Labscale Evaluation of Immobilization Media

Prepared for the

U.S. Department of Energy
Office of Electricity Delivery and Energy Reliability

Under Cooperative Agreement No. DE-EE0003507
Hawai‘i Energy Sustainability Program

Subtask 3.4: Biomass

Submitted by

Hawai‘i Natural Energy Institute
School of Ocean and Earth Science and Technology
University of Hawai‘i

September 2014

Acknowledgement: This material is based upon work supported by the United States Department of Energy under Cooperative Agreement Number DE-EE0003507.

SUMMARY

The performance of a three-phase up-flow integrated high-rate anaerobic digestion reactor system utilizing corn cob biochar as biofilm support was characterized at 10- liter total scale on waste-trap grease wastewater at defined hydraulic retention times (HRT) and packing densities. Performance was evaluated in terms of chemical oxygen demand (COD) reduction, total volatile organic acid variability, and total suspended solids reduction, as well as headspace gas composition and production rate. High resolution images of biofilms revealed extensive biofilm coverage and microbial analysis of their underlying composition revealed evidence of broad populations of anaerobic bacteria that ferment organic substrates to produce acetate, ethanol, and hydrogen as major end products as well as archaeal populations that produce methane gas. Under conditions of a two day HRT and organic loading rate of 9.75 kg COD m^{-3} d^{-1} total and soluble COD reductions of 92 and 95% were achieved with TSS reductions of 75%. The total volatile organic acids in the feed were almost completely digested and a total gas production rate of 6.1 m^3 m^{-3} d^{-1} was achieved with a methane composition of 73%.

Project Elements

Table of Contents

Results

PROJECT DESCRIPTION

The performance of an integrated three-phase high-rate anaerobic digestion system comprised of an initial continuous stirred tank reactor followed by two biochar filled column reactors connected in series was characterized at relatively low hydraulic retention times (HRT) when fed grease trap waste wastewater rich in total volatile organic acids (TVOA). Corncob biochar has a very high pore density and good pore size distribution [1, 2]. Biochar has also been found to support microbial growth and retention of methanogenic archaea in studies and effective at processing wastewaters at relatively high organic loading rates (OLR), with efficient COD reduction and methane production at relatively short hydraulic retention times [3].

PROJECT OBJECTIVES

To show that high rate anaerobic digestion of non-sterile wastewater feed that is infused with natural bacterial flora can be achieved through use of biochar filled packed bed column reactors connected in series and fronted by an in initial hydrolysis reactor that buffers the system from disturbances in pH and temperature.

RESULTS

Packing density

The system was fed high-strength grease-trap waste wastewater and the impact of packing density characterized. Grease -rap waste wastewater media presents a significant challenge as components in food waste can be more difficult to metabolize as compared to the readily metabolized constituents of the synthetic media [4]. The effect of packing density was explored in order to test for the presence of clogging and channel flow that typically undermines the performance of packed bed configurations (as opposed to the separated disc configuration which minimizes this effect). The packing densities tested were 5.5 (filter discs) and 25 (packed bed) g l^{-1} and their relative performance characterized at an HRT of three days. This HRT was chosen to meet our industry collaborator's desire to process the wastewater in three days (or less). For these experiments the COD of the grease-trap waste wastewater ranged between 15.9 to 16.6, yielding an OLR (kg COD m^{-3} d^{-1}) that ranged between 5.3 and 5.53.

The COD reduction was found to be far greater at the higher packing density with reductions as high as 94% (total) and 97% (soluble) achieved. With the packed bed, most of the COD (82, 96 [s] %) was removed before the wastewater entered R2. Although R2 was not particularly involved in removing COD, it was responsible for reducing the majority of TSS. The *specific* COD reduction (kg COD m^{-3}) was comparatively two times higher across each reactor at the higher packing densities. This doubling of specific COD reduction was likely aided by the daily recycling of solids (and active biomass) from both reactors to the mixing tank. The pH shift across both R1 and R2 was higher in the packed bed configuration (from 6.0 to 7.17) as compared to the disc configuration (from 6.4 to 6.73) presumably due to significant uptake of TVOAs in the packed column as compared to the disc filter configuration. The uptake of VOAs resulted in a pH rise that correlated to an excellent reduction in TVOAs (99%) to levels nearly below the detection limit (44 mg L^{-1}), suggesting a greater mass of active biofilm microbes in the packed bed. The reduction in TSS was much more effective in the packed bed although a significant increase in TSS in the mixing (HYD) reactor was observed with the packed column system, presumably due to the daily recycle of bottoms from both column reactors.

The methane headspace composition was similar in the mixing tanks for both system configurations but was slightly higher (77 vs. 69 %) in R1 for the higher packing density and lower (70 vs. 75 %) in R2 for the lower packing density, suggesting that the microbial metabolism (and presumably microbial composition) was generally equivalent in both configurations. Volumetric methane production, however, was higher (3.3 vs. 2.16 m^3 m^{-3} d^{-1}) in the first mixing (HYD) reactor at the higher packing density, equivalent (2.22 vs. 1.91 m^3 m^{-3} d^{-1}) in R1, and lower (0.42 vs. 0.72 m^3 m^{-3} d^{-1}) in R2, although the total methane production increased from 5.94 to 4.79 m^3 m^{-3} d^{-1} for the higher packing density. Likewise, total volumetric gas production rate (calculated over all reactors) increased from 7.73 to 9.49 m^3 m^{-3} d^{-1}. These values indicate that the packed bed configuration was more efficient and that methane production would likely have been higher if not for the fact that the overall reduction in COD was nearly completed in R1, suggesting gas hold up at the higher packing density was not realized. This result was further verified by the consistency of gas production rate measured over long time periods (data not shown).

The methane yield per COD consumed was higher for the lower packing density (disc filter) as compared with the higher packing density (packed bed) configuration at 1.57 vs. 1.11 (m^3 $kgCOD^{-1}$). Consequently, the energy yield in terms of BTU produced per gram COD consumed was slightly higher for the lower packing density at 43.35 compared to 31.22 (BTU $kgCOD^{-1}$).

Effect of HRT

With confirmation that the packed column configuration performed well and in the absence of decreased gas production due to clogging or channel flow, its performance as a function of reduced HRT was evaluated. In general the system performed well, achieving total and soluble COD reductions of 81 and 94%, respectively, for an HRT of 1 day. Optimal performance in terms of COD reduction was achieved at a two day HRT, however, with COD reductions of 92 (total) and 95 (soluble) realized. This is considered a competitive result given the organic loading rate of 9.75 (two-day HRT) and 21.5 (1 day HRT) kg COD m^{-3} d^{-1}, respectively. Although performance in terms of COD reduction was certainly competitive at a 1 day HRT, the reduction in TSS at this rate dropped to 13%, a value significantly below reductions of 75 and 76% achieved at HRTs of 2 and 3 days. This sharp drop off in TSS reduction is largely seen as the break point where the packed bed columns became unable to retain biofilm entrained bacteria as well as undigested solids that were otherwise metabolized at the higher HRTs.

At all HRTs the reduction of TVOA as present in the wastewater feed was largely accomplished with levels as high as 98% being achieved. In general the reduction was more rapid and efficient at the highest HRT of 3 days (99%) with a modest decline in efficiency at an HRT of 1 day (91%). Nonetheless, TVOA concentrations as low as 80 mg L^{-1} (compared to the feed value of approximately 4,250 mg L^{-1}) at a two-day HRT indicated a robust anaerobic metabolism.

The percentage of methane in the reactor headspace generally increased through the system for all HRTs, with values as high as 78 – 81% achieved, suggesting vibrant cultures with no significant loss of active biofilm activity even at the lowest HRT (and highest interstitial up-flow velocity). Methane production rates from each reactor generally increased with decreasing HRT although one unexplained anomaly occurred at an HRT of two days wherein the methane gas production the from mixing (HYD) reactor was actually lower than that measured from the first column reactor. Likewise, the volumetric COD consumption rate (kg COD m^{-3} d^{-1}) was higher for all reactors as the HRT decreased, reaching levels as high as 8.9, 14.6, and 16.2 (s) kg COD m^{-3} d^{-1} for the HYD, first, and second column reactors, respectively, at a 1 day HRT. Total energy yields (BTU produced per gram COD removed) gradually decreased from 31.22 to 18.64 as the HRT decreased from three to two days, but then rose back up to 25.11 BTU kg COD^{-1} as the HRT decreased again to 1 day.

At a two-day HRT, the total COD of the digester effluent was reduced by 92% and the soluble component of the COD by 95%. At one day HRT the entire reactor volume was required to achieve a total COD reduction of 81% and a soluble COD reduction as high as 94%. The TSS of the waste stream was reduced, however, dropped to below 15%, suggesting that the digester had reached a break point capacity despite the soluble COD still being almost entirely digested.

Biochar colonization

SEM images of pure biochar reveal a highly porous surface consisting of macro pores of roughly 50 microns diameter where corn kernels once sat. The surfaces of the macro pores were lined with micro pores of approximately five micron diameter. The overall roughness suggests a surface that is conducive to bacterial colonization and growth of biofilm. This was visually confirmed from images of biochar samples removed from R1 that were densely blanketed with biofilm.

Microbial Community Structure

To more accurately characterize the microbial community within the biofilms, samples were taken from both the first and second column reactors. The two column reactors exhibited identical bacterial community structure as revealed by 16S rRNA gene-based PCR-DGGE analysis. Samples obtained at different dates from the same reactors also exhibited highly similar microbial structure, indicating that stable bacterial communities were maintained in the biofilms over time. The two column reactors also contained several major archaeal populations that are shared between the two columns. The archaeal community structure also exhibited limited change, as indicated by the similar banding patterns over time.

DNA sequence information was obtained to determine the phylogenetic affiliation of some major bacterial and archaeal populations detected by PCR-DGGE. The nine major bacterial populations include six *Firmicutes*, two *Thermotogaceae*, and one *Spirochaetes*. All the *Firmicutes* populations are *Clostridia,* a group of anaerobic bacteria that ferment organic substrates to produce acetate, ethanol, and hydrogen as major end products [5-7]. The two *Thermotogae* populations are also likely important contributors to the anaerobic digestion process, as most cultured species of *Thermotogae* are obligate fermenters of sugar and other complex organics and produce lactate, acetate, ethanol, and hydrogen as major end products [8-11] [12, 13]. As one would expect for anaerobic digesters, all of the archaeal populations are methanogens, including two *Methanobacteriales* and two *Methanomicrobia*.

REFERENCES

1. Antal, M.J., K. Mochidzuki, and L.S. Paredes, *Flash carbonization of biomass.* Industrial & Engineering Chemistry Research, 2003. **42**(16): p. 3690-3699.
2. Wang, L., M. Trninic, O. Skreiberg, M. Gronli, R. Considine, and M.J. Antal, *Is Elevated Pressure Required To Achieve a High Fixed-Carbon Yield of Charcoal from Biomass? Part 1: Round-Robin Results for Three Different Corncob Materials.* Energy & Fuels, 2011. **25**(7): p. 3251-3265.
3. Lehmann, J., M.C. Rillig, J. Thies, C.A. Masiello, W.C. Hockaday, and D. Crowley, *Biochar effects on soil biota - A review.* Soil Biology & Biochemistry, 2011. **43**(9): p. 1812-1836.
4. LaPara, T.M., C.H. Nakatsu, L.M. Pantea, and J.E. Alleman, *Stability of the bacterial communities supported by a seven-stage biological process treating pharmaceutical wastewater as revealed by PCR-DGGE.* Water Research, 2002. **36**(3): p. 638-646.
5. Yang, S.T., I.C. Tang, and M.R. Okos, *Kinetics of Homoacetic Fermentation of Lactate by Clostridium formicoaceticum.* Appl Environ Microbiol, 1987. **53**(4): p. 823-827.
6. Weimer, P.J. and J.G. Zeikus, *Fermentation of cellulose and cellobiose by Clostridium thermocellum in the absence of Methanobacterium thermoautotrophicum.* Appl Environ Microbiol, 1977. **33**(2): p. 289-97.
7. Stevenson, D.M. and P.J. Weimer, *Expression of 17 genes in Clostridium thermocellum ATCC 27405 during fermentation of cellulose or cellobiose in continuous culture.* Appl Environ Microbiol, 2005. **71**(8): p. 4672-8.

8. Briones, A.M., B.J. Daugherty, L.T. Angenent, K. Rausch, M. Tumbleson, and L. Raskin, *Characterization of microbial trophic structures of two anaerobic bioreactors processing sulfate-rich waste streams.* Water Res, 2009. **43**(18): p. 4451-60.

9. Munro, S.A., S.H. Zinder, and L.P. Walker, *The fermentation stoichiometry of Thermotoga neapolitana and influence of temperature, oxygen, and pH on hydrogen production.* Biotechnol Prog, 2009. **25**(4): p. 1035-42.

10. Lakhal, R., R. Auria, S. Davidson, B. Ollivier, A. Dolla, M. Hamdi, and Y. Combet-Blanc, *Effect of Oxygen and Redox Potential on Glucose Fermentation in Thermotoga maritima under Controlled Physicochemical Conditions.* Int J Microbiol, 2010. **2010**: p. 896510.

11. Huber, R. and M. Hannig, *Thermotogales.*, in *The Prokaryotes*, M. Dworkin, Editor. 2006, Springer: New York. p. 899.

12. Romano, R.T., R. Zhang, S. Teter, and J.A. McGarvey, *The effect of enzyme addition on anaerobic digestion of JoseTall Wheat Grass.* Bioresour Technol, 2009. **100**(20): p. 4564-71.

13. Van Ooteghem, S.A., S.K. Beer, and P.C. Yue, *Hydrogen production by the thermophilic bacterium Thermotoga neapolitana.* Appl Biochem Biotechnol, 2002. **98-100**: p. 177-89.