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RESEARCH HIGHLIGHTS

- A GAC-coated membrane prevented enzyme washout and improved dye removal by a fungal MBR.
- Both suspended culture and the immobilized enzyme on the membrane degraded dye.
- A combined effect of HRT, membrane flux and amount of GAC on removal was observed.
- Under the same dye loading, longer HRT yielded better removal efficiency.
- Membrane fouling caused deterioration of the removal efficiency.

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Abstract
Additional granular activated carbon (GAC) layers on the membrane module within a whole cell fungal membrane bioreactor (MBR) set up to treat dye wastewater was effective in minimizing enzyme washout and in improvement of decoloration (degradation of the dye). Supporting batch tests and continuous monitoring of the quality of bioreactor-supernatant and membrane-permeate revealed that biodegradation was effected by both the suspended culture and the dynamically immobilized enzyme on the GAC-coated membrane. In a control MBR, the efficiency of dye removal was variable; in contrast, 85% to near complete removal of dye was achieved using the MBRs equipped with a membrane with additional GAC layers. A combined critical effect on dye removal efficiency of hydraulic retention time (HRT), instantaneous membrane flux and the amount of GAC coating was observed. Plausible explanations are presented for novel observations such as the achievement of better removal efficiency in the case of the longer HRT, even though the dye loading under different HRTs was kept the same by varying its concentration in the synthetic wastewater. The effect of membrane fouling on removal efficiency is highlighted and approaches to achieve stable long term performance are discussed.

Keywords: enzymatic membrane reactor; granular activated carbon (GAC); hollow fiber module; membrane bioreactor (MBR); dye wastewater; white-rot fungi

1. Introduction

The application of submerged membrane bioreactor (MBR) processes in municipal and industrial wastewater treatment has grown substantially in recent years owing to several advantages including excellent effluent quality, low sludge production, small foot print, robustness and flexibility for future expansion [1-2]. MBR systems are particularly attractive for treatment of recalcitrant wastewater where long sludge retention times (SRT) that facilitate physical retention and subsequent hydrolysis, are critical to achieving biological degradation of pollutants. Researchers have put forward novel modifications to conventional designs of MBR in order to enhance removal performance. Such modified designs include integrated anoxic/aerobic MBR, biofilm MBR, thermophilic MBR, bio-augmented MBR, and so on [3]. The membrane is an integral part of MBR, performing the role of solid-liquid separation, that is, they retain suspended solids (SS) as well as the soluble materials adsorbed on the SS, and thereby enhances removal compared with conventional activated sludge processes (CAS). To date, most MBR research has addressed membrane fouling mitigation (to enhance hydraulic performance of the membrane) and treatment performance improvement of the bioreactor separately. Membrane fouling mitigation strategies include modification of membrane surface and module design, alteration of mixed liquor characteristics and tuning operating conditions [4-5]. Notably, modification of surface properties of membranes to be used in MBR has been attempted mainly from the point of view of fouling mitigation [5-6].

In contrast to the membrane in a whole cell MBR, that in an enzymatic membrane reactor (EMR) is modified to act as supports for enzyme immobilization. Thus, the membrane takes part in catalytic degradation of pollutants with simultaneous downstream separation of the transformation products [7-8]. Investigations regarding application of enzymatic membrane reactors for wastewater treatment have been mainly carried out on low strength wastewater with limited total organic carbon (TOC) and total suspended solid (TSS) loadings [9]. Notably, the stabilities and catalytic properties of enzymes immobilized on membranes are dramatically affected by high strength wastewaters [10]. Gradual loss of enzymatic activity due to various physical, chemical and biological inhibitors under wastewater conditions is inevitable.

We envisaged that modification of membrane modules to dynamically immobilize pollutant degrading enzymes on membranes within a whole cell MBR may bring about the added advantages of continuous enzyme production (by microbes) and prevention of washout of enzyme. The resultant continuous application of enzyme, minimizing washout may mean undisrupted operation even under conditions where denaturation of enzyme would occur over time. Some studies have reported that addition of an adsorbent column downstream from the enzymatic membrane reactor resulted in improved removal performance [11]. In line with the above discussion, utilization of an adsorbent (e.g., GAC) as an enzyme immobilization support on membranes in whole cell MBR may be further beneficial for enhanced removal of contaminants from wastewater.

The proposed concept can be tested using a fungal bioreactor system which is known to suffer from unstable biological performance due to the limitations, namely bacterial contamination destabilizing fungal activity [12], and loss of the extracellular enzymes and mediators with discharged water [13]. It is worth noting that fungal reactors have been studied to find an alternative to treat various hazardous chemicals including dyes which are not degraded in bacteria-dominated conventional activated sludge. In contrast to the numerous reports on the excellent degradation capacity of pure fungus cultures or the relevant extracellular enzyme in small-scale batch-tests, there exist only a few studies which report dye degradation performance in continuous reactors[14]. Among the few studies concerning continuous fungal reactors, only a handful has explored dye degradation under non-sterile environments [14-18]. Only a few studies [13-14] so far have specifically investigated the potential of prevention of enzyme washout from continuous fungal reactors by applying simultaneous activated carbon adsorption within bioreactor. It is clear that systematic studies on enhancement of fungal degradation of dyes in continuous reactors under non-sterile environments are imperative. In this context, the proposed GAC-coated membrane may be useful.

In order to address these research gaps, the current study explored the efficacy of a GAC-coated membrane within a fungal MBR in improving performance of hazardous dye removal in wastewater treatment. Preliminary investigations showed that additional GAC layers on the membrane module enhanced decoloration and substantially decreased enzyme washout. Long-term investigations were carried out to ascertain the factors governing the performance

of the membrane module. The effect of membrane fouling on removal efficiency was also highlighted. This study: (i) demonstrates the application of enzyme-immobilization on membranes within a whole cell membrane bioreactor for the first time, and (ii) suggests a potential solution to unstable removal performance of fungal reactors used for treating recalcitrant wastewater.

2. Experimental

2.1. Microorganism, chemicals and synthetic wastewater

The white-rot fungus *Coriolus 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 (Acid Orange II, 33-100 mg L^{-1}) and starch (2 g L^{-1}) – two common components in real textile wastewater – and other nutrients into tap water. The other components of the synthetic wastewater were as follows: 0.1 g L^{-1} urea, 2 g L^{-1} KH_2PO_4, 0.099 g L^{-1} $CaCl_2$, 1.025 g L^{-1} $MgSO_4 \cdot 7H_2O$, 0.001 g L^{-1} thiamine and 1 mL L^{-1} trace elements. Stock trace elements solution was prepared by dissolving 0.125 g $CuSO_4 \cdot 5H_2O$, 0.05 g H_2MoO_4, 0.061 g $MnSO_4 \cdot 5H_2O$, 0.043 g $ZnSO_4 \cdot 7H_2O$, 0.082 g $Fe_2(SO_4)_3 \cdot 14H_2O$ in 1 L of Milli-Q water [19]. Acid Orange II (Sigma-Aldrich Co., USA) is a low molecular weight (350 g mol^{-1}) soluble, orange dye (peak absorbance at 481 nm). Granular activated carbon (GAC, F400-OS) was received from Calgon Mitsubishi Chemical Corporation, Japan. According to the manufacturer, the average particle size and pore diameter of the GAC was 1.1 mm and 2.44 nm, respectively. The GAC possessed a BET surface area, micropore volume and mesopore volume of 793 m^2 g^{-1}, 0.363 cm^3 g^{-1}, 0.123 cm^3 g^{-1}, respectively.

2.2. Batch test description

2.2.1 Harvesting crude enzyme

Pure cultures of fungi were grown into an agglomerated mass [20] in synthetic wastewater (section 2.1) excluding dye. Following the confirmation of secretion of the target amount of enzyme (see section 2.5), the medium was harvested as 'crude enzyme' by filtration using a 0.45 μm cellulose acetate filter. In order to capture the effect of co-existence of other organics along with enzyme on dye degradation, enzyme solutions possessing enzymatic activity (E) and total organic carbon (TOC) of 7-98 μM min^{-1} and 62-138 mg L^{-1},

respectively, were obtained by varying the dose of starch (main contributor of TOC) and the incubation period. Accordingly, the extent of dye degradation (see section 2.2.3) was assessed as a function of E/TOC ratio, in addition to absolute E.

2.2.2 Incubation of enzyme-preloaded GAC with dye

Two 100 mL conical flasks containing previously washed GAC (2 g) were set up. Initially, both flasks contained 70 mL Milli-Q water. The flasks were autoclaved to eliminate microbial contamination and cooled to room temperature. In the test flask, water was replaced by the same volume of crude enzyme. The contents of both flasks were then stirred (70 rpm) using a magnetic stirrer for 12 h The respective media from the flasks were decanted. The amounts of enzyme and TOC adsorped to GAC were estimated from the difference of their respective levels in the original crude enzyme solution and the spent, decanted media. Dye solution (70 mL of 1 g L^{-1}) was added aseptically into the control (only GAC) and enzyme-preloaded GAC flasks and stirred for one day for complete decoloration of the media.

2.2.3 Estimation of dye degradation

At the end of the experimental period, the decolored liquid media were decanted and methanol (80 mL) was added to each flask. In order to enhance dye extraction from the activated carbon, the flasks were placed on a hot-plate stirrer to apply strong stirring (150 rpm) and intermittent heating at one minute on/off intervals. Within a few minutes after the mixtures started boil, heating was discontinued and the methanol solution along with the extracted dye was collected by filtration using a 0.45 μm cellulose acetate filter. This sequence was repeated several times till the the amount of dye extracted was negligible (as judged by the absorbance of the media at 481 nm, see section 2.5). Under these experimental conditions, the dye extraction efficiency for GAC was 95%. Dye degradation resulting from enzymatic activity was estimated from the difference in the amount extracted from flasks containing only GAC and those containing enzyme-preloaded GAC. The degradation percentage calculated in this way was multiplied by the extraction efficiency and the resultant value was reported as percentage minimum dye degradation.

2.3. GAC-coated membrane module

A spacer-filled bundle (diameter = 4.5 cm, height = 22 cm) of micro-porous (0.4 μm), hydrophilically treated polyethylene hollow-fibers (surface area = 1.07 m^2) obtained from Mitsubishi Rayon, Japan, was utilized in this study. 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]. GAC-coated meshes were wrapped around the membrane module to obtain the GAC-coated module.

Water-resistant silicon adhesive was applied over a 15 cm x 20 cm piece of nylon mesh (#34; 1 mm x 1mm) in a way such that its openings were not completely blocked, and the mesh remained sufficiently porous. GAC (8 g) was then spread over the mesh and allowed to bond with the adhesive overnight. The GAC-coated mesh was then submerged into Milli-Q water for several hours to moisten the GAC. Initially, two such GAC-coated meshes were wrapped around the membrane module. An additional layer of GAC-coated mesh was introduced at the later part of the study (section 3.3, Run R2— V, VI and R3— IV, V). Figure 1 shows the GAC-coated mesh assembly and the operation concept.

[Figure 1]

In practice, the membrane is operated under an intermittent suction mode (providing a relaxation period), and periodic backwashing is performed to retard fouling. However, in this study as the reactor volume was very small (section 2.4) compared to the high membrane surface area of the module utilized, periodic in situ backwashing would dilute the reactor media. Therefore, no backwash was performed during any specific run, but rather long relaxation periods (24 - 465 min depending on the run, see section 2.4), were applied. Specific details for individual experiments are given in the relevant sections of the text. After each run (four weeks) the membrane module was lightly squeezed by hand to return the loosely trapped mixed liquor into the reactor. The module was then softly backwashed ex situ with permeate under a flux of 0.29 L m^{-2} h^{-1} for 30 min without dismantling the GAC-coated mesh assembly from over the membrane. Only a negligible amount of GAC was observed to be detached from the mesh during the mild backwashing. A new run was initiated by returning the thus cleaned module to the bioreactor. In order to assess the effect of fouling on removal performance, the final run with R2 (see section 2.4) was extended beyond four weeks. Further details about this particular run are available in section 3.5.

2.4. Design and operating conditions of the MBR

The GAC-coated membrane as described in section 2.3 was submerged in a 2 L glass reactor (diameter = 8.5 cm, height = 34.5 cm) with a working volume of 1.5 L. A diffuser supplied continuous air from the bottom of the reactor with an intensity of 5 L min^{-1} for complete mixing to facilitate supply of dissolved oxygen to the microbes. The temperature of the reactor was controlled at 29 ± 1 °C. *C.* v*ersicolor* (10 g dry wt.) aseptically grown to an agglomerated form for two weeks in several 1 L Erlenmeyer flasks (each containing 500 mL of the synthetic media) was inoculated into synthetic media (5 L) and then allowed to grow further under aeration. The MBR was inoculated with prepared stock sludge to obtain a mixed liquor suspended solids (MLSS) concentration of 8 g L^{-1}. Notably, after inoculation, no attempt was made to avoid bacterial intrusion from the environment to the stock sludge. Therefore, the stock sludge that was used to inoculate the MBR was composed of a mixed microbial community of fungi and bacteria [14].

For the first part of the study (section 3.2) three such MBRs (R1: without GAC coating, R2 and R3: with GAC coating) were operated in parallel. All three MBRs were inoculated with the same stock sludge. R1 and R2 were operated under sequencing batch mode with three cycles of suction (10 min)/ fill (5 min)/ idling (465 min) periods each day. It is noteworthy that sequencing batch operation mode was initially chosen to obtain a sludge of granular morphology [21], which has been reported to enhance fungal decoloration [20]. In order to capture any effect of operation mode on decoloration, R3 was operated in continuous mode (6 min suction/ 24 min idling period). The MBRs were operated in parallel under the same dye loading of 0.1 g $L^{-1}d^{-1}$ and HRT of 1 day. Owing to poor performance, the operation of R1 was discontinued after the initial trial (section 3.2).

Further detailed investigation about the influence of different factors (hydraulic retention time (HRT), dye loading, instantaneous membrane flux and amount of coated GAC) was carried out in conjunction with R2 and R3 (section 3.3). Because membranes within MBRs are operated under intermittent suction modes, the actual (instantaneous) flux is always greater than the calculated average flux. In this study, under the same HRT, the average flux imposed on the membrane in each MBR was the same (0.02 and 0.06 L m^{-2} h^{-1} in case of a

HRT of 3 and 1 day(s), respectively). In order to study the effect of instantaneous flux under the same average flux, the instantaneous flux of R3 was altered (0.29 L m^{-2} h^{-1}) from that applied to R2 (0.8-2.8 L m^{-2} h^{-1}) by effecting different intervals of suction and idling periods (section 3.3). While studying the effect of HRT, the dye (and TOC) loading was maintained by changing the concentrations of all the components in the synthetic wastewater. In order to study the combined effect of instantaneous flux (L m^{-2} h^{-1}) and the amount of GAC on the membrane surface (g m^{-2}), a parameter named 'instantaneous loading' on GAC ((L m^{-2} h^{-1})/ (g m^{-2})) was introduced.

Each of the runs lasted for four weeks. The final run of R2 (R2-VI, section 3.3) was extended beyond four weeks to study the effect of fouling on removal performance. Except for the MLSS sample and the small amount of MLSS washed out during ex situ backwash of the membrane, no sludge was withdrawn from the MBRs. Specific details about each run have been provided in the relevant sections of the text.

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). The activity of fungal enzyme (laccase) was measured by monitoring the change in absorbance at 468 nm due to the oxidation of 2,6-dimethoxy phenol (DMP) at room temperature over 2 min. Enzymatic activity was calculated from the molar extinction coefficient, $\varepsilon = 49.6\ mM^{-1}\ cm^{-1}$ [22] and expressed in μM substrate per minute (detection limit = 0.0045 ± 0.00074). Membrane-permeate samples were analyzed as collected, while samples collected from within the MBR were centrifuged (2150 × *g*) to obtain the supernatant and then analyzed for color, TOC and enzyme activity. The relative abundance of fungi/bacteria in mixed liquor suspended solids (MLSS) was estimated following the size-based fractionation method of Jasti et al. [23] using samples that were diluted 20-fold. 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. Enzymatic dye degradation on activated carbon

Co-adsorption of enzyme and dye on GAC and subsequent enzymatic dye degradation was confirmed by the dye degradation batch tests. Depending on the initial conditions, up to 50% dye degradation was achieved within the incubation period (Figure 2). It is worth noting that the crude enzyme solution used here contained, in addition to enzyme, other residual components of the growth media which can also adsorb onto GAC. In order to capture the effect of the possible co-existence of other organics, the dye degradation efficiency in Figure 2 has been plotted with reference to the initial ratio of enzymatic activity (E) and TOC in the crude enzyme solution and the E/TOC actually pre-adsorbed onto GAC. It is evident from the E/TOC plot that coexistence of other organics significantly interfered with enzyme adsorption onto GAC. Furthermore, by comparing the dye degradation efficiency with the absolute amount of adsorbed E and E/TOC ratio, respectively, it appears that E/TOC ratio, and not the absolute amount of E, is more important in dye degradation. For the catalytic function of the enzyme to proceed, the target substrate (dye in this case) needs to bind to the active site of the enzyme [24]. Interference of other organics may render the enzyme unavailable for interaction with the target compound (dye), explaining the observed importance of E/TOC.

[Figure 2]

Interestingly, within the tested range of adsorbed E and E/TOC, the dye degradation efficiency on GAC did not vary significantly, indicating that high enzymatic activity is not required for a high level of decoloration. This observation is in line with that of Blanquez et al. [15]. It is, however, important to note that the interference of co-existent organics in adsorption of enzyme onto GAC and subsequently with dye degradation may have greater implications in continuous bioreactors where, due to enzyme washout and destabilization as a result of contamination with bacteria, the net amount of E present within the bioreactor may be orders of magnitude lower (section 3.2) than that used in the batch test. Nevertheless, the batch test served the prime purpose of confirmation of enzymatic dye degradation on GAC.

3.2. Enzyme washout prevention and decoloration improvement by GAC-coated membrane

Of the two MBRs operated under sequencing batch mode (section 2.4), the performance of R1, without any GAC-coating on the membrane, was much worse than that of R2 which contained a GAC-coated membrane. Detection of enzyme in the membrane-permeate (Figure 3a) confirmed enzyme washout from both the MBRs. However, in the absence of any GAC-coating on the membrane, the extent of enzyme washout was greater in R1. Notably in the case of R1, the enzymatic activity both in the bioreactor and the membrane-permeate sharply dropped to a non-detectable level within five days. This was accompanied by a sharp increase in the absorbance corresponding to the orange dye (481 nm) of membrane-permeate to a value of 2 (corresponding to only 65% removal) within five days (Figure 3b) which continued to deteriorate further in the following days (data not shown), confirming the detrimental effect of enzyme washout on dye removal in line with previous studies [13-14, 20]. On the other hand, the enzymatic activity within R2 reduced to a level lower than the initial value, but did not drop to an undetectable level. The enzyme washout from R2 (indicated by level of enzyme in permeate) also stabilized to a lower value than the initial, and the color in the membrane permeate stabilized at an absorbance of 0.9 (85% removal). The comparison of performance of R1 and R2 confirmed that the GAC coating on the membrane was successful to a certain extent to prevent enzyme washout from R2, which favorably influenced dye removal.

[Figure 3]

Interestingly, in contrary to our initial expectation regarding better removal by granular sludge under sequencing batch mode operation (see section 2.4), both the enzymatic activity and decoloration efficiency of R3 (continuous mode) was significantly better than that of R2 (sequencing batch mode) (Figure 3). Sequencing batch operation necessitates withdrawal of a large volume of medium at a time, and in that case application of a higher instantaneous flux is inevitable. This, in turn, may increase the leakage of enzyme from the GAC layer on the membrane, and subsequently deteriorate decoloration efficiency. Although in line with the initial expectation, the sludge in R2 did appear more granulated (data not shown); any probable positive effect of such sludge granulation may have been offset by the detrimental effect of higher instantaneous flux. An inverse relationship of flow-rate and the operational stability of the fungal enzyme tyrosinase, immobilized on a polyacrylamide-based support, was reported by Vilanova et al. [25]. Furthermore, at high flow-rates the degree of oxidation of 2,6-dimethoxyphenol by immobilized laccase in a fluidized-bed system was observed to decrease [26], and in another study, an inverse relationship among flow-rate and the

operational stability of a laccase-immobilized polyethersulphone membrane reactor was reported [27]. In the current study, both the enzymatic activity in the bioreactor and decoloration in R2 improved significantly after 50% reduction in instantaneous flux (by increasing the 'suction' period and decreasing the 'idling' period), while keeping all other parameters (e.g., HRT, dye loading) the same (Figure 3, beyond 'day 15'). This observation provided further evidence in support of the hypothesis that higher instantaneous flux (higher instantaneous loading on GAC) has a detrimental influence on enzyme washout prevention and decoloration.

Short-term enhanced organics removal due to instantaneous adsorption on adsorbent-coated membrane has been reported in certain studies dealing with secondary wastewater or surface water with low total suspended solid (TSS) and TOC [28-29]. The influence of biodegradation was not addressed in those studies. On the other hand, by utilizing a powdered activated carbon (PAC) precoated coarse pore mesh in conjunction with a bioreactor, Ye et al. [30] could sustain the chemical oxygen demand (COD) removal efficiency at a level comparable to that achieved by a microfiltration membrane. Enhanced removal of phenol in a hollow fiber membrane reactor with entrapped microbes was achieved when GAC was incorporated during synthesis of the membrane [31]. On the other hand, pre-immobilization of various enzymes on membranes has been reported to facilitate conversion of hazardous chemicals concomitantly with the separation of the insoluble by-products [32-35]. The long-term stable performance of R3 (Figure 3) suggests that the proposed design combines the advantages of adsorbent-precoated membranes (adsorption) and enzyme immobilized membranes (biodegradation).

3.3. Factors affecting performance of GAC-coated membrane

Since preliminary comparison of the performance of R2 and R3 under the same HRT and dye loading indicated the influence of instantaneous flux on dye removal performance, an in-depth experimental scheme was designed to systematically monitor the effect of the factors namely, instantaneous flux, amount of GAC-coating on the membrane, HRT and dye loading. Table 1 summarizes the relevant data.

[Table 1]

For a given wastewater composition the dye loading increases with the decrease in HRT. Effect of dye-loading on the performance of various types of bioreactors has been well-documented in the literature [36-38]. However, there appears to be no study that mentions influence of HRT under the same dye loading. As noted in section 2.4, in order to study the influence of HRT under the same dye loading, the dye (and TOC) loading under various HRTs was maintained by changing the concentrations of all the components in the synthetic wastewater. Under the tested ranges of HRT (1-3 days) and dye loading (0.033-0.1 g L^{-1} d^{-1}), the removal performance was more dependent on HRT. For instance, under the same dye loading of 0.1 g L^{-1} d^{-1}, R2 exhibited better removal efficiency when the HRT was extended from 1 to 3 days (Run R2-II vs. R2-IV); however, under the HRT of 3 days, virtually no difference in removal efficiency was observed when a lower dye loading was applied (Run R2-III vs. R2-IV). The fact that the removal efficiency of R2 was lower in case of the shorter HRT even though the dye loading in the feed was kept the same, suggested that the utilized amount of GAC was not sufficient to completely prevent the leakage of enzyme from the reactor under the applied instantaneous flux. Indeed, under the same HRT (1 day) and dye loading (0.1 g L^{-1} d^{-1}), the removal performance was observed to improve when the amount of GAC was increased from 14.95 to 22.43 g m^{-2} (Run R2-II vs. R2-VI). Ye et al. [30] also reported better retention capacity of a composite module when the thickness of PAC coating on a coarse pore mesh was increased.

On the other hand, in line with the preliminary observations (section 3.2) , under the shorter HRT (1 day) the performance of R2 (instantaneous flux of 0.8-2.8 L m^{-2} h^{-1}) remained significantly worse than that of R3 (instantaneous flux of 0.29 L m^{-2} h^{-1}), even when the amount of GAC on membrane in both the MBRs was increased (Run R2-VI vs. R3-V). Notably, irrespective of the dye loading and amount of GAC, both R2 (Runs R2-III—V) and R3 (Runs R3-II—IV) accomplished almost complete dye removal when a HRT of 3 days was applied.

The difference in performance of R2 and R3 can be further addressed in reference to Figure 4 which portrays the combined critical effect of HRT, membrane flux and amount of GAC on removal efficiency. In this study, the instantaneous loading on GAC (membrane flux/ GAC amount over membrane surface area) under the same HRT was varied by changing the membrane flux (and accordingly adjusting the suction/ idling period) and/ or the amount of

GAC. Figure 4 shows that the effect of instantaneous loading on GAC on removal performance was more prominent in case of the shorter HRT. Shorter HRT meant withdrawal of larger volume of treated water by application of higher instantaneous membrane flux. This consequently implied a higher probability of enzyme leakage from the GAC layer on the membrane. In line with this argument, it is likely that, during each suction period, a bulk amount of enzyme was washed out under higher instantaneous flux operation of R2. A greater extent of enzyme leakage, therefore, was the main reason for the deterioration of removal performance of R2 as compared to R3.

[Figure 4]

In the system under study, biodegradation can occur both by the suspended culture and the immobilized enzyme on the GAC-coated layer over the membrane. Enzyme leakage prevention therefore results in a greater net enzymatic activity in the bioreactor. Therefore, in all the cases when the membrane-permeate quality was improved, a simultaneous improvement of the bioreactor performance (supernatant quality) was observed (Figure 5). The order of the MBR experimental runs is shown in Table 1, and reveals a reproducible trend of supernatant quality improvement/deterioration with the change of instantaneous loading on GAC. This confirms that the supernatant quality improvement was due to reduction of enzyme washout and not due to other factors influencing biological performance; all other conditions were kept the same during all experimental runs to avoid any alteration of biological activity. Although fungal laccase has been recurrently shown to be involved in the degradation of recalcitrant compounds, it may be involved in a wider range of other physiological functions involving interaction with other microorganisms [39-40]. An ncrease in laccase activity was observed during interactions between different white-rot fungi [39]. Furthermore, previous studies have reported on bacterial destabilization of fungal activity [12, 17, 41]. It is likely that fungal laccase also plays an antibacterial role. Therefore, prevention of enzyme washout would imply availability of adequate amounts of enzyme to simultaneously minimize bacterial disruption and complete dye degradation. Further studies will be needed to clarify this aspect.

[Figure 5]

3.4. Effect of GAC-coated membrane on TOC and UV absorbance reduction

Dye bath effluent constitutes only a minor part of TOC in textile wastewater. In addition to the nutrient-deficient dye bath effluent that is relatively resistant to biodegradation, different other streams of wastewater, which contain relatively readily biodegradable organics in high concentration, may originate from a textile mill [42]. Simultaneous achievement of color and TOC removal is, hence, indispensable.

In line with dye removal performance, the TOC removal performance was also significantly influenced by the instantaneous membrane flux and the amount of GAC on the membrane surface (Table 2). Notably, any increase in GAC content or reduction in applied flux resulted in enhanced TOC removal which was greater than could be expected due to improvement in dye removal only. For instance, under an instantaneous flux of 0.29 L m^{-2} h^{-1}, when the amount of GAC was increased from 14.95 to 22.4 g m^{-2}, the permeate absorbance decreased from 0.225 (4 mg L^{-1} dye) to 0.03 (0.5 mg L^{-1} dye) (Table 1), implying a TOC removal improvement of 2 mg L^{-1}. At the same time, the TOC in the permeate dropped from 16 to 3 mg L^{-1} (Run R3-I vs. R3-V, Table 2). This observation suggested that although the main aim of application of GAC was to improve dye removal, other organics, in addition to dye, were also adsorbed onto GAC and were subsequently biodegraded. Our results are in line with that of Li et al. [43] who reported that under competition with other organics in the synthetic wastewater, only a portion of added PAC into MBR was effectively utilized for the target micropollutant adsorption. Nevertheless, of special interest in the current study was the excellent diminution of UV absorbance, which confirmed the removal of UV-absorbing aromatic fragments following the degradation of the chromophoric group of the dye (Figure 6).

[Table 2]

[Figure 6]

3.5. Membrane fouling and its influence on removal performance

Thiruvenkatachari et al. [29] reported that membrane fouling of a microfiltration membrane fed with secondary treated wastewater was effectively minimized by pre-coating it with PAC. While treating surface water, Galijaard et al. [44] observed that a dynamic layer of certain adsorbent materials can be beneficial for long-term restoration of the permeability. By pre-coating a coarse pore (56-μm) terylene filter cloth with PAC, Ye et al. [30] demonstrated a

similar hydraulic performance of the tested module to a microfiltration module. The main purpose of this study was to assess the MBR performance-enhancement due to the utilization of a GAC-coated membrane. Although the experimental design of this study was not specifically aimed at assessing the fouling mitigation capacity of the GAC coating over the membrane, some important inferences regarding membrane fouling can be made from the relevant interesting observations made throughout the study.

In agreement with other reports [29, 44] the GAC layer over the membrane did not increase the intrinsic membrane filtration resistance (data not shown). Under the operating and cleaning conditions of the membrane module as depicted in section 2.3, the TMP across the composite module during all the runs remained stable at 3 kPa. However, during the extended final run with R2, the TMP across the module was observed to increase to 35 kPa, indicating significant fouling (Table 3). During the previous runs, one ex situ backwash after each run was sufficient to keep the TMP stable. However, once severe fouling had occurred owing to the absence of any backwash for an extended period, the TMP could not be reinstated to its original level by a mild backwash. A notable deterioration of the permeate quality (Table 3) was also observed at this stage. Both TMP and removal efficiency were restored to their respective original states when the composite module was returned to the reactor after cleaning the GAC layers and the module by ex situ water-jet rinsing (Table 3). Evidently, sludge deposition over GAC layer hampered enzymatic dye degradation by reducing the contact of enzyme and dye.

Observations conducted after dismantling the GAC layers revealed that while only a small amount of sludge accumulated over the outer-most GAC layer, a considerable amount of sludge was deposited within the inner layers and also over the main module (Figure 7). Nevertheless, owing to the compactness of the main module, only a moderate amount of sludge intruded into its core. This is in line with our previous reports on spacer-filled hollow fiber modules [19, 45]. The effect of fouling on TMP and removal performance means that periodic backwashing would be required to mitigate fouling and also to maintain the removal efficiency. It is important to note that in contrast to negligible GAC detachment during mild backwash, about 4% GAC was lost during the ex situ water jet rinsing. Under the operating conditions of this study, periodic (every four weeks) mild ex situ backwashing could maintain stable operation; however, in a scaled up system more frequent and vigorous backwashing may be necessary, and in that case the stability of the GAC-coat would be crucial. Future

studies will need to formulate stable GAC-coating techniques. In this context, wrapping the module with activated carbon cloth [46] may be a viable alternative.

[Table 3]

[Figure 7]

4. Conclusions

This study demonstrates for the first time that additional GAC layers on the membrane module within a whole cell fungal MBR treating dye wastewater is effective in enzyme washout prevention and decoloration improvement. The proposed design combines the advantages of the adsorbent-precoated and enzyme immobilized membranes, respectively, with that of a whole cell bioreactor in that it utilizes an adsorbent (GAC) as the support for dynamic immobilization of extracellular enzyme secreted by the whole cell microbes in suspension. Detailed investigations revealed the combined critical effect of HRT, instantaneous membrane flux and the amount of GAC coating on removal efficiency. Under the tested design conditions, beyond a certain instantaneous loading on GAC (instantaneous membrane flux/ GAC amount over membrane surface area), the removal efficiency reduced to some extent. Approaches to achieve stable long term performance have been highlighted. This study manifested a strategy to improve overall removal efficiency by minimizing enzyme washout from the bioreactor. Investigation is underway to combine the proposed design with ways (e.g., manipulation of morphology) to improve enzyme secretion and biodegradation capacity of the microbes.

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TABLES

Table 1: Operational parameters and performance during different runs

MBR	Run #	HRT (Day)	Dye loading, ($g\ L^{-1}\ d^{-1}$)	GAC coating on membrane, ($g\ m^{-2}$)	Instantaneous flux ($L\ m^{2}\ h^{-1}$)	Instantaneous Loading on GAC ($L\ m^{-2}\ h^{-1}$)/ ($g\ m^{-2}$)	Average absorbance	
							supernatant	permeate
R2	I	1	0.1	14.95	2.8[a]	0.19	2.1	0.9
	II	1	0.1	14.95	1.4[b]	0.094	1.8	0.6
	III	3	0.03	14.95	0.8[c]	0.054	0.972	0.01
	IV	3	0.1	14.95	0.8[c]	0.054	1.1	0.04
	V	3	0.1	22.43	0.8[c]	0.036	0.71	0.02
	VI	1	0.1	22.43	0.8[d]	0.036	0.88	0.1
R3	I	1	0.1	14.95	0.29[e]	0.02	1.3	0.225
	II	3	0.03	14.95	0.29[f]	0.02	0.687	0.01
	III	3	0.1	14.95	0.29[f]	0.02	0.76	0.04
	IV	3	0.1	22.43	0.29[f]	0.013	0.427	0.02
	V	1	0.1	22.43	0.29[e]	0.013	0.45	0.03

Note: Under the same HRT, the instantaneous membrane flux of R2 and R3 were different owing to different intervals of suction and idling period.

[a]Three cycles of [suction (10)/ fill (5)/ idling (465)]$_{min}$ each day, [b]three cycles of [suction (20)/ fill (5)/ idling (455)]$_{min}$ each day, [c]one cycle of [suction (35)/ fill (5)/ idling (440)]$_{min}$ each day, [d]three cycles of [suction (35)/ fill (5)/ idling (440)]$_{min}$ each day, , [e]6 min/24 min (suction/ idling period), [f]3min/ 45 min (suction/ idling period). The terms 'suction', 'fill' and 'idling' refer to the period of withdrawing treated water (membrane under operation), adding wastewater into the bioreactor (membrane idle) and biological reaction (membrane idle), respectively.

Table 2: TOC removal during different runs

Run #	GAC on membrane, $g\ m^{-2}$	Instantaneous membrane flux, $L\ m^{-2}\ h^{-1}$	TOC in permeate, $mg\ L^{-1}$
R3-I	14.95	0.29	16
R3-V	22.4	0.29	3
R2-I	14.95	2.80	76
R2-VI	22.4	0.80	26

TOC loading = 0.94 $g\ L^{-1}$; HRT = 1 day

Table 3: Influence of membrane fouling and cleaning on removal performance of R2[a]

Condition	TMP, kPa (suction/idling period)	Absorbance[b] in permeate
Clean	3/3	0.1
Fouled	35/3	0.45
After ex situ water-jet rinsing	3/3	0.1

[a] Extended run R2-VI (see Table 1), [b]481 nm

FIGURES

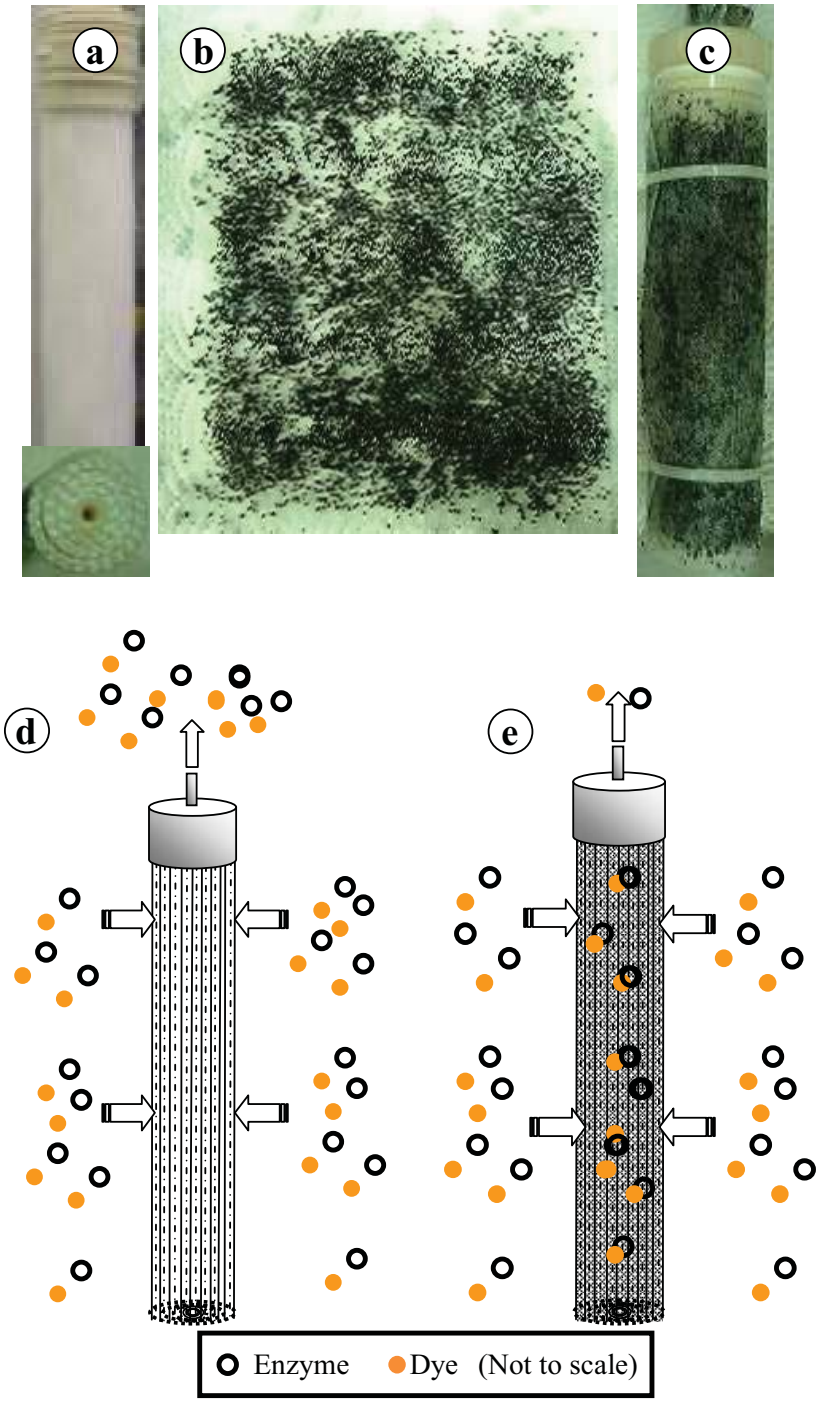

Figure 1: GAC-coated membrane assembly and the operation concept. (a) Bare hollow fiber membrane, (b) GAC-coated nylon mesh, (c) GAC-coated nylon mesh wrapped around membrane module, (d) Schematic of unrestricted washout of soluble dye and enzyme through membrane-permeate, (e) Schematic of prevention of dye and enzyme leakage through their co-adsorption on to GAC-coated mesh around membrane and subsequent dye degradation

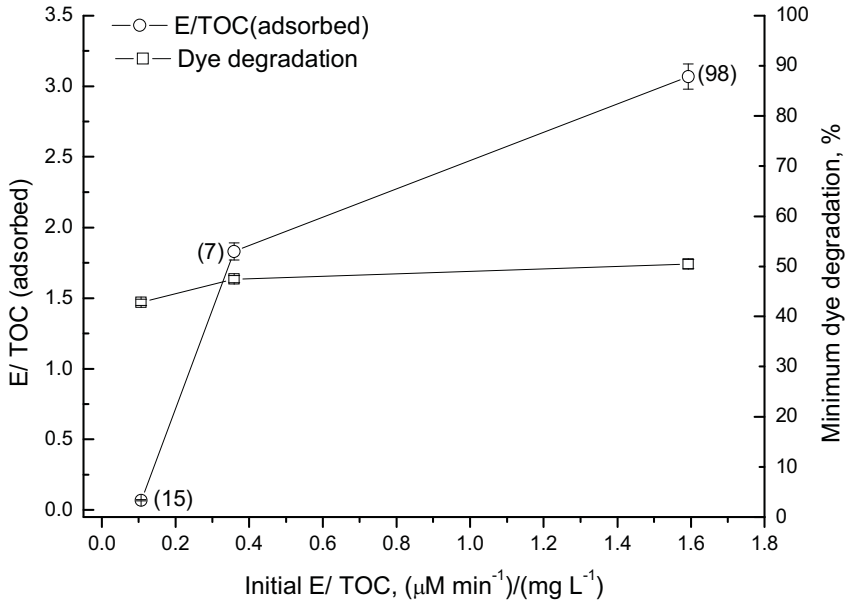

Figure 2: Enzymatic dye degradation on GAC.
Values in parentheses indicate absolute enzymatic activity corresponding to the adjacent E/TOC ratio. To pre-adsorb enzyme, GAC was incubated first with crude enzyme solutions possessing enzymatic activity and TOC of 7-98 μM min^{-1} and 62-138 mg L^{-1}, respectively, and then with dye. Minimum dye degradation (%) = [1- % extracted dye x η], where η = extraction efficiency (51%). The error bars indicate standard deviation of two replicates. Some error bars are too small to notice.

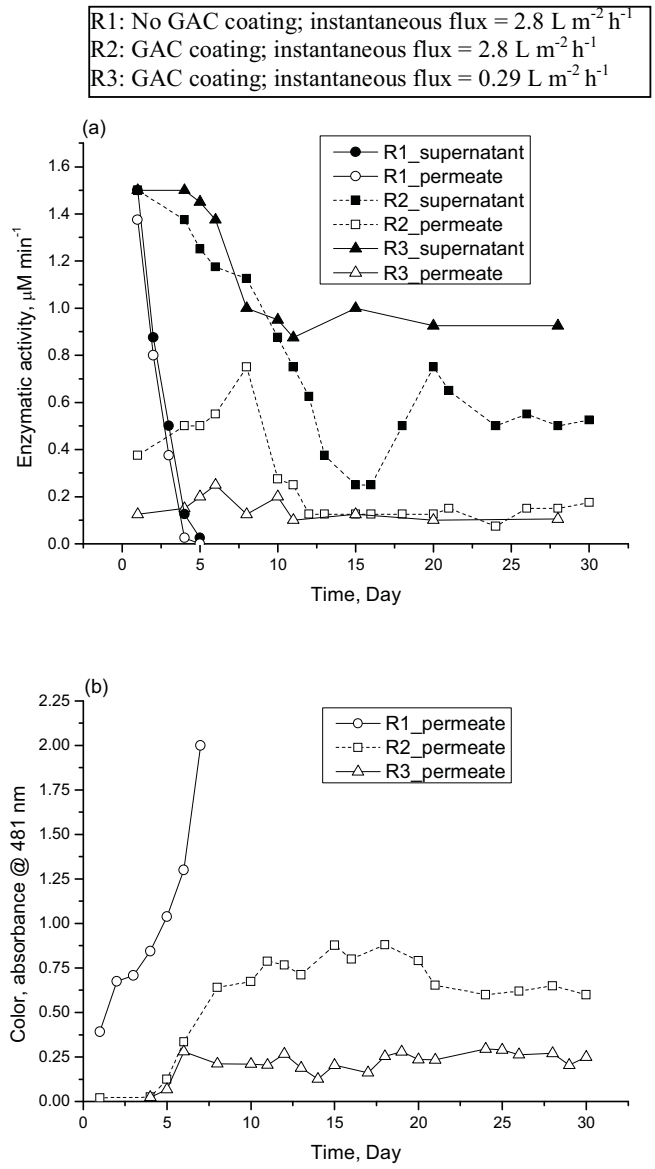

Figure 3: Enzyme washout prevention and decoloration improvement by GAC-coated membrane. (a) Variation in enzymatic activity in supernatant and permeate. (b) Color in permeate. All three MBRs were operated under the same HRT (1 day), average flux (0.06 L $m^2 h^{-1}$) and dye loading (0.1 g L^{-1} d^{-1}). R1 and R2 were operated under the same instantaneous flux (2.8 L $m^{-2} h^{-1}$), but the membrane in R1 was devoid of any GAC-coating. Like R2, R3 contained a GAC-coated membrane (14.95 g GAC m^{-2}), but the instantaneous flux of R3 was altered (0.29 L $m^2 h^{-1}$) from that of R1 and R2 by applying different intervals of suction and idling periods (for detailed operating conditions see runs R2-I, II and R3-I in Table 1). Each data point represents average of two measurements.

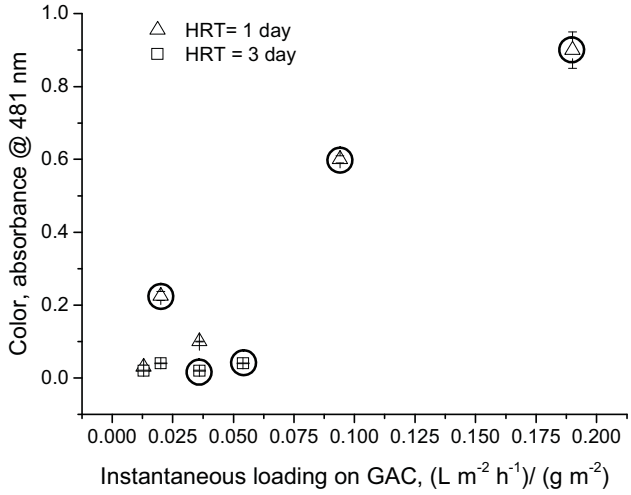

Figure 4: Combined effect of HRT and instantaneous loading on GAC (instantaneous flux/ amount of GAC on membrane) on decoloration. Data from R2 and R3 under the same dye loading (0.1 g L^{-1} d^{-1}) have been plotted (see runs R2: I-II, IV-VI and R3: I, III-V in table 1). Encircled data points belong to R2. The error bars indicate standard deviation between 16 samples collected over the four week operation period of each run.

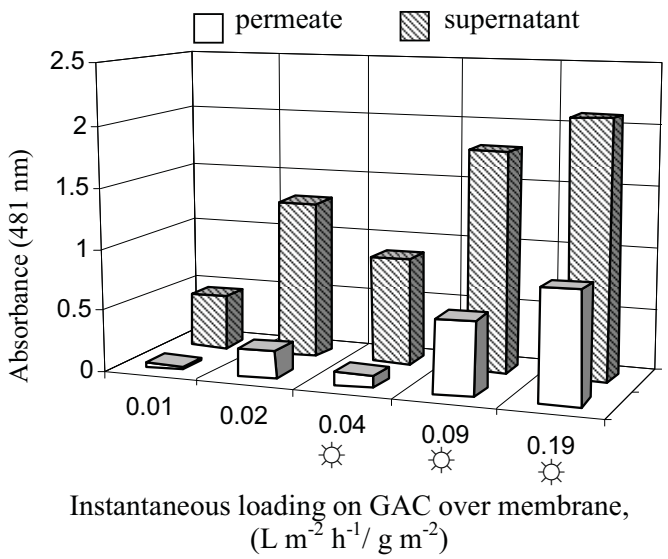

Figure 5: Variation of both supernatant and permeate absorbance under different loadings on GAC (instantaneous flux/ amount of GAC on membrane). Data pertaining to both R2 and R3 under an HRT of 1 d and dye loading of 0.1 g L^{-1} d^{-1} have been plotted (see runs R2-I, II, VI and R3-I, V in Table 1). ☼-marked data belong to R2. The values indicate average of 16 samples collected over the four week operation period of each run.

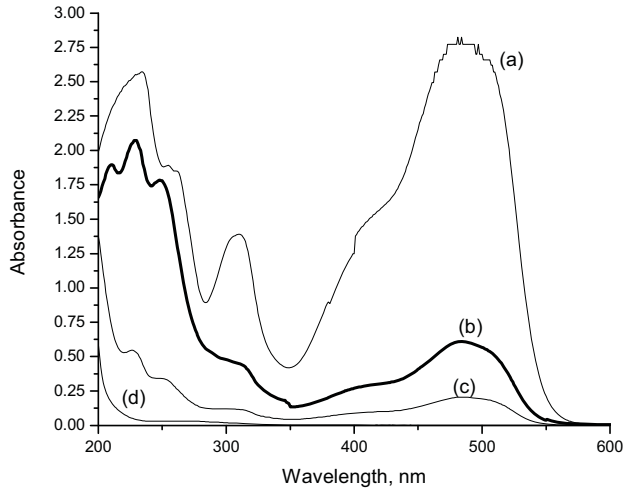

Figure 6: UV-visible spectra of permeate solutions. Data from R2 and R3 under the same HRT (1 d) and dye loading (0.1 g L^{-1} d^{-1}), but different instantaneous loadings on GAC have been plotted. (a) Spectrum of 50 mg L^{-1} dye solution as a reference, (b)-(d): Spectra corresponding to instantaneous loading ((L m^{-2} h^{-1})/(g m^{-2}))of 0.19 (R2-I), 0.02 (R3-I) and 0.013 (R3-V), respectively.

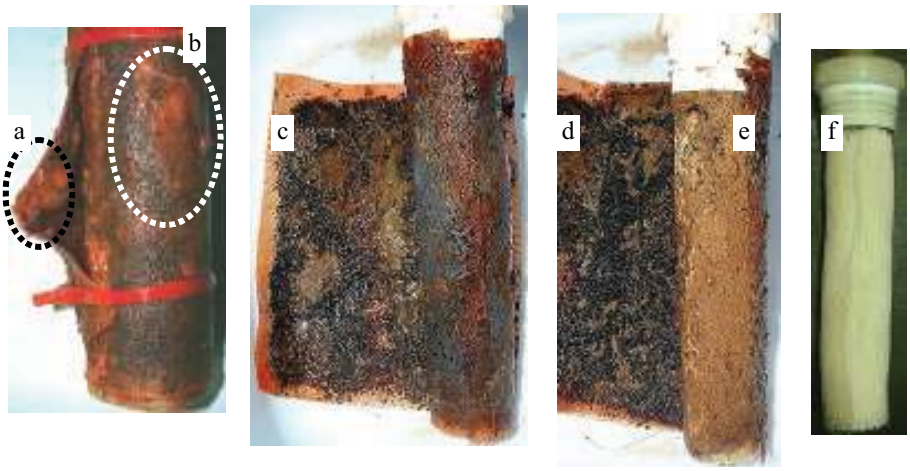

Figure 7: Membrane fouling under long-term operation in absence of periodic cleaning (**a**) Sludge deposition within GAC-layers, (**b)** moderate deposition on the outer surface of the composite module, (**c**) 1st GAC layer, (**d)** Inner-most GAC layer, (**e)** Surface-fouling of the main module, (**f)** Membrane-surface after *ex-situ* water-jet cleaning