Closed-Loop Water Purification System Utilizing an Algae Membrane Photobioreactor for the International Space Station

Alexandra A. Smith

NASA- KSC

Biotechnology Specialist

Fall Session

November 16, 2018

Abstract

The International Space Station (ISS) currently operates on an open-loop water purification and distribution system. This system is only 75% efficient with a 25% water loss that must be resupplied on a quarterly basis through cargo resupply missions (Pickett, M.). This system is not feasible for long-duration spaceflight missions. A closed-loop water purification system aboard the ISS can benefit water reclamation and become the water purification system for long-term space flight. The primary sub-systems integrated within the proposed closed loop system include an anaerobic digestion membrane bioreactor and an algae membrane photobioreactor. The processes mimics the abilities of natural biological systems naturally. The water that flows from the algal membrane photobioreactor is rich in nutrients providing a feed source for plants aboard the ISS or can be further processed for potable water.

1.0 Introduction

A bioreactor is a closed system in which organisms, such as bacteria and algae, are grown for biotechnology purposes. For water purification purposes, microorganisms within a bioreactor consume constituents within the wastewater. Terrestrially, wastewater is purified through multiple processes. A similar tactic is taken for this project. The project consists of a sequence of subsystems each naturally designed with a constituent removal procedure.

1.1. Anaerobic Membrane Bioreactor (AnMBR)

The first subsystem is the anaerobic membrane bioreactor (AnMBR). TheAnMBR is designed to break down solid waste matter (such as fecal) and raw wastewater via anaerobic digestion (Pickett, M.). By employing this

microcosm, solid waste matter and wastewater are broken down into anaerobic digested wastewater. The anaerobic digested wastewater will be prepared synthetically to replicate the AnMBR effluent and with realistic margins in mind to emulate a crew of four members living aboard the ISS. The synthetically modified ersatz simulates characteristics of wastewater generated on a transit mission and on early planetary base (Pickett, M.).

1.2 Algae Membrane Photobioreactor (MPBR)

The MPBR is the second subsystem used to house cultivated algae, and is the focus of this work. Once the algae reached optimal density and inoculated into the MPBR, the modified ersatz wastewater formulation is pumped into the reactor for processing. The algae then utilize the high levels of ammonia and phosphate as nutrients and further consume the constituents. The slurry pumps into porous tubing (the membrane), trapping the algae inside and allowing the water molecules to filtrate through the membrane and be pumped out of the reactor as permeate. The subsequent information and testing procedure is relevant only to the MPBR.

2.0 Objectives

Inoculate photobioreactor with algae.

Produce synthetic wastewater analogous to that created on the ISS for testing

Determine threshold dosage of wastewater on different alga species via biological testing.

Test permeate via Ion Chromatography for algae nutrient removal efficiency.

Research into symbiotic relationships between bacteria and alga.

Data collection to determine feasibility of technology implementation.

3.0 Technical approach

3.1 MPBR inoculation

Three species of algae obtained from the UTEX culture collection of algae, *Chlorella vulgaris*, *Chlorella sorokiniana*, and *Chlorella protothecoides* were cultured into separate flasks containing100 mL of deionized (DI)

and proteose media. The cultures grew robustly and acquired biomass within the flasks throughout several weeks (Figure 1).

Figure 1. Algae cultures.

C. vulgaris was determined, based on biomass and absorbency readings represented in Table 1, to be ideal for inoculation of the MPBR. The MPBR's inoculation was based on the volume of the reactor, approximately 4.5 L, and desired density of the culture at inoculation, which was 0.30 absorbency. Equation 1 shows each algal culture's representation within the inoculums. Equation 2 was used to determine the volume of culture needed in the MPBR to be successful. The inoculum proved to be hearty and 170 mL was saved for further testing or in event that the *C. vulgaris* in the MPBR was unsuccessful and needed to be re-inoculated.

Table1. Spectrometry readings of algal cells at 680 nm

Volume of sample	Optical Density at 680nm	Representation in total sample
1. 700mL	0.870 A	0.419
2. 100mL	0.529 A	0.060
3. 100mL	0.422 A	0.060
4. 170mL	0.624 A	0.102

5. 600mL	0.730 A	0.360
Total: 1670 mL		

[(0.807)(0.419)+(0.529)(0.060)+(0.422)(0.060)+(0.624)(0.102)+(0.730)(0.360)]= 0.7477

Equation 1

$C_1V_1=C_2V_2$

(0.25)(4.5L)=X(0.7477)

X= 1.50 L of *C. vulgaris* for inoculation

Equation 2

Throughout the week the optical density of the algae in the MBPR was taken to monitor culture health. The rise in absorbance indicates growth as shown in Table 2.

Table 2. Cell health monitoring after the algae inoculation into the MPBR.

Date	C. vulgaris	C. sorokiniana	MPBR
		680nm	
9/27/2018	0.805	0.308	0.909
9/28/2018			1.038
10/1/2018	0.698	0.463	1.205
10/2/2018			1.232
10/3/2018	1.068	0.74	1.172

3.2 Biological screening: batch reactors

C. vulgairs and *C. sorokiniana* were grown in batch reactors for biological screening in order to select a preferred alga for further testing moving forward. This testing intended to set a protocol for a dosing procedure for

the MPBR, generate data on the algae's ability to exploit ammonia and phosphate as nutrients, and to establish fail point (maximum dosage rate that the algae species can tolerate before dying).

Biological testing took place in two separate batch reactors each containing a different species: *C. vulgaris* in reactor 1 and *C. sorokiniana* in reactor 2.The batch reactors dose protocol began with 100ml of modified ersatz wastewater. Dosing increased every week by 150 ml and will do so until a fail point is established. The batch reactors were sampled before and after each dose to determine nutrient accumulation and algal utilization of constituents. The dissolved oxygen (DO) was closely monitored as well as pH by probes as indicators of culture growth and overall health.

Figures 1 and 2 characterize a 10 day set of modified ersatz wastewater dosing of the batch reactor tanks. Figure 1 indicated that reactor 1, *C. vulgaris*, utilized the PO_4 as it was added to the batch reactor. The figure also showed NH_3 was consumed, not completely, by the algae as it was added into the tank, as the NH_3 rose to around 80 mg/L.

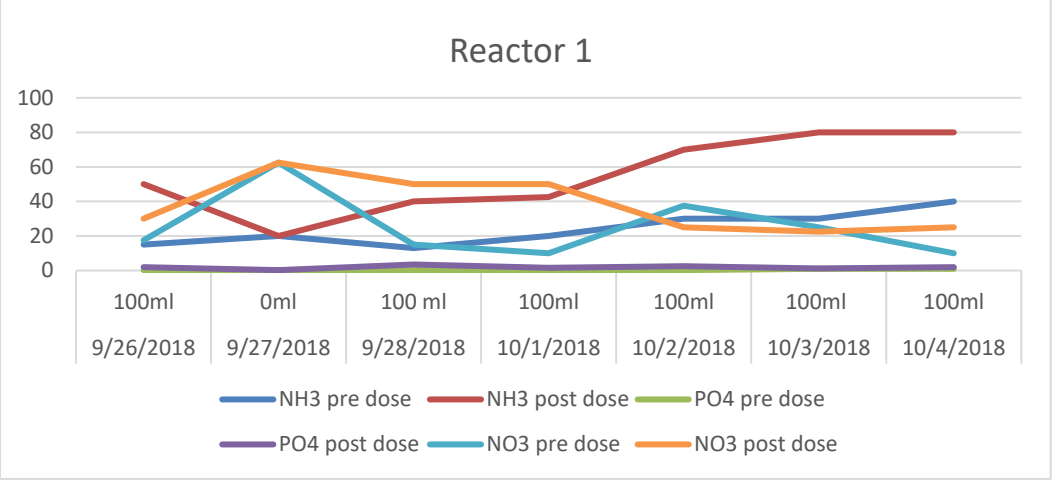

Figure 1. 10 day dosing results of batch reactors.

Figure 2 showed that reactor 2, containing *C. sorokiniana*, utilized PO_4 as it was added to the batch reactor as well as NH_4. Note a steady rise of NO_3 within the system.

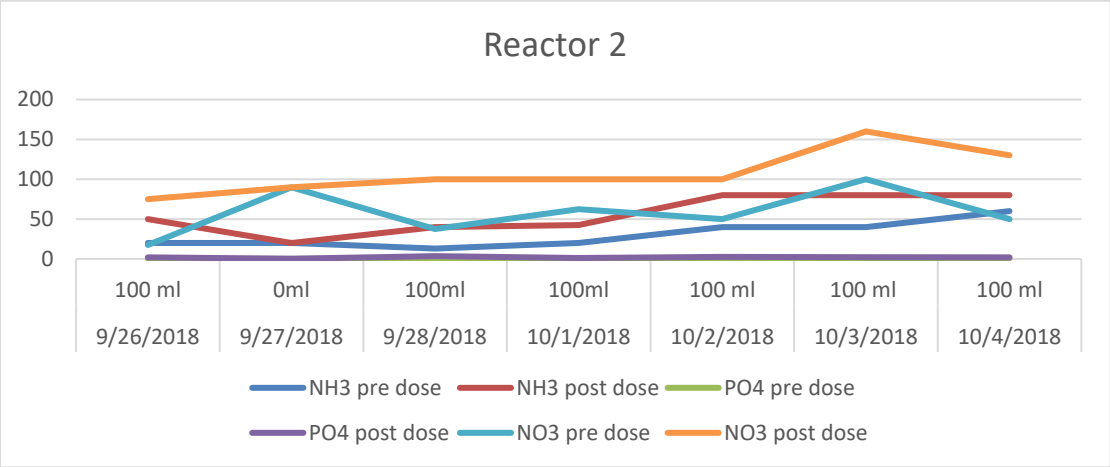

Figure 2. A 10 day set of dosing results batch reactors.

3.3 Nitrification

Algae is not known to biologically convert NH_3 to NO_3 (Pickett, M.). Reactor 2 seemingly utilized NH_3as it was added to the reactor, but due to the ascending levels of NO_3, the hypothesis that algae used NH_3 from wastewater for energy and biomass could not be confirmed. The oxidation of nitrogen and occurrence of NO_3 could be explained by nitrifying bacteria that began proliferating within the system without being deliberately inoculated. To test this theory a biological activity reaction test, (also known as BART), was conducted to confirm the presence of nitrifying bacteria.

20 mL of algae was taken from Reactor 1 and 2 and pipetted into separate BARTs. After an incubation period of 5 days the BARTs were observed for bacterial occurrence. Reactor 1 was observed to have red deposits in a pink solution. In accordance to the user manual there was a moderate population of bacteria (> 102 and < 105 nitrifiers/mL) forming a major component in bacterial flora. Reactor 2's test presented dark red deposits and dark

red solution indicating a dominant population of bacteria thus explaining the higher levels of NO_3 forming within batch reactor 2.

4.0 Schedule:

The project development and experimental testing that occurred Fall 2018 for the MPBR follows the information outlined in Table 3 below.

Table 3. MPBR Schedule Fall 2018

Task:	**Start Date:**	**End Date:**
Project plan	9/3/18	9/7/2018
Project research and alga inoculation of the MPBR	9/10/2018 11/2018	9/14/2018 11/2018
Effluent water testing and media preparation	9/17/2018	12/14/2018
Batch reactor modified ersatz wastewater dosing	9/21/2018	12/14/2018
Nutrient testing kits and ion chromatography testing	9/21/2018	12/14/2018
~~DNA sequencing or Gene banking~~	~~TBA~~	~~TBA~~
~~Data collection on algae genome and Bioinformatics~~	~~TBA~~	~~TBA~~
Nitrifying bacterial testing	10/5/2018	12/14/2018
50:50 dilution feed of secondary wastewater to the MPBR	10/15/2018	10/19/2018
Full concentration feed of secondary wastewater to the MPBR	10/22/2018	TBA
~~Project research on genetically modifying alga DNA to suit the needs of the project~~	~~TBA~~	~~TBA~~

5.0 Experiment Continuance and Expansions

The MPBR will continue to be tested by CO_2 delivery concentrations updates, media modification to change concentration, and membrane gas delivery to better suit future microgravity environments. The batch reactors will continue to be dosed and data acquired. Some experimental expansions considered are techniques for transporting

algae to space (such as drying and dormancy issues), MPBR effluent use for plant feed, utilization of algae as biofertilizer, and possible genetic modification of algal cells to better exist in microgravity/ less lighting requirements/ higher levels of constituent consumption. Valuable parameters of this and other algal experiments with wastewater treatment and water loss recovery are ongoing and will be investigated further in order to provide valuable insight for future space water purification systems.

6.0 References

Pickett, Melanie. MPBR Experimental Plan. V1.0. 7/5/2018.