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Cytotoxicity bioremoval achieved by a submerged membrane bioreactor operated at pilot scale for the treatment of surfactant wastewater

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ABSTRACT

The feasibility of reclaiming an anionic surfactant-rich wastewater with a submerged membrane bioreactor was investigated in a pilot scale plant of 60 L in the frame of the FP7 BioNexGen project. The MBR was operated continuously for eight months. The effects of the HRT (from 3 to 1 d) were evaluated regarding COD and anionic surfactant removal efficiencies. At a reduced HRT of 32 h, the MBR achieved high COD and anionic surfactant removal efficiencies (83.73 and 98.13%, respectively). Considering the complexity of the wastewater processed, the MBR was very stable and achieved high organic removal efficiency at organic loading of 1.5 g COD l^{-1} d^{-1} and HRT of 32 h. The evaluation of MBR potential to reduce the cytotoxicity of the investigated wastewater was studied at HRT of 32 h and OLR of 1.5 g COD L^{-1} d^{-1}. The toxicity bioremoval was assessed by the MTT assay on MCF-7 breast cancer cells.

Keywords: Submerged membrane bioreactor; Ultrafiltration membrane; Wastewater treatment; Anionic surfactants; Cytotoxicity bioremoval

1. Introduction

Increased water consumption for both industrial and domestic purposes has led to a shortage of good quality surface and groundwater resources and to an increase in the costs of water and wastewater treatments [1]. As a consequence of this shortage, it would be prudent for any rational water management authority to secure the purest water sources for direct human consumption and to encourage the reuse of processed water for industrial applications [2]. Surfactants are a group of compounds used daily in huge amounts mainly in household applications and as industrial cleaning agents [3]. In Tunisia, anionic surfactants are very common pollutants found in water, with more than 100,000 m^3/year of surfactant-containing wastewater released, coming mainly from detergent and cosmetic industries as well as daily uses, such as washing and cleaning.

Many methods have been developed for the extraction and removal of anionic surfactants from water by both physicochemical and microbiological techniques. Physicochemical processes included the application of adsorption on activated carbon and coagulation/

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precipitation procedures for the removal of anionic and non-ionic surfactants from wastewater. It was found that these methods were more effective for anionic surfactants than for non-ionic surfactants, the average removal being 67.4 and 31.7%, respectively [4].

Various destructive techniques have also been applied for the removal of anionic surfactants from waters. The effect of aqueous ozonation on the decomposition of anionic, cationic and non-ionic surfactants has been established many times. The results were earlier reviewed [5]. The oxidative treatment of p-toluenesulfonic acid using hydrogen peroxide has been reported too [6]. Gamma irradiation has also been proposed for the destruction of non-ionic and anionic surfactants in industrial wastes [7]. However, such physical treatment methods do not represent real degradation but only a displacement of the xenobiotic substances in a concentrated waste volume [8].

The application of microbiological systems using various pure and mixed cultures has been also reported for the enhancement of the decomposition rate of surfactants because of their simplicity and relatively low cost. It has been further established that the biodegradation of anionic surfactants was more rapid in mixed cultures than in isolated ones. However, for a biological treatment the surfactant concentration in the medium cannot exceed 1,000 mg L^{-1} due to its toxicity towards micro-organisms and foaming in aerated bioreactors [9]. Recent research in biological treatment of domestic and municipal wastewaters focused on membrane bioreactors (MBR) as a breakthrough in wastewater treatment. MBR processes combine biological degradation and membrane filtration in a single-step, compact process and allow a more flexible control of operational parameters. The main industrial applications of this technology are in food and beverage, pharmaceutical, cosmetic and textile industries as well as in laundries. The technical feasibility of this technology has been demonstrated through a large number of small- and large-scale applications [10–14]. Indeed, MBRs provide large molecule recovery, complete retention of all micro-organisms and increase in sludge concentration and more interestingly a complete disinfection of treated water [15]. However, membrane fouling is a major drawback of this technology [16], requiring more energy for backwashing and making the system less efficient.

The main factors affecting MBR processes are the operational parameters including, sludge retention time (SRT), biomass concentration, HRT, pH value and temperature of wastewater. In relation to SRT, the biomass concentration is very important in micropollutant degradation. Sorption of micropollutants is favoured by the high biomass content and the sludge composition inside the bioreactor. In this way, several reports were dedicated to study the possible improvement of MBR technology for higher surfactant biological degradation and lower energy consumption [17–19].

Although the research on the fate and removal of surfactants from wastewater has made consequent progress, one can still notice a lack of studies on cytotoxicity of surfactant-containing wastewaters. In addition, using only physicochemical analysis, it is impossible to predict the toxic properties of complex wastewater samples, especially if synergistic or antagonistic effects between the components occur. An alternative methodology to characterise the toxicity of wastewater samples are biological tests, which produce a global response to the complex mixture of chemicals without any prior knowledge of the mixture composition or its chemical properties. In this study, breast cancer MCF-7 cells have been used for toxicity evaluation of surfactant wastewater. In addition, MBR-treated effluents were tested to determine the MBR ability to reduce cytotoxicity response in MCF-7 cells.

2. Methods

2.1. Anionic surfactant-rich wastewater

Wastewater samples were collected from Henkel Alki industry. This factory is located in Sfax (Tunisia) and rejects about 10 m^3 per day. Wastewater was collected in 20 L tanks and stored at 4°C. The characteristics and the average composition of raw wastewaters are given in Table 1.

2.2. Experimental set-up

The same MBR was previously described for the treatment of cosmetic wastewater with consideration of microbial community dynamics [20]. The schematic experimental set-up is shown in Fig. 1. The aerobic

Table 1
Physicochemical characteristics of raw wastewater

Parameter	Average
pH	5.51
TKN (mg L^{-1})	107.37
EC (mS cm^{-1})	2.61
Anionic surfactants (g L^{-1})	3.31
TSS (g L^{-1})	2.34
Soluble COD (g L^{-1})	13.57
BOD_5 (g L^{-1})	1.62

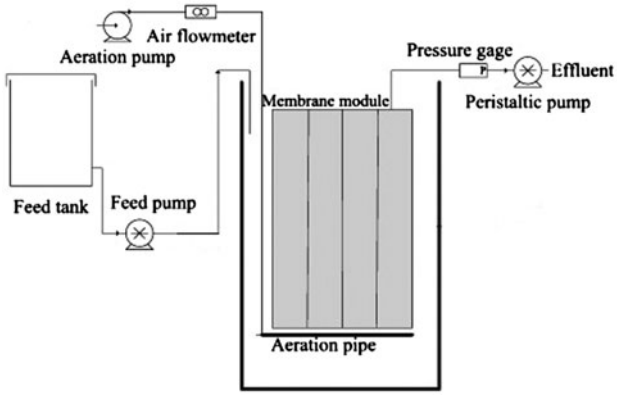

Fig. 1. Schematic diagram of the experimental set-up.

submerged membrane bioreactor consisted of a tank having a working volume of 60 L and coupled to a submerged cross-flow ultrafiltration flat sheet membrane module (Microdyn-Nadir GmbH, Germany) with a total effective filtration area of 0.39 m^2, a cut-off of 150 kDa and an operating transmembrane pressure ranging between 70 and 350 mbar. The seed sludge was obtained from the full-scale aerobic wastewater treatment plant (WWTP) of Henkel industry. The starting mixed liquor suspended solids (MLSS) concentration was 2.65 g L^{-1} and the aeration rate range from 1 to 2 m^3 h^{-1}. The temperature varied from 15 to 29°C. The MBR was operated continuously for eight months. During the treatment process, the OLR was increased from 0.25 to 2 g COD $L^{-1}d^{-1}$.

2.3. Analytical methods

pH and electrical conductivity were determined using a pH meter model Istek–NeoMet and a conductivimeter model CONSORT C 831, respectively. Hyamine colorimetric method was used for estimating the anionic surfactant content in wastewaters. Soluble COD was estimated as described by Knechtel [21]. BOD_5 was determined by the manometric method with a respirometer (BSB-controller Model 620 T (WTW)). Total Kjeldahl nitrogen content (TNK) was determined as described by Kjeldahl [22]. MLSS, Total Suspended Solids (TSS) were measured as the Standard Methods for examination of water and wastewater [23].

2.4. MTT cell viability assay

Influent sample and its corresponding permeate were collected in order to evaluate the MBR potential for cytotoxicity bioremoval under the operating conditions studied (OLR 1.5 g COD l^{-1} d^{-1} and HRT of 32 h). All samples were centrifuged at 4,500 rpm for 20 min. The pellets were removed and the supernatants were sterilized by filtration through a 0.45 μm filter (Millipore). MCF-7 breast cancer cells were routinely maintained in 75-cm^2 tissue culture flasks containing Roswell Park Memorial institute RPMI 1640 medium supplemented with 10% of foetal bovine serum and 1% of penicillin. Cells were incubated at 37°C in 95% air and 5% CO_2 incubator. Pass-cultures were carried out at 70–80% of confluence at a 1:2 ratio using 0.25% trypsin-1 mM ethylene diaminetetraacetic acid. Cells were then plated onto 96-well plates at 2,000 cells per well in 100 μL of medium and allowed to attach for 24 h. The filter-sterilized samples at final concentrations of 1, 5, 10, 20, 50 and 100% were introduced to the cell culture medium. The cells were incubated for 48 h, after which, cell number was assessed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay as follows: 10 μL of 5 mg mL^{-1} MTT was added to each well followed by incubation for 4 h. Sodium dodecyl sulphate (SDS, 10%) was then added at 100 μL per well, followed by incubation for another 24 h. The absorbance was then determined at 570 nm using a microplate reader ELX 800. The results are presented as percentages of the negative control [24].

3. Results

3.1. Anionic surfactant-rich wastewater characteristics

The wastewater was collected from the equalization tank of the WWTP of the cosmetic company (HENKEL, Sfax, Tunisia), characterized and stored at 4°C until feeding. The characteristics and the average composition are given in Table 1. As it can be seen in this table, the TKN concentration was very low. Consequently, urea was added continuously in the medium for reaching a COD/N ratio of 20 in order to have adequate biomass growth [9].

3.2. COD and surfactant removal efficiencies

The changes of substrate concentrations expressed as soluble COD and AS are shown in Fig. 2. The average operating conditions and the removal efficiencies of the MBR are summarized in Table 2.

The MBR was continuously fed with surfactant wastewater and the OLR was increased gradually during the operational period to reach 2 g COD L^{-1} d^{-1}. The respective HRT was decreased from 3 d at the start-up to 1 d at the end of the operational period. The biomass concentration inside the MBR increased from 3 to 6.17 g L^{-1} after two months of running systems. When no sludge was discharged from the MBR, such concentrations are lower than these of the

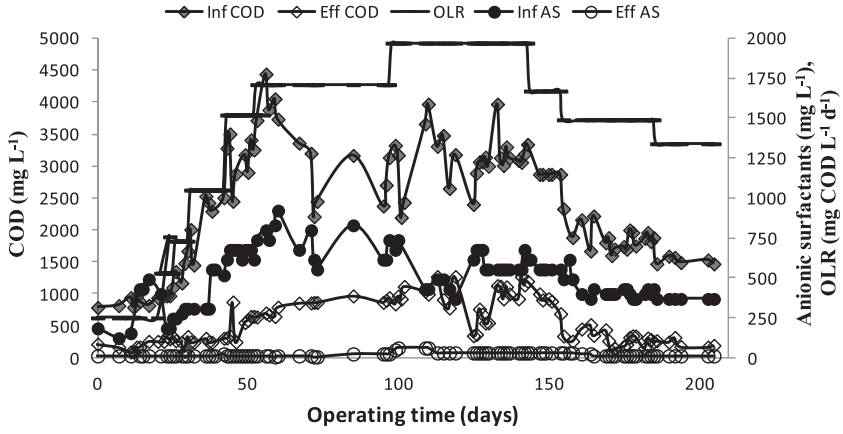

Fig. 2. Variation of the COD and AS of influent (inf) and effluent (eff) during MBR treatment.

Table 2
Average operating conditions and removal efficiencies of the MBR

Time (days)	Stage	OLR (g CODL^{-1} d^{-1})	COD removal efficiency (%)	AS removal efficiency (%)
0–20	Acclimatation	0.25 ± 0.02	79.49 ± 7.72	94.52 ± 3.19
21–25	I	0.53 ± 0.03	69.66 ± 3.17	94.65 ± 1.71
26–30	II	0.73 ± 0.11	83.20 ± 6.67	95.64 ± 0.47
31–42	III	1.05 ± 0.18	86.25 ± 2.33	96.47 ± 1.06
43–52	IV	1.52 ± 0.11	83.73 ± 5.81	98.13 ± 0.09
53–97	V	1.71 ± 0.23	74.92 ± 7.27	97.95 ± 1.12
98–143	VI	1.97 ± 0.15	67.80 ± 7.59	93.46 ± 2.67
144–154	VII	1.67 ± 0.06	67.96 ± 4.85	94.52 ± 0.21
155–185	VIII	1.49 ± 0.09	84.02 ± 4.22	95.89 ± 1.60
186–205	IX	1.34 ± 0.04	84.99 ± 3.75	96.67 ± 0.00

conventional biological treatment. This might be due to the composition of the surfactant wastewater, which contains a variety of bactericidal substances from shampoo, body soap or other cleaning agents.

As it can be seen in Fig. 2 the COD and AS values of the MBR effluent are significantly lower than these of the influent. Mean values of 98% for anionic surfactant removal were obtained. Furthermore, the MBR removed 84% of the influent COD at a HRT of 32 h and COD loading rate of 1.5 g COD L^{-1} d^{-1} (Table 2). Although the fluctuations of COD and AS values in the inlet are great (from 790 to 4,400 mg L $^{-1}$and 122 to 829 mg L^{-1}, respectively) the MBR responded very well and efficiently buffered the changing influent composition.

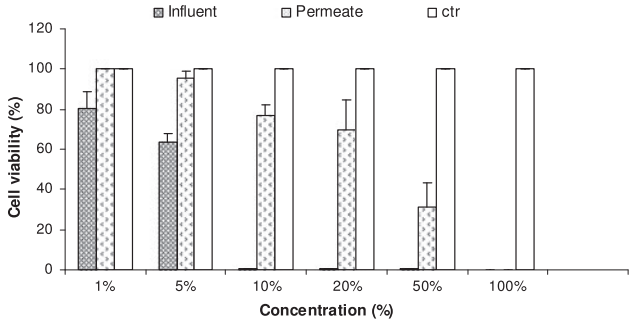

Fig. 3. Cell viability variation in MCF-7 cells treated with MBR influent and permeate samples.

3.3. Cytotoxicity bioremoval

Fig. 3 illustrates the data obtained from MTT experiments where the cytotoxicity was evaluated by measuring viability (death, <100% in Fig. 3) of the treated cells (in contact with MBR samples) compared to controls (untreated cells without MBR samples = 100% in Fig. 3) as described previously [9]. Viability was measured quantitatively by the colorimetric tetrazolium (MTT) assay and based on the metabolic activity of viable cells. The MTT assay is used to quantify live and actively metabolizing cells that can reduce the yellow MTT to

the purple formazan product through the action of mitochondrial and cytoplasmic enzymes. Thus, this approach provides a real indication of the ability of an MBR to reduce the cytotoxic effect of the investigated wastewater; such information is obtained through the percentage of viable cells when exposed to influent and effluent MBR samples.

The cell viability assay results clearly showed that raw wastewaters elicited cytotoxic effect in a dose-dependent manner compared to 100% viability of the control (Fig. 3). Indeed, significant cytotoxic effect was detected following the treatment of MCF-7 cells with raw wastewater samples and even at low concentrations of 1 and 5%, influents introduced to cell culture medium induced a total mean cell growth of 80.36 and 63.46%, respectively. Interestingly, significant reduction in the cytotoxic effect was observed for cells exposed to effluent samples, especially at concentrations below 5% (Fig. 3). Thus, these results proved the ability of the MBR to partially remove the toxicity of untreated wastewater.

The above findings suggest that advances in wastewater treatment using an MBR can provide a suitable process for lowering effluent toxicity before discharge into the aqueous environment. However, a tertiary treatment is necessary if complete elimination of toxicity is targeted. In this way, further studies on the capacity of reverse osmosis and nanofiltration membranes in cytotoxicity removal need to be performed.

4. Conclusion

The MBR system achieved high-removal efficiencies for anionic surfactant and COD and exhibited a good performance for cytotoxicity reduction. The resulting permeate was of high quality which suggests its reuse in the industrial process of detergent, cosmetic and cleaning industries. However, a tertiary treatment is necessary if complete elimination of toxicity is targeted.

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Abbreviations

BOD — biological oxygen demand
COD — chemical oxygen demand
EC — electrical conductivity
HRT — hydraulic retention time
MBR — membrane bioreactor
OLR — organic loading rate
TKN — total Kjeldal nitrogen
TSS — total suspended solids

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