^- (mg/L)	0.4±0.5	2.4±2.0	-	0.2±0.2	17±2	

Remark: average ± standard deviation (SD), sample number (N) = 11 for each run, ND: not detected, * denotes a significant difference between runs ($p < 0.05$)

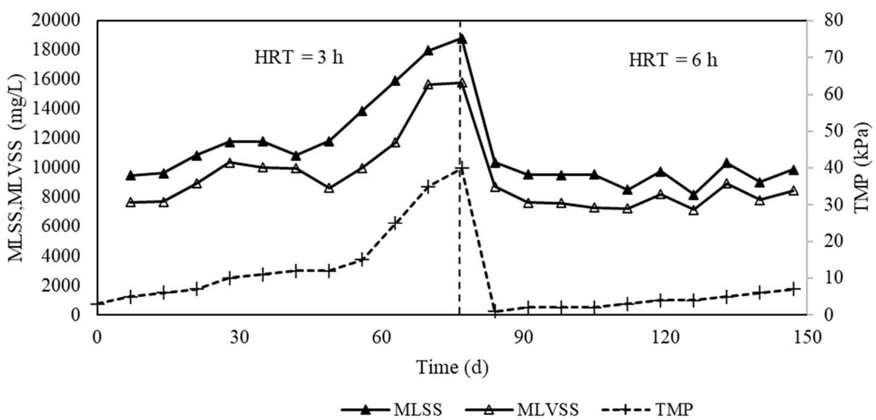

Figure 2 Variations of mixed liquor suspended solids and transmembrane pressure in the MBR.

The extension of HRT from 3 to 6 hours also helped improving the membrane filtration condition in the MBR. At an HRT of 3 hours, transmembrane pressure (TMP) was gradually increased to 40 kPa, at which membrane cleaning is required after 2.5 months of reactor operation. This TMP increase due to particulate fouling was accelerated by an increase in MLSS, as solid cake attachment on the membrane surface was clearly observed. When the MBR was re-operated at an HRT of 6 hours, the TMP was limited to less than 10 kPa during the same operation period when the MLSS was maintained at about 10 g/L. Based on this MBR performance, it was revealed that an HRT of 6 hours is the most appropriate condition for this MBR operation.

3.2 Coliform Removal in MBR

Table 2 shows a comparison of total coliform and *E. coli* removals in the MBR operated at different HRTs. While the influent contained total coliforms and *E. coli* ranging from 2.5 x 10^7 to 1.6 x 10^9 and from 1.6 x 10^6 to 6.3 x 10^7 CFU/L, they were reduced by the MBR treatment by 6.7 and 6.2 log at an HRT of 3 hours and by 6.4 and 6.1 log at an HRT of 6 hours. Their removals stayed relatively stable during the operation. The results suggest that HRT maintained in the MBR as well as membrane fouling did not significantly affect total coliform removal and the effectiveness of *E. coli* removal in the MBR was found proportional to that of total coliforms.

Table 2 Total coliform and *E. coli* removals in the MBR.

Samples	Total coliform (log CFU/L)		E. coli (log CFU/L)	
	Range	Average ± SD	Range	Average ± SD
Run 1: HRT 3 h				
Influent	8.2-9.2	8.9±0.4	6.3-7.8	7.2±0.6
Effluent	1.8-2.8	2.2±0.4	0.3-1.8	1.0±0.6
Removal	5.7-7.3	6.7±0.7	4.9-7.4	6.2±1.0
Run 2: HRT 6 h				
Influent	7.4-9.0	8.3±0.6	6.2-7.7	6.9±0.7
Effluent	1.8-2.1	1.9±0.1	0.4-1.1	0.8±0.3
Removal	5.3-7.2	6.4±0.7	5.2-6.7	6.2±0.8

Remark: sample number (N) = 5 for each HRT, there was no statistical difference between total coliform and *E. coli* removal between runs ($p > 0.05$)

Alaboud [17] found that the coliform removal in an MBR was not influenced by HRT or MLSS concentrations. It was reported that 5 to 6 log removal of total coliforms, which is comparable to that observed in this study, but lower removal of fecal coliforms (2.1 to 3.1 log) was achieved, even though an ultrafiltration membrane (0.04 μm) was used. Zhang and Farahbakhsh [18] also reported better coliform removal in an MBR than conventional AS with tertiary treatment by more than 1 log unit. After the MBR treatment, there were still 60 to 600 CFU/L of total coliforms and 1 to 64 CFU/L of *E. coli* contained in the effluent despite using a smaller membrane pore size (0.04 and 0.1 μm) than that of this study (0.4 μm). One of the possible reasons for the higher microbial penetration observed in the previous study could be due to its higher membrane flux employed (34 L/m^2/h). Tsutsui and Urase [19] reported the passage of bacteria through microfiltration when membranes with 0.4 μm or larger pore size were used.

3.3 Coliform Removal during Disinfection

Figure 3 shows the reduction of total coliforms and *E. coli* during disinfection experiments using AS and MBR effluents. The examined AS effluent contained total coliforms and *E. coli* ranging from 3.3 x 10^5 to 3.4 x 10^5 and from 5.4 x10^4

to 6.8 x 10^4 CFU/L, while the MBR effluent contained 3.4 x 10^3 to 5.0 x 10^3 and 2.1 x 10^2 to 1.4 x 10^3 CFU/L, respectively. When chlorine and ozone doses were varied at a contact time of 30 min, total coliforms in the AS effluent were much reduced when subjected to the presence of both disinfectants but only ozone at a high concentration of 10 mg/L could eliminate them. Meanwhile, *E. coli* could be completely eliminated by ozone at a 4 mg/L dose. The absence of solid particles in the MBR effluent yielded higher efficiencies of disinfection. Complete removals of total coliforms and *E. coli* could be achieved at 6 to 10 and 2 to 3 mg/L of chlorine, while it required only 3 to 4 and <1 mg/L of ozone to achieve the same results. Apparently, ozone was more effective than chlorine for coliform removal, especially when solid particles were absent from the samples.

When contact time was varied at a constant disinfectant dose of 2 mg/L, it was found that chlorine could not completely eliminate total coliforms and *E. coli* in the AS effluent, even after 1 hour of contact time. It was also noticed that chlorine became ineffective after 30 min of contact time, as the cell concentrations remained relatively unchanged afterwards. For the MBR effluent, total coliforms remained after 60 min, but *E. coli* was completely eliminated at 10 to 20 min contact time. The use of ozone was more effective for both effluent samples. Both total coliforms and *E. coli* disappeared after exposure to ozone of 2 mg/L at 3 min contact time. Hazaa, *et al.* [20] reported an optimum ozone dosage of 2.5 mg/L to achieve a 90% reduction of indicator microorganisms. They reported an optimum contact time of 8 min for heterotrophic bacteria, total coliforms, fecal coliforms, and *Streptococcus faecalis* contained in raw water of a drinking water treatment facility, while complete removal could be achieved with a filter.

From the above results, it was revealed that the disinfection efficacy of coliform bacteria was highest for MBR effluent exposed to ozone. For complete *E. coli* elimination, the use of ozone at 1 mg/L and 30 min contact time or 2 mg/L and 3 min contact time would be required. Therefore, a CT value of 30 mg.min/L is proposed for effective disinfection in this case. Nevertheless, the use of chlorine could also achieve the same results but requires a high dose of 2 to 3 mg/L at 30 min contact time or 2 mg/L at 10 to 20 min contact time, but it would demand a higher CT value of 40 to 60 mg.min/L.

The difference in CT value required for both disinfectants mainly comes from their different disinfection power and the time required to form the desire disinfectant product in the water. Oh, *et al.* [13] reported a much lower ozone (3 mg/L) than chlorine (30 mg/L) concentration to achieve 90% removal of antibiotic resistant bacteria. Meanwhile, the time required for reaching a nominal concentration of chlorine in water could be up to 35 to 40 min when sodium hypochlorite was used as disinfectant [10].

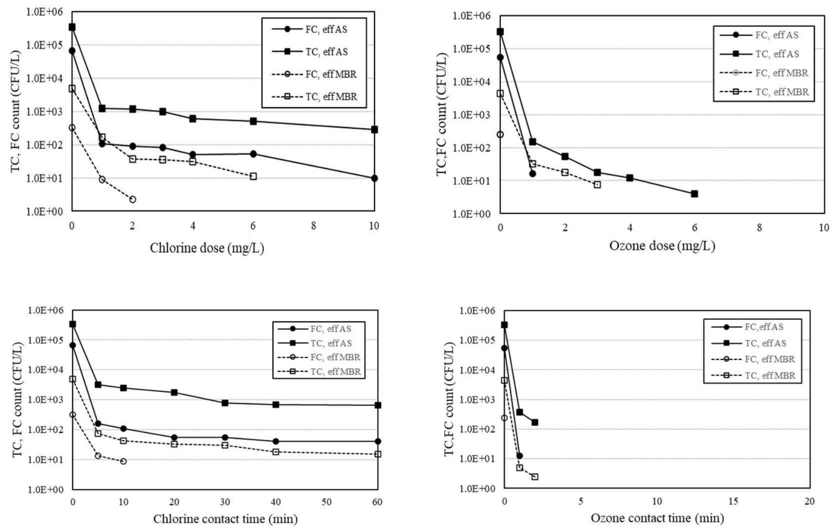

Figure 3 Variation of total coliform and *E. coli* counts during disinfection experiments (upper left and right: varied chlorine and ozone dose at 30 min contact time, lower left and right: varied contact time at chlorine and ozone dose of 2 mg/L, discontinuation of a line means no detection in subsequent dose or contact time).

4 Conclusion

Effective coliform removal from hospital wastewater was achieved during its treatment in an MBR and disinfection units. The majority of total coliform and *E. coli* up to 6 log reduction took place in the MBR. In order to completely eliminate *E. coli*, disinfection of the MBR effluent using ozone at a CT value of 30 mg.min/L was required. The use of typical chlorination could also achieve the same results, but a higher CT value of 40 to 60 mg.min/L is then recommended. Higher disinfection efficacy in solid-free MBR effluent than that of activated sludge effluent was observed.

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