



# Anaerobic fluidized bed digestion of primary and thickened waste activated sludges



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## HIGHLIGHTS

- Activity of three attached anaerobic microbial groups was characterized.
- A new method for measurement of acetogenic and acidogenic bacteria was developed.
- Activity-based SRTs were 1.9–4.4 times longer than VSS-based estimates.
- VSS based detachment rates were 3.2–9.8 times higher than activity-based rates.

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## ABSTRACT

Two lab-scale anaerobic fluidized bed bioreactors (AnFBRs) treating primary sludge (PS) and thickened waste activated sludge (TWAS) were studied to explore biofilm microbial characterization. COD and VSS removal for PS showed 62% and 63%, respectively at an organic loading rate (OLR) of 18 kg COD m<sup>-3</sup> d<sup>-1</sup> and an hydraulic retention times (HRTs) of 2.2 days. Similarly, TWAS was treated at 56% COD removal and 50% VSS reduction efficiencies at an OLR of 12 kg COD m<sup>-3</sup> d<sup>-1</sup> and an HRT of 4 days. Furthermore, the specific bacterial community activity tests showed a significant difference between solids retention times (SRT) based on general VSS and retention times based on the activity of methanogenic, acidogenic, and acetogenic microbes. While SRT based on VSS measurements in the PS-AnFBR were 3.3 days, the activity-based retention times varied from 12.5 to 16.6 days. Similarly, in the TWAS-AnFBR, the SRT based on VSS measurements were 5.0 days, and the activity-based retention times ranged from 9.3 to 10.8 days. The BioWin model simulation predicted that conventional anaerobic digestion processes achieved lower VSS reduction efficiencies than the AnFBR process at the same SRT.

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## 1. Introduction

Anaerobic digestion is a preferred treatment process for organic wastes due to its low nutrient requirements, low biomass yield, and additional biogas (hydrogen, methane) production [1]. Anaerobic digestion has been tested successfully on both municipal wastes, and industrial effluents i.e. olive oil mill, protein waste [2,3]. Anaerobic digestion of primary sludge (PS) and thickened waste activated sludge (TWAS) is often limited by slow biodegradation rates ensuing from slow biomass hydrolysis, and resulting in low solids destruction efficiencies of less than 50% despite long retention times [1].

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Fluidized bed reactors have been used in various biotechnological applications utilizing low suspended solids streams e.g. treating food-processing, digesting paper industry wastewater, and purifying fermentation wastewater [4,5]. The mesophilic anaerobic fluidized bed reactor (AnFBR) with zeolite as carrier media (425–610 μm) developed by Nakhla and coworkers [6], achieved up to 88% TCOD and 78% TSS removal at an organic loading rate (OLR) of 29 kg COD m<sup>-3</sup> d<sup>-1</sup> during the treatment of thin stillage with a TCOD of 130 g L<sup>-1</sup> and TSS of 47 g L<sup>-1</sup> [6]. In addition, the AnFBR has been recently demonstrated for the digestion of primary sludges [7] with a TSS destruction efficiency of 82% at an OLR of 9.5 kg COD m<sup>-3</sup> d<sup>-1</sup>.

In the fluidized bed systems using carrier media, biofilms have a critical role for optimal performance. In biofilm reactors, the development of the biofilm is determined by the difference between biofilm growth and detachment processes. Biofilm growth mainly relies on the carrier characteristics such as particle size, sphericity,

### Nomenclature

TCOD	total chemical oxygen demand	TWAS	thickened waste activated sludge
SCOD	soluble chemical oxygen demand	SRT	solids retention time
TSS	total suspended solid	HRT	hydraulic retention time
VSS	volatile suspended solid	AnFBR	anaerobic fluidized bed reactor
VFA	volatile fatty acids	SMA	specific methanogenic activity
HDPE	high-density polyethylene	SAdA	specific acidogenic activity
PS	primary sludge	SAtA	specific acetogenic activity

porosity, density, and specific surface area (SSA) [8]. The detachment of biofilm is usually contributed by abrasion (surface biofilm loss caused by particle collision), erosion (surface biofilm loss caused by shear stress), sloughing (the periodic loss of large biofilm patch), and predator grazing (outer surface biofilm consumed by protozoa) [8].

Additionally, the growth rate of bacteria also influences distribution of microbial community in the biofilm. Egli et al. [9] using fluorescent in situ hybridization (FISH) observed that the biofilm layer from a rotating biological contactor biofilm treating high ammonium wastewater, comprised aerobic nitrifiers on the outer layer of the biofilm and anammox bacteria in the inner layer. Similar observation was also reported by Vlaeminck et al. [10] who operated a granular sludge reactor with autotrophic biomass treating synthetic wastewater at a nitrogen loading rate (NLR) of 84 g  $\text{NH}_4\text{-N m}^{-3} \text{d}^{-1}$ . Vlaeminck and coauthors [10] addressed that the structure of the biofilm layer from inside to outside was in the following order: anammox bacteria ( $\mu_{\text{max}} = 0.1 \text{ d}^{-1}$ ), nitrite oxidizing bacteria (NOB), and ammonium oxidizing bacteria (AOB) ( $\mu_{\text{max}} = 0.14\text{--}1.44 \text{ d}^{-1}$ ). These findings clearly suggest that in the autotrophic biofilm, the slowest growing bacteria grow deep in the biofilm and are thus sheltered from hydrodynamic forces. Mozumder et al. [11] also studied the impact of substrate concentration on the bacterial distribution in a granular sludge reactor and found that in the absence of organics, the relatively few heterotrophic bacteria grew behind the autotrophic AOB and NOB bacteria. However, in the presence of organics, the fast growing heterotrophs became the majority on the outer surface of the biofilm. The aforementioned studies of aerobic biofilms clearly demonstrated that in multi-species biofilms, the slow-growing bacteria are present in the inner biofilm layers.

The structure of anaerobic biofilms is distinctively different from aerobic mixed-culture biofilms of heterotrophs and nitrifiers where the culture interaction and interdependency is not as strong. The various bacterial groups in anaerobic biofilms feed off the products generated by the other cultures and hence it is anticipated that the acidogenesis grow on the outside of the biofilm while the methanogens grow on the inside of the biofilm. Studies of the structure of anaerobic biofilms are limited with most of the studies focusing on the spatial distribution of active organisms along the reactor rather than the distribution inside the biofilm. Bull et al. [12] observed that methanogens mainly grew attached to the carrier surface while acidifiers tend to appear in the suspended phase when testing an anaerobic fluidized bed reactor with glucose solution at an HRT of 5 days and OLRs ranging from 6 to 18 kg  $\text{COD m}^{-3} \text{d}^{-1}$ . Kuba et al. [13], using zeolite as support media in an anaerobic fluidized bed treating volatile fatty acids (VFAs) based synthetic wastewater at an OLR of 4 kg  $\text{COD m}^{-3} \text{d}^{-1}$ , claimed that not all of the attached biomass were active methanogens.

Similarly, Hidalgo and Garcia-Encina [14] carried out specific methanogenic activity (SMA) tests only on the attached biomass

in a methanogenic fluidized bed reactor fed with acetic acid and found higher specific methanogenic activity at the top of the fluidized bed than at the bottom. Andalib et al. [7] observed a much lower detachment rate for methanogens than other biomass, resulting in a methanogenic SRT to overall biomass SRT ratio of 3.7:1 in an AnFBR reactor treating municipal biosolids. Kuo et al. [15], using biochemical hydrogen potential (BHP) on attached and suspended biomass from AnFBR treating kitchen wastes mesophilically at an HRT of 7.3 days and OLR of 1.1 kg  $\text{COD m}^{-3} \text{d}^{-1}$ , determined that the concentration of hydrogen-producing bacteria in suspension is 2.5 times on the carrier media, implying that the acidogenic bacteria grew primarily in suspension.

While anaerobic microbial activity in biofilm reactor has been assessed using SMA test [7,13,14], the activity of other anaerobic microbial groups have been scantily evaluated in the literature, presumably due to the common perception that methanogenesis is often the rate limiting anaerobic process. However, this postulation is not valid for solids digestion which is hydrolysis-limited [16]. Moreover, as the aforementioned study by Andalib et al. [7] highlighted that the activity-based methanogenes SRT was 3.7 times longer than overall biomass SRT, more precise microbial conditions of anaerobic biofilm systems can be assessed through the activity-based SRT estimation rather than general VSS-based SRT calculation. Thus, activity tests for other anaerobic microbes than methanogenes such as acidogenesis and acetogenesis microbes are also necessary to evaluate the different activity-based SRT of such microbial species.

In light of the scarcity of information in the dispersed literature on the structure of anaerobic biofilms, and limited tools for quantification of various microbial groups, the main objectives of this study were to develop a methodology to estimate active biomass SRT, and evaluate the attachment/detachment characteristics of the various anaerobic microbial groups.

## 2. Materials and methods

### 2.1. System description

Two identical lab-scale AnFBRs, demonstrated in Fig. 1, were tested for digestion of PS and TWAS. Each plexiglass reactor contained a 16-liters main anaerobic column (3.6 m height, 8.9 cm long and 5.1 cm width) and a liquid–solid separator from which the digested sludges was separated and circulated to the bottom of the AnFBR for fluidization. A wet tip gas meter connected to the top of the column was used to measure the biogas flow rate. A mesophilic temperature of 37 °C is uniformly maintained throughout the reactor by a water bath (IncuMax™ WB20C, USA). A 10-liter container with mixer was used as a feed tank, from which sludges were pumped to the bottom of the column by a peristaltic pump (Masterflex I/P, Masterflex AG, Germany). pH was checked and controlled manually every day. Approximately 3 kg high-density polyethylene (HDPE) media (600–850  $\mu\text{m}$ ) were

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