

Novel bioaugmentation strategy with a syntrophic enrichment for enhanced digestion (SEED) system for maximizing methane yield from municipal sludge

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Introduction

Municipal sludge is becoming a global environmental problem due to its increasing production and high content of organic pollutants, pathogens, and heavy metals. The development of alternative strategies for sludge treatment is a priority for waste utilities worldwide. Anaerobic digestion (AD) is a common technology for the reduction of organic pollutants, the destruction of pathogenic microorganisms and the subsequent production of renewable energy in the form of biogas from organic waste. However, organic removal efficiencies in AD are limited to 50-65% for complex substrates, such as municipal sludge. In addition to the complex molecular structure of municipal sludge, including microbial cells, intra- and extra-cellular polymeric substances, challenges around retaining slow-growing, fastidious archaea in bioreactors also contribute to low organic conversion efficiency in AD. The syntrophic microbial community is the main driver in the AD system. Despite the lower population of methanogenic archaea compared to fermentative bacteria, they play a vital role in methane synthesis. With particular attention to microbial syntrophy, the present research aimed to develop a novel Syntrophic Enrichment for Enhanced Digestion (SEED) system to grow a dense population of syntrophic bacteria and archaea as a side-stream, compact, fixed-film bio-incubator. The SEED system included a high-performance (activated) carbon cloth (CC) as a biocompatible surface for enhanced microbial attachment. The study also developed a novel bioaugmentation strategy to periodically transfer anaerobic cultures grown with a readily biodegradable substrate in the SEED system to a conventional anaerobic sludge digester to accelerate biogas recovery rate and extent from municipal sludge.

Material and methods

The experimental design (Fig. 1) consisted of four thermophilic ($55 \pm 1^\circ\text{C}$) semi-continuous flow, continuously stirred tank reactors (CSTR) with mechanical mixers: SEED system with activated CC; Control digester; Test digester; and an acid phase (AP) reactor, all operated over 304 days. As mentioned earlier, the main objective of the SEED system was to grow and then supply methanogen-rich microbial cultures to the “Test” or the bio-augmented AD. The Test reactor was then compared with the “Control” reactor utilizing municipal sludge, representing a conventional (non-bioaugmented) sludge digester at a typical wastewater treatment plant.

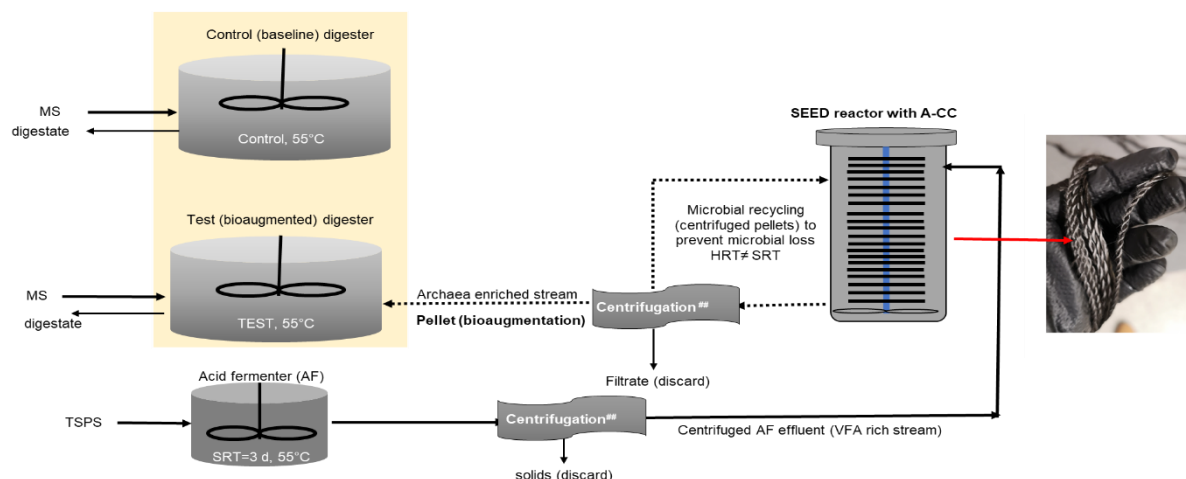


Fig. 1. Schematic flow diagram of the proposed bioaugmentation process with activated CC

The substrate type and design/operating conditions of all four reactors were different. The SEED reactor (working volume of 5-L) was fed intermittently (every 2 days) with a soluble, volatile fatty acid (VFA)-rich substrate, which was obtained by acidifying thickened screened primary sludge (TSPS) in an AP fermenter (working volume of 6-L) under sludge retention time (SRT) of 3 days, corresponding to an organic loading rate (OLR) of 20 g chemical oxygen demand (COD)/L/d. The effluent of the AP reactor was centrifuged at 3,500 revolutions per minute (rpm) for 10 minutes, and only the soluble (supernatant) phase of the effluent was used as the substrate for the SEED system to accelerate the culture growth rates and to prevent premature clogging of activated CC surfaces with influent particulate matter. The main reason for the intermittent feeding of SEED was to provide sufficient time for syntrophic cultures to grow on activated CC. The commercial CC was obtained from FibreGlast Developments Corporation (USA), and a two-step CC activation process was applied in the laboratory for surface functionalization for enhanced biomass retention. During step-1, CC was subjected to an acid pre-treatment in 2 M H_2SO_4 for 24 hours, then in 20% HNO_3 solution for 24 hours to remove impurities, followed by rinsing the acid-treated CC several times with deionized water. In step-2, the acid-treated CC was dried in an oven at 80°C overnight, followed by calcination in air at $525 \pm 25^\circ\text{C}$ for 3 hours in a muffle furnace to improve the porosity and add oxygen doping thereby improving the biocompatibility of CC (Kumar et al., 2022). The resulting activated CC was braided in a rope form to be utilized in SEED as biomass carrier (Fig. 1) at a packing density of

1.48 g activated CC per g volatile solids of substrate, determined by preliminary biochemical methane potential assays (Kumar et al., 2022). During SEED optimization to make this side-stream reactor as compact as possible, hydraulic retention time (HRT) was gradually decreased to 16, 8 and finally maintained at 5 days, when the sloughing/bioaugmentation of cultures grown on activated CC started. During bioaugmentation cycles (every 8-15 days), the speed of the mechanical mixer in the SEED reactor was increased from 60 to 120 rpm for 1 hour to mechanically slough off the slime layer on CC and cultures in effluent were transferred to the Test digester (Fig. 1). Outside the bioaugmentation cycles, the effluent of the SEED was centrifuged (3,500 rpm for 20 min), and the settled pellets were returned to the SEED reactor to maintain a dense population in the reactor, which allowed for SRT to exceed HRT. Test and Control digesters were semi-continuously fed (once a day, 7 days a week) with mixed sludge. Both sludge digesters were first operated at 20-d SRT (OLR of 3 g COD/L/d) for 200 days, and the SRT was gradually reduced to 10-d (OLR of 6 g COD/L/d) over 90 days to assess the effectiveness of bioaugmentation at various SRTs. Two strategies, Liquid Bioaugmentation (LB) and Pellet Bioaugmentation (PB), were implemented. During LB, 30% volume of the Test digester was replaced with effluent carrying microbial cultures from the SEED reactor, however during PB, the effluent from the SEED was first centrifuged (3,500 rpm for 10 minutes), and only the recovered microbial pellets were transferred to the Test reactor, which demanded much smaller (0.7-1.6%) volume replacement in the Test digester to keep liquid volume/SRT constant.

Results and discussion

During 20-d SRT, LB resulted in process fluctuations in the Test digester due to a significant volume (30%) of biomass that had to be withdrawn/replaced for each bioaugmentation cycle. Performance parameters, such as daily biogas volume, headspace methane content and COD removal of the Test digester, started to decline drastically. After three consecutive LB cycles, the Test digester was not recovering, the LB bioaugmentation strategy was abandoned to prevent reactor failure, and PB strategy was initiated (Fig. 2a). The PB positively impacted AD performance and was used for the subsequent bioaugmentation. The main goal with PB was to transfer a dense population of active microbial biomass in a small volume. Hence, the COD of the centrifuged pellets from the SEED reactor was chosen as a critical performance parameter to determine the frequency of bioaugmentation. From reactor monitoring, it was established that the COD of pellet obtained from SEED effluent above 3,500 mg/L accumulates enough biomass concentration equivalent to 1-2% of the Test reactor volume. At pellet COD \geq 3,500 mg/L, the microbial biomass and biofilm attachment on the CC was confirmed using fluorescence microscopy using FilmTracer™ SYPRO® Ruby biofilm stain and DAPI stain (Fig. 2b and Fig 2c).

The impact of AD bioaugmentation at different SRTs can be seen in Fig. 2a. The improvements in Test digester as a result of bioaugmentation in terms of daily specific biogas production (ml per g COD added) reached 33% and 88% at SRTs of 20 days and 10 days, respectively, with respect to the Control digester. In terms of organic removals, at the 20-day SRT, the average COD removal of the Test digester was 74% compared to 66% of the Control digester. When the SRT was shortened to 10 days, both digesters experienced slight reduction in COD removals due to the doubled OLR; however, the Test digester still performed better (Test: 70%, Control: 64%). The results indicated that PB facilitated stable operation at shorter HRT and positively affects reactor performance with an increase in biogas/methane yield.

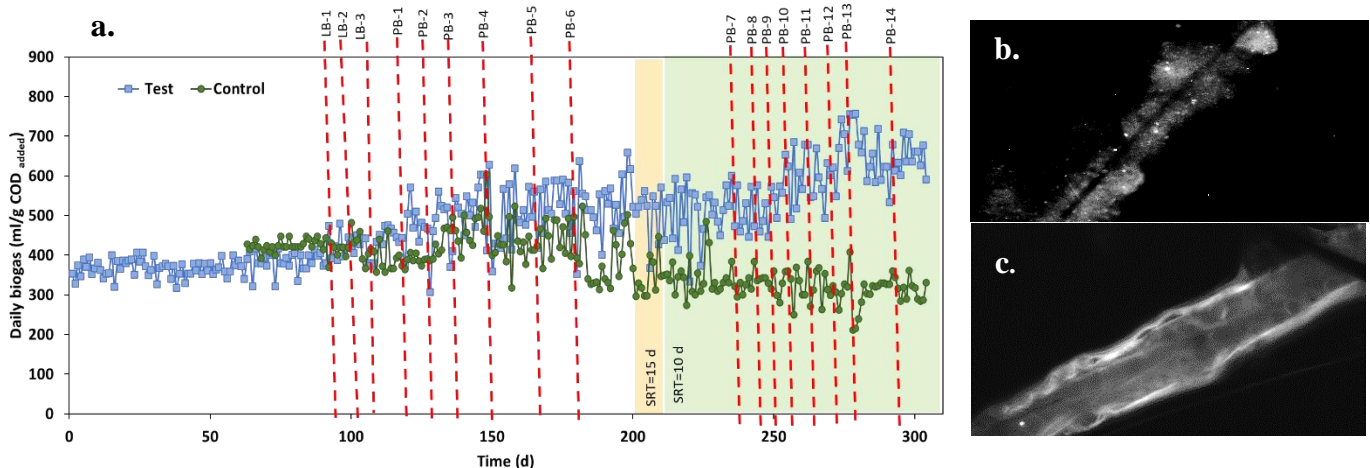


Fig. 2a. Daily specific biogas production from Test and Control AD, **b-c.** Fluorescent microscopic image of biofilm on CC

Conclusion

The thermophilic SEED system with activated CC is a novel syntrophic bacteria and archaea incubator that can be utilized to bioaugment conventional anaerobic sludge digesters. The pellet bioaugmentation developed allows for active microbial biomass to be transferred to AD at a shorter HRT, thereby reducing the size of the AD needed to process a large amount of waste. A synergistic interaction of activated CC with syntrophic cultures in the SEED incubator reactor enhances microbial symbiosis and can be implemented for any existing AD system as a lateral flow retrofit model without modifying the existing infrastructure.

References

Kumar, V., Kieft, B., Devi, P., Hallam, S.J., Eskicioglu, C., 2022. Two-step activated carbon cloth enhances microbial interactions and methane production during anaerobic digestion of municipal sludge. *Renew. Energy* 196, 366–374. <https://doi.org/10.1016/j.renene.2022.06.133>