Life Sciences in Space Research 15 (2017) 32–42

Contents lists available at ScienceDirect

Life Sciences in Space Research

journal homepage: www.elsevier.com/locate/lssr

Coupling of anaerobic waste treatment to produce protein- and lipid-rich bacterial biomass

Lisa M. Steinberg, Rachel E. Kronyak, Christopher H. House*

Department of Geosciences and Penn State Astrobiology Research Center, 220 Deike Building, Penn State University, University Park, PA 16802, United States

ARTICLE INFO

Keywords:
Anaerobic digestion
Methanotroph
Single-cell protein
Alkaline pH
Thermophile
Alkaliphile

ABSTRACT

Future long-term manned space missions will require effective recycling of water and nutrients as part of a life support system. Biological waste treatment is less energy intensive than physicochemical treatment methods, yet anaerobic methanogenic waste treatment has been largely avoided due to slow treatment rates and safety issues concerning methane production. However, methane is generated during atmosphere regeneration on the ISS. Here we propose waste treatment via anaerobic digestion followed by methanotrophic growth of *Methylococcus capsulatus* to produce a protein- and lipid-rich biomass that can be directly consumed, or used to produce other high-protein food sources such as fish. To achieve more rapid methanogenic waste treatment, we built and tested a fixed-film, flow-through, anaerobic reactor to treat an ersatz wastewater. During steady-state operation, the reactor achieved a 97% chemical oxygen demand (COD) removal rate with an organic loading rate of 1740 g d^{-1} m^{-3} and a hydraulic retention time of 12.25 d. The reactor was also tested on three occasions by feeding ca. 500 g COD in less than 12 h, representing 50x the daily feeding rate, with COD removal rates ranging from 56–70%, demonstrating the ability of the reactor to respond to overfeeding events. While investigating the storage of treated reactor effluent at a pH of 12, we isolated a strain of *Halomonas desiderata* capable of acetate degradation under high pH conditions. We then tested the nutritional content of the alkaliphilic *Halomonas desiderata* strain, as well as the thermophile *Thermus aquaticus*, as supplemental protein and lipid sources that grow in conditions that should preclude pathogens. The *M. capsulatus* biomass consisted of 52% protein and 36% lipids, the *H. desiderata* biomass consisted of 15% protein and 7% lipids, and the *Thermus aquaticus* biomass consisted of 61% protein and 16% lipids. This work demonstrates the feasibility of rapid waste treatment in a compact reactor design, and proposes recycling of nutrients back into foodstuffs via heterotrophic (including methanotrophic, acetotrophic, and thermophilic) microbial growth.

1. Introduction

Long-duration manned missions for space exploration require the development of advanced life support systems that can effectively recycle water and nutrients from waste to fulfill crew needs. Currently, physicochemical treatments are used on the ISS to recover water from urine, and to stabilize solid waste for return to Earth, with different collection and treatment strategies applied for liquid and solid human wastes (Schneider et al., 2016). Gaps in time between wastewater generation and processing requires that the wastewater be stabilized prior to treatment, typically with strong oxidizers and acids followed by distillation and filtration (Schneider et al., 2016; Yamashita and Wheeler, 2014). Currently, solid human wastes are dried and stabilized after collection, then returned to Earth (Schneider et al., 2016). These waste management methods are unsustainable for long-term transit missions, which will require the recapture of nutrients from waste and recycling back into food (Do et al., 2016). Proposed methods to achieve nutrient recapture from solid wastes include physicochemical treatment such as incineration (Caraccio et al., 2014; Hintze et al., 2013), "wet" mineralization by electromagnetically activated hydrogen peroxide (Kudenko et al., 2000), pyrolysis or acid extraction (Gribovskaya et al., 1996), or biological treatment through composting (Ushakova et al., 2008) or vermiculture (He et al., 2010). Scenarios for the recapture of nutrients from urine include direct application for plant fertilizer (Feng and Wu, 2006; Salisbury et al., 1997), or aerobic biological water treatment with nitrifying bacteria to couple carbon mineralization to CO_2 with ammonia oxidation to nitrate (Lasseur et al., 2010; Nelson et al., 2009).

Generally, waste management strategies have not employed methanogenic anaerobic biological processes due to concerns about the time required for organic matter mineralization, as well as safety concerns regarding methane production (Godia et al., 2004; Lasseur et al.,

* Corresponding author.
E-mail address: chrishouse@psu.edu (C.H. House).

http://dx.doi.org/10.1016/j.lssr.2017.07.006
Received 17 April 2017; Received in revised form 26 June 2017; Accepted 18 July 2017

2010). The European Space Agency's MELiSSA pilot plant uses thermophilic anaerobic digestion of solid waste that seeks to suppress methanogenesis, thereby producing primarily ammonium, carbon dioxide, fatty acids, and minerals (Godia et al., 2004; Lasseur et al., 2010). These products are then provided to photoheterotrophic and nitrifying microbial reactors, the growth of cyanobacteria, and eventually applied as fertilizer for plants (Godia et al., 2004; Lasseur et al., 2010). Despite the potentially dangerous nature of methane, atmosphere regeneration technology on the ISS uses a Sabatier reactor to form water and methane from H_2, produced from electrolysis, along with carbon dioxide (Greenwood et al., 2015). Long-term mission strategies propose to continue use of this strategy for atmosphere regeneration (Do et al., 2016). Additionally, another strategy proposed for recapturing nutrients from solid waste is a two-step incineration called Trash-to-Gas which produces methane and water that are then applied towards atmosphere regeneration (Caraccio et al., 2014; Hintze et al., 2013). As methane is already being used during atmosphere regeneration, biological methane production during waste treatment is a lower-energy alternative to incineration.

Anaerobic waste treatment proceeds more slowly than aerobic treatment, but high-rate anaerobic digesters achieve rapid waste treatment rates in a compact design (Tauseef et al., 2013). High-rate, or fixed-film, anaerobic digesters employ a solid matrix colonized by anaerobic bacteria with waste material pumped over and through the colonized media, so that treatment proceeds by a combination of mechanical filtering and biological treatment. In this way, the hydraulic and solids retention times in the reactor are separated so that bacteria and particulate matter are retained in the reactor, allowing longer treatment times, while treated water exits (Tauseef et al., 2013). In addition to rapid treatment and compact size, fixed-film reactors are resistant to organic loading rate changes (Chua et al., 1997; Di Berardino et al., 2000; Dupla et al., 2004), toxins (Ghaniyari-Benis et al., 2009; Karim and Gupta, 2006), ammonia (del Pozo et al., 2000; Dupla et al., 2004; Wang et al., 2011), and even intermittent operation (del Pozo et al., 2000), thus reducing operation complexity. Finally, anaerobic digestion is effective for pathogen removal during waste treatment (Rovirosa et al., 2004; Saddoud and Sayadi, 2007). Anaerobic digestion is advantageous in a LSS as it avoids the need for oxygen during waste treatment.

Besides atmosphere regeneration, a second potential use for methane in a LSS is for food production, especially a high protein food supplement. *Methylococcus capsulatus* is an aerobic methanotroph, which was successfully grown as a protein- and lipid-rich animal feed supplement by Dansk Bioprotein A/S (Odense, Denmark), and fed to mink, pigs, chicken, and salmon with no ill effects (Berge et al., 2005; Bothe et al., 2002; Skrede et al., 1998; Skrede et al., 2003). Subsequently, Dansk BioProtein was acquired by Norferm DA (Norway), which was subsequently acquired by Calysta (Menlo Park, CA), which is currently producing BioProtein for animal feed. Additionally, *M. capsulatus* is capable of nitrogen fixation, and produces nitrite from ammonia (Bergmann et al., 1998; Dalton, 1977), which would allow food production and nitrification reactions to be performed in the same reactor. The use of microbial cells as a human food supplement is not unprecedented as the MELiSSA project grows cyanobacteria for atmosphere regeneration and crew food (Godia et al., 2004; Lasseur et al., 2010). Additionally, the cultivated microbial biomass could be used to grow other proposed foods including insect pupae and fish (Yamashita and Wheeler, 2014).

Here we propose two components for incorporation into an advanced life support system coupling anaerobic waste treatment to food production (Fig. 1). The first compartment consists of a fixed-film, flow-through anaerobic digester treating liquid and solid human waste to produce CO_2 and methane, as well as ammonium from urea and protein degradation. The treated effluent pH is raised (e.g. with NaOH) to liberate dissolved ammonium as ammonia and to convert CO_2 to bicarbonate and carbonate salts. The ammonia and methane gas are fed to a second reactor, along with oxygen, to grow a methanotrophic biomass for food. The methanotrophic biomass uses the ammonia for protein production, but also converts excess ammonia to nitrite, thereby performing the first step in producing nitrate as a plant fertilizer. Alternatively, the nitrite-rich effluent from the methanotrophic reactor could be used to support secondary treatment of the anaerobic digester effluent using an alkali- and halotolerant acetotrophic denitrifier, isolated during this study, producing additional cultivated microbial biomass. The use of a high pH, high salt environment presumably inhibits pathogen growth. Our components couple waste treatment to food production without the need for separate collection of solid and liquid waste, effectively mineralizes organic carbon to CO_2 for autotrophic growth of plants or cyanobacteria, avoids oxygen consumption during waste treatment, and provides a valuable use for the methane produced during waste treatment.

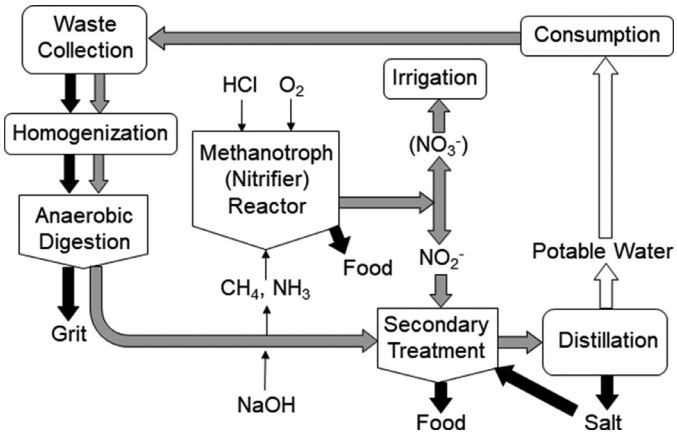

Fig. 1. Proposed waste treatment and food production compartments for incorporation in an advanced life support system. The flow of solids, wastewater, and potable water are shown with black, gray, and white arrows, respectively. Human solid and liquid waste are collected together and homogenized before primary treatment within a fixed-film, flow-through anaerobic digester. Insoluble, inorganic material is removed from the reactor as grit, and reactor effluent is treated with a strong base (e.g. sodium hydroxide, or NaOH) to liberate ammonia (NH_3) and remove carbon dioxide (CO_2). Methane (CH_4) and NH_3 are fed to a methanotrophic reactor where excess NH_3 is converted to nitrite (NO_2^-) by the methanotroph. Hydrochloric acid (HCl) is used to balance the pH of this reactor. A nitrifier can also be included in this reactor, thus converting the NO_2^- to nitrate (NO_3^-), which would serve as irrigation water for plants. Alternatively nitrite can be used for secondary waste treatment of the anaerobic digester effluent with an alkali- and halotolerant heterotrophic denitrifier. Salts from the distillation of the treated effluent to produce potable water can be returned to the secondary waste treatment reactor to maintain a high-salt environment, or electrolysis can be used to generate the acid and base to use for pH control. The heterotrophic biomass of the denitrifying reactor may be used as a feed supplement for fish or grubs.

2. Materials and methods

2.1. Construction and operation of a fixed-film anaerobic digester

A 6 ft (183 cm) long fixed-film anaerobic digester was constructed from 4 in (10.2 cm) diameter threaded cPVC tubing with bushings on the ends to reduce the diameter to accommodate a ¼ in (0.635 cm) ID barbed hose fitting (Fig. 2). Tubing and bushings were purchased from McMaster-Carr (Princeton, NJ), and are sold in English units; therefore, both English and metric units are provided here. Holes of 4.45 cm diameter were drilled every 30.5 cm to allow sampling from the interior of the reactor, and were plugged with #10 neoprene stoppers. The reactor was packed with size S/T Biofilter balls (Marineland, Blacksburg, VA) with a 1.9 cm diameter. The volume of the reactor was approximately 17.5 L without the Biofilter Balls, and approximately 12.25 L when packed with Biofilter balls, however the volume was not tested after the biofilm was established. Wastewater was pumped through the reactor at a flow rate of 45 mL/min using a peristaltic pump and