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Bioaccumulation of metals and granular sludge development in a newly-inoculated high rate anaerobic reactor

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ABSTRACT

For treatment of wastewater from soft drink production, a full scale (546 m^3) expanded granular sludge bed (EGSB) reactor was inoculated with granular sludge previously used to treat wastewater from paper and food processing industries. During startup and subsequent phases, there were significant changes observed in granule composition, performance, and population distribution. Microbial, compositional and functional development of granular sludge during a six month time period immediately following inoculation was investigated. Reaction kinetics and fluorescence in situ hybridization (FISH) analysis were used to describe population dynamics and evolution of methanogens and fermentative microbes within the granules. Bioaccumulation of elements was quantified and analyzed statistically with respect to operational parameters; calcium and sulfate deficiencies in the new wastewater likely contributed to granule disintegration and eventual reactor failure. Second generation granules formed from the novel conditions exhibited characteristics from multiple granulation models.

1. Introduction

High rate anaerobic technology offers a proven method for energy recovery and advanced stabilization of a wide range of industrial and municipal wastewaters. The success of a high rate system is strongly correlated with its ability to retain active biomass (i.e. granules) within the reactor during high hydraulic/organic loading periods, a phenomenon which is reflected in the difference between organic and sludge loading rates (OLR/SLR) in high rate reactors such as the upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB) reactor designs. This decoupling of hydraulic retention times (HRT) and sludge retention times (SRT) contrasts with conventional systems such as completely stirred tank reactors (CSTR), which rely on large reactor volumes and are limited by low growth rates of anaerobic microbes, particularly methanogens. Low excess sludge loads and small reactor volumes, when combined with energy production, lead to an overall savings of about 1 kWh of fossil energy per kg COD removed when compared with conventional aerobic activated sludge systems, depending on aeration technology (Van Lier et al., 2015). To avoid long startup times and costly delays in efficient operation, newly installed reactors are often seeded with inoculum from industries treating similar wastes; however, pressures exerted on granules due to substrate and operational changes can lead to performance inhibition, granule disintegration and biomass washout (Alphenaar, 1994; McHugh et al., 2003). Reducing start-up time and reaching stable operation in high-rate anaerobic reactors requires understanding the granulation and disruption mechanisms of anaerobic sludge, including physiochemical characteristics such as morphology, porosity, and settleability (Jiang et al., 2016; Liu et al., 2009). The stability of formed granules is process-dependent and depends on a number of operational parameters (e.g. upflow velocity, HRT, etc.) and reactor design; however, wastewater type is perceived to be the most influential factor (Connelly et al., 2017; Schnürer, 2016). Establishing the relationship between morphological characteristics and bioreactor performance is thus beneficial for monitoring and control of bioreactors (Liu et al., 2009).

1.1. Sludge granulation

The efficiency of a UASB/EGSB reactor is in large part related to the quality of residing biofilms, which attach to sludge particles, and the efficiency of wastewater-sludge contact (Abbasi and Abbasi, 2012). No single species is able to fully degrade organics and thus each granule contains multiple distinct species of bacteria and archaea; the most pivotal relationship in these microecosystems is that between the

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obligate hydrogen producing acetogens and hydrogen utilizing methanogens. This association (syntrophy) partially regulates the entire digestion process through interspecies hydrogen transfer by effectively maintaining hydrogen partial pressure at sufficiently low levels to create conditions in which critical intermediate reactions such as butyrate and propionate oxidation are exergonic. Without efficient hydrogen transfer, such reactions remain endergonic which leads to buildup of volatile fatty acids (VFA) and subsequent pH reduction to inhibitory levels. The agglomeration of biomass into granules encourages interspecies hydrogen transfer, which in turn accelerates the rate of methane conversion in granules up to a factor of 10 when compared to sediments and sewage sludge (Santegoeds et al., 1999).

Provided sufficient and consistent upflow velocities and non-inhibiting substrate, microflora will spontaneously amalgamate into granules roughly 0.1 mm–5 mm in diameter in a process called auto-immobilization. Granulation is a natural process which can proceed under psychrophilic, mesophilic, and thermophilic conditions (Van Lier et al., 2015), and is initiated through transportation, adsorption and adhesion of cells to the surface of inert matter, other cells, or organic precipitates which over time will lead to the formation of aggregates (Schmidt and Ahring, 1996; Schnürer, 2016). In subsequent stages and under suitable conditions the formed aggregates grow by cellular multiplication and form dense biofilms, which become nearly spherically shaped over time as a result of hydraulic shear forces of upflowing liquid and gas. The granulation process may be accelerated through introduction of inert carriers (which allow adhesion of suspended biomass) (Pol et al., 2004) and supplementation of certain ions.

In addition to macronutrients such as carbon, nitrogen, phosphorus and sulfur, micronutrients (e.g. trace metals) are essential for microorganism growth and other biochemical and physiological processes. Microelements are present in granules within either the enzyme system (as part of a cofactor) or are non-enzymatic in nature (Zandvoort et al., 2006), for instance in microbial respiration processes involving electron transfer bound to cell walls or extracellular electron acceptors, as in the case with Fe(III)- and Mn(IV)-reducing bacteria (Lovley, 1993).

Sodium (Na^+) is essential for anaerobic bacteria and archaea, presumably because it aids the enzymatic mechanism behind the synthesis of adenosine triphosphate (ATP), or the oxidation of nicotinamide adenine dinucleotide (NADH) (Appels et al., 2008), but at high concentrations it may cause biomass inhibition (Turcios et al., 2016a). Although high concentrations of Na^+ inhibit biogas formation (Turcios et al., 2016b), interactions with other elements may occur (Chen et al., 2008). Other cations (e.g. Ca^{2+}, Mg^{2+}, K^+) have also shown inhibitory effects on biogas production in a charge-dependent manner, possibly by inhibiting the Na^+ export channel necessary for the final methanogenic reaction. Therefore, an appropriate ratio between these cations can offset the inhibitory effects of one another, as well as help to avoid the inhibition of biogas production due to ammonia and VFA (FitzGerald et al., 2015). For example, excessive ion content in the cytoplasm of living cells should be avoided and this is apparently achieved by mechanisms that regulate K^+/Na^+ selectivity.

Granule size and mechanical strength of granules are in part regulated by cell lysis of internal microbes due to substrate limitation, which creates hollow cores within granules. Such void spaces may also induce biomass washout due to trapped gases causing granule flotation (Beeftink, 1987). In cases where granule size and substrate penetration are not properly balanced, as would usually be the case (at least initially) for granular sludge undergoing transitional substrate loading regimes, granule quality will be negatively affected (Alphenaar, 1994; Dolfing, 1985; Kosaric et al., 1990). In particular, sulfide in the liquid phase of anaerobic digesters, produced through competitive activity of sulfate reducing bacteria (SRB), is known to bind selectively to iron; if iron is not present in sufficient concentrations, other essential metal ions (e.g. Ni^{2+}, Co^{2+}, Mo^{2+}, Mn^{2+}) can precipitate into sparingly soluble metal sulfides ($Me^{2+} + S^{2-} \rightarrow MeS\downarrow$) (Rosenwinkel et al., 2015). This may result in micronutrient deficiencies which significantly reduce granular productivity (in terms of methane) and robustness, as well as increased S concentrations in the granule.

Granule characteristics (including performance) are primarily determined by operational characteristics such as organic/sludge loading rates (OLR/SLR), upflow velocity, temperature, pH and alkalinity, reactor hydrodynamics, and composition of input wastewater, including trace metals and metal ions (Abbasi and Abbasi, 2012). Due to the sheer number of variables and interdependencies, however, there exist no comprehensive models or theories which sufficiently predict granule formation (and associated performance) under variable conditions. Such limitations in the existing literature create uncertainties in reactor response to new substrate conditions, which may lead to inefficient design and/or operation of high-rate anaerobic treatment systems. In order to document the transitional characteristics of granular sludge (including full scale performance), in this work granule development in an inoculated reactor treating new wastewater was studied in depth using full scale and lab scale data, including kinetics, elemental analysis, and fluorescence in situ hybridization (FISH) for sludge samples (S1–S6).

2. Materials and Methods

Reactor operation, granular sludge characteristics and performance are described in detail in a previous publication (Cuff et al., 2018). Briefly, after seeding the EGSB reactor with adapted inoculum from the pulp and paper (56% by oDM) and food processing (44% by oDM) industries, operation began in March 2016 and gradually reached a consistently high degradative performance, with COD reduction and methane production maintained above 90% and 1000 $m^3 \cdot d^{-1}$, respectively. After ca. 80 days reactor performance dropped sharply; after a period of inhibition it was decided to reduce organic loading and the reactor was dosed with iron ($FeCl_2$) and micronutrients; reactor performance gradually recovered until COD degradation reached consistent values over 85% after day 125 with a maximum daily CH_4 production over 2200 $m^3 \cdot d^{-1}$. Sludge samples (S1–S6) were taken at roughly monthly intervals which corresponded to operational phases “pre-startup”, “adaptation”, “pre-inhibition”, “inhibition”, “recovery”, and “stable”, respectively.

2.1. Granule and elemental analysis

Dry matter (DM) and organic dry matter (oDM) content were determined according to DIN EN 12880 (2001) and DIN EN 12879 (2001) respectively. Microscopy was performed using a Zeiss Stemi 2000-C microscope, Sony ICC1 camera and Zeiss-Software AxioVision 4.9. Elemental analysis was performed via inductively coupled plasma optical emission spectrometry (ICP-OES) (iCAP 6000 ICP Spectrometer, Thermo Fisher Scientific Corporation) and the procedure was conducted as described by Turcios et al. (2016a, 2016b). Briefly, about 50 g of homogenized material (i.e. sludge) was dried at 105 °C for 24 h, then ground to a fine powder (MM 400, Retsch GmbH, Haan, Germany). About 20 mg of each sample was incinerated for 8 h in a muffle furnace (M104, Thermo Fisher Scientific Corporation). Next, 1.5 ml per sample of 66% nitric acid was added. After 10 min at room temperature, 13.5 ml of ultrapure water was added. The solution was filtered (0.45 μm filters, Carl Roth) and stored in vials before analysis at 4 °C.

2.2. Methanogenic activity test

The procedure for the methanogenic activity test was conducted according to VDI 4630 protocol (2016) with slight modifications as described in Cuff et al. (2018). Granular sludge was sampled (at reactor height of 3 m), sieved and analyzed along a 225 day investigative period; a total of six batch tests/sludge characterizations were carried out at roughly monthly intervals. Sludge samples were exhausted at 37 °C for a minimum of 7 days before test start in order to reduce gas