Integration of effective microorganisms and membrane bioreactor for the elimination of pharmaceutical active compounds from urine for safe reuse
Hussein I. Abdel-Shafy and Mona S. M. Mansour

ABSTRACT

The present study aims to investigate the efficiency of integrated effective microorganisms (EM) and membrane bioreactor (MBR) for eliminating pharmaceutical active compounds (PhACs) from urine. Natural urine was separated using a ‘diversion toilet’. The toilet users were under medication with some pharmaceuticals, namely levofloxacin (LEF), ibuprofen (IBP) and atorvastatin (ATV). For correlation, three MBR pilot-scale sequences were examined. In Sequence 1, the MBR was used without pre-treatment. In Sequence 2, EM was added as pre-treatment in the mixing tank. The effluent was further treated with the MBR. In Sequence 3, EM was added directly to the activated sludge of the MBR. The results showed that Sequence 1 could decrease the PhACs from 10 mg/L to 1.5 mg/L, 0.5 mg/L and 0.9 mg/L for LEF, IBP and ATV, respectively. Sequence 2 exhibited remarkable improvement in PhACs removal. The overall residual concentration reached 0.7, 0.13 and 0.28 mg/L for LEF, IBP and ATV, successively. Applying Sequence 3 gives higher removal efficiency, where the residual concentration of LEF, IBP and ATV decreased to 0.50 mg/L, 0.10 mg/L and 0.22 mg/L, respectively. It was concluded that the contaminated urine was efficiently treated by adding EM directly to the activated sludge of the MBR, and the treated urine can be safely used as fertilizer.

Key words | effective microorganisms, membrane bioreactor, pharmaceuticals compounds, safe urine reuse, urine separation

INTRODUCTION

Pharmaceutical active compounds (PhACs) are consumed in high quantities worldwide. The expectations are that these amounts will continuously increase because of improving health care systems and longer life expectations of people. Administered pharmaceuticals are excreted by humans as a parent compound or metabolite. Approximately 70% of pharmaceuticals are excreted with urine (metabolites, conjugates) from the human body and 30% with feces (Lienert et al. 2007). Usage of urine for soil fertilization includes the risk of transferring pharmaceutical residues to agricultural fields. Little is known of the fate of pharmaceuticals regarding their accumulation in soils, transfer to groundwater, and incorporation by plants (Abdel-Shafy & Abdel-Sabour 2006). The uptake of pharmaceuticals by plants is of major interest when crops are fertilized with urine.

The presence of certain pharmaceuticals in ground and surface waters is a serious environmental problem because these compounds are biologically active and could potentially affect non-target and susceptible species (Rabiet et al. 2006; Vieno et al. 2007). Pharmaceutical residues in the environment have the potential to elicit deleterious effects on some organisms such as crustaceans (Lange et al. 2006) and amphipods (Borgmann et al. 2007). Because most pharmaceuticals are relatively polar, their adsorption to soil or particulates could be of little importance; hence, most of these compounds are mobile in the environment. Persistent polar pharmaceuticals may reach drinking water sources...
and may become a serious problem in places that depend highly on recycled water, such as in France (Rabiet et al. 2006), the United States (Sedlak et al. 2000), Egypt (Abdel-Shafy et al. 2008) and Australia (Al-Rifai et al. 2007). Most pharmaceutical substances are, by nature, biologically active and hydrophilic, in order that the human body can take them up easily, and persistent, to avoid degradation before they have a curing effect. Depending on the pharmacology of a medicinal substance, it will be excreted as a mixture of metabolites, as unchanged substance, or conjugated with an inactivating compound attached to the molecule (Abdel-Shafy & Mansour 2013a, 2013b).

Separating the collection and processing of human urine is gaining interest for three important reasons:

1. Human urine (yellow water) contains the largest fraction of nutrients: nitrogen (80%), phosphorus (50%) and potassium (70%) emitted from households (Vinnerås & Jönsson 2002). These could be used, after an appropriate treatment if required, as fertilizers in agriculture.

2. To reduce the amounts of residual PhACs that are currently discharged through sewer overflows as well as wastewater treatment plants (WWTPs) that are not designed to efficiently eliminate these compounds.

3. Disconnection of the urine stream (or part of the stream) from the sewer would enable energy saving at WWTPs, this is spent on nitrification of ammonium, mainly originating from urine (Wilsenach & Loosdrecht 2006; Abdel-Shafy & Mansour 2013a, 2013b).

On the other hand, the membrane bioreactor (MBR) is a system that combines the biological treatment of microorganisms and the membrane separation process, which replaces the secondary clarifiers into a single step (Abdel-Shafy et al. 2005). The influent or feed water is mixed with the biomass, and this mixture is filtered through the membrane, separating the biomass from the treated water. There are several advantages of using the MBR. The main benefit of an MBR over conventional activated sludge (CAS) is that the amount of suspended solids remaining in the effluent of the MBR is much lower than in CAS, resulting in a better quality treated water. The low turbidity of the effluent water makes it more amenable to further treatment. Another benefit of an MBR is its inherently high sludge age, which allows for slow-growing bacteria development, leading to enhanced degradation of some compounds, such as trimethoprim (Perez et al. 2005; Abdel-Shafy & El-Khateeb 2011). A recent study reported the elimination of six selected acidic pharmaceuticals from municipal wastewater by an activated sludge system and two MBRs using different sludge retention times, and compared it to the removal in CAS (Kimura et al. 2007). Earlier publications reported varying removal efficiencies of several pharmaceuticals in MBRs (Bernhard et al. 2006; Snyder et al. 2007).

In addition, effective microorganisms (EM) were developed during the 1970s at the University of Ryukyus, Okinawa, Japan (Sangakkara & Higa 2000). EM were developed initially to enhance soil activity for the purpose of increasing the crop yield. Studies have suggested that EM may have a number of applications, including agriculture, livestock, gardening and landscaping, composting, bioremediation, cleaning septic tanks, algal control and household use (Abdel-Shafy et al. 2014). Sludge treated with EM is used as fertilizer. Meanwhile, wastewater treated with EM is used in crop production as it is enriched with beneficial microorganisms (Abdel-Shafy et al. 2014). EM contain selected species of microorganisms, which can be commonly found in many ecosystems including lactic acid bacteria, yeasts, actinomycetes, photosynthetic bacteria, and other types of organisms (Sangakkara & Higa 2000). These were blended in a molasses or sugar medium and maintained at a low pH under ambient conditions (Surasak & Labteephanao 2008). The EM preparation contains numerous enzymes, which can decompose the organic matter in an environmentally-friendly manner and ensure the survival and growth of the microorganisms both in the soil and in other environmental media (Grabas et al. 2009). EM detoxify contaminated water and are ideal for biological balance. EM convert a degraded ecosystem to one that is productive and contains useful microorganisms. EM have been effectively employed as a pre-treatment for greywater (Abdel-Shafy et al. 2014).

The aim of the present study is to investigate the efficiency of the MBR on the one hand and the efficiency of combining EM with an MBR on the other hand, to eliminate PhACs from raw contaminated urine. The study was carried out on a pilot scale. The studied pharmaceuticals are levofoxacin (LEF), ibuprofen (IBP) and atorvastatin (ATV). The necessary trans-membrane pressure difference was applied by the water head above the membrane (gravity flow) to save the required energy. The detection of such selected PhACs throughout the

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**References:**


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*Note: The references are not explicitly provided in the text but can be inferred from the context and cited within the document.*
present study was conducted by high performance liquid chromatography mass spectroscopy (HPLC MS/MS).

MATERIALS AND METHODS

The present study was carried out continuously for six months using a pilot scale MBR for the elimination of PhACs from contaminated urine. A urine diversion toilet (Figure 1) was implemented in the National Research Centre pilot plant in Cairo, Egypt.

Urine was directed through a piping system to a collection tank. The toilet users were under medication with LEF, IBP and ATV and not any other drugs.

The studied PhACs were LEF, IBP and ATV extra pure standard (98% assay) which were purchased from Merck, Germany.

The concentration of drugs in the raw contaminated urine as well as through all the study periods was determined using a Water 2795 HPLC equipped with a Quattro Ultima® MS/MS detector and a column: water X Terra® C18, 3.5 μm, 10 cm, 2.1 mm. Ionization: electro-spray positive (ES+). Acquisition: MRM mode, unit resolution. The mobile phase consisted of (A) 0.3% formic acid and 0.1% ammonium format, and (B) 1:1 acetonitrile:methanol. The solvent program was gradient; the injection volume was 5 μL.

The characteristics of the urine were also determined in all collected samples, namely raw urine, after EM treatment and the MBR effluent, including chemical oxygen demand (COD) (total and dissolved), biological oxygen demand (BOD), nitrates, nitrites, total phosphates, K, Na and CA. In this respect, the analytical procedures as described by Standard Methods for the Examination of Water and Wastewater (APHA, AWWA & WEF 2005) were used.

EM

EM was purchased in a liquid phase from the Ministry of Agriculture of Egypt, which consists of Lactobacillus, Pseudomonas, Aspergillus, Saccharomyces and Streptomyces. The selected formulated bacterial consortium comprised of the isolated bacterial strains acts in a synergistic way and is capable of degrading the easily associated organic compounds present in sewage wastewater. This consortium is capable of effectively reducing the pollution load of the sewage water within the desired discharge limits. The use of such specific consortia can overcome the inefficiencies of the conventional biological treatment facilities currently operational in sewage treatment plants. The present EM were previously examined for the treatment of sewage water by different investigators (Grabas et al. 2009; Dhall et al. 2012; Abdel-Shafy et al. 2013, 2014). Based on the fact that urine is usually associated with sewage water, it was important to examine the same selected formulated bacterial consortium for the elimination of pollutants from urine.

The optimum formulating conditions of EM were determined by culturing microbes at temperatures of 28 and 37 °C, pH from 6.5 to 8.0 and molasses concentration from 1 to 15%. Different incubation periods ranging from 1 to 7 days were studied to determine the optimum period in terms of the growth of all the five organisms. The effect of incubation period on the pH of EM at constant temperature was carried out at a constant temperature, namely 37 °C.

Figure 1 | Urine diversion toilet.
EM characterization by morphological and biochemical studies was conducted. Different tests were carried out for biochemical studies such as catalase, oxidase, sugar fermentation, triple sugar iron, hydrolysis and urease tests according to Cappuccino & Sherman (1996).

Isolation, characterization and formulation of EM

The materials and all supplies of the EM were purchased from the Ministry of Agriculture of Egypt (MAE) in the form of liquid phase.

Isolation of EM

This was carried out by the MAE. The obtained colonies were subcultured to obtain pure culture as described by Cappuccino & Sherman (1996).

Characterization of EM

The isolates were identified by morphological and biochemical studies. Biochemical tests such as catalase test, oxidase test, IMViC test, sugar fermentation tests, triple sugar iron test, urease test and hydrolysis tests, as described by Cappuccino & Sherman (1996), were performed by the MAE.

Formulation of EM

The formulation was carried out by the MAE as follows. The isolated microorganisms were cultured together in a medium (molasses) at various pH, temperature and concentration of molasses. The optimal physical conditions for formulating EM were analyzed by culturing microbial consortia at a pH of 6.5–8, temperature of 28 and 37 °C, and at various concentrations of molasses.

Determination of EM optimum dose used for contaminated urine treatment

Experimental batch studies were conducted at room temperature, in which different doses of the liquid EM were stirred with the contaminated urine. The examined EM doses ranged from 22.5 to 180.0 gVS/L.

MBR pilot plant sequences in a continuous feeding system

The MBR pilot plant was erected in the experimental area of the National Research Centre. The contaminated urine (ACU) was treated using three different sequences. A schematic diagram of the treatment sequences is shown in Figure 2. The three sequences are as follows:

- The ACU was treated directly by the MBR without any addition of EM (Sequence 1).
- The ACU was mixed first with the pre-determined optimum dose of EM in the mixing tank. The mixture was incubated for 3 days, after which effluent was further treated with the MBR (Sequence 2).
- The pre-determined EM optimum dose was added to the activated sludge of the MBR to enhance the degradation efficiency (i.e. ACU was treated directly with an MBR that was enhanced by EM (Sequence 3).

Weekly samples were collected after each step for the detection of the PhACs level as well as the physical and chemical characteristics. A mixing tank was used to homogenize the EM dose with the contaminated urine (ACU). The mixing tank was made of polyvinyl chloride. The dimensions of the tank were 1.00 m height, 1.00 m width and 1.00 m length (Figure 2). The predetermined EM optimum dose was added to the ACU in the mixing tank. The mixed effluent was further treated with the MBR (Sequence 2).

A pilot-scale MBR unit was used in this study which was equipped with a submerged membrane module. The average pore size was 0.378 μm to guarantee a reliable separation of bacteria and all particulate material. This unit comprised an extended aeration device with air diffusers located on the bottom, where air was supplied by a blower in order to ensure the required level of oxygen for the biological oxidation and to facilitate membrane securing. Therefore, the membrane module was connected to a micro gear pump capable of reversing speed, serving both as a permeate and backwash pump. The filtration cycle, i.e. permeate production time and backwash duration, were controlled by a timing device. Fouling on the surface of the plate and frame module was controlled through tangential flow along the membrane surface. The necessary trans-membrane
pressure difference was applied by the water head above the membrane (gravity flow), since a high value of this parameter is considered as crucial for the removal of the micro-pollutants. Fouling on the surface of the plate and frame module was controlled through tangential flow along the membrane surface. Membrane backwashing was conducted monthly during the entire experimental work to avoid any fouling problem. The necessary trans-membrane pressure difference was applied by the water head above the membrane (gravity flow) (Judd 2004). The water overhead was 1.4 m to ensure the flow of the treated effluent. The performance of an MBR was monitored for six months to investigate the operational stability of the system and the removal efficiency of the target compounds. Also, a 22 L tank was available to receive permeate, which was finally reused for irrigation. Samples were taken on a weekly basis. The specification of the studied MBR is as follows (Abdel-Shafy & El-Khateeb 2011):

- membrane surface, m² (0.6),
- number of membranes (8),
- resistance/pH range (1.5–10),
- resistance/H₂O₂ (NaOCl), ppm (3,000–5,000 (normal 500),
- resistance/temperature, °C (<50),
- resistance/pressure, mWS (Max. 1–3 (1.02 mWS = 10 kPa)).

It is worth mentioning that the MBR reactor was regulated to receive wastewater five times a day. At the moment of receiving such influent, vigorous aeration for 15 minutes occurred, followed by a great decrease in the rate of aeration. The purpose was to enhance the nitrification and de-nitrification process. In the meantime, the ammonia and any gases that could possibly be formed through the aeration and/or nitrification/de-nitrification process could be released from the MBR unit, mainly because the top of the system was not completely sealed.
RESULTS AND DISCUSSION

During the entire MBR operation period, the characteristics of the raw urine exhibited both diurnal and slight seasonal variation. This is mainly due to variation during the different seasons as well the relative diet habits in winter, summer and the holidays. The characteristics of urine are given in Table 1.

EM as provided by MAE

For EM optimum formulation conditions, the results show that at 27 and 37°C *Pseudomonas* species are able to grow in a wide range of pH from 4 to 10. *Streptomyces* also exhibit growth at pHs ranging from 5 to 10 and at temperatures ranging from 15 to 42°C. Therefore the optimum condition was 37°C and pH 8, which is suitable for all five studied organisms. Different molasses concentrations ranging from 1 to 15% were investigated to study the growth of the EM microbial consortia. It was found that the lowest concentration of molasses gave the highest growth of EM (i.e. decreasing the concentration of molasses increases the growth of EM). By increasing the molasses concentration, the survival of EM decreases as an indication of growth inhibition. This may be due to the osmotic pressure of molasses liquid. The results indicated that 1–3% concentration is favorable for EM growth. Therefore the concentration of 3% was selected as the optimum condition of EM at which all the five studied organisms were observed.

The incubation period showed great effect on the EM microbial consortia. The optimum period was 3 days. Increasing the incubation period inhibits the microorganism growth. This may be attributed to change in pH, depletion of nutrients and accumulation of toxic end products. Results showed that increasing the incubation period from 1 to 5 days decreases the pH from 7 to 2.95. Increasing the incubation period to 7 days did not show any further decrease in the pH. This may be because the organisms utilized all the available energy, thus there was no further growth of the EM organisms. Meanwhile, these organisms could not survive at such an acidic pH. At 3 days incubation period the pH decreased to 3.80.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Natural human urine</th>
<th>Artificially contaminated urine</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>N=12 Min 5.0 Max 6.3 Average 5.95 (±0.25)</td>
<td>N=12 Min 5.5 Max 6.8 Average 6.2 (±0.20)</td>
</tr>
<tr>
<td>CODT (mgO2/l)</td>
<td>N=12 Min 6,286 Max 7,152 Average 6,755 (±90)</td>
<td>N=12 Min 8,350 Max 8,500 Average 8,450 (±98)</td>
</tr>
<tr>
<td>CODD (mgO2/l)</td>
<td>N=12 Min 4,248 Max 4,270 Average 4,255 (±70)</td>
<td>N=12 Min 4,425.5 Max 4,590 Average 4,478.5 (±90)</td>
</tr>
<tr>
<td>BOD5 (mgO2/l)</td>
<td>N=12 Min 1,894 Max 2,198 Average 2,026 (±68)</td>
<td>N=12 Min 1,800 Max 2,090 Average 2,020 (±63)</td>
</tr>
<tr>
<td>TP (mg/l)</td>
<td>N=12 Min 529 Max 580 Average 547 (±40)</td>
<td>N=12 Min 490 Max 600 Average 550 (±53)</td>
</tr>
<tr>
<td>NO3 (mg/l)</td>
<td>N=12 Min 3.8 Max 4.5 Average 4.2 (±0.22)</td>
<td>N=12 Min 3.5 Max 6 Average 4.4 (±0.11)</td>
</tr>
<tr>
<td>NO2 (mg/l)</td>
<td>N=12 Min 15.0 Max 19.1 Average 16.36 (±0.81)</td>
<td>N=12 Min 15 Max 20 Average 18 (±0.52)</td>
</tr>
<tr>
<td>NH3 (mg/l)</td>
<td>N=12 Min 197 Max 229 Average 212 (±0.52)</td>
<td>N=12 Min 195 Max 242 Average 217 (±41)</td>
</tr>
<tr>
<td>K (mg/l)</td>
<td>N=12 Min 13,700 Max 13,870 Average 13,786 (±98)</td>
<td>N=12 Min 13,650 Max 13,900 Average 13,790 (±96)</td>
</tr>
<tr>
<td>Na (mg/l)</td>
<td>N=12 Min 14,580 Max 14,700 Average 14,660 (±99)</td>
<td>N=12 Min 14,600 Max 14,800 Average 14,670 (±95)</td>
</tr>
<tr>
<td>Ca (mg/l)</td>
<td>N=12 Min 5,800 Max 5,725 (±50)</td>
<td>N=12 Min 5,200 Max 6,000 Average 5,720 (±90)</td>
</tr>
<tr>
<td>LEF (mg/l)</td>
<td>– – – 0.0</td>
<td>– – – 10</td>
</tr>
<tr>
<td>IBP (mg/l)</td>
<td>– – – 0.0</td>
<td>– – – 10</td>
</tr>
<tr>
<td>ATV (mg/l)</td>
<td>– – – 0.0</td>
<td>– – – 10</td>
</tr>
</tbody>
</table>

N* – Number of samples, CODT – Total chemical oxygen demand, CODD – Dissolved chemical oxygen demand, BOD – Biological oxygen demand, TP – Total phosphates, NO3 – Nitrates, NO2 – Nitrites, NH3 – Ammonia, K – Potassium, Na – Sodium, Ca – Calcium, LEF – Levofloxacin, IBP – Ibuprofen, ATV – Atorvastatin.
For characterization of EM by morphological and biochemical studies, the results show the following:

- **Lactobacillus** species are white, mucoid, Gram-positive, rods and non-motile. Oxidase, urease and catalase test results are negative. Glucose, lactose and sucrose fermentation test results are positive.
- **Saccharomyces** species are creamy white, mucoid, smooth and ovoid budding cells. Glucose, lactose and sucrose fermentation test results were positive.
- **Streptomyces** species are white, dry, powdery, filamentous rods and Gram-positive. Casein, tyrosine and xanthine hydrolysis test results were positive.
- **Pseudomonadas** species are fluorescent, mucoid, Gram-positive, rods and motile. In addition the results exhibit positive tests for catalase, oxidase, urease and citrate utilization. Negative results were obtained for the glucose, lactose and sucrose fermentation tests. The characterization result of the triple sugar iron test shows both alkaline butt and alkaline slant.
- **Aspergillus** species are black, fuzzy mat conidia arising from conidiophores. Glucose, lactose and sucrose fermentation test results were negative.

**Effect of different EM doses on the treatment of contaminated urine in batch experiments**

Varying doses of EM ranging from 22.5 to 180.0 gVS/L were studied to determine the optimum dose for treatment of the contaminated urine. The results showed that increasing the EM dose increases the removal rate of the PhACs, BOD and COD. The optimum dose was found to be 135.0 gVS/L, at which the elimination rate was 35%, 40%, 50%, 45% and 60% for LEF, IBP, ATV, COD and BOD, respectively.

**MBR continuous flow pilot plant**

A hydraulic residential time of 2.4 d for the MBR was found to be sufficient for treating the contaminated urine.

Treatment of the contaminated urine by MBR, without the addition of EM (Sequence 1), is illustrated in Figure 2(b) and the results are given in Table 2. The PhACs removal reached 85%, 95% and 91% for LEF, IBP and ATV respectively. The residual concentration decreased from 10 mg/L to 1.5, 0.5 and 0.9, successively. These results indicate that the MBR is capable of eliminating the PhACs from the contaminated urine. On the other hand, removal of COD and BOD reached 94% and 93% respectively. The level of nitrates in the final effluent ranged from 0.49 to 0.77 mg/L, with an average value of 0.62 mg/L (Table 2). Meanwhile, the concentration of nitrates in the MBR effluent ranged from 1.99 to 3.08, at an average of 2.52 mg/L. These results (Table 2) indicate that an efficient elimination of nitrates and nitrites at the rate of 85.9% and 86% respectively was achieved via the MBR treatment. The level of total phosphorus (TP) in the MBR effluent ranged from 0.49 to 0.77 mg/L, with an average of 0.62 mg/L (Table 2). Meanwhile, the concentration of nitrates in the MBR effluent ranged from 1.99 to 3.08, at an average of 2.52 mg/L. These results (Table 2) indicate that an efficient elimination of nitrates and nitrites at the rate of 85.9% and 86% respectively was achieved via the MBR treatment. The level of total phosphorus (TP) in the MBR effluent ranged from 309 to 464.7 mg/L, with an average of 399.3 mg/L. Elimination of TP was the lowest of all the studied parameters, the rate ranging from 26.0 to 28.6% with an average of 27.4%.

For treatment of the contaminated urine by EM followed by MBR (Sequence 2), the pre-determined EM optimum dose in the mixing tank, namely 90.0 gVS/L, was added to the contaminated urine using a dosing pump. EM was employed as a pre-treatment for the purpose of decreasing the amount of residual PhACs, thus reducing the load on the successive treatment step. Remarkable removal efficiency was achieved in terms of LEF, IBP and ATV (Table 3). The corresponding removal rates were 30, 35 and 45% respectively. The residual concentration decreased from 10 mg/L to 7.0, 6.5 and 5.5 successively. The level of COD and BOD in the feed of the MBR permeate during the study period was 5,070 and 869 mg/L.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>N</th>
<th>Min</th>
<th>Max</th>
<th>Average</th>
<th>% of removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEF (mg/L)</td>
<td>12</td>
<td>1</td>
<td>2</td>
<td>1.5</td>
<td>85</td>
</tr>
<tr>
<td>IBP (mg/L)</td>
<td>12</td>
<td>0.35</td>
<td>0.55</td>
<td>0.5</td>
<td>95</td>
</tr>
<tr>
<td>ATV (mg/L)</td>
<td>12</td>
<td>0.5</td>
<td>1.8</td>
<td>0.9</td>
<td>91</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>12</td>
<td>350</td>
<td>520</td>
<td>507</td>
<td>94</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>12</td>
<td>90</td>
<td>202</td>
<td>141.5</td>
<td>93</td>
</tr>
<tr>
<td>Nitrates (mg/L)</td>
<td>12</td>
<td>0.49</td>
<td>0.77</td>
<td>0.62</td>
<td>85.9</td>
</tr>
<tr>
<td>Nitrite (mg/L)</td>
<td>12</td>
<td>1.99</td>
<td>3.08</td>
<td>2.52</td>
<td>86</td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td>12</td>
<td>22.7</td>
<td>31.8</td>
<td>28.2</td>
<td>87</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>12</td>
<td>309.1</td>
<td>464.7</td>
<td>399.3</td>
<td>27.4</td>
</tr>
</tbody>
</table>

N* = Number of samples, COD = Chemical oxygen demand, BOD = Biological oxygen demand, TP = Total phosphates, NO 3 = Nitrates, NO 2 = Nitrites, NH 4 = Ammonia, LEF = Levofoxacine, IBP = Ibuprofen, ATV = Atorvastatin.
successively (Table 3). By adding EM to the contaminated urine, a moderate removal rate was achieved for both COD and BOD, namely 40% and 57% respectively (Table 3). On the contrary, a low percentage of removal was reached for the elimination of NH₃, NO₃, NO₂ and TP, namely 12.3, 11.4, 22.2 and 10.5 successively (Table 3). The mixing tank effluent was further treated with the MBR, where the average removal of LEF, IBP and ATV was 90%, 98% and 95% respectively (Table 3). The final residual concentration of these PhACs decreased to 0.7, 0.13 and 0.28 mg/L for LEF, IBP and ATV successively. These results indicate efficient removal of the studied PhACs. The best eliminated drug among all the studied PhACs was IBP (98%), with an average final concentration in the effluent of 0.13 mg/L. The results in Table 3 show a remarkable improvement in the final treated contaminated urine. The overall accumulated removal rate was 93%, 98.7% and 97.2% for LEF, IBP and ATV, respectively. Meanwhile, an excellent removal rate was achieved by the MBR for COD and BOD at the rate of 98% each (Table 3). However, the residual COD was still 101 mg/L. For NH₃, NO₃, NO₂ and TP, the elimination reached 88.7%, 84.6%, 86.1% and 26% respectively (Table 3). The overall accumulated removal was 98.8%, 99%, 90.0%, 86.4%, 89.2% and 33.8% for COD, BOD, NH₃, NO₃, NO₂ and TP, respectively. This corresponds to a residual concentration of 101, 17.4, 0.60, 1.95 and 364 mg/L respectively in the final treated effluent.

For treatment of the contaminated urine by EM added directly to MBR (Sequence 3), the addition of EM was from the top of the reactor and this was conducted by a dosing pump. The results are given in Table 4. The residual concentration of LEF, IBP and ATV was 0.50 mg/L, 0.10 mg/L and 0.22 mg/L respectively. The removal rate was 95%, 99% and 97.8%, respectively. The elimination percentage of COD, BOD, NH₃, NO₃, NO₂ and TP increased to 98.9%, 99.1%, 93.8%, 90.5%, 93% and 40% respectively. The

### Table 3 | Efficiency of EM followed by MBR on the removal of PhACs from the contaminated urine sample

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>Concentration (average)</th>
<th>% of removal</th>
<th>Concentration (average)</th>
<th>% of removal</th>
<th>Overall % of removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEF (mg/L)</td>
<td>12</td>
<td>7</td>
<td>30</td>
<td>0.70</td>
<td>90</td>
<td>93</td>
</tr>
<tr>
<td>IBP (mg/L)</td>
<td>12</td>
<td>6.5</td>
<td>35</td>
<td>0.13</td>
<td>98</td>
<td>98.7</td>
</tr>
<tr>
<td>ATV (mg/L)</td>
<td>12</td>
<td>5.5</td>
<td>45</td>
<td>0.28</td>
<td>95</td>
<td>97.2</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>12</td>
<td>5,070 (±81)</td>
<td>40</td>
<td>101.1</td>
<td>98</td>
<td>98.8</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>12</td>
<td>869 (±72)</td>
<td>57</td>
<td>17.4</td>
<td>98</td>
<td>99</td>
</tr>
<tr>
<td>Nitrates (mg/L)</td>
<td>12</td>
<td>3.9 (±0.1)</td>
<td>11.4</td>
<td>0.60</td>
<td>84.6</td>
<td>86.4</td>
</tr>
<tr>
<td>Nitrite (mg/L)</td>
<td>12</td>
<td>14 (±0.4)</td>
<td>22.2</td>
<td>1.95</td>
<td>86.1</td>
<td>89.2</td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td>12</td>
<td>190.3</td>
<td>12.3</td>
<td>21.7</td>
<td>88.7</td>
<td>90.0</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>12</td>
<td>492 (±27)</td>
<td>10.5</td>
<td>364.1</td>
<td>26.0</td>
<td>33.8</td>
</tr>
</tbody>
</table>

N* = Number of samples, COD = Chemical oxygen demand, BOD = Biological oxygen demand, TP = Total phosphates, NO₃ = Nitrates, NO₂ = Nitrites, NH₃ = Ammonia, LEF = Levofloxacin, IBP = Ibuprofen, ATV = Atorvastatin.

### Table 4 | Efficiency of MBR containing EM on the removal of PhACs from contaminated urine sample

<table>
<thead>
<tr>
<th>Parameters</th>
<th>N</th>
<th>Min</th>
<th>Max</th>
<th>Average</th>
<th>% of removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>LEF (mg/L)</td>
<td>12</td>
<td>0.30</td>
<td>0.70</td>
<td>0.5</td>
<td>95</td>
</tr>
<tr>
<td>IBP (mg/L)</td>
<td>12</td>
<td>0.20</td>
<td>0.30</td>
<td>0.10</td>
<td>99</td>
</tr>
<tr>
<td>ATV (mg/L)</td>
<td>12</td>
<td>0.10</td>
<td>0.34</td>
<td>0.22</td>
<td>97.8</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>12</td>
<td>89.70</td>
<td>96.30</td>
<td>93.0</td>
<td>98.9</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>12</td>
<td>14.10</td>
<td>18.40</td>
<td>16.3</td>
<td>99.1</td>
</tr>
<tr>
<td>Nitrates (mg/L)</td>
<td>12</td>
<td>0.24</td>
<td>0.60</td>
<td>0.42</td>
<td>90.5</td>
</tr>
<tr>
<td>Nitrite (mg/L)</td>
<td>12</td>
<td>0.90</td>
<td>1.6</td>
<td>1.25</td>
<td>93</td>
</tr>
<tr>
<td>Ammonia (mg/L)</td>
<td>12</td>
<td>12.7</td>
<td>15.2</td>
<td>13.4</td>
<td>93.8</td>
</tr>
<tr>
<td>TP (mg/L)</td>
<td>12</td>
<td>210</td>
<td>230</td>
<td>220</td>
<td>40</td>
</tr>
</tbody>
</table>

N* = Number of samples, COD = Chemical oxygen demand, BOD = Biological oxygen demand, TP = Total phosphates, NO₃ = Nitrates, NO₂ = Nitrites, NH₃ = Ammonia, LEF = Levofloxacin, IBP = Ibuprofen, ATV = Atorvastatin.
corresponding residual concentration was 93.0, 16.3, 13.4, 0.42, 1.25 and 220 mg/L for COD, BOD, NH3, NO3, NO2 and TP successively. It is worth mentioning that the addition of EM directly to the activated sludge of the MBR has the advantage of eliminating one step in the treatment process (Figure 2(c)). This means eliminating space, including the consumption of energy, operation and maintenance processes.

CONCLUSIONS

The MBR process is an excellent tool and is technically feasible for removing PhACs from urine. The MBR improves the physical–chemical characteristics of urine in terms of COD and BOD. The combination of EM and the MBR proved to be effective in removing PhACs from contaminated urine. Adding EM directly to the activated sludge of the MBR as one step is more feasible than adding it to a mixing tank as a pretreatment. It proved to be more efficient in the removal of PhACs from urine. The efficiency of the combined EM and MBR pilot study system will guide decision makers on a potential full-scale application and safe reuse of treated urine for agricultural purposes.

RECOMMENDATION

It is essential to remove the PhACs from sewage water or the separated urine before any application for agricultural or soil purposes due to the impact on soil and plants. The combination of EM and MBR is successful and efficient for the removal of PhACs.

REFERENCES

Kimura, K., Hara, H. & Watanabe, Y. 2007 Elimination of selected acidic pharmaceuticals from municipal wastewater by an

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