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An innovative membrane bioreactor (MBR) system for simultaneous nitrogen and phosphorus removal

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1   **Abstract**

2   Membrane filtration was integrated with a post-denitrification process to form an innovative

3   membrane bioreactor (MBR) system for effective organic degradation and nutrient (N and P)

4   removal. The system comprised of an aerobic tank, an anoxic tank, an intermediate

5   sedimentation tank, and a membrane filtration tank. The sedimentation tank functioned not

6   only as a rough settler for sludge-water separation before membrane filtration, but also as an

7   anaerobic chamber for P release. While half of the influent flowed into the aerobic tank, the

8   other half was fed into the anoxic tank to favor the proliferation of phosphorus accumulating

9   organisms (PAOs). The experiment was conducted continuously for about 430 days. With a

10 short overall treatment time of less than 10 hrs for municipal wastewater, the MBR-based

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process could achieve the total organic carbon, total nitrogen, and total phosphorus removals of around 94%, 85%, and 87%, respectively. The growth and activity of PAOs in the MBR system were evidenced by the significant P release in the anaerobic chamber followed by the luxury P uptake in the membrane tank. With the DAPI and PAO$_{mix}$ probe staining, the increases of PAOs and polyhydroxybutyrate (PHB) in sludge during the experiment were well observed under the fluorescent microscope.

1. Introduction

Eutrophication has been recognized as one of the most serious water pollution problems. Wastewater discharge brings nutrients, both nitrogen (N) and phosphorus (P), into the receiving water, causing nutrient enrichment and algal blooms. For effective water environment protection, a number of nutrient removal processes have been developed for N and/or P removal from wastewaters [1]. Biological nitrogen removal involves the combined nitrification by autotrophic bacteria under aerobic conditions and denitrification by heterotrophic bacteria under anoxic conditions, while enhanced biological phosphorus removal (EBPR) is achieved through P luxury uptake by phosphorus accumulating organisms (PAOs) together with sludge discharge [2]. Biological N removal can be achieved by either pre-denitrification or post-denitrification [3]. Compared to the pre-denitrification process, post-denitrification would allow complete N removal [3], although the external carbon source usually needs to be added into the anoxic reactor. More recently, it was reported that simultaneous N and P removal could be achieved by placing an anaerobic tank before the

aerobic tank (i.e. post-anoxic type), for which external carbon sources might not be required [4]. Although P removal was primarily driven by stored polyhydroxybutyrate (PHB) and other organic substances, it was apparent that a portion of PAOs was able to utilize nitrate as the electron acceptor [4].

Membrane bioreactor (MBR) is an attractive process that has been increasingly used for biological wastewater treatment. With membrane filtration replacing the conventional clarifier, MBR possesses a number of merits such as biomass enrichment, small footprint, ensured sludge-effluent separation, easy manipulation of the hydraulic and sludge retention times (HRT and SRT) and, most importantly, excellent effluent quality with little organic and solid contents [5, 6]. However, an MBR tank without anaerobic and anoxic variations cannot be simply used for nutrient removal in wastewater treatment. There is a need to develop innovative MBR systems that incorporate membrane separation into the biological treatment process for N and P removals.

In comparison to organic degradation, biological nutrient removal is more difficult to achieve with the MBR, and P is the most difficult one to remove [1, 7, 8]. Incorporation of membrane filtration into various processes for simultaneous N and P removal has been attempted in the past decade [7, 8]. The system variations reported include sequencing batch reactors [10, 11], the continually-operated pre-oxic system [8, 9, 12, 13], and post-oxic denitrification type [4, 14]. For large-scale applications, the MBR has been used conventionally to replace the final clarifiers for solids-effluent separation in common nutrient removal activated sludge processes (e.g., the anaerobic-anoxic-oxic (A/A/O) process) (Table 1). These large-scale MBR systems often adopt a long treatment time or a low filtration rate for wastewater treatment and nutrient removal. Besides, although nutrient removal can be achieved in these systems, it has been found that denitrification sometimes may complicate

3

60 the EBPR as denitrifiers compete with PAOs for the limited carbon source and hence affect

61 the stability of the system performance [14].

62 Similar to any membrane separation process, membrane fouling in MBR is inevitable. A

63 number of studies has suggested that the membrane fouling rate in MBR correlates well with

64 the sludge concentration [5, 6]. Thus, lowering the sludge concentration in the membrane

65 chamber would help mitigate the fouling problem to some extent. However, the settleability

66 of sludge has not been fully utilized for regulating the sludge concentration before membrane

67 filtration in the MBR-based nutrient removal systems [10, 18]. Moreover, when membrane is

68 used to retrofit or upgrade existing biological wastewater treatment systems, some of the

69 secondary clarifiers would often become obsolete.

70 In the present study, we developed a new system to integrate membrane filtration with

71 the post-denitrification process for simultaneous N and P removal. The membrane chamber

72 was connected to an intermediate sludge settler. As such, the sedimentation tank not only was

73 used as a rough settler for sludge-water separation before membrane filtration but also

74 provided an anaerobic zone to allow the proliferation and function of PAOs for P removal

75 purpose. In addition to the development of an innovative and effective MBR wastewater

76 treatment system for simultaneous N and P removal, PAO abundance and the P concentration

77 profile throughout the system were characterized, and the long-term performance of the

78 system was evaluated.

79

80 **2. Materials and Methods**

81 *2.1   Experimental set-up*

82 An MBR wastewater treatment system (Fig. 1) was developed and operated for the

83 experimental study on the simultaneous removals of organic pollutants, nitrogen, and

84 phosphorous. The system was a modification of the post-denitrification process that

4

85 comprised of an aerobic tank, an anoxic tank, an intermediate sludge settler, and a membrane

86 filtration tank. In addition to biomass concentration, the rough sludge settler also provided an

87 anaerobic zone for P release. In the MBR tank, a polyethylene hollow-fiber membrane

88 module (0.4 μm pore size, 0.075 m^2 effective area, Mitsubishi Rayon) was immersed. The

89 working volumes of the aerobic, anoxic, settling, and membrane tanks were 2, 1.4, 1.5, and

90 1.6 L, respectively. Aeration was provided through fine air diffusers from the bottom in the

91 aerobic tank and the membrane filtration tank, while sludge in the anoxic tank was suspended

92 by a paddle mixer at 30 rpm. Half of the feed wastewater was pumped into the aerobic tank

93 and the other half into the anoxic tank. The sludge recirculation ratios from the settling tank

94 to the aerobic tank and from the membrane tank to the anoxic tank ranged from 300 to 400%

95 (Table 2).

96   The feed consisted of 90% synthetic wastewater prepared according to a classic recipe

97 [19] for typical municipal wastewater and 10% actual domestic sewage collected from a local

98 municipal wastewater treatment plant (Stanley Sewage Treatment Plant, Hong Kong). The

99 raw sewage was expected to supply trace elements for the biomass growth. The carbon source

100 in the synthetic wastewater was a mixture of 90% NaAc and 10% glucose, and the N and P

101 sources in the feed were supplied with NH_4Cl and a mixture of KH_2PO_4 and NaH_2PO_4,

102 respectively. The variations of the wastewater influent in chemical oxygen demand (COD)

103 and COD:N:P ratio are summarized in Table 2. $NaHCO_3$ was added to the feed at 50 mg/L or

104 higher to keep the pH between 6.5 and 7.5. Effluent was withdrawn through the membrane by

105 a suction pump (MasterFLEX, Cole-Parmer) that was set off for 2 min (for membrane

106 relaxation) every 10 min. Membrane fouling was indicated by the trans-membrane pressure

107 (TMP) increase, which was monitored with a manometer in mm Hg. The TMP increased

108 gradually with time from an initial value of about 5 mm Hg (0.67 kPa) to around 600 mm Hg

109 (80 kPa) and then, the fouled membrane was washed thoroughly with running tap water to

5

110   restore its permeability [20].

111

112   *2.2   MBR wastewater treatment experiment*

113   The wastewater treatment experiment was conducted in four phases in the laboratory at

114   room temperature (~25 ^{o}C) (Table 2). A short initial phase, termed as Phase 0, was recorded

115   for the original performance of the system in terms of organic and nutrient removals.

116   Afterwards, the system was operated and optimized for more than 400 days for enhanced

117   biological P removal together with N removal. The operation could be divided into three

118   phases according to the P remvoal performance in relation to the different COD:N:P ratios

119   and/or recirculation ratios (Table 2). From Phase I to Phase II, the P removal efficiency

120   improved significantly. Phase III was operated for a long period to demonstrate the stable

121   operation of the system and its nutrient removal performance. In addition, the organic content

122   or the COD:TN:TP ratio decreased in the influent in Phase III compared to Phase II to

123   increase the difficulty of biological nutrient removals. During this phase, the recirculation

124   ratio was increased as an adjustment to maintain a high nutrient removal efficiency (Table 2).

125   The treatment performance was evaluated in terms of the removal efficiencies of the total

126   organic carbon (TOC), total N (TN), and total P (TP) as well as the P concentration profile in

127   the liquid phase of the sludge suspensions through different tanks.

128

129   *2.3   Microscopic examination of PAOs*

130   The composition and spatial distribution of the microbial community of the sludge in

131   relation to PAO-based P removal were examined under a fluorescent microscope after

132   staining. DAPI (4′,6′-diamidino-2-phenylindole) staining on all cells was performed

133   following the method described by Kawaharasaki et al [21] using a filtered DAPI solution (10

134   mg/mL in 25 mM Tris-HCl buffered saline, pH 7.0). For a sludge sample taken from the

6

aerobic tank, it was homogenized briefly using a vortex mixer (Maxi Mix II, Thermolyne) to break up large flocs. The dispersed biomass was then air-dried on a slide and stained with the DAPI solution. After 10 min of staining, the slide was washed using a phosphate buffer saline (PBS) solution and then air-dried at room temperature. The sample was examined under a fluorescent microscope (Eclipse, Nikon) with a 100 W high-pressure mercury lamp and a filter set MWU (Olympus Optical, excitation 330-385 nm). The DAPI-DNA fluorescence appeared to be blue white, while the fluorescence of either DAPI-poly-P or DAPI-lipid was bright yellow.

Fluorescence *in situ* hybridization (FISH) technique was also employed to characterize PAO in the sludge following the method described by Fu et al [22]. A sludge sample was first homogenized briefly using the vortex mixer and then placed in a hybridization well on a gelatin-coated microscopic slide plate. The PAO_{mix} probes (comprising the equal amount of probes PAO_{462}, PAO_{651}, and PAO_{846} (TechDragon, Hong Kong)) were used to target PAO species. Meanwhile, EUB338 was used to target all eubacteria in the sludge sample. The sample after staining was examined under an epifluorescent confocal laser scanning microscope (CLSM) (LSM Pascal, Zeiss, Thornwood). The combined use of DAPI staining and FISH also would display the abundance of PHB in the sludge [23].

2.4   Analytical methods

The influent and effluent of the MBR system were sampled twice a week for determination of the overall treatment performance in terms of the removals of the organic (TOC), total nitrogen (NH_4^+-N and NO_3^--N), and total phosphorus (PO_4^{3-}-P). In addition, the sludge suspension was also sampled twice a week from each of the tanks and chambers for detail analysis. For the sludge samples, the suspensions were filtered through a 0.4-μm polycarbonate membrane (25 mm, Osmonics) and the filtrates were analyzed. The TOC

160 concentration was determined by a TOC analyzer (IL550 TOC-TN Analyzers, Lachat) using

161 the combustion-infrared method. Ammonia nitrogen (NH_4^+-N) was analyzed using the

162 electrochemical method with an ammonia electrode and a potentiometer (920A, ORION).

163 Nitrate (NO_3^--N) was analyzed by an UV/VIS spectrophotometer (Lambda 25, Perkin Elmer)

164 according to the Standard Methods [24]. The liquid-phase P concentration in the sludge

165 suspension was analyzed using a spectrophotometer (UV/VIS Lambda 12, Perkin Elmer) in

166 accordance to the Standard Methods [24]. The dissolved oxygen (DO) concentration in each

167 chamber was determined by a DO probe (97-08-99, Orion) with an electrometer (920A,

168 Orion). Mixed liquor suspended solids (MLSS) for the sludge concentration and COD for the

169 organic concentration were measured following the Standard Methods [24].

170

171 **3. Results and Discussion**

172 *3.1   Organic and nitrogen removal*

173   The MBR wastewater treatment system was operated for over 430 days under various

174 conditions, including variations in food-to-microorganism (F/M) ratio, COD:TN:TP, and

175 internal recirculation ratio, as summarized in Table 2. The MBR system performed

176 consistently well in organic degradation and nitrification. In detail, the TOC removal

177 efficiency was more than 94% throughout the experiment (Fig. 2), even that a volumetric

178 organic loading rate up to 720 mg COD/L-d. The effluent contained a low organic

179 concentration with a TOC below 5 mg/L, which was not affected significantly by the

180 variations in operation. The organic content in the supernatant of the anoxic tank was

181 constantly below 25 mg TOC/L. Thus, it is deduced that the major portion of the influent

182 organic was degraded in the aerobic and anoxic tanks, with an average removal efficiency of

183 around 85%. The additional organic removal was attributable to the step of membrane

184 filtration, due to its effective retention of organic solutes [25, 26]. Moreover, the membrane

185 interception helped keep a relatively high biomass concentration in the MBR system, which

186 made the system less sensitive to the changes in operation [27, 28]. Meanwhile, the effluent

187 after membrane filtration was of high quality with a SS concentration of less than 1 mg/L.

188   The average NH_4^+-N removal efficiency of the MBR system was about 92%, indicating

189 sufficient biological nitrification (Fig. 2). The aerobic zone followed by the anoxic zone

190 formed a post-denitrification process for TN removal, and feeding the substrate into the

191 anoxic tank was proven to effectively facilitate denitrification. An increase of the nitrogen

192 loading rate or change of the COD/TN ratio did not significantly affect the NH_4^+-N removal

193 efficiency. At a high volumetric nitrogen loading rate from 70 to 80 mg N/L-d, the average

194 TN removal efficiencies were 79.2±4.3, 78.8±3.4, 83.7±3.0, and 73.2±1.3% for Phases 0, I, II,

195 and III, respectively. However, the C:N ratio in the wastewater influent was important to the

196 denitrification efficiency. As the C:N ratio decreased from 54:5 to 46.5:5 in Phase III, the

197 effluent TN content increased considerably, due likely to the insufficient carbon source in the

198 influent for denitrification. The recirculation ratio appeared to be another important factor to

199 the TN removal result [7, 14, 29]. As NO_3^--N counted for around 90% of the TN residue in

200 the MBR effluent, the TN removal efficiency by denitrification could be further improved if a

201 higher recirculation ratio was adopted.

202

203 *3.2 Biologically-enhanced phosphorus removal*

204   The TP removal was no more than 40% during the start-up phase, or Phase 0 (Fig. 3),

205 which also suggested that The P removal via assimilation was clearly below 40%. The P

206 concentration in the supernatant of sludge at the outlet of the anaerobic tank (i.e. the rough

207 setter, DO < 0.05 mg/L) was not significantly higher than that in the aerobic MBR suspension.

208 This implies that the PAO community was not well developed in the system. For the EBPR

209 process, P release by PAOs under anaerobic condition is crucial to the luxury P uptake in the

subsequent aerobic stage [7, 30, 31]. Thus, conditions that favor PAO growth and anaerobic phosphorus release should be provided. As such, a longer anaerobic period (to ~200 min) and a shorter SRT (to ~10 d) were adopted from day 31 (i.e. Phase I). The anaerobic P release was evidently improved gradually in the intermeidate sedimentation chamber, and the TP removal efficiency increased to nearly 50% with an average effluent TP concentration of about 5.2 mg/L. In order to enhance PAO accumulation further, the COD/TP ratio was increased to more than 50 in Phase II. During this period, the TP removal efficiency increased gradually to over 75% and the effluent TP concentration decreased eventually to 2.2 mg/L. The phenomena of P release in the anaerobic phase (intermediate sedimentation chamber) and P uptake in the aerobic phase (MBR tank) could be well observed. By the end of Phase II at a volumeric phosphorus loading rate of 15 mg P/L-d, a fairly low effluent TP concentration of less than 2.0 mg/L was attained and the average P removal efficiency reached to 87.1% (Fig. 3).

In Phase III, the organic content (COD concentration) in the wastewater influent was reduced by more than 10%, resulting in lower COD/TN and COD/TP ratios (Table 2). However, with a higher recirculation ratio applied ($4Q_{in}+4Q_{in}$), the TP removal efficiency increased to over 88% with an average effluent TP concentration of around 0.8 mg/L. Although decreased slightly, the TN removal rate was still around 80% (Fig. 2). The experimental results showed that the MBR system developed in this study is highly effective for sufficient organic degradation and simultaneous nutrient (N and P) removal. In this regard, this MBR system with a short treatment time (HRT<10 hr) and a high pollutant loading rate (720 mg COD/L-d, 77 mg N/L-d and 15.4 mg P/L-d) is comparable to or more effective than the MBR systems reported by others [11, 32] for simultaneous N and P removal. Moreover, the long-term experimental operaiton also evidenced the stable performance of the MBR-based treatment process. During the stable operation in Phase III, the specific nutrient

removal rates could be estimated for the sludge in different reactors or tanks, and the results summarized in Table 3 include the specific nitrification, denitirfication, P release and P uptake rates.

Phosphrate release in the anaerobic zone is crucial to the PAO function for P-removal, and its performance is affected considerably by the form of the carbon source [7, 31]. In the anaerobic chamber (intermediate settler), acetate in the influent could be readily uptaken by PAO cells, leading to the activation of acetyl-CoA. Two molecules of acetyl-CoA could condense to form acetoacetyl-CoA, which would be transformed eventually to poly-b-hydroxybutyrate (PHB) [7, 33]. Subsequently, in the aerobic stage with a rather low organic concentration, PAOs could use PHB as the carbon and energy sources to grow and to assimilate P forming poly-P [31, 32]. The configuration and operation of the wastewater treeatement process shown in Fig. 1 was apparently favorable to the growth of PAOs in the MBR system. In addition, a sufficient residence time of the sludge in the anaerobic chamber would ensure the conversion of readily degradable substrates to PHB, which is essential to the luxury P uptake in the aerobic stage [34, 35].

Phosphorus was then removed in the MBR via luxury P uptake and sludge discharge. The intermediate settler functioned not only for sludge-water separation, but also as an anaerobic chamber for P release. As half of the influent was fed into the anoxic tank, the organic carbon would induce P release in the intermediate sludge settler. Subsequently, luxury P uptake took place in the MBR to achieve P removal from the liquid phase. The P contents in the liquid phases of the sludge mixture in different tanks were analyzed when a high TP removal efficiency was maintained in Phase III (Fig. 4). With an influent P concentration of 8 mg/L, the liquid-phase P concentrations in the aerobic tank, rough settler, and membrane chamber were 6.7, 21.5, and 1.5 mg/L, respectively. In view of the sludge flow from the rough settler to the aerobic tank and membrane chamber, it becomes apparent that anaerobic P release and

260   aerobic luxury P uptake occurred well in the system. The liquid-phase P contents showed the

261   characteristic P distribution profile of the EBPR process. The membrane filtration also played

262   a role in polishing the effluent for P removal, as indicated by a lower TP concentration in the

263   effluent (0.8 mg/L) than that in the MBR sludge supernatant (1.5 mg/L). Comparing Phase III

264   with Phase 0 for TP removal, it can be estimated that more than 56% of phosphorus (112 mg

265   P/d) was removed by the luxury P uptake of the sludge, whilst the other less than 40 mg P/d

266   was probably utilized for biomass growth.

267

268   *3.3   PAOs and their role in phosphorus removal*

269   PAO accumulation and function are crucial to the P removal capability of the

270   MBR-based EBPR system. The PAO growth in sludge during the experiment was examined

271   by microscopic observations. The aerobic sludge flocs in different test phases (0, I, II, and III)

272   were stained by DAPI and the specific PAO probes (PAO$_{mix}$) [11, 31, 34]. After DAPI

273   staining, PAOs appeared as bright blue spots and polyhydroxybutyrate (PHB) appeared to be

274   yellow dots. As for the FISH images of the sludge labeled with the PAO probe, PAOs

275   appeared to be yellow clusters, as the probe would bind coccobacilli [35]. In the start-up

276   phase, the activated sludge (Fig. 5a$_1$ and a$_2$) showed only a little PAO and PHB signals. With

277   the development of the system for P removal, the PAO abundance in the sludge flocs

278   increased gradually. For the sludge in Phase I during which the MBR exhibited a moderate P

279   removal, the PAO and PHB contents increased considerably (Fig. 5b$_1$ and b$_2$).

280   In relation to the improvement of P removal in Phases II and III, the clusters of PAO

281   probes binding with the distinct and uniform yellow cells can be well observed (Fig. 5c$_2$ and

282   d$_2$). Moreover, large PHB granules were displayed by the bright yellow spots (Fig. 5c$_1$ and d$_1$),

283   which confirmed further the PAO activities. The apparent PAO content and PHB density

284   correlated well with the P removal performance of the EBPR system. For example, compared

285 to the early sludge samples, the sludge flocs in Phase III had more compact and larger PAO

286 clusters. It is believed that the massive PAO clustering signified the great P release and

287 uptake activities of the sludge under the anaerobic and aerobic conditions, respectively [2,

288 34].

289

3.4 Sludge concentration profile

291 The membrane fouling in MBR is caused mainly by sludge depostion on the membrane

292 surface [6, 20, 26]. Previous studies have shown that the membrane fouling rate in MBR is

293 affected to a certain extent by the bulk sludge concentration [20, 36, 37]. It would be

294 beneficial to the membrane fouling reduction if the sludge concentration in the membrane

295 chamber could be maintained at a low level (e.g. < 6 g MLSS/L) [28]. The present MBR

296 system employed a rough settler for sludge-water separtion before the membrane chamber.

297 This would therefore help lower the sludge concentration in the membrane chamber while

298 increase the MLSS contents in other tanks to favor biological treatment activities. During the

299 operation of the MBR system for over 400 days, the average sludge concentration in the

300 membrane chamber (3 g MLSS/L) was found to be even lower than that in the aerobic tank

301 (3.6 g/L) (Fig. 6). If the rough settler was not employed, the membrane chamber would have

302 the highest sludge concentration in comparion to other tanks, which would likely lead to

303 more rapid membrane fouling [6, 36, 37]. During the stable operation in Phase III, the

304 overflow from the rough settler into the MBR had a sludge concentration (MLSS) of around

305 1.9 mg/L. This was considerably lower than the sludge concentration (~3.0 g/L) in the

306 upstream bioreactors. Because of the membrane separation, the effluent was free of solids and

307 sludge in the MBR tank was concentrated to about 2.4 g/L. The sludge mixture was

308 recirculated at a rate of $4Q_{in}$ (Q_{in} =16.6 L/d). Meanwhile, for a stable operation and effective

309 P removal, the sludge mixture was discharged from the MBR at a rate of about 0.7 L/d.

13

4. Conclusions

- An innovative MBR system was developed for effective organic degradation and nutrient removal in wastewater treatment. The system integrated membrane filtration with the post-denitrification activated sludge process, and the wastewater influent was split into the aerobic tank and the anoxic tank. A rough settler was used not only for sludge-water separation before membrane filtration but also as an anaerobic chamber for P release.

- The MBR-based system performed remarkably well for simultaneous organic, N and P removals. With a short treatment time of less than 10 hrs, the system could achieve stable TOC, TN, and TP removal efficiencies of around 94%, 85%, and 87%, with effluent concentrations of less than 5, 6, and 1 mg/L, respectively.

- The proliferation and activity of PAOs in the MBR system were indicated by the significant P release in the anaerobic chamber and the luxury P uptake in the aerobic membrane chamber. PAOs and their P accumulating function were evidenced by the large PAO clusters and PHB granules under the fluorescent microscope after DAPI and PAOM probe staining.

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Table 1. Summary of a few large-scale MBR processes for biological nutrient removals in wastewater treatment

Process	Capacity (m³/d)	Membrane material	Operating conditions			Effluent quality			Membrane cleaning cycle	Features and limitations	Ref.
			HRT (h)	SRT (d)	MLSS in MBR (g/L)	COD (mg/L)	TN (mg/L)	TP (mg/L)			
A_1-A_2-O-MBR	2000	0.1 μm PVDF	13.8	50	5.4~8.2	14.2±5.8	8.6±3.4	0.3±0.2	1-2 weeks	High quality reclaimed water; but a low filtration flux.	[15]
Post-denitrification +MBR	200	PVDF	38	20	4	20.1±14.8	24.6±3.6	n.a.	weekly	Stable operation; but a long HRT.	[16]
A_1-A_2-O-MBR	60,000	0.04 μm, PVDF	17.3	20	7.00–8.50	15~20	15~25	0.30~0.50	n.a	Stable pollutant removal; but a long HRT and low filtration flux.	
A_1-A_2-A_2-O-MBR	50,000	0.1 μm, PVDF	12~13	25~32	5.65~10.44	8~53	4.20~22.70	0.03~1.45	n.a.	Treatment of both municipal and industrial wastewater; but less stable pollutant removal.	[17]
A_1-A_2-O-A_2-MBR	20,000	0.4 μm PVDF	33~38	27~37	7.07~11.59	3~36	3.02~13.10	0.03~0.67	n.a.	Treatment of both municipal and industrial wastewater; but a long HRT.	
A_1-A_2-O-MBR	20,000	0.04 μm PVDF	10~14	14	3.24~12.00	8~91	3.42~18.66	0.05~0.89	n.a.	High resistance to the seasonal shocking; but low filtration flux.	

Note: A_1 - anaerobic; A_2 - anoxic; O - oxic (aerobic); n.a.- not available.

Table 2. The experimental condition in different phases for the MBR nutrient removal study.

Phase	Duration (d)	SRT (d)	HRT (hr)	COD (mg/L)	TOC (mg/L)	F/M (g COD/g MLSS-d)	TN (mg/L)	COD:TN:TP	Recirculation rate
0	30	> 12	11.6	320	120	~0.32	40	32:4:1	$3Q_{in} + 3Q_{in}$
I	83	~ 10	11.6	320	120	~0.22	40	32:4:1	$3Q_{in} + 3Q_{in}$
II	153	~ 10	9.3	432	160	~0.26	40	54:5:1	$3Q_{in} + 3Q_{in}$
III	165	~ 10	9.3	372	140	~0.28	40	46.5:5:1	$4Q_{in} + 4Q_{in}$

Table 3. Specific biological N and P removal rates estimated for the sludge in different reactors during the stable operation in Phase III.

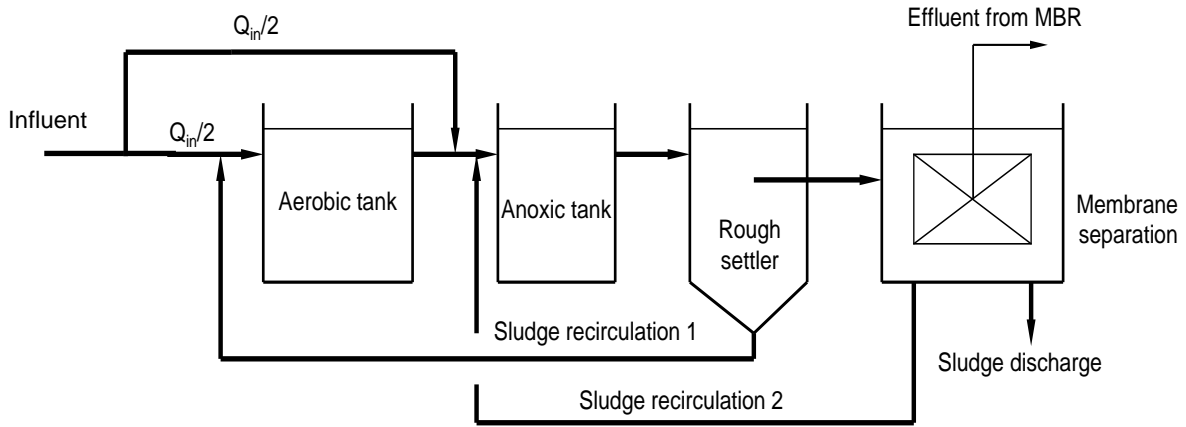| Specific nutrient removal rate	Aerobic tank	Anoxic tank + sludge settler		MBR
Volume (L)	2.0	1.4	1.5	1.6
HRT (hr)	2.9	2.0	2.1	2.3
MLSS (g/L)	3.0	3.0	3.0	2.4
Nitrification (mg N/g MLSS-d)	45			70
Denitrification (mg N/g MLSS-d)		100		
P release (mg P/g MLSS-d)		180		
P uptake (mg P/g MLSS-d)				230

Figure captions

Fig. 1.　Schematic diagram of the new MBR system for organic degradation and simultaneous N and P removal.
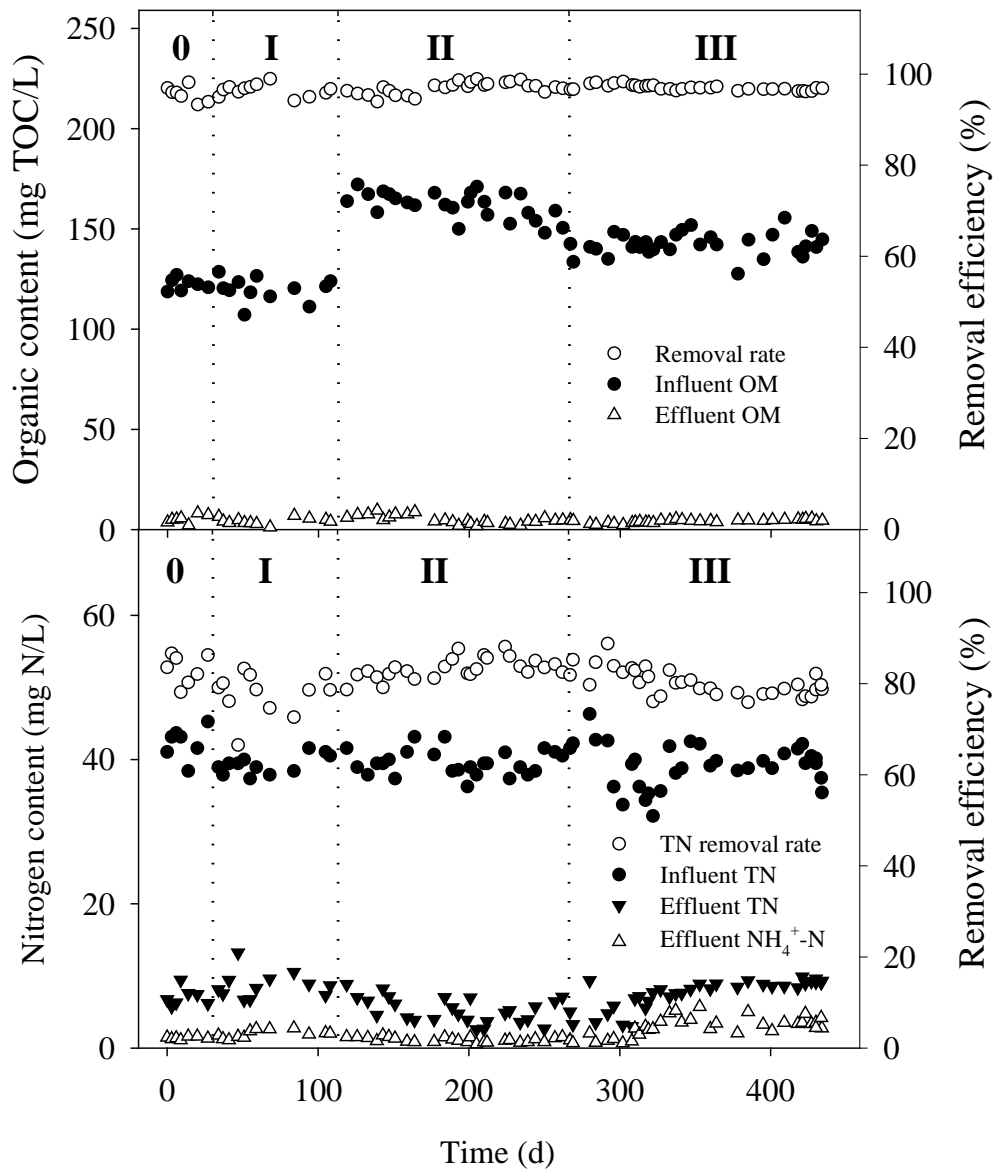
Fig. 2.　Organic and total nitrogen removal performance of the MBR system (OM: organic matter measured by mg TOC/L).
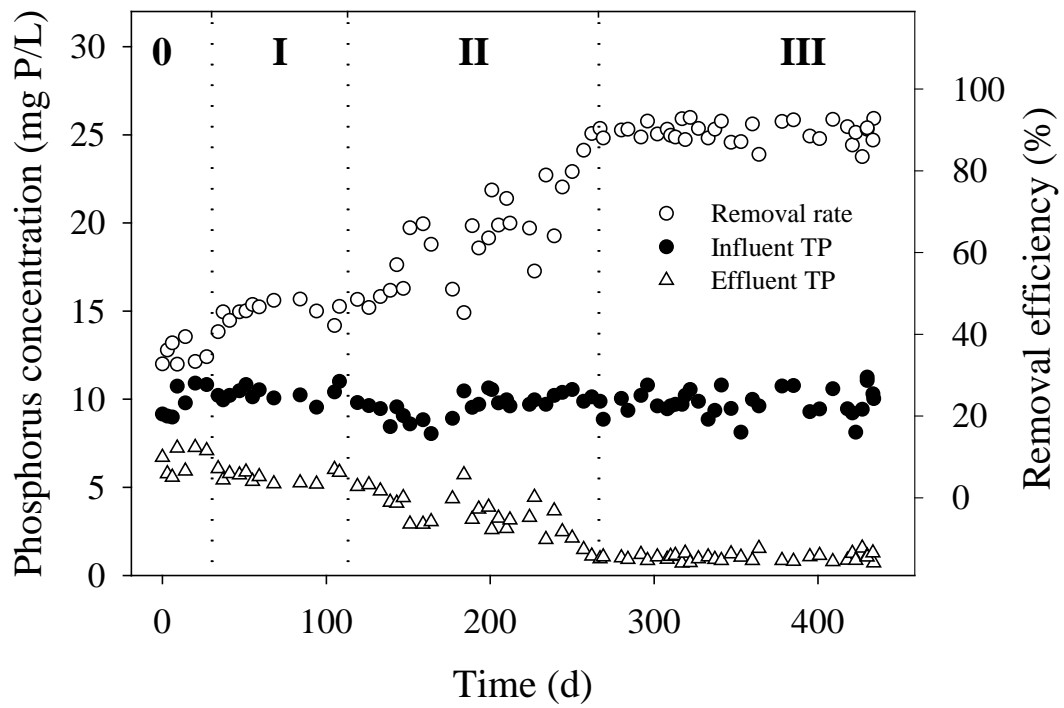
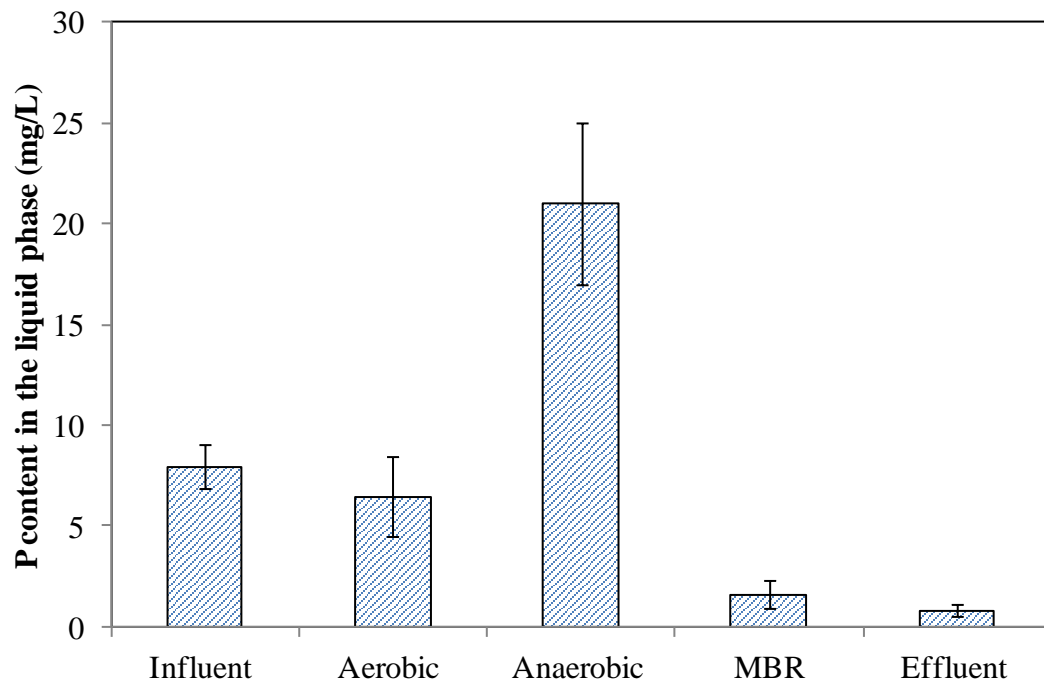Fig. 3.　Total phosphorus removal in different phases (Phase 0, I, II, and III) of the MBR experiment.

Fig. 4.　Change of the phosphate concentration in the liquid phase of the sludge in different tanks of the MBR system during Phase III operation.

Fig. 5.　Microscopic images of the PAOs in the MBR sludge after (1) DAPI staining (bright blue spots - live bacteria stained by DAPI; yellow dots - PHB stained by DAPI) and (2) FISH-CLSM observation (green spots - bacteria stained by EUB338; yellow area - PAO stained by PAO_{mix}): (a) seed sludge in Phase 0, (b) sludge flocs in Phase I, (c) sludge flocs in Phase II, and (d) sludge flocs in Phase III.

Fig. 6.　The MLSS concentrations of the sludge in the aerobic tank and MBR chamber.

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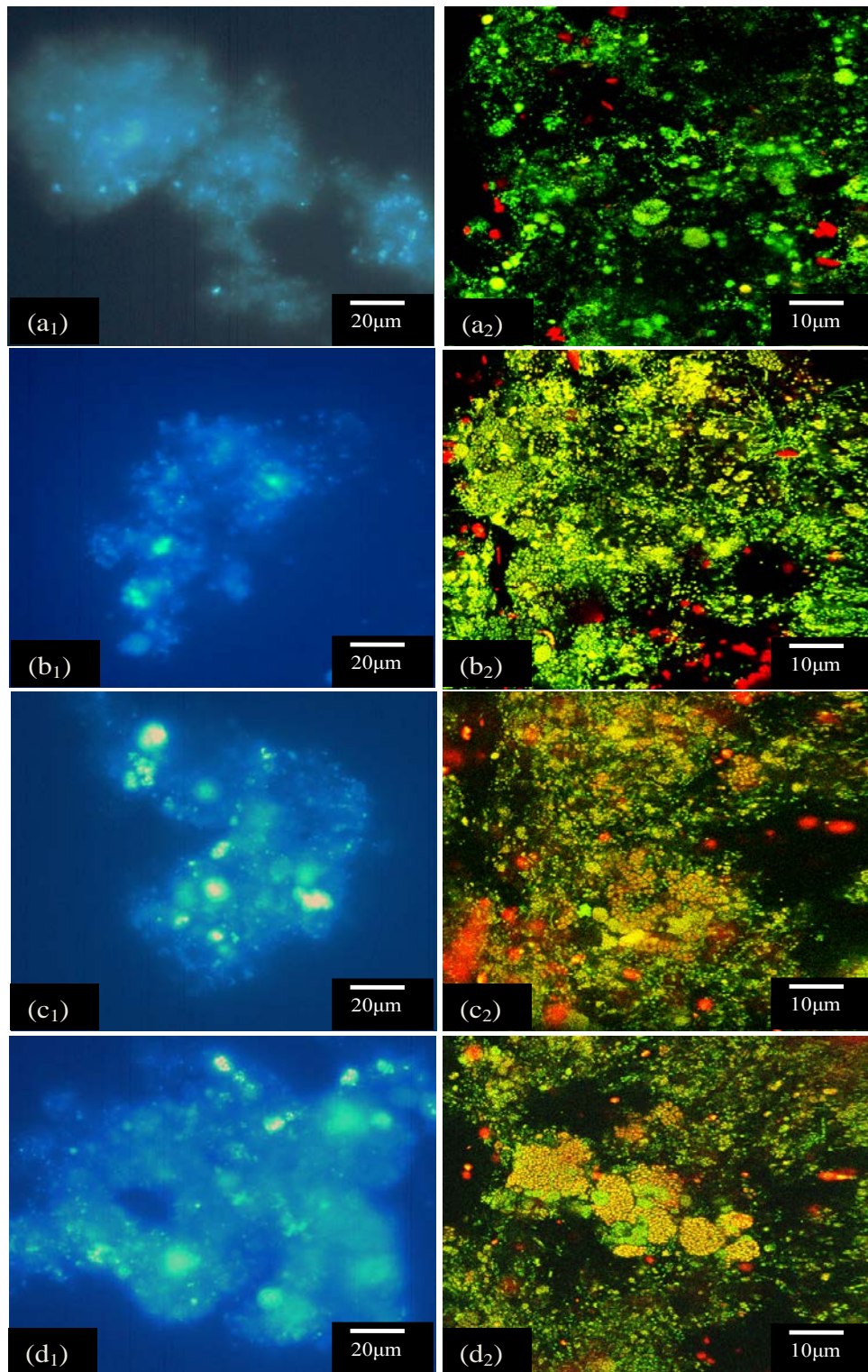
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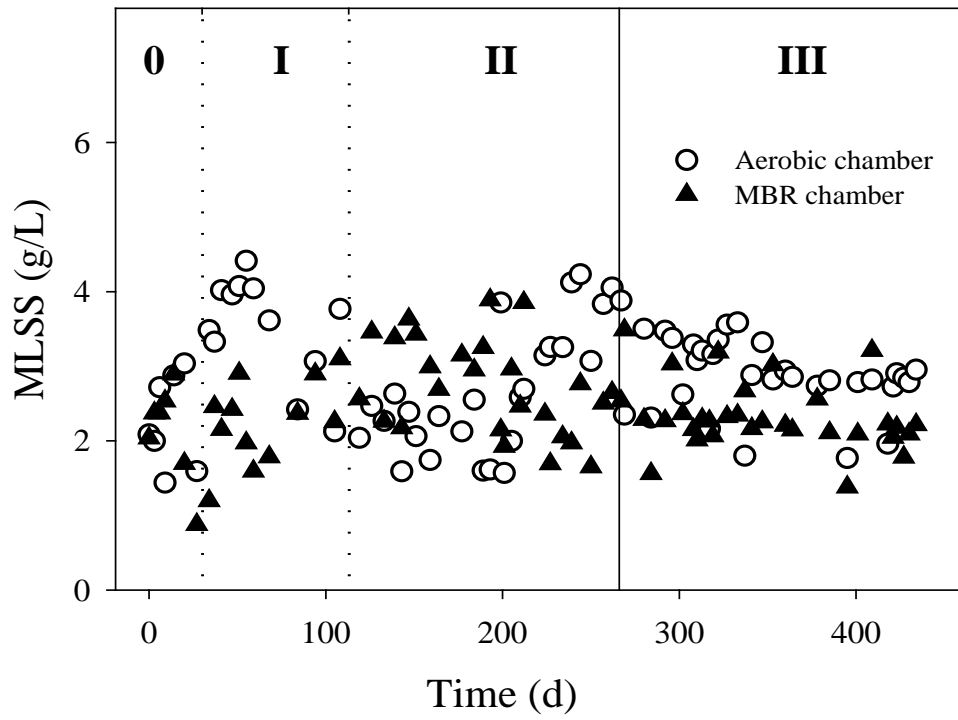
Fig. 6. The MLSS concentrations of the sludge in the aerobic tank and MBR chamber.