2008

Removal of structurally different dyes in submerged membrane fungi reactorâ€"Biosorption/PAC-adsorption, membrane retention and biodegradation

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Keywords

GeoQUEST

Disciplines

Life Sciences | Physical Sciences and Mathematics | Social and Behavioral Sciences

Publication Details

Hai, F. Ibney., Yamamoto, K., Nakajima, F. & Fukushi, K. (2008). Removal of structurally different dyes in submerged membrane fungi reactorâ€"Biosorption/PAC-adsorption, membrane retention and biodegradation. Journal of Membrane Science, 325 (1), 395-403.

Removal of structurally different dyes in submerged membrane fungi reactor—biosorption/ PAC-adsorption, membrane retention and biodegradation

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Abstract

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Keywords: Dye; Fungi; Submerged Membrane Bioreactor; Non-sterile; Powdered activated carbon; Textile wastewater

1. Introduction

Over the past decade, submerged membrane bioreactor (MBR) processes have experienced unprecedented growth in domestic and municipal wastewater treatment owing to several advantages including excellent effluent quality, low sludge production, small foot print, and flexibility in future expansion [1,2]. Application of MBR technology for industrial wastewater treatment has also gained attention because of the robustness of the process.

Textile wastewater is a complex and highly variable mixture of many polluting substances including dye [3]. As dyes are seldom degraded by aerobic bacteria, rather low removal of soluble dyes is achieved in aerobic MBRs only through adsorption to the sludge, that too at the expense of frequent sludge withdrawal [4]. Some studies, therefore, propose a lengthy alternative of sequential anaerobic (decoloration)/ aerobic (detoxification) MBR [5].

It is interesting to note in this context that unlike bacteria, aerobic white-rot fungi can degrade wide varieties of recalcitrant compounds including textile dyes by non-specific extracellular enzymes [6]. Regrettably, in contrast to the numerous reports on excellent degradation capacity of pure fungi culture in small scale batch-tests, there exist only a few studies which

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report dye degradation performance in continuous reactors. The problems associated with continuous fungal reactors include rather slow fungal degradation requiring long hydraulic retention time [7], excessive growth of fungi causing reactor-clogging [8], bacterial contamination destabilizing fungal decoloration [9], and loss of the extracellular enzymes and mediators with discharged water [10].

Of major concern is the fact that deteriorated degradation rates due to destabilization of fungal enzymatic activity or the secreted enzyme itself [9,11] are observed under bacterial contamination. However, among the few studies concerning continuous fungal reactor, only a handful explored dye degradation under non-sterile environment [9,12-15]. To date, only the latest two studies [14,15] have reported long-term stable decoloration under non-sterile conditions. Hai et al. [14] developed a submerged membrane fungi reactor and demonstrated long-term (50 days), excellent decoloration (99%) of a selected dye along with good total organic carbon (TOC) removal capacity under a hydraulic retention time (HRT) of 15 h. However, they did not directly address the effect of bacterial contamination on fungal decoloration. On the other hand, by combining the strategies of nutrient-limited condition and periodic addition of fresh biomass, recently Blanquez et al.[15] demonstrated long-term (110 days) stable decoloration (78%) of another dye by an air-pulsed bed bioreactor under an HRT of 48 h. Nevertheless, once exposed to real textile wastewater —bearing a mixture of three dyes and more heavily contaminated with bacteria— the process achieved much lower (40-60%) levels of decoloration. Surprisingly, so far none except one study [10] has specifically investigated the potential of prevention of enzyme washout from continuous fungal reactors. It is clear from the above discussion that more systematic studies on enhancement of fungal degradation of chemically different dyes in continuous reactor under non-sterile environment are an imperative.

We envisaged that MBR, with its well-documented inherent advantages [1,2], can cope up with the aforementioned difficulties (high biomass concentration, bacterial disruption, enzyme washout). For instance, the cake layer on the membrane may lead to significant retention of soluble dye, thereby de-coupling the dye retention time and HRT of the reactor. This mechanism may play a role in reduction of enzyme washout as well. Addition of adsorbent within MBR may enhance this process. Direct addition of activated carbon in conventional activated sludge system has been explored in certain studies to obtain removal of COD and color from textile wastewater in a single step with no additional physicochemical treatment [16]. However, frequent addition of fresh activated carbon is inevitable in such systems. On the other hand, some recent studies have reported slightly improved treatment of different biodegradable wastewater in PAC-amended MBR [17,18]. However, report on PAC-added MBR (utilizing either conventional bacteria-dominated activated sludge or fungi) for the recalcitrant textile dye wastewater treatment is apparently lacking.

The objective of this study was to investigate the long-term removal performance of two chemically different dyes in a membrane-coupled fungi reactor under non-sterile conditions. In order to explain the performance of MBR, the decoloration capacity of pure fungus as well as MBR-sludge and the extent of decoloration through direct membrane filtration were assessed in batch tests. The effect of dye structure and its capacity to adsorb on biomass, on any component of the wastewater or on PAC added into the MBR were discussed. Special focus was given on the role of membrane in overall dye removal (biosorption/PAC-adsorption,

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cake layer filtration, biodegradation) in the MBR. This study on one hand provides insight into the strategies to enhance fungal dye degradation under non-sterile environment, and, on the other hand demonstrates successful application of a unique version of MBR.

2. Experimental

This study involved experiments employing a synthetic textile wastewater in a 12 L PVC bioreactor within which a spacer-filled hollow-fiber module was submerged. The MBR was initially inoculated with pure fungi culture; however, it was operated without any special control of bacterial intrusion. Batch tests with pure fungi culture, MBR sludge, crude enzyme solution and PAC provided valuable information to explain the MBR performance.

2.1 Microorganism, chemicals and synthetic wastewater

The white-rot fungus *C. versicolor*, NBRC 9791 obtained from the NITE Biological Resource Center (NBRC), Japan was used for this study. A nutrient-sufficient synthetic wastewater was prepared by adding dye (either of two azo dyes: Poly S119, Acid Orange II in 100 mg L^{-1} or both in 50 mg L^{-1}) and starch (2 g L^{-1})—two common components in real textile wastewater—along with urea (0.1 g L^{-1}) and other nutrients [19] into tap water. A significant portion of the poorly soluble starch in the wastewater remained in suspended form. During batch tests, Milli Q water instead of tap water and higher concentrations of starch (4.5 g L^{-1}) and urea (0.4g L^{-1}) were used. Also, during batch tests starch was in soluble form as the final solution was autoclaved. Poly S119 is a polymeric dye, while Acid Orange II is a low molecular weight (350) dye (Fig. 1). Both the dyes were water-soluble and provided orange color (peak absorbance: Poly S119=472 nm, Orange II= 481 nm); however, Acid Orange II showed higher peak absorbance per unit concentration. 1 g L^{-1} high purity powdered activated carbon, PAC (Darco G-60, mesh size 100-325) was added in the MBR on day 110 of continuous operation. Dyes and PAC were purchased from Sigma-Aldrich Co., USA.

2.2 Operating conditions of membrane module and direct filtration test

A spacer-filled bundle (Diameter=4.5 cm, Height= 22 cm) of micro-porous (0.4μm), hydrophilically treated polyethylene hollow-fibers (surface area=1.07 m^2) obtained from Mitsubishi Rayon, Japan was utilized in this study (Fig.2). Spacer was introduced within the module to obtain appropriate compactness under which the fiber arrangement would remain relatively undisturbed, thereby minimizing intrusion of sludge and also providing regular backwash channels [19]. Short-term (24 hrs) direct filtration test with wastewater, where the filtrate was returned to the reactor, was conducted under the operating conditions (flux, aeration) same as that applied during MBR operation. The module was operated under a selected average flux of 1.27×10^{-7} m^3 m^{-2} s^{-1} (0.46 L $m^{-2}h^{-1}$) with 5 min on/off mode. Pulsed backwash with permeate (flowrate=1.67 mL s^{-1}, duration=3 s per 10 min) and periodic backwash with NaOCl solution containing 500 mg Cl L^{-1} (100 mL m^{-2} membrane surface; twice/ week) were applied during MBR operation. In addition, intermittent surface aeration was provided with a specially designed diffuser (intensity= 2.5L air min^{-1}, duration=1min per 30 min). Details about the design principle of the module and the corresponding moderate consumption of chemical and air for cleaning (despite their rather frequent application) have been documented elsewhere [19].

2.3 Design and operating conditions of the bioreactor

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A cylindrical, PVC bioreactor (working volume = 11.8 L) was used in this study (Fig.3). Continuous air from the bottom of the reactor was supplied through a diffuser with an intensity of 5 L min^{-1} for complete mixing and supply of dissolved oxygen to the microbes. Concentrated synthetic wastewater was diluted with tap water and then added into the reactor by pumps controlled by a water level controller. The temperature of the reactor was controlled at 29±1° C. The system was first inoculated with 2.5 gm *C. versicolor* (dry wt.) grown for two weeks in 1 L Erlenmeyer flasks each containing 500 mL of the culture media (section 2.1). The reactor was then kept under aeration for 2 weeks after which the continuous operation under an HRT of 1 day was initiated. The dye and TOC loading during the MBR operation were 0.1 and 0.944 g $L^{-1}d^{-1}$, respectively. Except for sampling, no sludge was withdrawn from the MBR.

2.4 Batch test description

For pure culture test, 300 ml conical flasks containing 200 ml of culture media (section 2.1) with 0.1 g L^{-1} dye were aseptically inoculated with 0.1 gm (dry wt.) *C. versicolor* previously grown in agglomerated form within colorless media. The flasks were then incubated for 2 weeks at the optimum growth temperature of 28 °C on a shaker (BR-300LF, Taitec reciprocal bio-shaker, Japan) at 80 rpm. Same amount of sludge was collected from MBR on day 100 of continuous operation, washed with Milli-Q water and incubated under identical conditions. Samples (0.3 mL) were collected daily, diluted with Milli-Q water and then analyzed.

Following confirmation of secretion of considerable amount of extracellular enzyme, the colorless media, in which fungi were grown, was filtered (0.45μm cellulose acetate filter, Advantec, Japan) and utilized as crude enzyme solution for *in vitro* decoloration test. 5 mL dye solution was aseptically incubated with previously harvested 5 mL crude enzyme solution (final solution activity = 5.2 μM min^{-1}) in 50 mL tubes and then subjected to reciprocal shaking (80 rpm). Tubes were harvested at 2.4, 6, 12, 18 and 24 hrs to assess decoloration.

Dye sorption (in absence of biodegradation) on fungi was estimated under growth-inhibiting conditions [20]. For this test, fungi, grown previously in colorless media, were aseptically washed with Milli-Q water and incubated under a temperature of 10°C in dye solution devoid of any other substrates. The test was continued for a week within which media decoloration stabilized. The adsorbed amount was estimated from the extent of media decoloration. On the other hand, the amount adsorbed on actively growing fungi during pure culture batch decoloration test was estimated after harvesting the biomass and extracting dye from biomass using methanol.

For batch test with PAC, 1.5 gm of PAC, placed into 100 mL Milli-Q water within a 200 mL flask, was autoclaved, cooled down to room temperature, and then water was replaced by the same volume of crude enzyme. The flask was placed under stirring (70 rpm, magnetic bar) over night. The control flask was prepared in the same way, but in this case water was not replaced with enzyme. After 24 hrs, the respective media from the flasks were decanted off and 100 mL of 1 g L^{-1} Orange II dye solution was added. The flasks were then again placed under stirring for 24 hrs to allow complete adsorption of dye onto carbon. After that the liquid media was decanted off from the flask and then dye was extracted from PAC using methanol.

2.5 Analytical methods

TOC was measured with a Total Organic Carbon analyzer (TOC-V, Shimadzu, Japan). Color measurements were carried out using a spectrophotometer (U-2010, Hitachi, Japan). Membrane-permeate samples were analyzed as collected, while samples collected from within MBR was centrifuged under 2150 x *g* to obtain the supernatant and then analyzed for color and TOC .Fungal enzymatic (laccase) activity was measured by monitoring the OD468-change due to the oxidation of 2,6-dimethoxyphenol (DMP) at room temperature over 2 min. Enzymatic activity was calculated from the molar extinction coefficient, ε =49.6 mM^{-1} cm^{-1} [21] and expressed in μM substrate per minute (Detection limit =0.0045±0.00074). The relative abundance of fungi/bacteria in mixed liquor suspended solids (MLSS) was estimated following the method of Jasti et al. [22] using 20 times diluted samples. Transmembrane pressure (TMP), as an indicator of membrane fouling, was continuously monitored using a vacuum pressure gauge (GC 61, Nagano Keiki Co. Ltd., Japan).

3. Results and discussion

3.1 Batch tests with biomass and PAC

The main purpose of this study was to investigate the long-term dye removal performance of the fungal MBR under non-sterile conditions. Nevertheless, batch tests with pure fungus, MBR-sludge, crude enzyme solution and PAC were conducted to provide evidences in support of our interpretations regarding the MBR performance.

3.1.1 Degradation by pure fungi culture

The decoloration of Orange II proceeded faster than that of Poly S119 (Fig.4). The specific removal rates of Orange II and Poly S119 were 284 and 124 mg dye (g dry biomass wt)$^{-1}$ d^{-1}, respectively. While virtually complete decoloration of Orange II solution was achieved in 4 days, not more than 90% decoloration of Poly S119 solution was achieved even after 2 weeks. It should be noted furthermore that, at the end of 2 weeks the adsorbed amounts of Orange II and Poly S119 on actively growing biomass were 0.1 and 8.2 mg dye (g dry biomass wt)$^{-1}$, respectively (Table 1). In absence of biosorption, the Poly S119 solution would bear an additional color equivalent to 7 mgL^{-1} dye. Separate biosorption experiments conducted with same amount of inactive biomass (kept under growth-limiting condition) also confirmed the substantial and negligible biosorption capacities of Poly S119 and Orange II, respectively.

3.1.2 In vitro enzymatic degradation

Like in pure culture test, Orange II was degraded faster by crude enzyme solution (Fig. 5). The specific removal rates of Orange II and Poly S119 were 19.5 and 6.3 mg dye (enzymatic activity)$^{-1}$ d^{-1}, respectively. In 1 day, about 55 and 15% of Orange II and Poly S119 were degraded from their respective 100 mgL^{-1} solutions. Each of the dyes exhibited faster removal when the concentration of that dye was lowered to 50 mgL^{-1}. It is interesting to note that, decoloration, although to a much lesser extent, proceeded even when the utilized enzyme solution possessed 10 times lower activity (data not shown). Depending on the type of dye, culture media and the fungus strain under consideration, contradictory reports on capacity of crude extracellular suspension to decolorize dye solution exist in literature [15,23,24]. In our study, extracellular suspension showed moderate to significant degradation of the tested dyes. More importantly, the *in vitro* test confirmed that compared to the polymeric dye, Orange II is more amenable to fungal degradation.

3.1.3 Activity of MBR sludge

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The activity of agglomerated pure fungi culture (Fig. 4b) and the bacteria-rich disintegrated MBR-sludge were compared in batch tests using Orange II dye. This investigation was carried out following achievement of incomplete removal of that dye in the MBR. We observed moderate decoloration, no (undetectable) enzymatic activity and much faster rate of TOC consumption (Figure 6 a,b) by the MBR-sludge, which clearly demonstrated bacterial influence on fungal activity.

3.1.4 PAC adsorption

We conducted batch tests with enzyme-soaked PAC in order to substantiate our hypothesis that simultaneous PAC adsorption within MBR may bring about improved decoloration and enzymatic activity. Under the applied conditions, complete decoloration of the Orange II dye solution occurred in case of both untreated and enzyme-soaked PAC. However, at the end of the test, only 6% dye could be extracted from enzyme-soaked PAC, whereas 51% dye was extracted from regular PAC (Table 2). It was clear from the difference of extracted amount of dye from regular and enzyme-soaked PAC that enzymatic dye degradation indeed occurred on PAC following co-adsorption of dye and enzyme onto PAC.

3.2 Removal of dyes by membrane filtration alone

As is common in case of MBRs, a microfiltration (MF) membrane, which is not expected to remove soluble fractions by its own, was used in this study. Nevertheless, it was considered interesting to assess the extent of dye removal during direct filtration of only the dye solution or the complete synthetic wastewater.

Adsorption of the dyes on the membrane itself was negligible. In case of the polymeric dye (Poly S119), during direct filtration of only the dye solution and the synthetic wastewater, about 56% and 97% dye removals, respectively, were observed (Table 3). The corresponding values in case of the lower molecular weight (350) dye (Orange II) were only 4.2 and 20%, respectively. The usual belief is MF membrane can not remove soluble fractions by its own. However, Porter et al. [25] reported that trace contaminants would be sufficient to influence the passage of ions of low molecular weight and can cause organic microfilter to reject ionic dyes after a few hours of operation. We prepared solutions using tap water, and, hence, the dye removal observed during filtration of only the dye solution can be explained in the light of the study of Porter et al. [25]. On the other hand, Capar et al. [26] contended that high removal of color from a mixture by MF may indicate the adsorption of dyes onto particulates which were retained on the MF surface. In this study, a significant portion of the poorly soluble starch in the wastewater was in suspended form (Total suspended solid, TSS= 2.6 g L^{-1}). The high removal of the Poly S119 dye during filtration of the synthetic wastewater may be attributed to its adsorption on starch and subsequent significant retention by the membrane. The role of starch is supported by the fact that the extent of removal in case of the complete synthetic wastewater and only the mixture of starch and dye were similar. On the other hand, acid dyes typically possess low molecular weight and very limited affinity to cellulosic fibers [27]. Apparently, lower extent of adsorption of Acid Orange II dye on starch led to its lower removal. Finally, it should be noted here that although encouraging extents of removal of certain dye during direct filtration of synthetic wastewater was observed, pore-blocking of membrane occurred under longer operation, and such fouling would restrict practical application of direct filtration.

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3.3 Treatment performance of continuous MBR (non-sterile)

3.3.1 MLSS concentration and relative abundance of fungi/bacteria

One of the advantages of MBR is maintenance of high MLSS concentration. Figure 7a shows the change of MLSS concentration during continuous operation of the MBR. In absence of any withdrawal of sludge from the reactor, the initial MLSS concentration of around 5 gL^{-1} rose up to 17.5 gL^{-1} by day 110 when PAC (1 gL^{-1}) was added in the MBR. The MLSS concentration almost stabilized at 20 gL^{-1} by day 150. Lesage et al. [28] also reported slight reduction in sludge production following PAC addition. Nevertheless, as detailed in the following sections, even under the high MLSS concentration of 20 gL^{-1}, the treatment performance was not disrupted. It should be also noted that following pure fungi inoculation, bacterial contamination occurred during the start-up period. However, size-based approximate fractionation of the MLSS indicated a stable relative abundance (66%) of fungi during continuous operation (Figure 7b). With the composition mentioned in section 2.1, the pH of the wastewater stood at 4.5. Without any control, the pH in the MBR varied in the range of 5.5 to 6. This slightly acidic pH may have contributed to some extent to the observed relative abundance of fungi.

3.3.2 Removal of Poly S119 dye possessing complex structure but high biosorption

In pure culture batch tests, the decoloration of Poly S119 solution was much slower than that of the Orange II dye (Fig.4). Intersetingly, under the applied dye loading of 0.1 g $L^{-1}d^{-1}$ (HRT= 1 day) over 99% removal of Poly S119 dye was consistently achieved in the continuous MBR (Fig.8, Table 4). Such excellent removal in MBR called for further investigation. Close observation of the quality of the reactor-supernatant (68.3% removal) and the membrane-permeate (99.1 % removal) revealed the significant contribution of the membrane to the overall removal performance. Apparently, the high adsorption of Poly S119 dye on biomass (Table 1) and starch (section 3.2) led to its subsequent excellent retention by the cake layer on the microfiltration membrane within the bioreactor. Maintenance of high MLSS concentration in MBR appears to be beneficial in this context. However, stable quality of the supernatant and the permeate over an operation period of 2 months with this dye confirmed that retained dye was subsequently biodegraded.

It is important to note that unlike in pure culture batch test, extracellular enzyme was undetectable in the MBR during this run (Fig.8a). Bacterial contamination in the MBR was evident (section 3.3.1). It is likely that fungal enzymatic activity or the secreted enzyme itself was destabilized by bacteria [9,11]. Continuous enzyme washout with effluent may have exacerbated the situation [10]. The molecular weight of Laccase has been reported to be in the range of 59-110 kDa [6], which is much smaller than the pore size of the microfiltration membrane utilized in this study. A few authors used ultrafiltration membrane in enzymatic membrane reactors to retain fungal enzyme [29]. In contrast, we originally expected that the cake-layer on the membrane may play a role; but that did not turn out to be an adequate strategy. Our sterile *in vitro* test (section 3.1.2) confirmed dye degradation even under very low enzymatic activity.. Janshekar et al. [30] reported decoloration under undetectable level of extracellular enzymatic activity in a batch stirred reactor. Also Svobodova et al. [24] contended that mycelium-associated enzyme is more important for dye degradation, and Blanquez et al. [15] reported that high enzymatic activity is not required for high level of decoloration. Apparently, in our study, the activity of fungi was just enough to decolorize Poly S119 but not strong enough to show extracellular enzymatic activity. Owing to biosorption

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and subsequent retention of dye by membrane, the dye degradation rate, although low, was enough to sustain overall excellent decoloration rate.

3.3.3 Removal of Orange II dye having simpler structure but low biosorption

Based on the excellent overall removal of the Poly S119 dye in the MBR even in absence of detectable level of extracellular enzymatic activity, it was expected that at least a similar extent of removal would be achieved in case of the Orange II dye, which possessed a much simpler structure (Fig.1) and was more amenable to fungal degradation (Fig. 4,5). Contrary to our expectation, an average overall removal of 93% was observed (Fig.8a, Table 4). The supernatant quality (82%) in case of this dye was better than that in case of the Poly S119 dye (68.3%), indicating that the degradation of Orange II dye in fact proceeded faster in the MBR. However, as the Orange II dye showed lower sorption on biomass, the cake layer on the membrane could not retain this dye as effectively as the Poly S119 dye. Accordingly lower overall removal of Orange II dye was achieved under the applied loading.

3.3.4 Improved Orange II removal after PAC addition in MBR

PAC was added into the MBR in order to improve the overall decoloration of Orange II dye—which exhibited negligible biosorption (Table 1) but adsorbed considerably on PAC. In line with our expectation, marked improvement in supernatant as well as permeate-quality (93 and 99.6 % removal, respectively) was observed. Stable decoloration of Orange II dye for an extended period of 1 month confirmed that the improvement in decoloration was not due to instantaneous adsorption on PAC only, but biodegradation occurred simultaneously. Figure 9 compares the changes in UV-visible spectra of the wastewater containing different dyes following MBR treatment. This is the first report on PAC-added MBR for treatment of recalcitrant textile dye wastewater.

Of further interest was the fact that within a few days of PAC addition, slight extracellular enzymatic activity appeared within the reactor. Some authors argue that high enzymatic activity is not required for high level of decoloration [15]. In that sense, transition of extracellular enzymatic activity from a moderate to substantial level may not bring about any significant change in total decoloration. However, improvement of the enzymatic activity from undetectable to moderate level was very encouraging as that may suggest enhanced biodegradation. Zhang et al. [10] previously reported improved decolorization due to adsorption and close contact of dye as well as extracellular enzyme on PAC. Our own sterile batch tests with crude enzyme solution also confirmed co-adsorption of dye and enzyme onto activated carbon and subsequent enzymatic dye degradation (section 3.1.4). These observations manifest that simultaneous PAC adsorption within fungi MBR can result in multiple advantages including adsorption of dye and prevention of enzyme washout which can eventually lead to enhanced degradation of dye.

3.3.5 Simultaneous removal of Poly S119 and Orange II dyes

Achievement of excellent decoloration of wastewater containing either Orange II or Poly S119 dye with/without simultaneous PAC adsorption in the fungi-MBR encouraged further investigations by incorporating both the dyes (each in 50 mgL^{-1}) into the wastewater.

As the absorbance per unit concentration of the Poly S119 dye is lower than that of the Orange II dye, the wastewater containing mixture of the two dyes exhibited slightly lower

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absorbance than the one containing only Orange II. The average permeate quality in terms of absorbance too appeared slightly better (Table 4) with this wastewater; however, the removal percentage was the same. The fact that there was no sign of permeate quality deterioration during the observation period of 1 month with this wastewater (total operation of 2 months with PAC addition) demonstrated the excellent performance of the proposed reactor.

3.3.6 TOC removal

Dye bath effluent constitutes only a minor part of TOC in textile wastewater. Unlike the nutrient-deficient hardly biodegradable dye bath effluent, different other streams of wastewater in a textile mill, namely, scouring and desizing effluent, usually contain high concentrations of relatively easily degradable organics [3]. Simultaneous achievement of color and TOC removal is, hence, indispensable.

In this study, the TOC loading was 0.944 g $L^{-1}d^{-1}$. The contribution of the dye(s) to total TOC was rather low (5%). Accordingly, the dye removal, which varied from 93.2% to 99.1%, did not affect the TOC removal in the course of the first 110 days. Consistently over 98% TOC removal (Table 4), corresponding to an average TOC concentration of 15 mgL^{-1} in the permeate was achieved during that period. The supernatant TOC concentration ranged around 30 mgL^{-1}. The overall average removal performance improved to over 99% after addition of PAC within the MBR. Simultaneous improvement in supernatant quality (>98% removal) was also observed. It is likely that other organics, in addition to dye, were also adsorbed on PAC [17] and were subsequently degraded. Excellent TOC removal along with decoloration manifested the superiority of the proposed reactor.

It is important to point out here that, as confirmed in batch tests (Figure 6b), pure fungi culture can obtain only a moderate TOC removal. Fungi can initiate the degradation of recalcitrant compounds (which are not amenable to bacterial degradation); however to complete the TOC removal, bacteria is also required. As demonstrated in our study, a reactor design which provides an optimum condition under which fungi attain dominance to some extent, but bacteria also remain functional would be appropriate. Perhaps the importance of "dominance of fungi" has been overemphasized in the available literature on fungi-based reactors.

3.3.7 Performance of the membrane in the bioreactor

The contribution of the membrane module to overall color and TOC removal has been discussed in the previous sections. This section will focus on the long-term filtration performance of the membrane in MBR.

Figure 10 depicts the slight variation of transmembrane pressure (TMP) in the course of operation, during which the MLSS concentration (without any withdrawal) varied from around 5 to 20 gL^{-1} (Figure 7). It is evident that under the cleaning strategies, the utilized module effectively resisted fatal fouling. The conventional hollow-fiber module design relies on somewhat free movement of the fibers under the scouring action of air bubbles to avoid accumulation of sludge. However, the conceptual expectation of complete removal following rather unrestricted entrance of sludge into module does not often come true. A previously developed [19] spacer-filled module was utilized in this study. Spacer was introduced within the module to minimize the intrusion of sludge by obtaining appropriate compactness under

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which the fiber arrangement would remain relatively undisturbed, thereby also providing regular backwash channels. The little amount of intruded sludge was backwashed through the bottom end of the module, while the sludge deposited on the surface was effectively cleaned by air-scouring. Accordingly, stable permeate flux could be maintained throughout.

It should be mentioned here that, slightly higher fluctuations of TMP were observed after addition of PAC in the MBR, especially at the initial stage. As confirmed by visual observation, this was due to intrusion of PAC to the core of the module. Several studies have reported improvement of membrane performance in MBR due to PAC addition. They attributed this improvement to reduction of some of the fouling components by adsorption on PAC, formation of highly porous and non-compressible cake layer on membrane and scouring effect of PAC [17]. On the other hand, Ng et al. [17] reported aggravated fouling in PAC-amended MBR in absence of sludge withdrawal and, accordingly, recommended periodic replenishment of spent PAC with fresh PAC. We did not encounter aggravated fouling during 2 months following PAC addition. Since in this study a differently designed module was utilized under a selected low flux and TMP variation was rather minimal throughout the operation period (with/without PAC), direct comparison of the observations made in this study to those in the previous studies would not be meaningful. However, the issue of sludge withdrawal from PAC-amended MBR deserves further attention in the future studies.

3.4 Insight into enhancement of dye degradation and suitability of MBR

Irrespective of the scale of investigation (in small flask or much larger reactor), the strategies that have been used so far to improve fungal decoloration under non-sterile environment include maintenance of low pH and nutrient (nitrogen)-limited condition [9,31], encapsulation of fungus in polyvinyl alcohol hydrogel beads [13], periodic addition of fresh biomass [15], and de-coupling of growth (sterile condition) and decolorization (non-sterile condition) stages [31]. None of the approaches on its own came out to be a long-term solution to the problem and practical application of some of those approaches are questionable from the cost and maintenance requirement points of view.

Results from our study indicate that means to de-couple the dye retention time and HRT of the reactor would allow satisfactory removal even under lower titer of fungal activity in presence of bacterial contamination, while simultaneously avoiding application of excessively long HRT. In the present study, this was achieved by coupling a membrane to the bioreactor and simultaneously applying PAC adsorption. In presence of PAC, the membrane submerged in the bioreactor contributed significantly to retention of dye and to overall decoloration in case of the dye with low biosorption (Orange II), while in case of the dye with high biosorption (Poly S119) the same was achieved even in absence of the adsorbent. Our study also stresses that instead of devoting all our efforts to elimination of bacterial contamination, we need to shift our focus to minimization of enzyme wash-out from continuous reactors. Both elongation of the retention time of dye and minimization of enzyme wash-out may be achieved by application of simultaneous adsorption within MBR.

While it is indispensable that new reports on enhancement of fungal activity under non-sterile conditions appear, one needs to consider other strategies to complement fungal degradation in order to achieve overall excellent dye removal in continuous reactors, as has been

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demonstrated in our study.

4. Conclusions

The long term performance of a submerged membrane fungi reactor with/without simultaneous PAC adsorption was observed while a synthetic textile wastewater containing either or both of two structurally different azo dyes was continuously fed. The specific conclusions drawn from this study are listed below,

- Preliminary batch tests with extracellular enzyme secreting pure fungi culture as well as sterile *in vitro* tests with crude enzyme solution confirmed the much slower degradation rate, but higher biosorption of the polymeric dye (Poly S119) as compared to the other azo dye (Acid Orange II) possessing a simpler structure.
- Direct filtration of the synthetic wastewater containing either of the two dyes achieved about 20% and 97% removal of Orange II and Poly S119 dye, respectively. However, pore-blocking was evident in case of direct filtration.
- A relative abundance of fungi (66%) without any specific control of bacterial contamination could be maintained in the MBR; however, enzymatic activity was below detection limit. Conversely, in absence of any withdrawal of sludge from the reactor, the MLSS concentration rose up to 20 g L^{-1} in the course of operation, but this did not disrupt the reactor operation.
- Excellent stable decoloration of the wastewater containing both the dyes was achieved with simultaneous PAC adsorption in the MBR. Comparison of the reactor-supernatant and the membrane-permeate qualities revealed the significant contribution of the membrane to the overall removal (biosorption/PAC-adsorption, cake layer filtration, biodegradation) of the dyes.
- In contrast to low TOC removal by fungi alone, the MBR containing mixed microbial community steadily achieved >98% removal, which improved further to >99% after PAC addition.
- Low-dose *in situ* chemical backwashing (500 mg Cl L^{-1}, 100 mL m^{-2}, twice/week) of the utilized spacer-filled hollow-fiber module enabled stable operation under the selected average flux (1.27×10^{-7} m^3 m^{-2} s^{-1}) and MLSS concentrations (up to 20 g L^{-1}).

This study, on one hand, provides insight into the strategies to enhance fungal dye degradation under non-sterile environment, and, on the other hand, demonstrates successful application of a unique version of MBR. However, a few further areas for optimization can be pointed out. For instance, probable enhancement of fungal decoloration by tuning the process parameters should be explored. Assessment of performance with a more representative wastewater comprising a mixture of several chemically diverse dyes along with other auxiliary textile chemicals would confirm the superiority of the proposed PAC-added fungal MBR. Also the issue of sludge withdrawal from PAC-amended MBR during prolonged operation deserves further attention in the future studies.

Acknowledgments

The authors would like to extend thanks to Japan Society for the Promotion of Science for the financial support (Project # 17106007) and to Mitsubishi Rayon Co. Ltd. for their supply of membrane modules.

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Figures

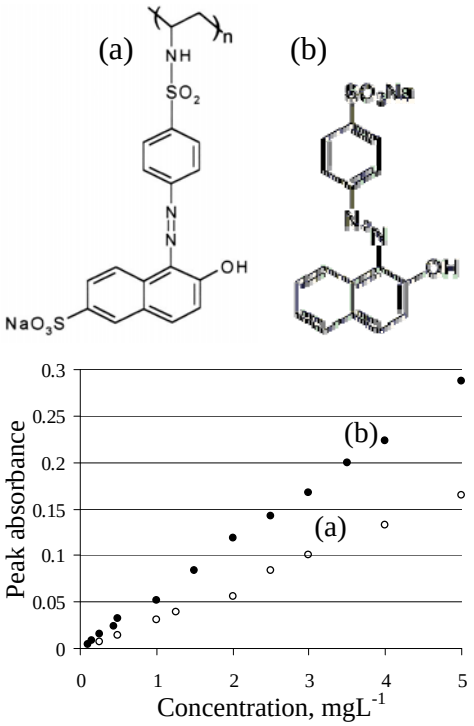

Fig.1 Chemical structures and peak absorbance vs. concentration graphs of Poly S119 (a) and Orange II (b) dyes.

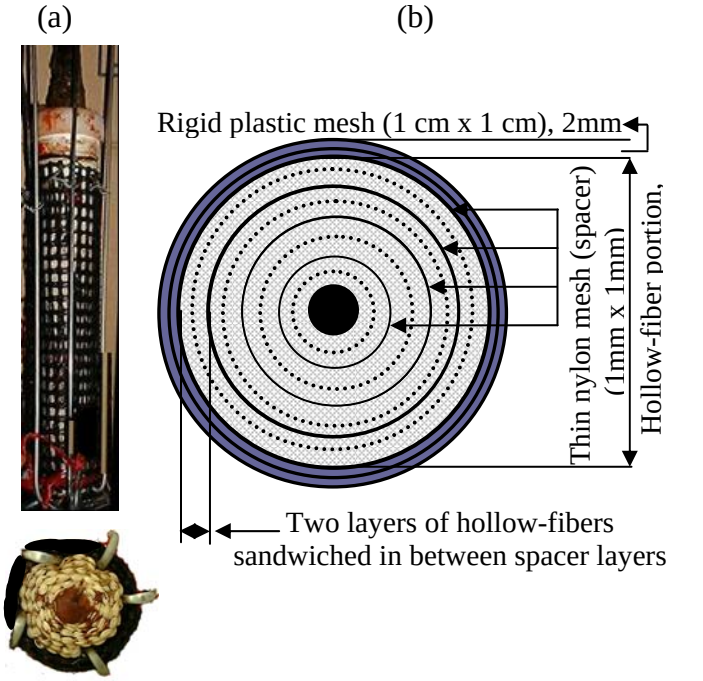

Fig.2 Spacer-filled hollow-fiber module
(a) Side and bottom views (surface-cleaning device visible)
(b) Enlarged schematic of fiber and spacer layer arrangement (surface cleaning device not shown in this case)

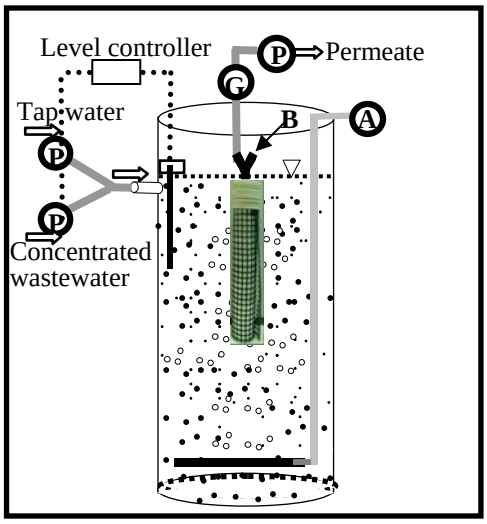

Fig.3 Schematic of laboratory setup
(**A**: Air pump, **B**: Backwash, **G**: Vacuum gauge, **P**: Pump)

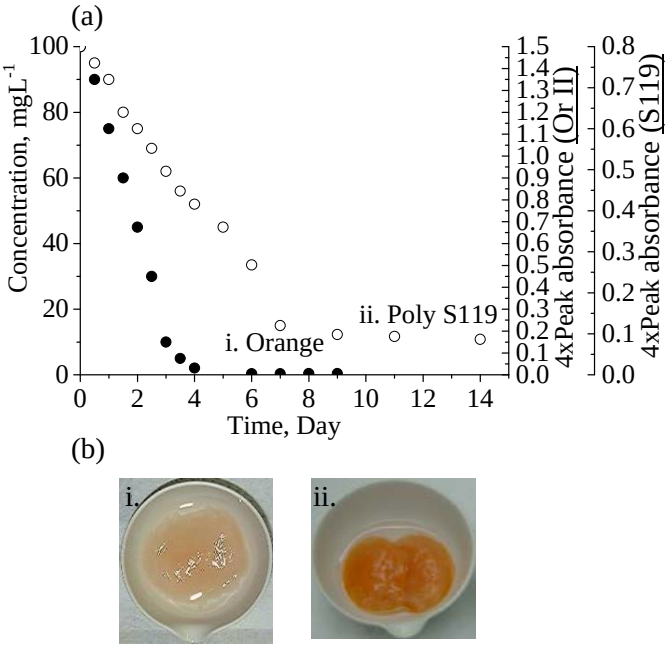

Fig.4 Removal of the dyes in pure culture batch test
(a) Decoloration of liquid medium
(b) Appearance of biomass at the end of batch test
(Linear relation of absorbance vs. concentration existed in the range shown here)

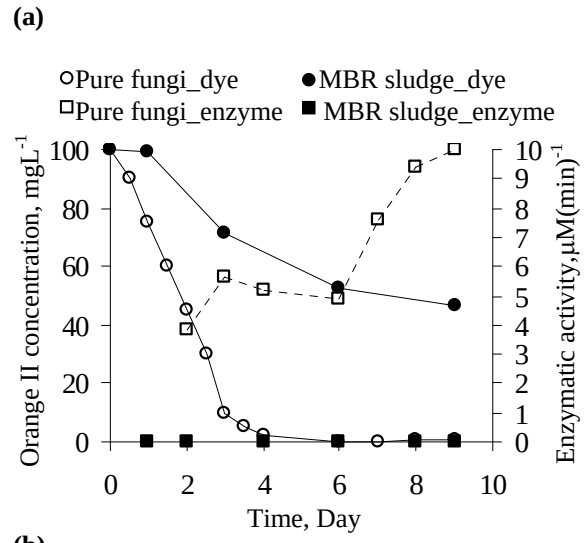

Fig.6 Activity of pure fungi culture and MBR sludge in batch test.
(a) Decoloration and enzymatic activity
(b)TOC removal

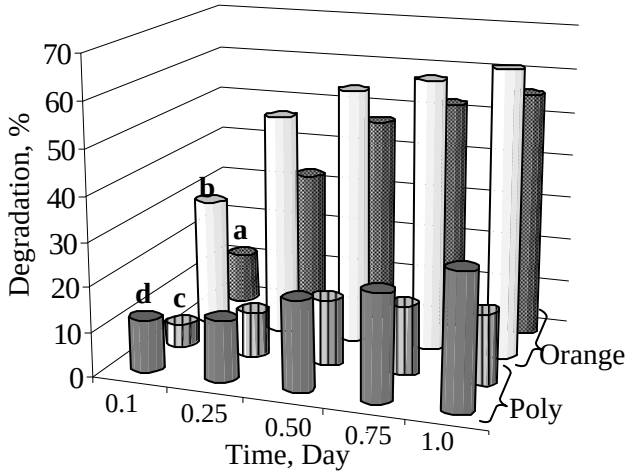

Fig.5 In-vitro enzymatic degradation of the dyes
a,**b**: Orange II (100, 50 mgL^{-1});
c,**d**:Poly S119 (100, 50 mgL^{-1})
(Initial enzymatic activity= 5.2 $\mu M(min)^{-1}$,
Loss of activity in one day= 60%)

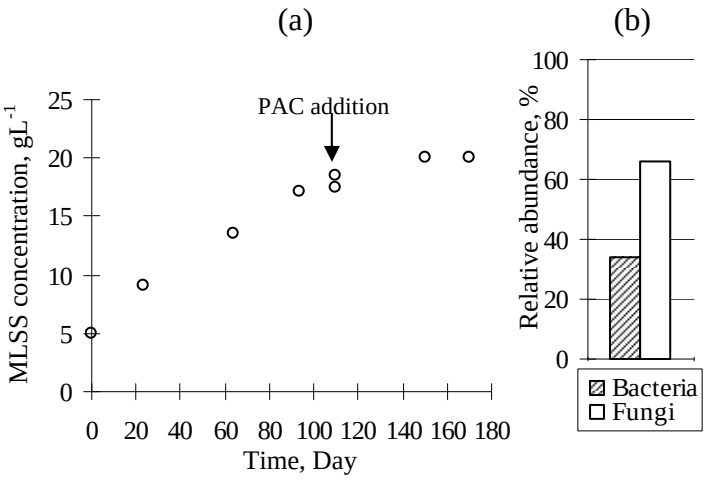

Fig.7 Change in MLSS concentration (a) and average relative abundance of bacteria/fungi in MLSS (b) during continuous operation of MBR.

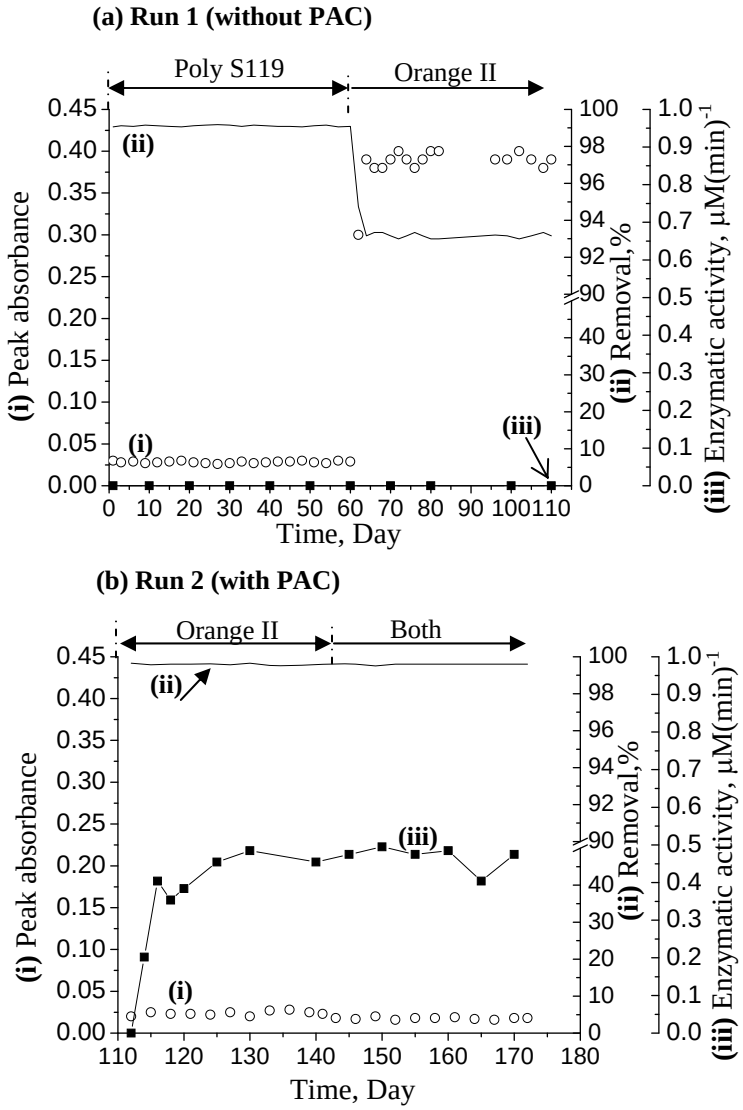

Fig.8 Decoloration and enzymatic activity throughout continuous operation of the MBR (i. Peak absorbance, ii. % dye removal, iii. Enzymatic activity)

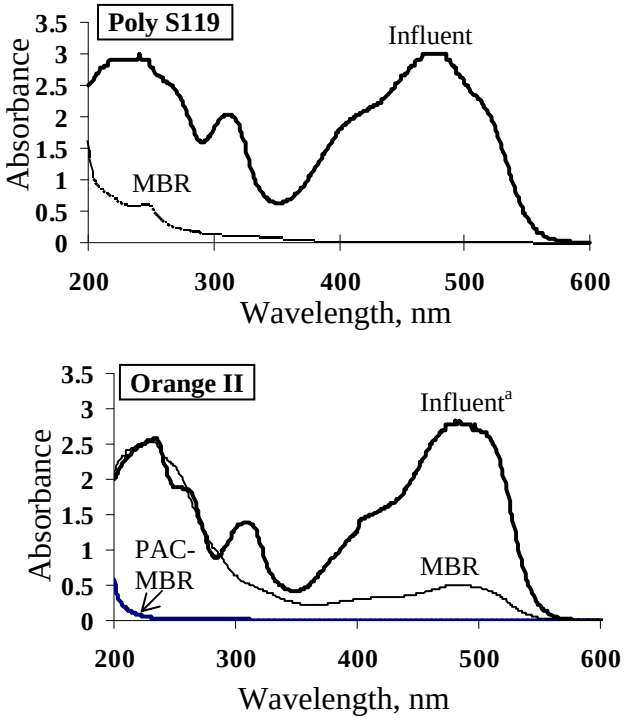

Figure 9 Typical observed change in UV-VIS spectra following MBR treatment of the dyes, evidencing excellent removal of Poly S119 even without PAC addition in MBR.
([a]Owing to very high absorbance per unit concentration of Orange II, the spectra corresponding to 50 mgL^{-1} has been shown for influent)

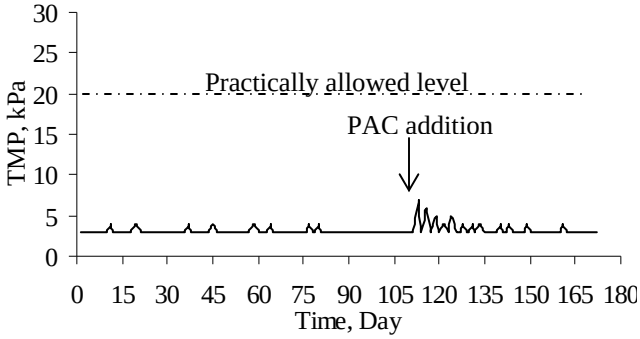

Fig.10 Minimal TMP variation during continuous operation of the MBR

Tables

Table 1. Adsorption of dye on fungi[a]

Dye	Adsorption, (mg dye/g dry biomass wt)	
	Inactive biomass	Active biomass[b]
Poly S119	10.41	8.2
Orange II	0.81	0.1

[a]0.17 g dry biomass wt.
[b]Data corresponding to day 14 in figure 4. Lower values compared to those for inactive biomass due to simultaneous biodegradation.

Table 2. Enzymatic dye degradation on PAC

Enzymatic activity, $\mu M(min)^{-1}$		Dye recovery, %		Enzymatic degradation on PAC[c], %
Original solution	Adsorbed on PAC[a]	Control[b]	Enzyme-soaked PAC[b]	
6.6	4.4	51	6	47.9

[a]Estimated from difference of original and spent enzyme solution
[b]100% decoloration (adsorption) for both cases
[c]Minimum degradation taking into account the dye extraction efficiency (51%)

Table 3. Removal of dye by membrane filtration only[a]

Dye	Removal of dye, %	
	Only Dye solution	Synthetic wastewater[b]
Poly S119	56	97[c]
Orange II	4.2	20

[a] 24 hrs batch test, adsorption on membrane negligible
[b] TSS=2.6 g L^{-1}; similar removal with (Dye+ Starch) mixture
[c] Pore-blocking occurred for longer operation

Table 4. Dye and TOC removal in the course of continuous operation of the MBR

Run #	Dye	Day	Average Absorbance		Average dye removal, %		Average TOC removal,%	
			Supernatant	Permeate	Supernatant	Permeate	Supernatant	Permeate
1[a]	Poly S119	1-60	1.00	0.03	68.3	99.1		
	Orange II	61-110	1.02	0.39	82	93.2	96.8	98.4
2[b]	Orange II	111-141	0.40	0.023	93	99.6		
	Both	142-172	0.31	0.018	93	99.6	98.3	99.5

[a,b]Without/with PAC addition in MBR