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Research paper



Removal of Synthetic Estrogens in Attached Growth System

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Abstract

Effective treatment of wastewater is crucial to reduce the impact of industry and human activities on the environment. Thus, a more efficient water treatment is needed to remove the components of waste, or the pollutants, faster. Hence in the case of this study, the aim was to investigate the use of bio-filters to remove ammonia-nitrogen and endocrine disrupters (17α-ethynylestradiol, the EE2, and mestranol, the MeEE2) in a batch culture experiment. The initial concentrations of 100 µg/L of EE2 and 100 µg/L of MeEE2 were prepared and added to the batch reactor with bio-filters. The bio-filters contained four concentrations of ammonia-nitrogen which were 35 mg/L, 65 mg/L, 85 mg/L and 100 mg/L. The results showed that with 35 mg/L of ammonia-nitrogen, the concentration of the EE2 and the MeEE2 was reduced to 30 µg/L showing 70% of them removed over the incubation period. Whereas with 65 mg/L of ammonia-nitrogen, the EE2 and MeEE2 were reduced to 27 µg/L and 22 µg/L, of which 73% of the EE2 was removed while the MeEE2, 78%. Thirdly, with 85 mg/L ammonia-nitrogen, the concentration of both oestrogens was reduced nearly the same, which was the EE2 16 µg/L and the MeEE2 14 µg/L. Here, 84% of the EE2 was removed and the MeEE2, 86%. Lastly, using 100 mg/L of ammonia-nitrogen, the concentrations of the EE2 and the MeEE2 were reduced to 11 µg/L and 14 µg/L, thus removing 89% of the former and 86% the later. These results displayed the highest removal of oestrogens in comparison with other experiments at different initial concentrations of ammonia-nitrogen in the attached growth system of bio-filters.

Keywords: *ammonia-nitrogen; bio-filters; mestranol; 17α-ethynylestradiol*

1. Introduction

Steroid hormones are naturally occurring organic substances, mainly produced by humans and animals, which are more known as human sexual hormones. Oestrogens are female sexual hormones produced in the female ovaries while the androgens, the male sexual hormones, are and produced in the testes. The most abundant oestrogens are 17β–oestradiol (E2) and oestrone (E1) [1] while the androgen is testosterone [2]. These hormones are excreted in the urine of humans or animals, normally in a conjugated form as soluble and inactive glucorinides [1]. This conjugated form is then become microbially-cleaved in the environment or wastewater treatment plants (WWTP) [3]. Alas these humans and animal's hormones can be endocrine disruptors, especially to water organisms, which have become a critical concern [4]. Worse still, as city populations have grown larger, their excreted hormones, of which some from those taking contraceptive pills, are entering the environment. They have become the effluent of the WWTPs [3]. To note, 17α–ethinylestradiol (EE2) and mestranol (MeEE2) are the major compounds of contraceptive pills, which are excreted in urine, that enhance the concentration of hormone-like substances in the WWTP effluent [4,5]. One (1) percent of all excreted oestrogens are EE2, of which 80% being the oestrogens, which are produced by women [6].

The concentration of oestrogens in water is mostly lower than 5 ng/L, whereas the concentration of oestrogens in the WWTP effluent can exceed 50 ng/L [7]. Due to the higher persistence of EE2 in the WWTP, the concentrations of synthetic oestrogens in the environment can be likened to the concentrations of natural oestrogens, although these synthetic hormones are emitted in smaller quantity [7]. For ease of understanding, in conventional wastewater treatment, nitrification occurs in the aeration tank of activated sludge systems. It is the two-step biological conversion of ammonia to nitrite and then to nitrate under aerobic condition [8]. In it, the microorganisms that perform the nitrification and denitrification were able to act on the inorganic forms of nitrogen (ammonia, nitrite and nitrate). Here, the nitrifying bacteria in the wastewater produce monooxygenase enzymes to co-metabolise the organic compounds aerobically [9,10]. The co-metabolism is a simultaneous degradation of two compounds, in which the degradation of the second compound (the secondary substrate) depends on the presence of the first compound (the primary substrate). In this case, the first substrate is ammonia-nitrogen while the second, the oestrogens (the EE2 and the MeEE2) [10].

The biotransformation, where the second compound is modified but not utilized for growth, is another important biodegradation [9]. The knowledge of co-metabolism transformations of the endocrine disrupting chemicals (EDCs), especially the EE2 and MeEE2, is essential to assess the potential effects of the compounds [11]. Hence this study focused on the use of K2 AnoxKaldnes packing material as bio-filters to remove ammonia-nitrogen and the oestrogens. These bio-filters act as an attached growth system of the nitrifying bacteria.

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2. Material and methods

This investigation of the batch culture for the nitrifying bacteria was done using bio-filter of Anox-Kaldnes packing materials provided from Veolia Environmental Services (UK). Then the removal of ammonia-nitrogen by the nitrifying bacteria and the degradation of oestrogens were assessed by the different concentrations of the ammonia oxidation in a batch culture system. The incubation period was nine (9) days.

2.1. Chemicals and materials

A nitrification medium was used whose formulation was as follows: Na_2HPO_4, 13.5 g; KH_2PO_4, 0.7 g; $NaHCO_3$, 0.5 g; $MgSO_4.7H_2O$, 0.1g; $FeCl_3.6H_2O$, 0.014 g; $CaCl_2.2H_2O$, 0.18 g and 1000 mL of distilled water [8]. The aliquots of 100 mL of medium were placed in 250 mL Erlenmeyer flasks. They were steriled at 121°C for 15 min. A steriled stock solution of $(NH_4)_2SO_4$ was prepared separately from the basal medium as ammonium oxidizers. The stock solution of $(NH_4)_2SO_4$ was added aseptically to provide the final concentration of 0.5 g/L. The pH of the final media was 8.0 [8].
The nitrifying bacteria were inoculated from the Centre for Sustainable Aquatic Research, College of Science, Swansea University. The chemicals for the nitrification medium, ammonia-nitrogen, the EE2 and the MeEE2 were purchased from Sigma Aldrich Co. Ltd, the United Kingdom.

2.2. Reactor configuration

The reactor configuration is shown in Figure 1. Four flasks were used of which each had 1 litre of nitrification medium. All the flasks were submerged in the water bath to gain an optimum temperature of 30°C. This was controlled by a thermal regulator (Figure 1). Each flask contained a bio-filter with 35 mg/L, 65 mg/L, 85 mg/L and 100 mg/L of ammonia-nitrogen. The ammonia-nitrogen was added separately.

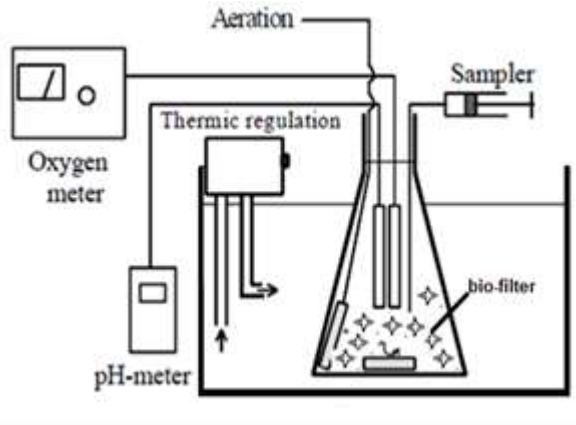


Fig. 1: Reactor Configuration

The aeration was provided using an aeration pump and the dissolved oxygen (DO) was monitored by an oxygen meter. The dissolved oxygen was observed with a dissolved oxygen probe on a daily basis to maintain its concentration above 6 mg/L in the reactor. The aeration allowed the submerged bio-filter to move constantly in the reactor medium. To maintain the pH of 8.0 a pH meter was used. The initial concentrations of 100 µg/L of EE2 and 100 µg/L of MeEE2 were prepared and added to the batch reactor with the bio-filters containing 35 mg/L, 65 mg/L, 85 mg/L and 100 mg/L of ammonia-nitrogen, respectively.

2.3. Instruments

The ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen concentrations were determined in a phenate method and ion-chromatography (IC) [12]. The EE2 and the MeEE2 were detected by a gas chromatograph-mass spectrometer (GC-MS) using method from [13] with some modifications [14]. The pH was monitored by a pH probe and the dissolved oxygen was observed by an oxygen meter.

3. Results and discussion

The performance of nitrifying bacteria in the immobilized batch culture system with bio-filter could be studied by the reduction of the synthetic oestrogens, in this case the EE2 and MeEE2. Here, the focus of the analysis was to note in percentage how much the EE2 and MeEE2 being removed by the nitrifying bacteria.

3.1. Reduction of oestrogens in 35mg/L of ammonia-nitrogen

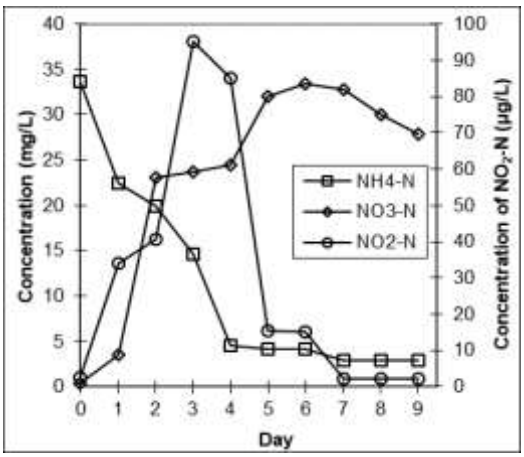


Fig. 2: The ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen in 35 mg/L ammonia nitrogen

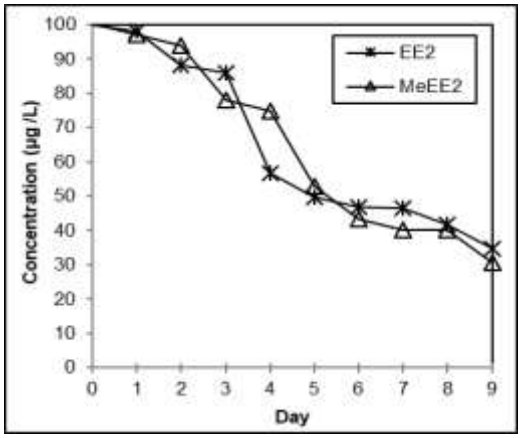


Fig. 3: Degradation of EE2 and MeEE2 in 35 mg/L of ammonia-nitrogen

Figure 2 shows the results of the ammonia oxidation in the moving bed batch reactor with the bio-filter in 35 mg/L of ammonia-nitrogen. It was found that the ammonia-nitrogen levels constantly decreased over time, with the build-up of the nitrate-nitrogen throughout 9 days. On Day 3, the nitrite-nitrogen concentration was at its peak which was 95 µg/L. It then was reduced after Day 5.

Figure 3 shows the amount of synthetic oestrogens EE2 and MeEE2 being removed where their concentrations are shown lower, which are close to 30 µg/L. Both oestrogens in the moving bed batch reactor with bio-filter were removed about 70% over the incubation period. By comparing the nitrification profile and that of the oestrogen degradation, its activity correlates with the reduction of the nitrite-nitrogen.

3.2. Reduction of oestrogens in 65 mg/L of ammonia-nitrogen

Figure 4 shows the batch reactor with bio-filter in 65 mg/L of ammonia-nitrogen. As shown, the concentration of the ammonia-nitrogen declines towards zero with the production of nitrate-nitrogen to the maximum of nearly 60 mg/L nitrate-nitrogen in 9 days. This shows that the nitrifying bacteria consumed most of the ammonia-nitrogen in the batch reactor in the ammonia oxidation.

In Figure 5, the levels of the synthetic oestrogens EE2 and MeEE2 decreases further compared to the batch reactor with the bio-filter in 35 mg/L ammonia-nitrogen. The EE2 and MeEE2 are shown reduced to 27 µg/L and 22 µg/L, respectively. An amount of 73% of EE2 is removed whereas the 78% of MeEE2 removed, which is slightly higher than the removal of the former.

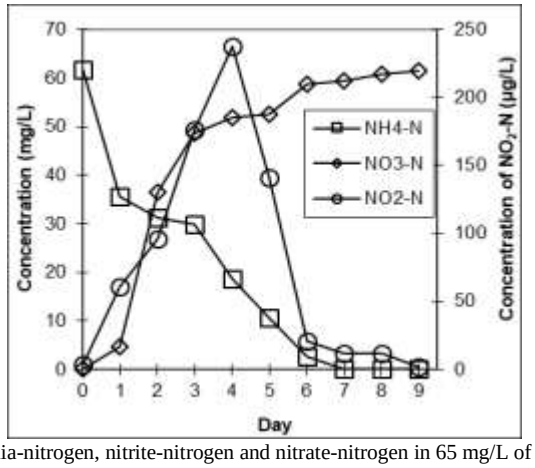


Fig. 4: The ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen in 65 mg/L of ammonia-nitrogen

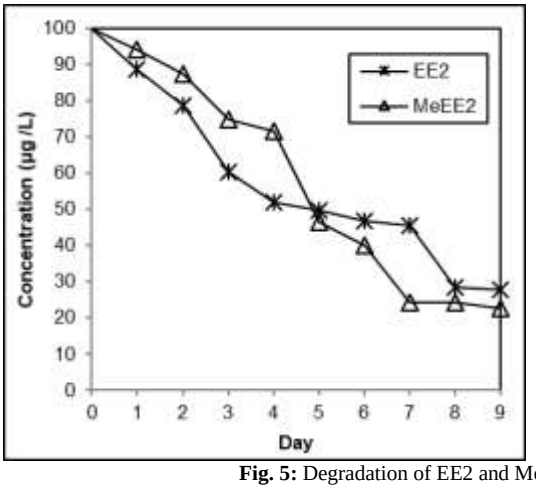


Fig. 5: Degradation of EE2 and MeEE2 in 65 mg/L of ammonia-nitrogen

3.3. Reduction of oestrogens in 85 mg/L of ammonia-nitrogen

Figure 6 shows the reduction of ammonia-nitrogen in the batch reactor of 85 mg/L ammonia-nitrogen. It shows the ammonia-nitrogen level decreases rapidly towards zero in 7 days. In these 7 days, it can be deduced that the nitrate-nitrogen was produced in the ammonia oxidation through the consumption of ammonia-nitrogen and nitrite-nitrogen by the ammonia oxidising bacteria and the nitrite oxidising bacteria in the batch reactor. The level of nitrate-nitrogen produced was nearly 80 mg/L at the end of the incubation period. Again, the most rapid decline of the oestrogens was during the nitrite-nitrogen degradation phase.

Figure 7 shows the degradation of EE2 and MeEE2 in 85 mg/L of ammonia-nitrogen in the batch reactor with the bio-filter. As shown in Figure 7, the MeEE2 decreases slowly compared to the EE2. However, towards the end of the incubation period, the concentrations of both oestrogens were nearly the same, which were 16 µg/L of EE2 and 14 µg/L MeEE2. The removal of both oestrogens was much higher than that in the 35 mg/L and 65 mg/L ammonia-nitrogen, which were 84% for EE2 and 86% for MeEE2, respectively.

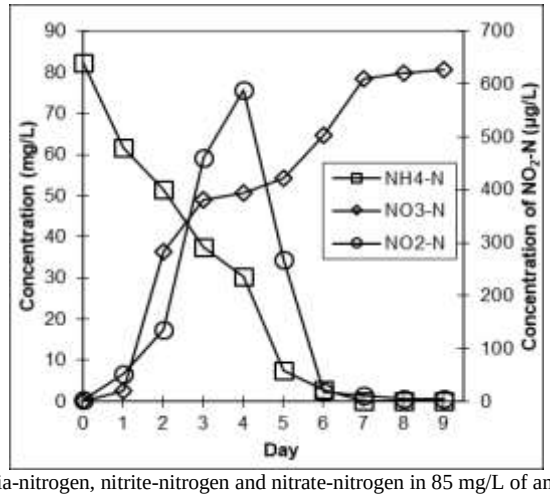


Fig. 6: Ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen in 85 mg/L of ammonia-nitrogen

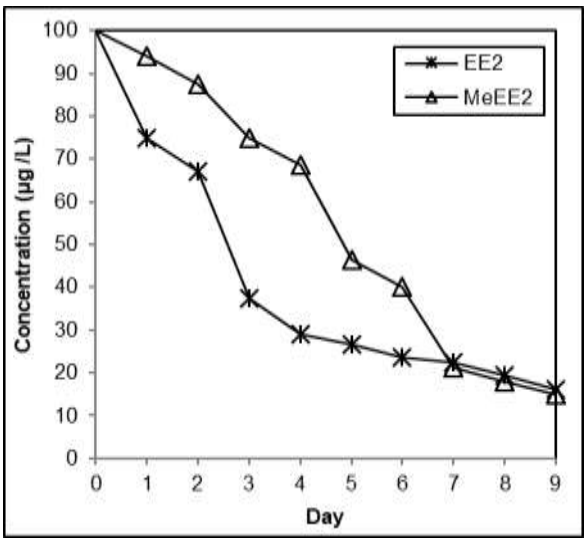


Fig. 7: The degradation of EE2 and MeEE2 in 85 mg/L of ammonia-nitrogen

3.4. Reduction of oestrogens in 100 mg/L of ammonia-nitrogen

Figure 8 shows the concentration of 100 mg/L of ammonia-nitrogen in the batch reactor with the bio-filter. The concentrations of ammonia-nitrogen reduce rapidly toward the end of the incubation period. The build-up of nitrate-nitrogen was nearly 100 mg/L, demonstrating that the ammonia oxidation process by nitrifying bacteria occurs at the highest rate.
Figure 9 shows the decreased levels of EE2 and MeEE2. The figure shows the same pattern of degradation of both synthetic oestrogens as shown in Figure 7. MeEE2 decreases slowly compared to EE2. Here, the concentrations of EE2 and MeEE2 are 11 µg/L and 14 µg/L, respectively, showing removals of 89% for EE2 and 86% MeEE2. This was the highest removal achieved compared to the other studies' results that employed different initial concentrations of ammonia-nitrogen in batch reactors with bio-filter.

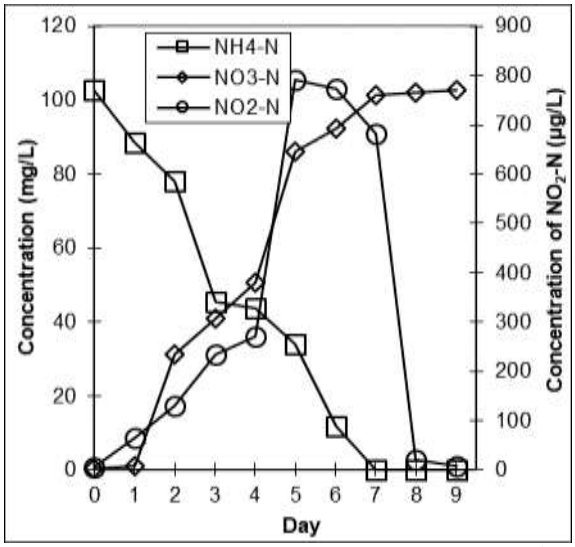


Fig. 8: Ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen in 100 mg/L of ammonia-nitrogen

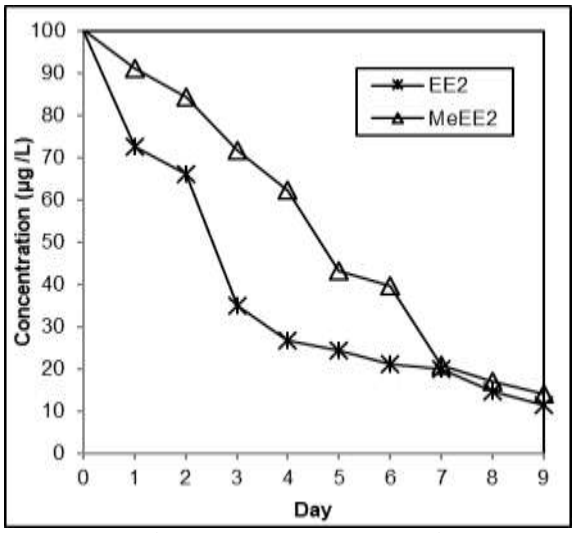


Fig. 9: Degradation of EE2 and MeEE2 in 100 mg/L of ammonia-nitrogen.

As can be seen in the results shown above, the experiment demonstrated that the enrichment of actively growing nitrifying bacteria in the batch reactor with the bio-filter is capable of degrading the EE2 and MeEE2 synthetic oestrogens. The experiment displayed that the degradation of both oestrogens increased when the concentration of ammonia-nitrogen increased to 85 mg/L or 100 mg/L. The removal of EE2 and MeEE2 was nearly 90% in all ammonia-nitrogen concentrations.
This study on the 1 litre batches were performed in an optimum condition suitable for the growth of the nitrifying bacteria [8]. This condition allowed more effective sorption of the substrate and offered a maximum allowable incubation time to observe the dynamics of the ammonia-nitrogen, nitrite-nitrogen and nitrate-nitrogen transformation within the batch culture system. Studies employing 1 litre batches showed that the rates of transformation were dependent on the ammonia-nitrogen concentration, and the kinetics were typical of those observed by other workers [15]. The saturation constant of the ammonia-nitrogen transformation was thought to be slightly high. The formation and disappearance of nitrite-nitrogen was observed and is a classic observation [16]. The levels of nitrite-nitrogen in the culture were dependent on the amount of ammonia-nitrogen added.
The results were also compared to the experiments of the batch culture in the absence of the bio-filter with the same concentration of ammonia-nitrogen of 35 mg/L, 65 mg/L, 85 mg/L and 100 mg/L of ammonia-nitrogen. However, the oestrogens were not included in the experiment without bio-filter [14]. This is due to the results for the percentage removals of all the concentration of ammonia-nitrogen were lower from the experiment with bio-filter [14]. Hence the present experiment provided insights into the importance of suspended carriers which combine the features of both fixed-growth and activated sludge systems.
In an immobilized system, the microbial community is largely attached on the surface of supported media and retained within the reactor as a biofilm. This microbial community can flourish and multiply better than when present in a suspension as free-floating cells or small flocs [17]. The immobilized system of supported media, compared to the absence of the packing material system, could support the de-

velopment of microbial biofilms of nitrifying bacteria on the surface of the carriers within the microenvironment which occurred in the batch reactor system. The movement of the suspended packing material in the reactor system maintains effective gas and nutrient transfer, since an ideal biofilm is relatively thin and evenly distributed over the carrier surface [18]. The turbulence in the batch reactor system from the aerator influences the substrate and oxygen transfer within the suspended carrier [19]. In contrast to the absence of the bio-filter in the batch culture system, the performance of the immobilized system with the bio-filter was much better. Higher ammonia oxidation was found in the experimental analysis of the immobilized packing material at different concentrations of ammonia-nitrogen compared to the batch culture without the suspended carrier [14].

As the ammonia-nitrogen removal increases with an increase of the ammonia-nitrogen concentration, hence showing a similar behaviour between the nitrifying bacteria in the suspended growth batch reactor without bio-filter [14] and the ones in the attached growth in the moving bed batch reactor with bio-filter in the present study. Both nitrifying bacteria cultures, i.e. in the batch reactor with bio-filter and the batch reactor without bio-filter, can consume ammonia-nitrogen up to 100 mg/L. Rapid consumption of ammonia-nitrogen was observed at an ammonia-nitrogen concentration of 100 mg/L for the nitrifying bacteria in the batch culture with and without bio-filter. This occurred since the bacteria in the serial batch enrichment culture had been grown in an initial ammonia-nitrogen concentration of 100 mg/L [20]. The system using bio-filters also showed that it was more effective than the one without bio-filter [14].

In the case of using the bio-filter, the results show that the nitrifying bacteria had the ability to transform oestrogens in batch culture. These substances were indeed degraded of which its amount influenced by the amount of ammonia-nitrogen used. There is some suggestion that the best degradation rate should coincide with the degradation of nitrite-nitrogen and the formation of nitrate-nitrogen [9,21]. Hence, the MeEE2 can be degraded over the EE2 in the high concentrations of ammonia-nitrogen [22]. The synthetic hormone EE2 is primarily removed in wastewater treatment plants (WWTP) by sorption. Here, the nitrifying biomass has been shown to be able of EE2 biodegradation [23]. The MBR systems can be achieved and successfully remove more than 90% EE2 [23]. The membrane bioreactor (MBR) technology, combined with an activated sludge system, was chosen to develop a community of autotrophic, nitrifying microorganisms. However, the disadvantages of this reactor system are predominantly due to the membrane fouling and the rejection efficiency of the membrane type depends on the physicochemical properties of the organic micro-pollutants [24].

To compare, the adsorption of estrogenic compounds, i.e. 17β-estradiol (E2), and EE2 on several powdered activated carbons (PAC) was investigated by [25]. The removal of the activated carbon for these estrogenic compounds was found to be efficient [25]. However, the removal of the activated carbon was controlled by the physiochemical properties of the organic pollutant and the type of the activated carbon used [26]. Further, the adsorption capabilities of the activated carbon depend on the activated carbon type. Hence, the service life of the activated carbon could affect the performance of the oestrogen removal rate [26]. The adsorption capacity of activated carbon decreased with an increase in the adsorbent concentration and the presence of others surfactant [27]. Here, the results with the batch reactor using bio-filter showed an excellent reduction in EE2 and MeEE2. This study demonstrated that the biodegradation of oestrogens in the batch culture system was approximately 90% for EE2 and MeEE2. Therefore, the bio-filter can be considered a reliable system based on the usage of small cylindrical carriers which move freely in the reactor system. These cylindrical carriers are specifically designed to provide a large surface area for the growth of attached microbial biofilms [28]. Furthermore, the microbial growth of the nitrifying bacteria was mainly confined to the surface of the moving bio-filter, especially within the sheltered internal surfaces [29]. This resulted in the formation of a stable and sheltered biofilm, protecting the microbes from alterations in the wastewater parameter characteristics, in this study attributed to organic pollutants, i.e. oestrogens. Besides promoting the microbial activity of nitrifying bacteria, the major advantages of the batch reactor using bio-filter also include reduced head losses and the elimination of backwashing costs as this bio-filter is moving constantly in the reactor medium with the aid of aeration system [30].

4. Conclusion

It can be concluded that removing synthetic oestrogens with bio-filter is reliable compared with the other type of removal techniques. The feasibility of using immobilized system can be considered as potential alternatives in removing the oestrogens as this technique has shown satisfying results. In addition, immobilized suspended carrier system has posed several advantages compared to other treatments which are a low maintenance and being more cost effective.

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