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Short Communication

Biohydrogen production from space crew's waste simulants using thermophilic consolidated bioprocessing

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ABSTRACT

Human waste simulants were for the first time converted into biohydrogen by a newly developed anaerobic microbial consortium via thermophilic consolidated bioprocessing. Four different $BioH_2$-producing consortia (denoted as C1, C2, C3 and C4) were isolated, and developed using human waste simulants as substrate. The thermophilic consortium C3, which contained *Thermoanaerobacterium*, *Caloribacterium,* and *Caldanaerobius* species as the main constituents, showed the highest $BioH_2$ production (3.999 mmol/g) from human waste simulants under optimized conditions (pH 7.0 and 60 °C). The consortium C3 also produced significant amounts of $BioH_2$ (5.732 mmol/g and 2.186 mmol/g) using wastewater and activated sludge, respectively. The developed consortium in this study is a promising candidate for H_2 production in space applications as *in situ* resource utilization.

1. Introduction

Longer duration and distance exploration of the extraterrestrial environment by humans pose challenges for waste management throughout the expedition. The human waste generated during the space mission is carried and stored inside a logistic module and then deorbited into the Earth's atmosphere for destruction (Hintze et al., 2013). However, a mission to Mars, one of NASA's deep space exploration programs, which aims to land humans on a planet for the first time, will be challenged by several logistical needs that include, but are not limited to, water, oxygen, nitrogen, clothing, waste collection, hygiene, healthcare and consumables (Lopez et al., 2015). Sending waste to Earth will not be feasible for such a long expedition. Hence, a sustainable solution for the waste generated is a crucial requirement.

NASA's research during the past decades to find a sustainable and economical solution for generated waste during extraterrestrial expeditions had led to several techniques, and ground-breaking discoveries. In 1967, researchers developed a dough-like gel called 'Monex' using human waste, as an emergency fuel source for spaceships. However, Monex had a much lower fuel efficiency than the prevalent rocket fuel at that time (Eberhart, 1967). Algae, cyanobacteria, and genetically modified yeasts are currently being studied to convert human urine onboard into 3-D printable plastics and nutritional omega-3 fatty acids (Blenner, 2015). The physiochemical conversion of the trash to gas (TtG), involving energy intensive high temperature (300–1000 °C) and high pressure of 5–220 atmospheres (Hintze et al., 2012), was also considered a viable option. However, the safety issues, operating conditions, and mass and volume of the TtG process, undermine the viability of its onboard usage.

$BioH_2$ production can be a sustainable alternative for the biotransformation of the human waste generated during space flights, which can be sustainable and economical. A $BioH_2$ production process is more eco-friendly and less energy intensive than thermochemical and electrochemical processes (Zeidan and Van Niel, 2009). The $BioH_2$ obtained from human waste can be used to provide electricity, and portable water for the inhabitants in a space station (Jain, 2009). The anaerobic fermentation installation can be integrated with the fuel cell system, and used to generate heat and electrical energy, with water as the byproduct. The combination of $BioH_2$ production with a hydrogen fuel cell can also circumvent the critical issue of the storage of H_2 in the space station. $BioH_2$ production at elevated temperature is advantageous compared to mesophilic fermentation due to higher kinetic and

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mass transfer rates, lower viscosity, better mixing, reduced microbial contamination risk and faster production rate (Verhaart et al., 2010). Several waste-to-biofuel processes utilizing various kinds of waste including office waste, kitchen waste, industrial effluent, and agricultural waste by thermophiles have already been reported (Guo et al., 2010; Lee et al., 2010; O-Thong et al., 2011; Roy et al., 2012).

To our best knowledge, no biological process has been examined for $BioH_2$ production using crew's waste generated during manned space missions. In this study, $BioH_2$ production by thermophilic consortia was carried out using human waste simulants, and then compared to the use of solid waste from waste water treatment plant. The main goal of this research work is to develop a lab-scale prototype bioprocessing system for cost-effective conversion of space crew's waste into biofuel using extremophiles. The long-term space missions, where energy generation and waste management are two major concerns, can benefit from the newly developed process of $BioH_2$ production from various wastes.

2. Materials and methods

2.1. Sampling and enrichment of thermophilic H_2 producing culture

The soil and water samples were originally collected from different sites: Consortium 1 (C1): Thermopolis Hot Springs, Wyoming; Consortium 2 (C2): Wastewater Treatment Plant, Rapid City, South Dakota; Consortium 3 (C3) and Consortium 4 (C4): Rapid City Landfill compost facility, South Dakota. The enrichment experiments were carried in 165-mL serum bottles sealed with butyl rubber septa and aluminum caps. The simulated feces contained (mass %): 6% cellulose, 3% polyethylene glycol, 11% peanut oil, 17% miso, 2% KCl, 1% $CaCl_2$, and 60% water. The composition of the urine simulant was (per liter): 5.20 g urea, 0.06 g taurine, 0.52 g creatinine, 0.10 g histidine, 0.17 g glutamic acid, 1.23 g ammonium citrate, 0.15 g ammonium formate, 0.07 g ammonium oxalate monohydrate, 2.31 g NaCl, 0.55 g $MgCl_2$ $6H_2O$, 0.22 g $KHCO_3$, 0.05 g K_2CO_3, 0.11 g KH_2PO_4, 0.54 g KCl, 0.74 g K_2SO_4, 0.02 g $CaCl_2$, 0.41 g Na_2SO_4 (Hintze et al., 2012). For enrichment 1% w/v of the feces waste simulant was added in the urine waste simulant as human waste simulants, and 0.2 g/L yeast extract was added as a nutrient supplement. The urine waste simulant is refereed as the minimal medium hereafter. The initial pH of the medium was adjusted to 7.0 by 6 M NaOH. The serum bottles with medium were sterilized at 121 °C for 15 min. Enrichment experiments were inoculated with 1% w/v soil sample, or 10 mL v/v liquid samples collected from the different sites such that the final reaction volume was 100 mL. After deaeration by flushing ultrapure N_2 gas for 15 min, the serum bottles were incubated at 60 °C in an incubator shaker (50 rpm) for 7 days.

2.2. DNA extraction and microbial diversity analysis

To study the microbial diversity of each consortium, DNA of enriched culture was extracted from the cells using QIAamp® DNA Mini Kit (Qiagen, Valencia, CA, USA) as per manufacturer's protocol. The microbial diversity analysis was performed using Illumina sequencing by Research and Testing Laboratory (Lubbock, TX, USA). The Illumina sequencing process is described in detail in the online Supplementary information (SI).

2.3. Hydrogen production

1% w/v of the feces waste simulant was added to the minimal medium to a final reaction volume of 100 mL in 165 mL anaerobic serum bottles. The serum bottles were prepared as explained in the Section 2.1. For inoculum preparation, enriched consortia were grown in the minimal medium with 0.1% (w/v) simulated solid feces waste as carbon and energy source and 10% (v/v) of it was used as inoculum. For later experiments, culture growing in serum bottles of completed experiments was used as the starter culture for inoculum preparation. When using pure sugar as carbon and energy source, the feces waste simulant was replaced by 1% (w/v) of glucose in 100 mL of the minimal medium in 165 mL serum bottles. The serum bottles were kept at 60 °C, and 50 rpm for 7 days. All the experiments were run in duplicates and appropriate controls, e.g. culture- and substrate-free, were prepared with each experiment.

2.4. pH and temperature optimization

The consortium producing maximum $BioH_2$ using human waste simulant medium was selected for further optimization. A broad pH range 5.0–9.0 with an increment of 1.0, and temperatures between 50 and 70 °C with an increment of 5 °C were chosen for optimization. To find the effect of the initial pH, fermentation was carried out at 60 °C, and then the optimal pH value obtained was used for the temperature optimization.

2.5. Hydrogen production using the waste water sludge under optimized conditions

The optimized conditions were used for H_2 production using wastewater pretreated sludge (PS) and waste activated sludge (WAS). The simulated solid feces waste in minimal medium was replaced by PS or WAS at the concentration of 1% (w/v) to final reaction volume of 100 mL in 165 mL serum bottles. To these serum bottles 10% (v/v) of actively growing consortium was added, and the bottles were kept at 60 °C and 50 rpm for 7 days.

2.6. Analytical methods

The analytical methods of gas composition, metabolites, CMCase activity, and statistical analysis are given in SI.

3. Results and discussion

3.1. Enrichment and characterization of the microbial diversity

Four thermophilic microbial consortia (C1, C2, C3 and C4) were developed by processing the samples obtained from a hot spring, wastewater reclamation center and landfill compost facility. After five serial transfers, the consortia producing H_2 as the dominant gaseous product during fermentation were obtained, and each culture was designated as the "Master Culture". No heat or chemical pretreatment was required to prevent methanogenesis in sustained thermophilic conditions during the enrichment process, which is one of the advantages of thermophilic consolidated bioprocessing (CBP) for $BioH_2$ production (Carver et al., 2012). No archaeal species were observed during the phylogenetic analysis of the master cultures confirming the absence of the methanogenic microbes. A total of 35, 29, 30 and 14 operational taxonomic units were identified for C1, C2, C3 and C4, respectively. Simple dominant microbial species were found in these thermophilic mixed cultures. Due to the high temperature effect, less variety of bacteria can be observed under thermophilic conditions, and high $BioH_2$ production by thermophilic consortia is associated with low microbial diversity of communities (Hasyim et al., 2011). *Thermoanaerobacterium* dominated the C1 (98.20%) and C3 (58.05%) consortia, whereas *Geobacillus* dominated the C2 (77.25%) and C4 (97.38%) consortia. In consortia C2 and C4, *Thermoanaerobacterium* (22.51% and 2.18%, respectively) were the second abundant microbial species after *Geobacillus*. *Caloribacterium* were the next abundant species after *Thermoanaerobacterium* in consortium C3. *Thermoanaerobacterium* spp. have been widely reported for $BioH_2$ production following ethanol/butyrate type fermentation under thermophilic conditions, and they can grow on a wide variety of complex and simple carbohydrates. Both *Caloribacterium* and *Caldanaerobius* were considered as components in $BioH_2$ producing consortia in the former