Long-term evaluation of membrane bioreactor inoculated with commercial baker’s yeast treating landfill leachate: pollutant removal, microorganism dynamic and membrane fouling

Gabriela C. B. Brito, Liséte C. Lange, Vera L. Santos, Miriam C. S. Amaral and Wagner G. Moravia

ABSTRACT

In this study, commercial baker’s yeast (Saccharomyces cerevisiae) was employed as a novel inoculum for a membrane bioreactor (MBRy). It was applied to landfill leachate (LFL) treatment to remove recalcitrant organic compounds as well as for the assimilation of recalcitrant compounds, since yeasts have a high ability to break such compounds down. The MBRy was inoculated with 10 g L⁻¹ of commercial baker’s yeast and was operated at a hydraulic retention time of 48 h and pH of 3.5. The specific air demand based on the membrane area (SADm) was maintained at 0.6 m³ h⁻¹ m⁻². The MBRy achieved chemical oxygen demand (COD), color, NH₃, and humic substances removal of 68, 79, 68, and 50%, respectively. Furthermore, the MBRy showed lower fouling potential, which can be attributed to the low extracellular polymeric substances production, as the formation of a cake layer was the major mechanism of membrane fouling. The work demonstrated that novel MBR is a promising technology for treating recalcitrant landfill leachate.

INTRODUCTION

Landfill leachate (LFL) is a complex mixture of inorganic and organic compounds. It is generated through the precipitation, infiltration, compaction, and degradation of waste mass at landfill sites (Kjeldsen et al. 2002). Leachate is characterized by high concentrations of biological oxygen demand (BOD), chemical oxygen demand (COD) and nitrogen, especially in the form of ammonium. It may also contain heavy metals, xenobiotic compounds, phenols, and other aromatic hydrocarbons (Lee et al. 2010). The synergistic, additive or antagonistic effects of the contaminants present in the leachate may lead to toxicity, carcinogenicity or estrogenicity (Kumari et al. 2016).

Thus, the intensive use of biological treatments alone is insufficient to treat the LFL effluent to the standards required by the legislation (Kurniawan et al. 2010). Membrane bioreactors (MBR), can operate with a high concentration of biomass and sludge retention time, as the membrane allows the complete retention of sludge in the bioreactor, resulting in a more efficient biological degradation system (Boonyaroj et al. 2012).

Alvarez-Vazquez et al. (2004) compared the MBR to conventional biological treatment of LFL, concluding that the MBR, in general, has a higher COD removal efficiency for older leachate. Similarly, Ahmed & Lan (2012) conducted a review on the use of MBR in the treatment of leachate from landfills. Their findings suggest that excellent removal of BOD and ammonia (over 90%) was achieved with low hydraulic retention time (HRT) and high organic load, compared to conventional biological systems. The COD removal efficiency ranged from 23 to 90%, mainly...
due to the age of the leachate and the operating conditions employed.

The bacterial sludge in aerobic and anaerobic condition commonly used in the MBR processes, has limitations with regard to the degradation of recalcitrant compounds in the leachate. About 45% and 40% of the initial COD concentration under aerobic and anaerobic conditions respectively are inert to biological degradation (Amaral et al. 2009). Thus, the use of other groups of microorganisms may provide better results. Fungi and yeasts have a high ability to breakdown and assimilate pollutants that are difficult to degrade (Harms et al. 2011). According to Jarboui et al. (2012), the genera Saccharomyces, Candida, Rhodotorula, Pichia, Yarrowia and Hansenula, among others, have been reported as being capable of degrading complex organic compounds that are difficult to degrade, such as n-alkanes, n-alkylbenzenes, cresols, crude oil, benzene, polycyclic aromatic hydrocarbons (PAHs), trinitrotoluene (TNT), etc. The application of yeasts in wastewater treatment has been tested in recent decades, with high treatment processes efficiency being observed.

In the study carried out by Dan et al. (2002), a MBR system that was seeded with yeast, obtained through the enrichment of sludge from a conventional wastewater treatment plant, showed a higher COD removal efficiency (60–85%) than one seeded with bacteria (40–76%). Moreover, the system with yeast showed a lower fouling rate of the membrane compared to the one with bacteria. Saccharomyces cerevisiae is the most commonly known species of all fungal groups, being the main yeast used in the production of beer, ethanol, wine, and bread (Griffin 1995). Furthermore, there are reports in the literature demonstrating its ability to treat industrial effluents.

In this scenario, the use of MBR inoculated with a yeast biomass for the treatment of LFL, with high concentrations of recalcitrant compounds, is promising and may represent a great contribution and innovation towards the treatment of effluents. Thus, the objective of this study was to evaluate the long-term use of MBR inoculated with commercial baker’s yeast (S. cerevisiae) in the treatment of LFL. In a previous study, the authors investigated the startup of an MBR using a granulated active dry commercial baker’s yeast and the results suggest the potential use of this inoculum to treat LFL. In this context, this study provides a consolidation of the perspective on the MBR use for LFL treatment by using commercial baker’s yeast as the inoculum. This strategy has the advantages of practicality in acquiring the inoculum and therefore in the startup of the reactors and the standardization of inoculum specifications.

### MATERIALS AND METHODS

#### Characteristics of the leachate

The landfill leachate used in this study was supplied from the sanitary landfill area in the state of Minas Gerais, Brazil. Raw leachate was collected from the equalization tank. The landfill leachate characteristics are shown in Table 1. The leachate was subjected to ammonia removal treatment by air stripping. The air-stripping reactor was fed with 10 m³ of raw LFL. The reactor had an HRT of 48 h and its pH was not adjusted. Aeration with an airflow rate of 40 m³ h⁻¹ was provided using an air compressor coupled to a coarse bubble diffuser.

#### Description of the MBR unit and the membrane module

The MBR and membrane module used for performing the tests were built by PAM Membranas Seletivas Ltda. The MBR had a submerged hollow fiber microfiltration (MF) membrane module made of poly(etherimide), with an average pore size of 0.5 μm, packing density of 500 m² m⁻³ and membrane area of 0.04 m². The membrane bioreactor consisted of three acrylic tanks (Figure 1): a biological tank that operated with an effective volume of 50 L, a 10 L membrane tank and a 20 L storage tank for the permeate. A diaphragm pump was used to promote both the MF and the backwash. In addition, there were some three-way solenoid valves, level sensors, needle valves for flow adjustment, rotameters to indicate permeate, backwash and air.

#### Table 1 | Average characteristics values of the raw landfill leachate

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Unit</th>
<th>Average</th>
<th>Range</th>
<th>Sample number</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>mg L⁻¹</td>
<td>3,605</td>
<td>2,033–5,429</td>
<td>65</td>
</tr>
<tr>
<td>BOD₃</td>
<td>mg L⁻¹</td>
<td>386</td>
<td>74–540</td>
<td>28</td>
</tr>
<tr>
<td>BOD₅/COD</td>
<td>–</td>
<td>0.09</td>
<td>0.03–0.1</td>
<td>28</td>
</tr>
<tr>
<td>TOC</td>
<td>mg L⁻¹</td>
<td>1,175</td>
<td>853–2,728</td>
<td>48</td>
</tr>
<tr>
<td>Color</td>
<td>uH</td>
<td>1,804</td>
<td>815–2,383</td>
<td>73</td>
</tr>
<tr>
<td>pH</td>
<td>–</td>
<td>8.51</td>
<td>8.06–9.47</td>
<td>65</td>
</tr>
<tr>
<td>Humic substances</td>
<td>mg L⁻¹</td>
<td>2,078</td>
<td>965–2,693</td>
<td>51</td>
</tr>
<tr>
<td>Total nitrogen</td>
<td>mg L⁻¹</td>
<td>1,810</td>
<td>1,009–2,336</td>
<td>48</td>
</tr>
<tr>
<td>Ammoniacal nitrogen</td>
<td>mg L⁻¹</td>
<td>1,311</td>
<td>844–1,815</td>
<td>48</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>mg L⁻¹</td>
<td>6,866</td>
<td>2,814–9,123</td>
<td>74</td>
</tr>
<tr>
<td>Chlorides</td>
<td>mg L⁻¹</td>
<td>2,463</td>
<td>1,155–4,010</td>
<td>24</td>
</tr>
</tbody>
</table>
flow, a manometer to indicate pressure and a skid with an electric panel for the automatic control of permeation and backwash operations.

**Biomass acclimatization and the operation of MBR**

Commercial baker’s yeast (*S. cerevisiae*) was used as the seed, which was purchased from Fleischmann®. Firstly, the granulated active dry yeast was rehydrated. 100 g of granulated active dry yeast and 30 g of Sabouraud Dextrose Broth (SAD) were added to 10 L of distilled water. The biomass suspension was aerated for 24 h before being allowed to settle. The supernatant was then discarded. The biomass was transferred to the biological tank of the MBR and was fed with LFL and Sabouraud Dextrose Broth solution for biomass acclimation, to allow the yeast to adapt to the recalcitrant compounds and the inhospitable characteristics of LFL, as well as to the operating conditions of the MBR. The biomass acclimation was based on the gradual decrease in the dilution of LFL and the subsequent reduction in the concentration of Sabouraud Dextrose broth. Initially, the MBR was fed LFL diluted in water at a concentration of 20% v/v added to 3 g L⁻¹ of SAD broth. Subsequently, the concentration of leachate was increased to 40, 60, 80 and 100% v/v, followed by a reduction in the concentration of SAD broth to 2, 1 and 0 g L⁻¹. The biomass acclimation was assumed to be complete when the COD removal and biomass concentration were stabilized, which took about 180 days.

During acclimation, the MBR was operated at ‘infinite’ sludge retention time (sludge removal only to carry out the analysis), which was set to 60 d after acclimation. The system was kept under aeration, at a pH of 3.5 and temperature between 25 and 30 °C. The initial concentration of *S. cerevisiae* was 10,000 mg L⁻¹. The permeate flow was 0.2 L h⁻¹ and the HRT was 48 h, corresponding to a useful biological tank volume of 9.60 L. To control the fouling, 15 s of backwash was applied for every 15 min of filtration. The specific air demand based on the membrane area (SADm) was maintained at 0.6 m³ h⁻¹ m⁻². The MBR operated with an average F/M and an organic load of 0.44 kg-COD kg⁻¹ MLSS d⁻¹ and 2.11 MBR kg-COD m⁻³ d⁻¹.
respectively (mixed liquor suspended solids – MLSS). The membrane underwent fortnightly maintenance cleanings with a 500 mg L\(^{-1}\) sodium hypochlorite solution for 4 h followed by a citric acid solution with a pH less than 2 for 20 min. The MBR was monitored for 227 d after acclimation in order to record the permeate flow rate, operating pressure and temperature and collect feed, permeate and sludge samples to evaluate their quality.

**Membrane fouling investigation**

Critical flux, permeability and membrane resistance were monitored for the membrane fouling investigation. Critical flux is the value of flux at which the membrane flux decreases to constant pressure values. In this work, the critical flux was determined periodically using the Transmembrane pressure-step method (Bacchin et al. 2006). The membrane resistance (Rm), static adsorption (Ra), pore blockade (Rpb) and the cake (Rc) formed were determined using the method of resistance in series proposed by Choo and Lee (Choo & Lee 1998).

**Analytical methods**

Analyses to determine the COD (5220 B), alkalinity (2320 B), ammonia (4500-NH\(_3\) B), color (2120 B), turbidity (2130 B), conductivity (2510 B), chloride (4500-Cl B), phosphorus (4500-P) and mixed liquor volatile suspended solids (MLVSS) (2540 E) were carried out in accordance with the recommendations of *Standard Methods for the Examination of Water and Wastewater* (APHA 2012). The concentration of humic substances was determined using the method of Lowry modified by Frolund et al. (1995). The CFU mL\(^{-1}\) count was determined by the serial dilution plating method (Tortora et al. 2003). SMP and extracellular polymeric substances (EPS) were extracted using the thermal treatment method described by Morgan et al. (1990). First, the sludge was centrifuged at 4,500 rpm for 10 minutes and the supernatant liquid, mainly consisting of SMP, was collected. The solids resulting from the centrifugation were re-suspended with 0.05% sodium chloride solution and heated at 80 °C for 10 minutes for EPS release. This new suspension was centrifuged again and the supernatant liquid, constituting mainly of EPS, was collected. The SMP and EPS fractions were characterized in relation to carbohydrate and protein content in accordance with the methods proposed by Dubois et al. (1956) and Lowry et al. (1951).

**RESULTS AND DISCUSSION**

**Acclimation**

The acclimation of *S. cerevisiae* biomass occurred at the MBR based on the gradual increase in concentration of the leachate medium (0, 20, 40, 60, 80 and 100%) and subsequent reduction in the concentration of Sabouraud Dextrose broth (3, 2 and 1 g L\(^{-1}\)). During this period, the change in concentration of the LFL in the feed of the MBR occurred approximately every 20 days. Table 2 shows the results for the acclimation period.

Contrary to expectations, the removal of color, COD and humic substances increased with increasing LFL in the feed, suggesting gradual biomass acclimation to the toxic recalcitrant compounds in the LFL. With reduction in the concentration of Sabouraud Dextrose broth, there was initially a reduction in the removal efficiency of COD, color and humic substances. However, the subsequent restoration of efficiency and greater stability of removal suggested a gradual adaptation of the microorganisms to the leachate compounds.

The MLVSS concentration decreased with increasing concentration of LFL in the feed during acclimation. Initially, there was a sharp drop in the MLVSS concentration, probably due to microbial shock owing to the inhospitable conditions generated by the xenobiotic compounds present in the LFL. However, it should also be noted that the activity of microorganisms that were resistant to these conditions ensured high efficiency of removal of organic matter.

For a better evaluation of biomass growth and characterization of the plating, the counting of colony forming units of microbial groups (CFU mL\(^{-1}\)) present in the sludge was carried out. With regard to the growth of *S. cerevisiae*, there was intense microbial contamination by other groups such as filamentous fungi and the group of non-filamentous colonies, indicating the presence of bacteria and wild yeasts. It is believed that these organisms also contributed to the organic matter in the LFL, although it is not possible to quantify the contribution of each group. In any case, it can be highlighted that even by inoculating the MBR for the LFL treatment with *S. cerevisiae* alone, it was possible to obtain a mixed sludge that was able to remove the organic matter in the LFL.

It is noteworthy that *S. cerevisiae* increased from 10\(^9\) CFU/mL to about 10\(^{12}\) CFU mL\(^{-1}\) with increasing concentration of leachate, confirming the rapid acclimation of biomass to the leachate. At the end of the acclimation
Table 2 | Results for the acclimation period

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sampling</th>
<th>20 ± 3 g L⁻¹ SAB</th>
<th>40 ± 3 g L⁻¹ SAB</th>
<th>60 ± 3 g L⁻¹ SAB</th>
<th>80 ± 3 g L⁻¹ SAB</th>
<th>100 ± 3 g L⁻¹ SAB</th>
<th>100 ± 2 g L⁻¹ SAB</th>
<th>100 ± 1 g L⁻¹ SAB</th>
<th>100 ± 0 g L⁻¹ SAB</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg L⁻¹)</td>
<td>Feed</td>
<td>4.312 ± 772</td>
<td>4.334 ± 953</td>
<td>4.185 ± 1145</td>
<td>6.425 ± 711</td>
<td>6.296 ± 1,459</td>
<td>3.668 ± 1.138</td>
<td>3.792 ± 751</td>
<td>2.865 ± 625</td>
</tr>
<tr>
<td></td>
<td>Permeate</td>
<td>1,159 ± 582 (72)</td>
<td>923 ± 724 (78)</td>
<td>1,390 ± 274 (64)</td>
<td>1,715 ± 540 (74)</td>
<td>1,329 ± 941 (79)</td>
<td>797 ± 364 (76)</td>
<td>1,169 ± 526 (68)</td>
<td>745 ± 209 (73)</td>
</tr>
<tr>
<td>Color (mg L⁻¹)</td>
<td>Feed</td>
<td>1,235 ± 396</td>
<td>1,443 ± 387</td>
<td>2,100 ± 631</td>
<td>4,433 ± 653</td>
<td>4,759 ± 898</td>
<td>3,729 ± 1,126</td>
<td>3,300 ± 1,142</td>
<td>2,188 ± 580</td>
</tr>
<tr>
<td></td>
<td>Permeate</td>
<td>346 ± 158 (70)</td>
<td>329 ± 130 (77)</td>
<td>310 ± 139 (84)</td>
<td>456 ± 136 (90)</td>
<td>499 ± 165 (79)</td>
<td>391 ± 148 (76)</td>
<td>425 ± 144 (68)</td>
<td>591 ± 180 (72)</td>
</tr>
<tr>
<td>Humic substance (mg L⁻¹)</td>
<td>Feed</td>
<td>676 ± 39</td>
<td>739 ± 35</td>
<td>706 ± 199</td>
<td>798 ± 213</td>
<td>825 ± 174</td>
<td>561 ± 54</td>
<td>453 ± 116</td>
<td>344 ± 135</td>
</tr>
<tr>
<td></td>
<td>Permeate</td>
<td>371 ± 102 (44)</td>
<td>292 ± 89 (60)</td>
<td>157 ± 66 (78)</td>
<td>203 ± 125 (76)</td>
<td>170 ± 59 (78)</td>
<td>185 ± 57 (66)</td>
<td>136 ± 79 (71)</td>
<td>58 ± 38 (83)</td>
</tr>
<tr>
<td>pH</td>
<td>Sludge</td>
<td>4.5 ± 2.6</td>
<td>3.5 ± 0.7</td>
<td>3.5 ± 0.6</td>
<td>3.5 ± 0.3</td>
<td>3.8 ± 0.4</td>
<td>3.4 ± 0.5</td>
<td>3.4 ± 0.6</td>
<td>3.9 ± 0.5</td>
</tr>
<tr>
<td>MLSS (g L⁻¹)</td>
<td>Sludge</td>
<td>10.0 ± 4.1</td>
<td>3.2 ± 0.9</td>
<td>5.1 ± 2.4</td>
<td>7.8 ± 2.8</td>
<td>7.5 ± 2.4</td>
<td>6.3 ± 2.1</td>
<td>6.7 ± 1.9</td>
<td>3.7 ± 0.9</td>
</tr>
<tr>
<td>MLVSS (g L⁻¹)</td>
<td>Sludge</td>
<td>8.4 ± 4.8</td>
<td>7.5 ± 0.8</td>
<td>7.1 ± 1.9</td>
<td>6.6 ± 2.6</td>
<td>6.3 ± 2.0</td>
<td>5.8 ± 1.6</td>
<td>5.0 ± 1.4</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td>S. cerevisiae (UFC 100 mL⁻¹)</td>
<td>Sludge</td>
<td>NM</td>
<td>8.8 ± 10¹⁰</td>
<td>3.1 ± 10¹⁰</td>
<td>6.5 ± 10¹</td>
<td>8.34 ± 10¹¹</td>
<td>5.0 ± 10¹²</td>
<td>1.6 ± 10¹²</td>
<td>5.0 ± 10¹¹</td>
</tr>
<tr>
<td>Filamentous fungi (UFC 100 mL⁻¹)</td>
<td>Sludge</td>
<td>NM</td>
<td>4.4 ± 10⁹</td>
<td>6.4 ± 10⁹</td>
<td>3.3 ± 10⁹</td>
<td>2.87 ± 10¹⁰</td>
<td>7.8 ± 10¹¹</td>
<td>1.1 ± 10¹¹</td>
<td>5.2 ± 10¹¹</td>
</tr>
<tr>
<td>Non-filamentous colonies (UFC 100 mL⁻¹)</td>
<td>Sludge</td>
<td>NM</td>
<td>1.0 ± 10⁵</td>
<td>8.7 ± 10⁷</td>
<td>1.8 ± 10⁷</td>
<td>9.72 ± 10⁸</td>
<td>2.8 ± 10¹¹</td>
<td>8.6 ± 10⁸</td>
<td>4.3 ± 10⁸</td>
</tr>
<tr>
<td>Permeability (L m⁻² h⁻¹ bar⁻¹)</td>
<td>-</td>
<td>50 ± 14</td>
<td>40 ± 17</td>
<td>47 ± 23</td>
<td>24 ± 7</td>
<td>27 ± 10</td>
<td>52 ± 12</td>
<td>48 ± 32</td>
<td>35 ± 17</td>
</tr>
</tbody>
</table>

*¹ removal efficiency in %.
*²For COD, the concentration of the feed was also considered the concentration of the Sabouraud broth.
*³Not measured.
period, during the process of withdrawing SAB broth, there was a slight decrease in the concentrations of *S. cerevisiae* and filamentous fungi, with a greater reduction in the concentration of other non-filamentous colonies. This fact corroborates the reduction in the removal efficiency of organic matter during the same period.

**Organic matter removal**

The COD concentration in the LFL ranged between 1,552 and 6,899 mg L\(^{-1}\), with an average value of 3,491 mg L\(^{-1}\) and standard deviation of 1,299 mg L\(^{-1}\) (Figure 2). The average value of the corresponding permeate COD was 1,139 mg L\(^{-1}\), with a standard deviation of 643 mg L\(^{-1}\), which gives an average removal efficiency of 68% and standard deviation of 11.74%. The average removal efficiency of COD obtained in this study is similar to that of the MBR with yeast, using leachate with prior removal of ammonia, as verified in the study by Wichitsathian et al. (2018) (about 73%). It should be noted that the yeast biomass used by the authors was probably already adapted to the compounds of the leachate, since it came from the sludge of the same wastewater treatment plant from which the leachate was obtained, unlike the biomass used in this study, which primarily consisted of a specific, exogenous yeast (*S. cerevisiae*).

Furthermore, the COD removal in the proposed system (MBRy) was superior to that obtained in the MBR using conventional sludge, operating under the same HRT and SRT of this study and using the same LFL (Amaral et al. 2016). According to the authors, the bacterial MBR showed organic matter removal of 44% (as COD). This result suggests that the greater COD removal in the MBRy occurred due to the yeast performance used as inoculum since the other microorganisms present in the LFL also contribute to COD removal in the conventional MBR. The COD removal of the MBRy was also superior to the systems that other authors have worked with, using LFL with similar characteristics. Saleem et al. (2018) observed an average TOC removal was 58 in an aerobic MBR. Galleguillos et al. (2011) reported that although conventional MBR exhibited high BOD and ammonia removal of 94% and 98% respectively, COD removal was rather low (approximately 40%) due to the high concentration of recalcitrant organics. In addition, Ahmed & Lan (2012) show that conventional MBRs achieve COD removal efficiencies ranging from 54 to 78%.

In order to evaluate the potential of yeast biomass to remove recalcitrant COD, in a previous study, inert COD assay was carried out using the method proposed by Germirli et al. (1991) for bacterial (sludge collected in an activated sludge plant) and yeast (collected in the MBR being studied) biomass (Brito et al. 2013). The LFL had about 40% of inert COD to the bacterial biomass (biodegradable and non-biodegradable COD concentration of 2,112 and 1,408 mg L\(^{-1}\) respectively), while for the yeast biomass the corresponding inert COD was 30% (biodegradable and non-biodegradable COD concentration of 2,464 and 1,056 mg L\(^{-1}\) respectively). These results show the higher potential of yeast biomass to degrade the organic matter present in the LFL. Considering that 30% of LFL COD is inert, the MBR inoculated with commercial baker's yeast has a theoretical COD removal efficiency of 97% (considering only the biodegradable fraction of the feed: Total COD – Inert COD).

The removal efficiency of COD was not always stable. There were some significant variations over short periods
of time. The low and high efficiency peaks were attributed mainly to variations in organic loads of ammoniacal nitrogen (NH₃-N) in the leachate, the F/M ratio and some instances of disruption of the permeate flow rate and the aeration tank of the biological system.

Nutrient removal

The LFL NH₃-N concentration ranged between 375 and 1,562 mg L⁻¹, with an average value of 799 ± 315 mg L⁻¹ (Figure 3(a)). The average value of the corresponding permeate NH₃-N was 400 ± 262 mg L⁻¹, which gives an average removal efficiency of 55 ± 19%. It can be considered that the average removal of NH₃ was promising, considering that the concentration of NH₃ in LFL is high and its removal is often problematic. In different MBR systems treating leachates, the average removal efficiency of NH₃ is highly variable, depending on the age of the landfill, the NH₃ load, the concentration of dissolved oxygen in the biological tank, climate, etc. (Alvarez-Vazquez et al. 2004; Ahmed & Lan 2012).

The average NH₃-N removal efficiency in the MBR in this study was higher than those presented in the similar works. Shaohua & Junxin (2006) reported that the removal efficiency of N-NH₃ from conventional sludge was about 30%. Wichitsathan et al. (2004) found NH₃ removal efficiency in the system with bacterial sludge equal to 40%, while it was equal to 43% in the system with yeast.

The average pH of the sludge in the MB Ry (3.7 ± 0.4) suggests that most of the NH₃-N found during the monitoring period was in ammonium cation (NH₄⁺) form, as ammonia (NH₃) forms when the pH is above 9.25. Under such conditions, ammonia removal by volatilization becomes negligible.

The MB Ry lacks optimal conditions for the growth of nitrifying organisms in the bioreactor. Therefore, the nitrogen removal in this system is due to its uptake by yeast. Yeasts are capable of using a wide variety of different organic and inorganic sources of nitrogen, with ammonia nitrogen as the most widely used form by fungi in general, which can be readily utilized (Carlile et al. 2001). Thus, once the high metabolism of these organisms is sustained, high ammonia nitrogen removal efficiency can be expected.
The phosphorus concentration in the LFL ranged between 19 and 58 mg L\(^{-1}\) with an average value of 36 ± 12 mg L\(^{-1}\) (Figure 3(b)). The average value of the corresponding phosphorus concentration was 14 ± 7 mg L\(^{-1}\), which gives an average removal efficiency of 57 ± 18%. In an MBR, the presence of the membrane may contribute to a higher removal of that nutrient, since it is responsible for phosphorus retention associated with particulate material and biomass. Dan et al. (2002) observed that the phosphorous absorption capacity of yeast sludge was two times greater than bacterial sludge.

**Characteristics of biomass and identification of morphotypes**

Figure 4(a) and 4(b) show the MLSS and MLVSS concentration and colony forming units of microbial groups present in the MBR sludge respectively. The MLVSS concentration in the MBR increases up to the 130th day of monitoring, demonstrating the occurrence of degradation of organic matter in the LFL by microbial groups present in the biological tank, helping the growth of the biomass, which is completely retained by the MF membrane. During the period between the 130th and the 180th day of monitoring, the MLVSS remained stable initially, after which a standard drop was observed, with subsequent stabilization of MLVSS values at around 6,606 ± 442 mg L\(^{-1}\), possibly due to the increase in organic load during the same period, which may have affected the microbial growth. The results of the Pearson correlation test suggest that there is a statistically significant negative correlation between feed COD and MLVSS (Pearson R = −0.67) during the period ranging from 180th to 230th day of monitoring for p-value <0.05.

It can also be observed that the increase in the total microbial concentration recorded by the CFU mL\(^{-1}\) count is about four times. This finding is supported by an increase of about five times for the MLVSS concentration. Despite
S. cerevisiae being the only species that was inoculated to the MBR and the pH being maintained at around 3.5 to favor yeast growth and to inhibit bacterial growth, other microorganisms, filamentous fungi and non-filamentous colonies, grow in the MBR sludge. These microorganisms may contribute to the degradation of LFL compounds. According to the results of the Kruskal-Wallis test, there was no difference in the concentration of S. cerevisiae and filamentous fungi during the acclimation period (MBR feed with LFL and no SAB) and the operation period (p = 0.5 and 0.1 for S. cerevisiae and filamentous fungi, respectively), while the concentration of non-filamentous colonies was statistically higher during the operation period than during acclimation (p < 0.02). Despite this difference, no statistical difference was observed in the COD removal (p = 0.8).

It was not possible to assess whether there was a difference in the MBR's performance with regard to ammonia removal during the acclimation stage and the standard operation stage. This can be attributed to the change in sludge composition, since the concentration of ammonia in the feed MBR varied widely during the standard operation phase. For this reason, the presence of a correlation between the ammonia feed concentration and the concentration of microbial groups identified in the biomass was confirmed. According to the Pearson correlation test, there is a statistically significant positive correlation between the ammonia feed concentration and the concentration of non-filamentous colonies (Pearson R = 0.87 and p-value <0.002), while no correlation was observed between S. cerevisiae and filamentous fungi and the ammonia feed concentration.

The non-filamentous morphotypes that were more abundant were selected for isolation and identification, along with S. cerevisiae. The yeast morphotypes were identified as Candida sp., Candida infanticola and Candida palmioleophila. The literature reports that the Candida genus in the sludge is able to degrade complex recalcitrant organic compounds (Harms et al. 2011) Therefore, these microorganisms may have assisted in the degradation of recalcitrant compounds from LFL.

Of the six bacterial morphotypes considered for the Gram staining technique, it was possible to sequence four nucleotides. These were identified as Bacillus sp., Alcaligenes sp., Alcaligenes faecalis and Enterococcus faecalis. These microorganisms were probably from the LFL, since they were indicative of fecal or environmental contamination, which in this case may have been from the soil used for grounding. In general, these bacteria are highly resistant to adverse conditions such as those present in the leachate.

Membrane fouling

The membrane permeability ranged from 10 to 75 L m⁻² h⁻¹ bar⁻¹, with an average value of 30 L m⁻² h⁻¹ bar⁻¹ and standard deviation of 16 L m⁻² h⁻¹ bar⁻¹ (Figure 5). Strict membrane fouling can be seen, even with the MBR operating at a permeate flux lower than the critical flux. The critical flux is one of the parameters that heavily influences fouling, since it is generally believed that operating below a critical flux can reduce the fouling rate (Amaral et al. 2015). Although there is fouling during subcritical
operations, it has already been established that MBRs that operate at a flux above the critical flux have a rather elevated fouling rate. The maintenance of critical flux at high values was guaranteed by the better filterability condition of yeast sludge. Fouling of the membrane could have been prevented was guaranteed by the better fouling rate. The maintenance of critical pore structure (internal fouling) of the membrane. The particles or macromolecules on the pores or within the internal (external fouling), or deposition and adsorption of small particulates as a result of reduced back-transport effect. It can lead to the formation of a sticky cake layer on the membrane surface, thereby increasing cake resistance.

The MBR inoculated with commercial baker’s yeast showed lower fouling potential compared with the results observed by Amaral et al. (2016), who investigated the use of a conventional MBR (inoculated with sludge from an activated sludge plant) to treat leachate from the same landfill, using the same membrane. According to the authors, the membrane permeability ranges from 3.4 to 22 L m\(^{-2}\) h\(^{-1}\) bar\(^{-1}\), with an average value of 8.3 L m\(^{-2}\) h\(^{-1}\) bar\(^{-1}\) and standard deviation of 5.2 L m\(^{-2}\) h\(^{-1}\) bar\(^{-1}\). The MLVSS concentration was 5,242 ± 3,483 mg L\(^{-1}\). The maintenance chemical cleaning was performed weekly for fouling control. The low fouling potential of yeast sludge was also observed by Dan et al. (2002) and Wichitsathian et al. (2004). In these studies, the MBR with sludge yeast presented an increased pressure over time, which was significantly lower than in the bacterial system, thus requiring less frequent chemical cleanings. It is noteworthy that according to respective authors, the sludge yeast reduced the rate of membrane fouling due to the specific characteristics of this biomass, such as large cells (2.5–3.9 mm), poor adhesion capacity, low sedimentation, low viscosity and low production of EPS.

Membrane fouling in an MBR is the result of accumulation of rejected particles at the membrane surface (external fouling), or deposition and adsorption of small particles or macromolecules on the pores or within the internal pore structure (internal fouling) of the membrane. The Pearson correlation test showed a statistically significant negative correlation between the MLVSS concentration and membrane permeability (Pearson R = −0.27) for a p-value of 0.02, while there was no correlation between the colony forming unit of microbial groups present in the MBR sludge (S. cerevisiae, filamentous fungi and no filamentous colonies) and membrane permeability, considering a p-value <0.05.

To evaluate the role of each portion of membrane resistance, the resistance-in-series model was applied (Table 3). Resistance due to the formation of the cake layer was the major mechanism of resistance. These results highlighted the importance of the cake layer in the development of membrane fouling. For biomass particulates with higher MLSS concentration and sludge viscosity, the net force towards the membrane surface can be developed on these particulates as a result of reduced back-transport effect. It can lead to the accumulation of more sludge particles and the formation of a sticky cake layer on the membrane surface, thereby increasing cake resistance.

The cake layer, as the predominant portion of the total resistance to filtration, was characterized by the composition and concentration of SMP and EPS (Figure 6). Note that the average concentration of SMP (SMPc = 701 ± 369 mg L\(^{-1}\) and SMPp = 428 ± 124 mg L\(^{-1}\)) was considerably higher than EPS (EPSc = 48 ± 42 mg L\(^{-1}\) and EPSp = 75 ± 39 mg L\(^{-1}\)). The low EPS concentration can be associated with the low fouling potential, observed in the MBR inoculated with yeast biomass compared to the MBR inoculated with bacterial biomass (EPSc = 48 ± 42 mg L\(^{-1}\) and EPSp = 75 ± 39 mg L\(^{-1}\)), since many authors consider EPS as the main factor responsible for the fouling of the membrane in an MBR (Meng et al. 2009; Wang et al. 2009; Johir et al. 2012). The Pearson correlation test showed a statistically significant positive correlation between the EPSp concentration and membrane fouling between chemical cleanings (TMP after chemical cleaning − TMP before next chemical cleaning)/time between cleanings (Pearson R = 0.28) for a p-value <0.05, while there was no correlation between the EPSc, SMPc and SMPp

### Table 3 | Membrane, adsorption, pore blocking and cake fouling resistance

<table>
<thead>
<tr>
<th>Operating time (days)</th>
<th>Resistance (m(^{-1}) × 10(^{11}))</th>
<th>Membrane</th>
<th>Adsorption</th>
<th>Pore blocking</th>
<th>Cake</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>25.9</td>
<td>9.1 (35.2%)</td>
<td>0.7 (2.6%)</td>
<td>2.5 (9.6%)</td>
<td>13.6 (52.6%)</td>
</tr>
<tr>
<td>84</td>
<td>64.0</td>
<td>14.0 (21.9%)</td>
<td>1.4 (2.1%)</td>
<td>6.0 (9.3%)</td>
<td>42.7 (66.7%)</td>
</tr>
<tr>
<td>113</td>
<td>120.0</td>
<td>15.0 (12.5%)</td>
<td>2.8 (2.3%)</td>
<td>6.2 (5.2%)</td>
<td>96.0 (80.0%)</td>
</tr>
<tr>
<td>148</td>
<td>75.0</td>
<td>8.9 (11.9%)</td>
<td>0.6 (0.8%)</td>
<td>0.5 (0.7%)</td>
<td>65.0 (86.7%)</td>
</tr>
<tr>
<td>197</td>
<td>70.6</td>
<td>10.9 (15.5%)</td>
<td>2.4 (3.4%)</td>
<td>3.2 (4.6%)</td>
<td>54.0 (76.6%)</td>
</tr>
</tbody>
</table>
concentration and membrane fouling. According to Dan et al. (2002) and Wichitsathian et al. (2004), the low concentration of EPS in the MBR with yeast sludge stems from the formation of flocs in such systems by physically inter-winding mycelia/pseudomycelium yeast, unlike the flocculation caused by biopolymers (EPS) in the conventional activated sludge process. The low concentration of EPS and the presence of filamentous fungi that agglomerate by inter-winding hyphae in the sludge, can be associated with the low formation of flocs in the sludge of the MBR inoculated with yeast compared to the conventional MBR sludge, as investigated by Amaral et al. (2016).

The lack of correlation between SMP and membrane fouling can be related to the cake layer (dynamic membrane) which acts as a barrier to filter out the SMP, thereby preventing them reaching the pores of the membrane and causing fouling. The cake layer formation in the MBRy is favoured by the large yeast cells, which physically attach themselves to the membrane surface. The high retention of carbohydrates and proteins reinforces that this cake layer acted as a dynamic membrane that also improved MBR performance. The molecular size of these carbohydrates and proteins is lower than the membrane pore size; consequently, the membrane size exclusion mechanism does not explain the high retention. The average concentration of carbohydrates and proteins in the permeate was 211 ± 128 and 185 ± 99 mg L⁻¹, respectively.

CONCLUSIONS

The commercial baker’s yeast proved to be an excellent source of inoculum for MBR, since the MBRy showed a good organic, nitrogen and phosphorus removal efficiency.

Even though the MBR was inoculated with an exogenous source of yeast (Saccharomyces cerevisiae), there was intensive contamination by wild filamentous fungi, bacteria
and yeasts. It is believed that these organisms also contributed to the removal of the organic matter.

The MBRy showed lower fouling potential, and cake layer formation was a major mechanism of membrane fouling.

The work demonstrated that the novel MBR is a promising technology to treat recalcitrant LFL.

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