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ORIGINAL PAPER

Biological treatment of a synthetic space mission wastewater using a membrane-aerated, membrane-coupled bioreactor (M2BR)

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Abstract This paper describes the membrane-aerated, membrane-coupled bioreactor (M2BR), which was developed for wastewater treatment during long-term space missions because it achieves aeration and biomass separation using components that are compatible with microgravity conditions. In the experiments described herein, the M2BR was used to treat a synthetic wastewater formulated by NASA to simulate the wastewater typically collected during space missions. The M2BR was able to achieve more than 90% removal of both chemical oxygen demand (COD) and total nitrogen when it was fed a modified NASA wastewater that had a 4:1 COD to nitrogen ratio. When the full-strength synthetic wastewater was fed to the M2BR (COD:N = 1), however, the nitrogenous pollutant removal efficiency was adversely affected because of either insufficient oxygen transfer to support nitrification (an air-fed M2BR) or insufficient electron donor to support denitrification (an oxygen-fed M2BR). In conclusion, the M2BR provides considerable promise for wastewater treatment during long-term space missions, although additional research is needed to identify the best approach to treat the space mission wastewater, which poses a unique challenge because of its low COD:N ratio.

Keywords M2BR · MABR · Membrane filtration · Microgravity · Space travel · Wastewater treatment

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Introduction

Potable water is a critical resource for human beings during long-term space travel. While spaceships are currently fueled by the combustion of H_2 and O_2, which generates sufficient quantities of potable water, future long-term space missions (e.g., to Mars) will utilize nuclear-based power, thus eliminating this source of water. Future long-term space missions, therefore, will need to treat and reuse as much water and wastewater as possible.

Wastewater treatment during long-term spaces missions poses unique challenges compared to analogous terrestrial systems. Wastewater treatment must produce a very high quality effluent without adversely affecting the habitability of the spacecraft. Another challenge is to design the entire process to be compatible with microgravity conditions. Conventional aerobic biological wastewater treatment, for example, depends on gravity to create buoyant air bubbles for aeration and on gravitational clarifiers to separate the biomass from the treated effluent.

We have proposed the membrane-aerated, membrane-coupled bioreactor (M2BR) to meet the challenge of wastewater treatment in space. The M2BR utilizes a gas-transferring membrane to provide bubbleless gas transfer to the wastewater [1, 6]. This membrane also serves as the support for a uniquely stratified biofilm that can contain active populations of both nitrifying and denitrifying bacteria [5, 22]. The M2BR also utilizes a second membrane to completely separate suspended cells from the treated effluent, thus providing an important hygienic safety constraint for astronauts living in a confined space for an extended period of time. This membrane filtration step is the most pertinent difference between the M2BR and other reactor designs that are currently being considered for wastewater treatment during long-term space travel [18, 20]. The retention of

high densities of bacterial cells within the bioreactor also helps produce a high quality effluent from a relatively small reactor while generating minimal quantities of residual biomass [4, 12, 13].

In this study, we tested the ability of the M2BR to treat a synthetic wastewater formulated by NASA to simulate the combination of urine, atmospheric condensate, and used hygiene water that would be generated during space missions. The NASA wastewater ersatz contains relatively high concentrations of both nitrogenous (700 mg N L^{-1}) and carbonaceous pollutants (chemical oxygen demand = 800 mg L^{-1}). Our goals for this study were to identify an appropriate start-up procedure so that the M2BR could achieve simultaneous nitrification/denitrification and to determine the limitations of the M2BR in treating this wastewater.

Materials and methods

Membrane bioreactors

The M2BR system consisted of a membrane-aerated bioreactor coupled to an external filtration membrane module (Fig. 1). The membrane-aerated bioreactor consisted of a 1.5 L polycarbonate chamber with two internally mounted membrane sheets (25 × 30 cm) for gas transfer. The membrane sheets were composed of numerous hollow fiber polyolefin membranes in a parallel configuration (outside diameter = 300 μm, interspacial distance = 300 μm; total membrane surface area = 0.118 m^2; Celgard LLC, Charlotte, NC). This gas-transferring membrane was supplied with air or pure oxygen at a flow rate of 200 mL min^{-1} at atmospheric pressure. The external membrane filter was a 0.2-μm pore size polysulfone microfiltration membrane cartridge (surface area 0.042 m^2; Amersham Biosciences Corp., Piscataway, NJ). Culture fluid was rapidly (residence time <5 min) removed from the bottom of the bioreactor, pumped through the membrane cartridge, and returned to the bioreactor. The feed medium was added into the bioreactor using a Masterflex variable-speed console drive pump (Cole–Parmer, Vernon Hills, IL). Permeate was pumped from the filter cartridge at a specific rate to maintain the culture volume (1,500 ± 50 ml) in the M2BR. The pH of the system was maintained at 7.5 using a pH controller (Eutech Instruments, Singapore) that fed 0.5 M Na_2CO_3 as needed.

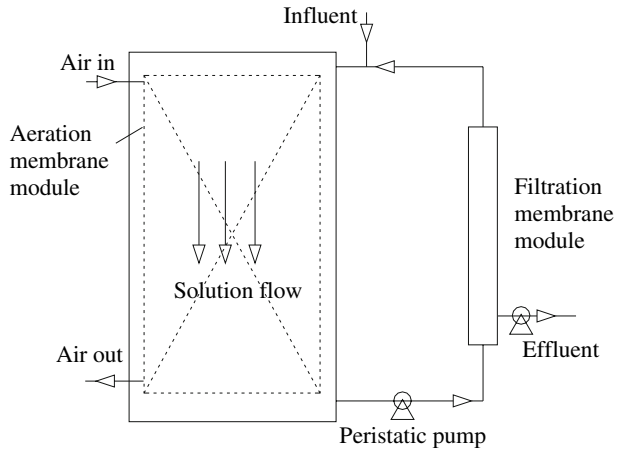


Fig. 1 Schematic diagram of the M2BR system used in this research

The synthetic wastewater used in this study was a modified version of the Early Planetary Base Wastewater Ersatz formulated by NASA (Table 1). This wastewater was used in dilute forms throughout our experiments so that the COD:N ratio could be manipulated. An inorganic growth medium was also used to enrich for nitrifying bacteria (i.e., to use an inoculum), which contained (per liter of deionized water): 943 mg ammonium sulfate, 100 mg sodium bicarbonate, 25 mg sodium phosphate, 30 mg potassium phosphate, 40 mg magnesium chloride, 60 mg calcium chloride, and 0.1 mL SL7 trace mineral solution [3].

The M2BRs were separately inoculated with heterotrophic and nitrifying bacteria. Heterotrophic bacteria were enriched by inoculating a 100 mL shake-flask containing the NASA wastewater ersatz with 1 mL of cells collected from the aeration tanks of the Metropolitan Wastewater Treatment Plant (St Paul, MN). Five milliliters of this culture were then used to inoculate the M2BRs. An analogous nitrifying enrichment culture was also used to inoculate the M2BRs.

Analytical methods

The oxygen transfer rate (OTR) of the aeration membrane was determined by obtaining a time profile of the oxygen concentrations in the reactor. The M2BR system was fully filled with deionized water to remove all of the air inside the reactor. Sodium sulfite was then added to deplete oxygen using cobalt chloride as a catalyst. Oxygen concentrations in the bulk water of the M2BR were then monitored to account for air leakage (negligible). Once membrane aeration was initiated, oxygen concentrations in the M2BR were recorded for up to 20 min. The OTR was then computed via a regression of the dissolved oxygen profile versus time.

Suspended biomass was measured as both optical density at 600 nm (OD_{600}) and particulate protein. Optical density was quantified using a Beckman DU 530 UV–Vis spectrophotometer (Beckman Coulter Inc., Fullerton, CA) with a 1 cm path length. Particulate protein was quantified using the Lowry method [17] with bovine serum albumin (BSA) as the protein standard. One OD_{600} unit

Table 1 Components of the modified NASA early planetary base wastewater ersatz

Component	Quantity (per L)
Shampoo (suave for kids)	275 mg
Ammonium bicarbonate	2.3 g
Ammonium hydroxide	300 mg
Ammonium citrate	370 mg
Ammonium formate	45 mg
Ammonium oxalate monohydrate	20 mg
Sodium chloride	690 mg
Potassium chloride	200 mg
Sodium bicarbonate	200 mg
Potassium phosphate monobasic	166 mg
Potassium sulfate	690 mg
Urea	160 mg
Lactic acid	80 mg
Creatinine	160 mg
Histidine	29 mg
Taurine	17 mg
Glutamic acid	51 mg
Glucose	78 mg
Acetic acid	34 mL
Benzoic acid	1 mg
Benzyl alcohol	5 μL
Ethanol	6.5 mL
Acetone	0.5 μL
Caprolactam	4 mg
Phenol	0.6 mg
N,*N*-dimethylformamide	0.7 μL
Ethylene glycol	3.3 μL
4-Ethyl morpholine	1.5 μL
Formaldehyde	3 μL
Formic acid	4.4 μL
Methanol	7.6 μL
1,2-Propanediol	2 μL
2-Propanol	2.4 μL
Propionic acid	15 μL

corresponded to 360 mg L^{-1} of particulate protein ($r^2 = 0.96$). Following the completion of each experiment, biofilm samples for community analysis were collected from the upper, middle, and lower sides of the outer face of both aeration membranes as well as the inner face of each membrane. Biomass that deposited on the bottom of reactor was also collected for community analysis.

Chemical oxygen demand (COD) was determined colorimetrically using low-range accu-Test vials (Bioscience Inc., Bethlehem, PA) using potassium hydrogen phthalate as a standard. Ammonia was measured using the Nessler method [7] using ammonium chloride as standard. Total Kjeldahl nitrogen (TKN) was measured using a Digesdahl Digestion Apparatus (Hach, Loveland, CO) followed by the Nessler method. Nitrite and nitrate were determined using a 761 compact ion chromatography system (Metrohm, Houston, TX) using sodium nitrite and sodium nitrate as standards. All assays were performed in triplicate. Data are presented as the arithmetic means; the standard deviations for all samples were <5% of the mean (data not shown).

Bacterial community analysis

Suspended biomass and biofilm samples were centrifuged and resuspended with 0.5 mL of lysis buffer (120 mM sodium phosphate buffer, 5% sodium dodecyl sulfate, pH 8.0). After three consecutive freeze-thaw cycles, cells were lysed during 90 min incubation at 70°C. Genomic DNA was extracted from those samples using the Fast DNA Spin Kit per manufacturer's instructions (Qbiogene, Vista, CA).

From the extracted DNA, the variable V3 region of the 16S rRNA gene was amplified by PCR using primers 338F [14] and 518R [19], with a GC-clamp attached to the forward primer [19]. DGGE was performed using a D-Code apparatus (BioRad; Hercules, CA). Equal amounts of PCR amplicons were loaded onto 8% (wt/vol) polyacrylamide gels (37.5:1, acrylamide:bisacrylamide) in 0.5 × TAE buffer [21] using a denaturing gradient ranging from 30 to 55% denaturant (100% denaturant contains 7 M urea, 40% (vol/vol) formamide in 0.5 × TAE). Following electrophoresis, gels were stained with SYBR Green I (Molecular Probes, Eugene, Oreg.; diluted 1:5,000 in 0.5 × TAE). Gels were visualized on a UV transilluminator and photographed with a digital CCD camera (BioChemi System; UVP, Inc., Upland, CA). Photographs were inverted and enhanced for contrast and brightness using Adobe Photoshop v6.0.

Specific bands were manually excised from the denaturing gel, suspended in 25 μL of sterile water, and incubated overnight. The entire PCR–DGGE protocol was repeated using this water as template until only a single band was detectable (2–4 iterations were typically required). A final PCR step was performed without the GC-clamp attached to the forward primer. These PCR products were purified (Geneclean II; Qbiogene) and used as template for nucleotide sequence analysis.

All nucleotide sequences were determined at the Advanced Genetic Analysis Center at the University of Minnesota using an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA). Nucleotide sequences were determined fully in both directions for each purified PCR–DGGE band using 338F and 518R as sequencing primers. Reported nucleotide sequences are the consensus of bi-directional sequence information without the original PCR primer sequences. Reference nucleotide sequences were obtained from the GenBank database [2]. Nucleotide

sequences were checked for possible chimeric sequences by manually splitting sequences into subsections to determine if the segments were from different phylogenetic groups. The nucleotide sequences obtained in this study have been deposited in the GenBank database under accession nos. DQ838089–DQ838095.

Results

Clean membrane oxygen transfer

Prior to the initiation of any of the M2BR experiment, the oxygen transfer rate (OTR) of clean gas-transferring membranes (using atmospheric air as a source of oxygen) was measured as 830 mg day^{-1} (SD = 13 mg day^{-1}; $n = 5$). Based on stoichiometric oxidation of COD and NH_3 in the NASA wastewater ersatz, the OTR needed to be approximately 4,800 mg day^{-1} for complete treatment at a hydraulic residence time of 24 h (assuming partial COD oxidation via denitrification). Previous research, however, has suggested that bacterial growth on the gas transferring membrane can increase the OTR [9, 23]. Recognizing that oxygen transfer could constrain M2BR operation, therefore, three different M2BR experiments were performed during which the nutrient loading, feed composition, and the oxygen content of the aeration gas (i.e., atmospheric air vs. pure oxygen) were varied.

First M2BR experiment

The first M2BR was fed the full-strength NASA wastewater ersatz for 66 days at a hydraulic residence time (HRT) of 24 h without biosolids removal (Fig. 2a). This experiment simulated the simplest start-up procedure for the M2BR. The effluent COD concentration steadily declined during the first 30 days of operation, after which the M2BR performance was reasonably consistent (mean COD removal efficiency = 87%). In contrast, no ammonia oxidation was observed throughout the entire experiment. Suspended biomass accumulated rapidly during the first 25 days, after which it gradually increased until the end of the experiment (Fig. 2b).

Second M2BR experiment

Since the first M2BR experiment failed to achieve nitrification, the second M2BR experiment involved a more complicated start-up procedure to ensure better performance. Based on our prior experience with membrane-aerated bioreactors [11], we attempted to establish an actively nitrifying biofilm on the gas-transferring membrane before introducing the NASA wastewater ersatz. The second

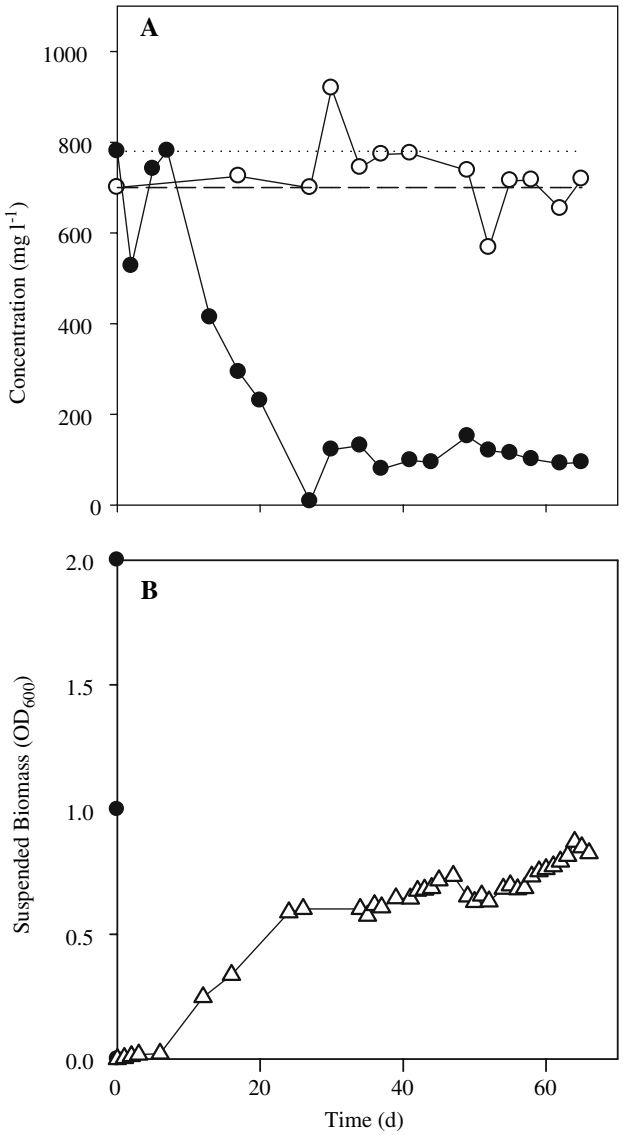


Fig. 2 **a** Effluent concentrations of COD (*filled circles*) and ammonia (*open circles*) during the first M2BR experiment. The *dotted* and *dashed lines* represent the influent concentrations of COD and ammonia, respectively. **b** Suspended biomass concentrations

M2BR experiment, therefore, involved four distinct phases of operation during which the composition of the feed solution was varied while the HRT was maintained at 24 h. The M2BR was initially fed an ammonia-only solution (Phase 1: 29 days; COD = 0 mg L^{-1}; NH_3 = 200 mg L^{-1} as N) to establish the nitrifying bacterial community, after which a dilute version NASA wastewater ersatz was fed to the M2BR to also establish the heterotrophic bacterial community (Phase 2: 23 days; COD = 220 mg L^{-1}; NH_3 = 170 mg L^{-1} as N). After high removals of both COD and NH_3 were observed, then the feed solution was changed to a more concentrated version of the NASA wastewater ersatz that had a COD:N ratio that would support simultaneous nitrification/denitrification in the M2BR

(Phase 3: 33 days; COD = 480 mg L^{-1}; NH_3 = 180 mg L^{-1} as N). During the final phase of the second M2BR experiment, the full-strength NASA wastewater ersatz was fed to the bioreactor (Phase 4: 20 days; COD = 780 mg L^{-1}; NH_3 = 700 mg L^{-1} as N).

During Phase 1, ammonia removal efficiency was initially low but eventually was sustained at >90% (Fig. 3a); an apparent stoichiometric conversion of the ammonia to nitrate was observed during this phase. During Phase 2, COD removal was consistently excellent (Fig. 3b) while the ammonia removal efficiency was sustained (Fig. 3a). The concentration of nitrate in the M2BR effluent decreased by about 35% during Phase 2 compared to Phase 1 (Fig. 3a), indicating that denitrification was occurring to a minor extent. During Phase 3, COD and NH_3 removal remained excellent, but a substantial reduction in the nitrate levels were observed in the effluent of the M2BR (Fig. 3a)—corresponding to better than 90% removal of both COD and total nitrogenous species. During Phase 4, ammonia concentrations in the effluent increased substantially (Fig. 3a), although COD removal remained excellent (Fig. 3b).

Although the majority of metabolic activity was assumed to occur in the biofilm, a substantial quantity of suspended biomass accumulated during the second M2BR experiment (Fig. 3c). Suspended biomass concentrations were very low throughout Phase 1, but then rapidly accumulated during Phase 2 and the first portion of Phase 3. For unknown reasons, a substantial decrease in suspended biomass was observed during Phase 3, after which suspended biomass levels rapidly increased for the remainder of the second M2BR experiment.

Following the second M2BR experiment, the bacterial communities of both the suspended biomass and the biofilm were analyzed by denaturing gradient gel electrophoresis of PCR-amplified 16S rRNA gene fragments (PCR–DGGE) (Fig. 4). The bacterial community of the suspended biomass (Lane 1) contained three dominant bacterial populations, each of which was found within the biofilm community (Lanes 2–10). The biofilm community, in contrast, was substantially more complex (>21 bands) and exhibited some spatial variation in community structure. Seven prominent bands were excised and purified from the denaturing gels. These bacteria were predominantly from the β-subdivision of *Proteobacteria* (Table 2).

Third M2BR experiment

Although the second experiment demonstrated that the M2BR could achieve excellent COD and total nitrogenous pollutant removal during Phase 3, a substantial decline in nitrification/denitrification efficiency was observed once the feed solution was switched to the full-strength NASA

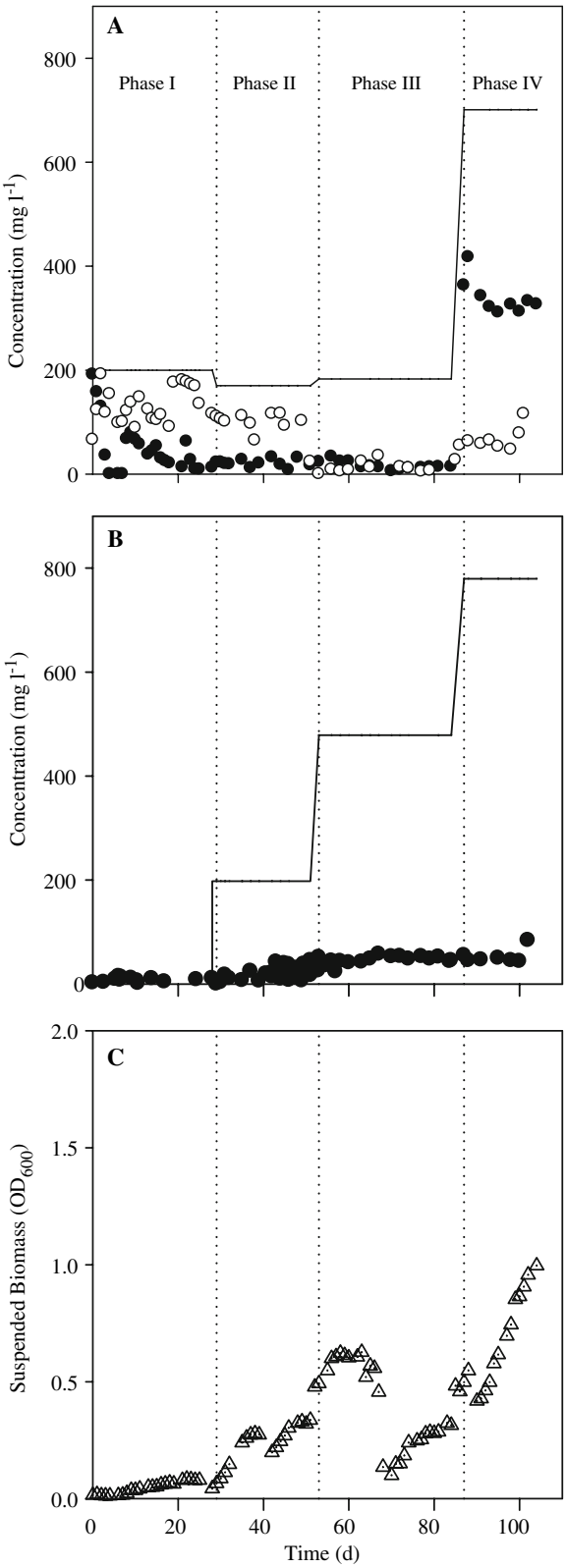


Fig. 3 **a** Effluent concentrations of ammonia (*closed circles*) and nitrate (*open circles*) during the second M2BR experiment. The *solid line* represents the influent ammonia concentration. **b** Effluent COD concentrations during the second M2BR experiment. The *solid line* represents the influent COD concentration. **c** Suspended biomass concentrations

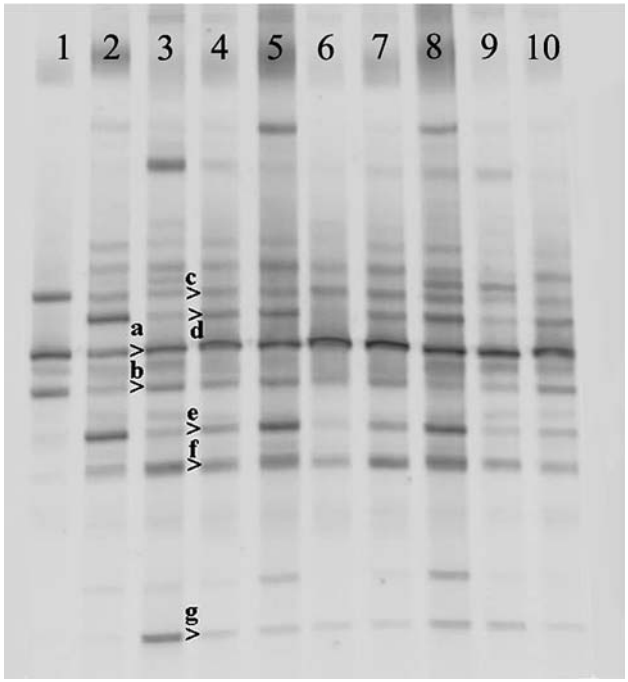


Fig. 4 Denaturing gradient gel electrophoresis (DGGE) of PCR-amplified 16S rRNA gene fragments of samples collected following the second M2BR experiment. Samples were collected from bulk solution (*Lane 1*), *top* (*Lanes 2, 5 and 8*), *middle* (*Lanes 3, 6, and 9*), and *bottom* (*Lanes 4, 7, and 10*) of the aeration membrane. Identified bands were excised, purified, and sequenced (see Table 2)

wastewater ersatz during Phase 4. We assumed that this decline in M2BR performance occurred because of the oxygen demand for treatment exceeded the OTR of the gas-transferring membrane. The third M2BR experiment, therefore, was performed to evaluate the performance of the M2BR using pure oxygen to increase the OTR to the bioreactor.

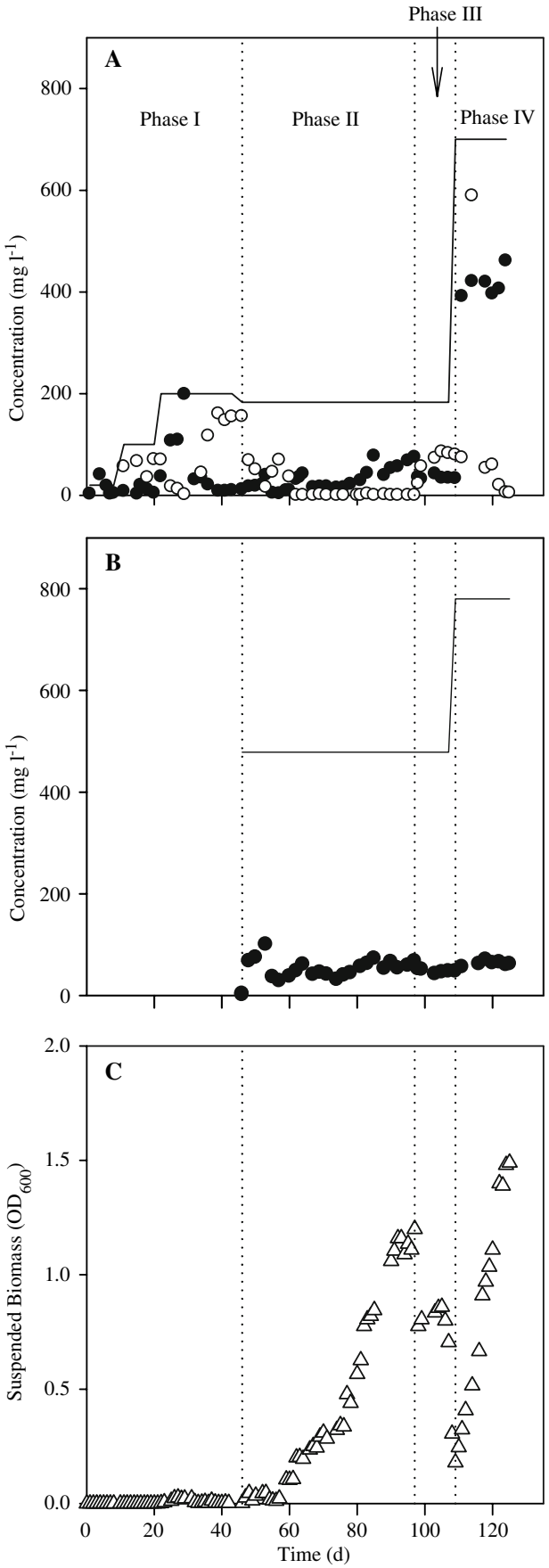
The third M2BR experiment also consisted of four distinct phases. The first phase involved the establishment of a nitrifying bacterial community (Phase 1: 46 days; COD = 0 mg L^{-1}; NH_3 = 200 mg L^{-1} as N). The second phase was intended to establish simultaneous COD and total nitrogen removal (Phase 2: 51 days; COD = 480 mg L^{-1}; NH_3 = 180 mg L^{-1} as N). During the third phase, pure oxygen was employed for membrane-aeration (Phase 3: 12 days; COD = 480 mg L^{-1}; NH_3 = 180 mg L^{-1} as N). The fourth phase was designed to test the ability of the M2BR to treat the full-strength NASA wastewater ersatz using pure oxygen to help meet the oxygen demand for treatment (Phase 3: 17 days; COD = 780 mg L^{-1}; NH_3 = 700 mg L^{-1} as N).

Biological nitrification initially proceeded much more slowly than the second experiment, but eventually the M2BR was able to almost completely nitrify (>95%) the ammonia in the influent wastewater (Fig. 5a). Once organic compounds were included in the influent (Phase 2), nitrate levels declined rapidly although ammonia levels slowly increased. In response, the HRT was increased to 36 h on day 54 and ammonia concentrations quickly decreased. After another week of operation, the HRT was reduced back to 24 h, causing another gradual increase in effluent ammonia concentrations. The HRT was then re-adjusted back to 36 h on day 65 and the ammonia removal efficiency remained around 90% until the HRT was reduced back to 24 h on day 78. The application of pure oxygen aeration improved the ammonia removal efficiency from 60 to 82%, leading to a simultaneous increase in nitrate concentrations. During Phase 4, the average effluent ammonia was 420 mg L^{-1} as N (removal efficiency = 40%) while nitrate concentrations declined.

Effluent COD concentrations remained low throughout the third M2BR experiment, independent of the specific reactor conditions (Fig. 5b)—similar to the second M2BR experiment. Suspended biomass levels also followed patterns similar to the first two M2BR experiments in which biomass concentrations remained low during Phase I but then increased steadily once organic compounds were included in the feed medium (Fig. 5c). Curiously, suspended biomass concentrations decreased steadily following the switch to pure oxygen aeration (Phase III), but then increased throughout Phase IV when the feed medium was switched to the full-strength NASA wastewater ersatz.

Table 2 The best phylogenetic match of the nucleotide sequences excised from prominent bands detected by PCR-DGGE during the second M2BR experiment (Fig. 4)

Band	Sequence length (bases)	Phylogenetic relationship		
		Most closely related sequence (GenBank accession no.)	Bacterial division	% Identity
A	158	Perchlorate reducing bacterium (AY530552)	*β-Proteobacteria*	99.4
B	133	*Pseudomonas saccharophila* (AJ746122)	*β-Proteobacteria*	94.8
C	159	*Acidovorax* sp. I-F1 (AY779520)	*β-Proteobacteria*	98.1
D	158	*Alicycliphilus* sp. (DQ342277)	*β-Proteobacteria*	100
E	158	*Comamonas testosteroni* (AY291591)	*β-Proteobacteria*	100
F	134	*Bradyrhizobium* sp (AY823225)	*α-Proteobacteria*	100
G	133	Uncultured *α-proteobacterium* (AY466779)	*α-Proteobacteria*	100



◀ **Fig. 5** **a** Effluent concentrations of ammonia (*closed circles*) and nitrate (*open circles*) during the third M2BR experiment. The *solid line* represents the influent ammonia concentration. **b** Effluent COD concentrations during the third M2BR experiment. The *solid line* represents the influent COD concentration. **c** Suspended biomass concentrations

Discussion

The goal of this research was to demonstrate that the M2BR could acceptably treat a NASA wastewater ersatz using components that were completely compatible with microgravity conditions. During Phase III the second M2BR experiment, we achieved this goal when COD and total nitrogenous pollutant removal efficiencies both exceeded 90%. This confirms our hypothesis that the M2BR, which contains components that are completely compatible with microgravity conditions, could be used during long-term space missions to treat a waste stream comprising urine, atmospheric condensate, and spent hygiene water.

Our research demonstrates that the gas transferring membrane is a key design constraint that will control the performance of the M2BR. The challenge with membrane-aerated biofilms for simultaneous nitrification and denitrification is to ensure that there is sufficient gas transfer to promote complete ammonia oxidation, but not so much gas transfer that the biofilm is fully penetrated with oxygen, thus inhibiting denitrification. In our experiments, identifying the optimum rate of gas transfer proved difficult in that oxygen transfer during many of our experimental conditions was either excessive (e.g., Experiment 2, Phase 2) or deficient (e.g., Experiment 2, Phase 4). To resolve this issue, future research is needed to calibrate mathematical models of membrane-aerated biofilms (e.g., [22]) with analogous experimental analyses (e.g., [5, 15]).

Our research also demonstrates that the membrane filter serves as a key hygienic constraint for astronauts that will need to live in close proximity to this biological treatment process throughout the duration of their long-term space mission. The relatively large quantity of suspended biomass that accumulated in the M2BR would have otherwise been discharged with the effluent. During space missions, this biomass would then have a substantially better opportunity to adversely affect astronaut health. It remains unclear, however, whether this suspended biomass helps catalyze pollutant removal or it is inactive biomass that is merely accumulating in the M2BR. Since all of the bacterial populations detected in the suspended biomass (Fig. 4; Lane 1) are also found in the biofilm (Fig. 4; Lanes 2–10), we conclude that the majority of this biomass is inactive. Additional research is necessary to further elucidate the biocatalytic importance of this bacterial biomass.

Our PCR–DGGE results also demonstrate substantial spatial heterogeneity of bacterial community composition within the membrane-aerated biofilm. This spatial heterogeneity could reflect differences in fluid shear within the M2BR, which then lead to different biofilm thicknesses; this is pertinent because membrane-aerated biofilms typically exhibited substantial variation in bacterial community composition as a function of biofilm depth [5, 15]. Alternatively, the spatial heterogeneity of bacterial community composition could reflect hydraulic short-circuiting through the M2BR; this would suggest that M2BR performance could be enhanced by improving mixing within the reactor. In both cases, additional research is needed to understand spatial heterogeneity in fluid shear within the M2BR.

Although our results have confirmed that the M2BR can simultaneously remove both carbonaceous and nitrogenous pollutants, the NASA wastewater ersatz poses a particularly difficult challenge because of its low COD:N ratio. Simply put, there is insufficient electron donor to allow for complete denitrification. Our previous experiments have demonstrated that a COD:N ratio approximately equal to four is needed for complete nitrogenous pollutant removal [10, 15]. To fully treat the NASA wastewater ersatz, therefore, an approach beyond the M2BR will be required. One alternative would be to supply electron donor in the form of gaseous hydrogen [8, 16]; another option would be to utilize a two-stage system in which one M2BR achieves strictly COD removal while a second stage utilizes some form of anaerobic ammonia oxidation, such as the CANON process [24], to remove the nitrogenous pollutants.

In conclusion, the M2BR has the capability of achieving simultaneous removal of carbonaceous and nitrogenous pollutants using components that are entirely compatible with microgravity conditions. For long-term space missions, the M2BR design is a particularly attractive bioreactor design because the membrane filter provides an additional hygienic constraint for the astronauts who must live in close proximity with a wastewater bioreactor. Additional research is needed, however, to identify the best approach to treat the NASA wastewater ersatz, which poses a unique challenge because of its low COD:N ratio.

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References

1. Ahmed T, Semmens MJ (1992) Use of sealed end hollow fibers for bubbleless membrane aeration: experimental studies. J Membr Sci 69:1–10
2. Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Rapp BA, Wheeler DL (2002) GenBank. Nucleic Acids Res 30:17–20
3. Biebl H, Pfennig N (1981) Isolation of members of the family Rhodospirillacaea. In: Starr MP, Stolp HG, Troper HG, Balows A, Schlegel HG (eds) The Prokaryotes. Springer, Berlin, pp 267–273
4. Chen R, LaPara TM (2006) Aerobic biological treatment of low-strength synthetic wastewater in membrane-coupled bioreactors: the structure and function of bacterial enrichment cultures as the net growth rate approaches zero. Microb Ecol 51:99–108
5. Cole AC, Semmens MJ, LaPara TM (2004) Stratification of activity and bacterial community structure in biofilms grown on membranes transferring oxygen. Appl Environ Microbiol 70:1982–1989
6. Côte P, Bersillon J-L, Huyard A (1989) Bubble-free aeration using membranes: mass transfer analysis. J Membr Sci 47:91–106
7. Daniels L, Hanson RS, Phillips JA (1994) Chemical analysis. In: Gerhardt P, Murray RGE, Wood WA, Krieg NR (eds) Methods for general and molecular bacteriology. ASM Press, Washington DC, pp 512–554
8. Edstrom JA, Semmens MJ, Hozalski RM, Clapp LW, Novak PJ (2004) Stimulation of dechlorination by membrane-delivered hydrogen: small field demonstration. Environ Eng Sci 22:281–293
9. Essila NJ, Semmens MJ, Voller VR (2000) Modeling biofilms on gas-permeable supports: concentration and activity profiles. J Environ Eng 126:250–257
10. Ghosh S, LaPara TM (2004) Removal of carbonaceous and nitrogenous pollutants from a synthetic wastewater using a membrane coupled bioreactor. J Ind Microbiol Biotechnol 31:353–361
11. Kappell AS, Semmens MJ, Novak PJ, LaPara TM (2005) A novel application of oxygen-transferring membranes to improve anaerobic wastewater treatment. Biotechnol Bioeng 89:373–380
12. Klatt CG, LaPara TM (2003) Aerobic biological treatment of synthetic municipal wastewater in membrane-coupled bioreactors. Biotechnol Bioeng 82:313–320
13. Konopka A, Zakharova T, Oliver L, Camp D, Turco RF (1996) Biodegradation of organic wastes containing surfactants in a biomass recycle reactor. Appl Environ Microbiol 62:3292–3297
14. Lane DJ (1991) 16S/23S rRNA sequencing. In: Stackebrandt E, Goodfellow M (eds) Nucleic acid techniques in bacterial systematics Wiley, West Sussex, pp 115–175
15. LaPara TM, Cole AC, Shanahan JW, Semmens M (2006) The effects of organic carbon, ammoniacal-nitrogen, and oxygen partial pressure on the stratification of membrane-aerated biofilms. J Ind Microbiol Biotechnol 33:315–323
16. Lee KC, Rittmann BE (2003) Effects of pH and precipitation on autohydrogenotrophic denitrification using the hollow-fiber membrane–biofilm reactor. Water Res 37:1551–1556
17. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275
18. McLamore E, Jackson WA, Morse A (2007) Abiotic transport in a membrane aerated bioreactor. J Membr Sci 298:110–116
19. Muyzer G, Dewaal EC, Uitterlinden AG (1993) Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl Environ Microbiol 59:695–700
20. Rector TJ, Garland JL, Starr SO (2006) dispersion characteristics of a rotating hollow fiber membrane bioreactor: effects of module packing density and rotational frequency. J Membr Sci 278:144–150
21. Sambrook J, Fritsch EF, Maniatis T (1989) Molecular cloning: a laboratory manual, 2nd edn. Cold Spring Harbor Laboratory, Cold Spring Harbor

22. Shanahan JW, Semmens MJ (2004) Multipopulation model of membrane-aerated biofilms. Environ Sci Technol 38:3176–3183
23. Shanahan JW, Semmens MJ (2006) Influence of a nitrifying biofilm on local oxygen fluxes across a micro-porous flat sheet membrane. J Membr Sci 277:65–74
24. Third KA, Paxman J, Schmid M, Strous M, Jetten MS, Cord-Ruwisch R (2005) Treatment of nitrogen-rich wastewater using partial nitrification and anammox in the CANON process. Water Sci Technol 52(4):47–54