Continuous stirred tank reactors in series: an approach to enhance the enzymatic hydrolysis and sludge reduction in anaerobic waste activated sludge digestion?

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Abstract

Hydrolysis is commonly regarded as the rate-limiting step in anaerobic digestion of waste activated sludge (WAS). To enhance the hydrolysis rate, in this study, a novel cascade anaerobic digestion system was developed consisting of small-volume reactors in series, instead of a conventional continuous stirred tank reactor (CSTR). The cascade system achieved a higher reduction in total chemical oxygen demand (TCOD) and increased methane production, applying a solids retention time (SRT) of 22 days. More importantly, a clear acceleration of the hydrolysis rate was found in the cascade system. The measured activities of total protease and cellulase in both systems revealed that the hydrolytic enzymatic reaction rates in each CSTR of the cascade system were generally higher than the activities in the conventional CSTR. Rate acceleration was ascribed to the applied higher organic loading rates in the small-volume CSTRs in series. The results indicate that the developed cascade system indeed is more effective for sludge digestion than the commonly applied conventional CSTR.

Key words: cascade system concept; hydrolytic enzymatic activity; waste activated sludge.

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Introduction

Waste activated sludge (WAS) is an inevitable by-product generated in biological wastewater treatment plants. Anaerobic digestion (AD) is an established technology for both the stabilisation of WAS and for the recovery of the biochemical energy stored in the sludge in the form of biogas. WAS usually contains (large) particulate organics, consisting of proteins, polysaccharides, lignocellulosic matter, and fats. Hydrolysis of these particulate organics into simpler derivatives is generally regarded the rate-limiting step in the sludge digestion. Hydrolysis mainly proceeds via microbial hydrolytic enzymatic reactions. Acceleration of these enzymatic reactions, or an increased activity of enzymes involved, results in an enhanced hydrolysis step (Kim et al., 2012). Previous studies have shown that WAS hydrolysis enhancement can be achieved by the addition of hydrolytic enzymes (Yu et al., 2013). However, the economic viability of this approach is not yet clear owing to the increased operational costs.

The expression and activity of hydrolytic enzymes are dependent on the substrate concentration, following Michaelis-Menten kinetics (Cornish-Bowden, 2013). For practice, this implies that the enzymatic reactions can be accelerated when higher organic loading rate are applied. However, in general, conventional CSTR reactors are operated at solids retention times (SRTs) between 20-28 days, resulting in extremely low non-hydrolysed substrate concentrations in the reactor broth. Increased substrate loadings of non-hydrolysed WAS can be attained by applying a plug-flow mode or a tank in series concept. Very likely, such mode of operation will also lead to a physical separation of methanogenesis and hydrolysis/acidogenesis, which possibly may lead to a higher stability, when overloading conditions are experienced.

Based on these theoretical considerations, a novel cascade anaerobic digestion system, using CSTRs in series, was proposed for WAS treatment. The objective of this research was to establish a cascade system aiming for a higher degree of sludge reduction compared to the conventionally applied CSTR systems, applying similar SRTs. If successful, the proposed approach will result in a significant drop in treatment costs, impacting the economy of WAS treatment.
Materials and methods

Reactor set-up and operation

Two CSTR systems were operated in parallel: 1) a cascade AD system comprised of 3 x 2.2 L CSTRs (R1, R2, R3) + a 15.4 L CSTR (R4), and 2) conventional reference CSTR with a working volume of 22 L. Both systems were operated at 35 ± 1 °C and an overall SRT of 22 days. A sludge recirculation system from reactor R3 to R1 was implemented to regulate the pH in R1, in order to achieve optimized conditions for enzyme production by microorganism. All reactors were seeded with anaerobic sludge collected from an anaerobic digester at the municipal wastewater treatment plant Harnaschpolder (Den Hoorn, the Netherlands). The feed sludge was the WAS from the same plant, and stored at 4 °C before use.

Analytical methods

Total chemical oxygen demand (TCOD) was measured in accordance with standard methods. Biogas compositions were measured using gas chromatography (GC) (7890A, Agilent, USA). Daily biogas volume produced was measured with a gas flowmeter (Ritter, Germany). The activities of total protease and cellulase (including cell-free and cell-attached fraction) (Zhang et al., 2007) were individually analysed by Pierce fluorescent protease assay kit (Thermo Fisher, USA) and MarkerGene fluorescent cellulase assay kit (MarkerGene, USA), using a 96-well microplate spectrophotometer (Synergy HTX, BioTek, USA) at 35 °C. The hydrolysis rate and specific hydrolysis rate were calculated by equations based on Wu et al. (2015):

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\text{Hydrolysis rate (g COD L}^{-1} \text{day}^{-1}) = \frac{\text{mass}_{\text{COD}} \text{ soluble} \cdot \text{day} \cdot \text{reactor volume}}{\text{mass}_{\text{COD}} \text{ soluble} \cdot \text{day}}
\]

(1)

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\text{Specific hydrolysis rate (g COD g VS}^{-1} \text{day}^{-1}) = \frac{\text{mass}_{\text{COD}} \text{ soluble} \cdot \text{day}}{\text{mass of VS within reactor}}
\]

(2)

Results and conclusion

This study was conducted for a period of 125 days. During the start-up phase, both the methane production and TCOD concentrations fluctuated (Figure 1). Both parameters more or less stabilised from day 69 onward, after which the cascade and reference system were both operated under steady state conditions for another 56 days. It was calculated that the TCOD removal efficiency of the cascade system was approximately 43 ± 6%, versus 38 ± 5% of the reference CSTR during the latter period. Interestingly, a TCOD reduction of about 26% was obtained by the reactor R1, R2 and R3 together, with an SRT of only 6.6 days in total. Besides, on average, the overall methane production was approximately 6.55 L/day for the cascade system and 5.86 L/day for the reference CSTR.

As for the hydrolysis rate in each reactor (Figure 2 left), the results show a clear increased hydrolysis rate in reactors R1, R2 and R3, whereas the hydrolysis rate in the reference CSTR was just in between the values of R3 and R4. When taking the volume of the reactors into account, it was found that the total hydrolysis rate of the cascade system was 12.21 g COD/day (or 0.56 g COD/L/day), which was 1.37 times higher than that of the reference CSTR, i.e. 8.92 g COD/day (or 0.41 g COD/L/day). The specific hydrolysis rate showed a similar trend as the hydrolysis rate (Figure 2 right). The observed increased hydrolysis rate in the cascade system was further evidenced by the activity of total protease and cellulase, in each reactor (Figure 3). The increased enzymatic activities in the cascade system can be ascribed to the increased substrate loadings in the tanks in series set-up. By transforming a large volume reactor to several small volume reactors in series, the organic loading rate of R1, R2, R3 and R4 could reach values as high as 23.16, 20.87, 18.76 and 2.47 g COD L·day⁻¹, respectively, whereas that of the reference CSTR was only 2.32 g COD L·day⁻¹. In the cascade system, the prevailing higher organic loading rates, apparently enhanced enzyme expression and enzymatic activity, boosting the overall hydrolysis rate of the system. As a consequence, a better TCOD reduction efficiency was achieved by the cascade system.

In the next steps of the experiment, both systems will be consecutively operated under SRTs of 15 days and 10 days. It is expected that under such short SRTs, operational performance becomes unstable in the conventional CSTR, possibly leading to process failure. In contrast, based on the above-described results, we expect a higher degree of stability in the cascade system.

Reference


**Fig.1** Variations of TCOD (left) and methane production (right)

**Fig.2** Distribution of the hydrolysis rate (left) and the specific hydrolysis rate (right)

**Fig.3** Distribution of the activity of cellulase (left) and protease (right)