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Hazardous events in membrane bioreactors – Part 3: Impacts on microorganism log removal efficiencies

Amos Branch [a], Trang Trinh [a], Guido Carvajal [b], Greg Leslie [a], Heather M. Coleman [b,c], Richard M. Stuetz [b], Jörg E. Drewes [d], Stuart J. Khan [b], Pierre Le-Clech [a,*]

[a] UNESCO Centre for Membrane Science and Technology, School of Chemical Engineering, University of New South Wales, NSW 2052, Australia
[b] UNSW Water Research Centre, School of Civil and Environmental Engineering, University of New South Wales, NSW 2052, Australia
[c] Nanotechnology and Integrated Bioengineering Centre, Ulster University, Jordanstown, Northern Ireland, United Kingdom
[d] Chair of Urban Water Systems Engineering, Technische Universität München, Garching, München, Germany

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ABSTRACT

Under normal operation, membrane bioreactors (MBRs) produce high quality effluent leading to common application in water recycling schemes. When hazardous events, outside of normal operation, occur strategies must be in place to ensure that microbially safe water is consistently produced. Limited literature is available to quantify the consequence of hazardous events on microorganism log removal value (LRV) by MBRs. As a result, current MBR risk management practices cannot be based on sufficient quantitative evidence. In this study, MBRs were subjected to hazardous events designed to reflect a range of potential severe feed water variations and process failures. During these challenge events, removal of four microbial indicator organisms representing viruses, bacteria and protozoa were quantified. Hazardous event impacts were benchmarked against the 5th percentile of normal process LRV, determined via probabilistic techniques. Severe feed water variation decreased the efficacy of bio-predation due to adversely affected activated sludge performance. However, overall LRV was preserved due to the onset of fouling, aiding rejection by the membrane. Overall FRNA bacteriophage LRV significantly decreased to 0.3 log units below the 5th percentile (LRV=4.1) after the NaCl shock, most likely due to inhibition of adsorption mechanisms. Fibre breakage resulted in a significant reduction in LRV, but was mitigated by suspended solids plugging the membrane lumen within 15 min. The findings presented here can inform risk management strategies for MBRs by permitting control strategy prioritisation according to quantified event consequences.

1. Introduction

Membrane bioreactors (MBR) are frequently used as a barrier in water recycling schemes, where a high quality effluent is desired and plant footprint is constrained [1]. Pathogenic microorganisms originating from sewage are the primary hazard in water recycling due to the potential for acute health effects from exposure to low dosages [2]. In water recycling applications, a thorough understanding of how pathogen removal performance is achieved by each treatment barrier is imperative. Any event compromising the pathogen removal efficiency must be detected and quantified to inform appropriate corrective action [3]. A hazardous event can be defined as an incident or situation leading to the presence of a hazard [4], in this context the loss of containment of pathogens by MBRs. Consideration of hazardous events is a key philosophy in the approach to water quality risk assessment used by the World Health Organisation (WHO) for the development of Water Safety Plans [5] and is described in the WHO Guidelines for Drinking Water Quality [6]. Current Australian water recycling guidelines are based on risk assessment and risk management considerations [4]. Previously, theoretical simulations of hazardous events [7] and shock loading of MBRs with chemicals [8,9] have been conducted, but without measurement of pathogen removal.

Direct measurement of pathogenic species in wastewater is often not feasible due to low and highly variable concentrations and complex analysis procedures [10]. As a result, indicator organisms are often chosen as surrogates for pathogens. A suitable

Abbreviations: CFU, colony forming units; C_{In}, influent microorganism density; C_{ML}, mixed liquor microorganism density; CP, *Clostridium perfringens*; C_{Perm}, permeate microorganism density; EC, *Escherichia coli*; FRNA, FRNA bacteriophage; IEP, iso electric point; IN, influent wastewater; LOD, limit of detection; LRV, log removal value; LRV_{MBR}, overall process LRV; LRV_{Bio}, LRV due to biopredation; MBR, membrane bioreactor; ML, mixed liquor (activated sludge); PDF, probability density function; PDT, pressure decay testing; PDR, pressure decay rate; Perm, permeate; PFU, plaque forming units; SEM, scanning electron microscopy; TC, total coliforms; WWTP, wastewater treatment plant

* Corresponding author.
E-mail address: p.le-clech@unsw.edu.au (P. Le-Clech).

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indicator organism should be selected such that it displays correlated or more conservative removal than the target pathogen [11]. Historically, clean water challenge testing of membranes for virus removal has been performed with laboratory grown cultures of the model virus MS2 bacteriophage (MS2) [12]. MS2 belongs to a genotype of the larger family of FRNA bacteriophages (FRNA). MS2 was previously shown to exhibit poor survival in surface waters and lower prevalence in sewage when compared to other FRNA genotypes [13]. This may indicate a tendency for MS2 to exhibit a less conservative removal in activated sludge than other genotypes of FRNA, indigenous to wastewater. FRNA have been investigated in several previous studies of log removal in MBRs [2,14–19]. FRNA was selected as an indicator of virus removal performance due to its small size (0.025 μm) [10] and low isoelectric point (IEP) (pH 3.9) [20]. With a diameter of 0.025 μm, FRNA presents a substantial challenge to removal via size exclusion by the membrane (pore diameter generally larger than 0.04 μm) and was chosen to model similarly sized pathogenic viruses present in wastewater such as, enterovirus (0.022–0.030 μm, IEP 4.0–6.4) and hepatitis A (0.027–0.028 μm, IEP 2.8) [21]. A low IEP (pH 3.9) relative to the typical operating pH of MBR (7–8) [22] reduced the likelihood of adsorption of FRNA to the membrane, as above pH 3.9 the virus particle carries a net negative charge [10]. Hence, FRNA was chosen as the virus indicator given, well-documented previous use and its conservative model properties. *Escherichia coli* (EC) and total coliforms (TC) were chosen to represent bacterial pathogens, due to their extensive historic use as faecal contamination indicators and as challenge organisms for membrane systems. *Clostridium perfringens* (CP) was selected as a surrogate for protozoa. The propensity of CP to form spores and withstand harsh environments has led to its use as a surrogate for *Cryptosporidium parvum* in disinfection studies [23]. Depending on the strain analysed, CP spore diameters range between 0.6 and 1.0 μm [24]. The smaller size of CP, relative to other protozoa (5–10 μm) [10], further supports its use as a conservative indicator in membrane challenge testing. Additionally, CP has been used as a challenge organism to represent protozoan removal in previous studies on MBR [2,15,25].

We have previously assessed the impact of hazardous events on key bulk water quality parameters (Part 1) [8] and trace organic chemical removal (Part 2) [26]. This study (Part 3) aimed to quantify the impact of hazardous events on removal of indicator microorganisms during MBR operation. Even under normal conditions, performances of wastewater treatment processes are inherently variable. Through the use of Monte Carlo simulation, hazardous events were evaluated with respect to process variability under normal conditions. Benchmarking against the magnitude of normal variability provides a realistic measure and ranking of hazardous event consequence. New knowledge has been provided as a result of this study that supports application of quantitative health risk management practices for MBRs in water recycling.

2. Experimental

2.1. Microbial analysis

CP, EC, TC and FRNA were analysed according to previously published culture methods [2]. In recent research, quantitative polymerase chain reaction (qPCR) has been applied for analysis of viruses that were not able to be cultured. qPCR can rapidly determine the concentration of viruses by replication of DNA tagged with fluorescent primers. Some concern has been raised over the ability of qPCR to indicate the subtle effect of activated sludge on microorganism viability, as DNA may not be altered sufficiently to prevent virus detection by qPCR [27,28]. Double agar layer (DAL) plaque counting methods for virus removal were seen to be advantageous in this study, as the changes in organism viability due to biopredation in activated sludge could be assessed. Data was reported in colony forming units (CFU) for bacterial indicators and plaque forming units (PFU) for phage per 100 mL volume of sample.

In preliminary trials, FRNA concentration measured in sewage was lower than expected. The low sewage concentration meant that minimal removal could be demonstrated, as FRNA was removed to below the limit of detection (LOD). Spiking a laboratory culture into the feed tank, grown from FRNA indigenous to the wastewater treatment plant (WWTP), increased FRNA concentration during the experiments. The laboratory culture was first extracted from the top of positive plates using tryptone water (Oxoid CM0087, UK). The extract was centrifuged (at 4400 RPM for 15 min) and filtered through 0.45 μm syringe filters (Sartorius, USA). The extract was then plated and incubated according to the double agar layer method used for analysis [2]. A second, more concentrated, solution was then extracted from the incubated plates, centrifuged, and excess bacteria filtered out with 0.45 μm gridded filter membrane (Millipore, USA). The re-incubation step was repeated until the resulting stock solution had a final concentration of approximately 10^9–10^{11} PFU (100 mL)$^{-1}$.

2.2. Operation and sampling

MBR trials were conducted for 5–6 days at a time using 30 L laboratory scale bioreactors. Short trial times featuring start up and equilibration for 2 days and monitoring recovery for up to 4 days were adopted, as an event that impacted water quality for longer than the observation period could be considered to have severe consequences to health, and warrant further study. Further details on MBR operation, sampling and the rationale for hazardous events selection are provided in a previously published paper (Part 1) [8] and a diagram is included in supplementary information. At the start of the experiment, 30 L lab scale MBRs were seeded with activated sludge from a larger 3.7 $m^3\ d^{-1}$ pilot MBR, operated continuously at the WWTP [29]. The solids retention time (SRT) of the larger pilot MBR was 30 days. The 30 L MBRs were operated at constant flux (10 L $m^{-2}\ h^{-1}$) and with a hydraulic retention time (HRT) of 24 h. The activated sludge compartment was aerated intermittently (15 min on/off cycles) to promote nitrification and denitrification. Fouling was mitigated by constant air scouring of the membrane compartment and relaxation for 10 min each day. Four bespoke membrane mini-modules (having a combined area of 0.13 m^2) were constructed from commercial hollow fibre membranes (Evoqua Water Technologies, AUS) and installed in each reactor. The membranes were made of polyvinylidene difluoride (PVDF), with an outer diameter of 1300 μm and a nominal pore size of 0.04 μm. Four 30 L MBRs were operated in parallel and fed with sewage from a common feed tank (200 L). The feed tank was refilled each day and spiked with a volume of laboratory cultured FRNA (Section 2.1), sufficient to achieve a concentration of 10^7–10^8 PFU (100 mL)$^{-1}$. The dosage of 10^7–10^8 PFU (100 mL)$^{-1}$ was chosen in order to maximise the chance of detecting FRNA in the permeate but not to exceed the recommended dosage of $10^{7.5}$ ($10^{6.5}$+LOD) to avoid artificially high LRVs due to aggregation [12]. An additional control against FRNA aggregation was buffering of the influent tank pH to between 7 and 8 by daily addition of sodium bicarbonate, as at higher pH aggregation is minimised [30]. Grab samples were taken once per day from the feed tank (influent – 0.5 L), activated sludge compartment (mixed liquor – 0.5 L) and permeate (1 L). Densities of microorganisms were measured on all influent (C_{In}), mixed liquor (C_{ML}) and permeate (C_{Perm}) samples.