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Syntrophic acetate oxidation during the two-phase anaerobic digestion of waste activated sludge: Microbial population, Gibbs free energy and kinetic modelling

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ABSTRACT

A system using a two-phase anaerobic configuration (mesophilic/thermophilic) was tested by feeding waste activated sludge (WAS). The first acidogenic stage presented a hydraulic retention time (HRT) of 3 days, while the second methanogenic stage had an HRT of 10 days. Both raw and ultrasonically pretreated WAS samples were utilized for the experiment. Previous Fluorescence in Situ Hybridization (FISH) observations, revealed that in the thermophilic phase, the acetoclastic methanogenesis was likely replaced by a nonacetoclastic pathway, namely, syntrophic acetate oxidation (SAO). A modified version of Anaerobic Digestion Model n°1 (ADM1), accounting for the SAO pathway, was implemented and calibrated. The proposed model addressed the relationship between the hydrogen concentration and Gibbs free energy and showed the thermodynamic feasibility of the SAO pathway, while simultaneously highlighting the role played by hydrogenotrophic methanogens in maintaining a sufficiently low hydrogen partial pressure so that the SAO was energetically feasible. The estimated energy loss was estimated to be approximately 20% due to the switch of the microbial pathway from acetoclastic methanogenesis to SAO.

1. Introduction

Anaerobic digestion (AD) is a biological process consisting of the conversion of complex organic substrates into a biogas composed of methane, carbon dioxide and hydrogen. This technology is traditionally considered a preeminent option for sewage sludge management, as it allows for the production of bioenergy as well as the reduction of disposal costs. AD is a complex process involving a series of biochemical pathways, such as disintegration/hydrolysis, acidogenesis, acetogenesis and methanogenesis (Siegrist et al., 2002). Hydrolytic and acido/acetogenic pathways require the cooperation of a wide variety of bacteria, while methane is produced by acetate or hydrogen via acetoclastic and hydrogenotrophic methanogens, respectively. In the traditional technological configuration, namely, the continuous stirred tank reactor (CSTR), all the biochemical steps simultaneously occur in an ideally mixed digester. This technological approach presents several drawbacks, as some of the biochemical pathways are in competition with one another; for example, expediting hydrolysis and acidogenesis could decrease the pH, thereby decelerating and even hampering the next acetoclastic step.

To overcome the technological limitations, different strategies have been reported in the literature. Pretreatments, e.g., microwaves, ultrasounds, mechanical, thermal and biological applications (Izumi et al., 2010; Braguglia et al., 2015; Lizama et al., 2017), have been applied to a wide variety of substrates. In particular, ultrasound has been widely investigated for sewage sludge pretreatment with the aim of disintegrating the structure of sludge flocs and releasing extracellular or intracellular organic substances, thus enhancing the digestion kinetics and performance (Braguglia et al., 2011; Park et al., 2013).

An option for improving the AD performance involves modifying the temperature regimen; indeed, the thermophilic anaerobic process (55 °C) offers several advantages, such as higher organic matter removal, lower hydraulic retention time (HRT) and higher

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methane yield (Micolucci et al., 2016). However, mesophilic (37 °C) anaerobic digestion is more widely applied at the industrial scale due to its better process stability and lower energy demand compared with the thermophilic process. Therefore, a temperature-phased anaerobic digestion (TPAD) system that combines both temperatures in the same process could bring out the advantages of both systems (Ge et al., 2010).

Conventional TPAD technology is based on a two-stage process, namely, a first thermophilic step with a short HRT, acting as a pretreatment to improve the hydrolysis rate, followed by a longer mesophilic step, aimed at achieving effective organic matter removal. The two-stage process may permit the separation of the hydrolytic and acidogenic steps from the methanogenesis; in the first stage, presenting faster kinetics, the organic matter degradation and the production of Volatile Fatty Acids (VFAs) are achieved via the action of a synergistic community of microorganisms, while in the second stage, acetoclastic methanogenesis can occur more effectively under higher pH conditions (Ge et al., 2010).

An in-depth insight into the structure and function of microbial communities will be fundamental for a better understanding of the different pathways occurring in the anaerobic environment. Only two genera of methanogens transform acetate to methane: *Methanosaeta* and *Methanosarcina* (De Vrieze et al., 2012). Both of these genera are sensitive to the toxicity caused by the presence of inorganic nitrogen, especially free ammonia nitrogen (FAN), with particular reference to *Methanosaeta* spp. (De Vrieze et al., 2012); indeed, under high FAN concentrations, acetoclastic methanogenesis is strongly inhibited, and the overall methane production is greatly affected (Batstone et al., 2002). Under these conditions, acetate degradation can proceed through an alternative pathway that is more tolerant to ammonia, known as syntrophic acetate oxidation (SAO) (Ho et al., 2013; Jiang et al., 2017). SAO involves the syntrophic activity of acetate-oxidizing bacteria and hydrogenotrophic archaea; the former microorganisms can oxidize acetate to hydrogen and carbon dioxide, and the methanogens can use the hydrogen for carbon dioxide reduction to methane, following the classical hydrogenotrophic step (Wett et al., 2014). SAO is thermodynamically unfavourable under standard conditions; in the thermophilic regimen, the Gibbs free energy (ΔG) is significantly reduced, and its value is dependent on the end product concentrations, particularly that of hydrogen (Hattori, 2008). Therefore, it is essential to maintain a low hydrogen partial pressure in the liquid phase to obtain a negative ΔG and to render the reaction energetically feasible. Hydrogen-scavenging archaea, such as *Methanothermobacter* spp., play a crucial role in achieving these conditions, as they are capable of continuously removing hydrogen from the environment (Gagliano et al., 2014).

Although a few SAO bacterial species have been isolated, there is limited knowledge about these organisms (Werner et al., 2014); isolated SAO bacteria cover several bacterial phyla (Westerholm et al., 2011), while culture-independent approaches identified putative SAO bacteria affiliated with *Clostridia* (Hao et al., 2015) and *Synergistes* group 4 (Ito et al., 2001).

Mathematical modelling is a powerful tool that can be usefully combined with microbiological investigations for examining the AD process; the most popular model in the literature is the Anaerobic Digestion Model n°1 (ADM1) (Batstone et al., 2002). The original ADM1 version, encompassing 19 biochemical processes, does not consider the nonacetoclastic pathway, whose importance under standard conditions is negligible compared to that of acetoclastic methanogenesis (Schink, 1997). However, in the literature, several modifications to the original ADM1 have been proposed to consider processes originally neglected (Batstone et al., 2015; Cassidy et al., 2017). In particular, the SAO pathway has been included in the ADM1 by Wett et al. (2014) for domestic sludge applications and by Rivera-Salvador et al. (2014) for simulating poultry litter thermophilic AD; the former incorporated both SAO and acetoclastic methanogenesis, whereas the latter disregarded the acetoclastic pathway, which was ultimately irrelevant compared with the nonacetoclastic pathway. In ongoing research, data from the monitoring of SAO bacterial activity are fundamental for updating the ADM1 model with the inclusion of the non-acetoclastic pathway (Batstone et al., 2015).

The two-phased AD configuration presented several points of concern, such as the necessity for preventing the pH drop in the first stage and the hydrogen accumulation (in presence of acetate oxidation) in the second one. These issues have been hardly addressed from a mathematical point of view; the model was used as a tool to a) provide the most appropriate feeding strategy for maximizing the methane yield in the first stage, b) detect the hydrogen concentration capable to render the SAO pathway thermodynamically feasible in the second stage and c) estimate the energy loss consequent upon the switch of the microbial pathway from acetoclastic methanogenesis to SAO.

In this paper, the semicontinuous anaerobic digestion of waste activated sludge in a two-stage configuration was modeled. The first step, performed under mesophilic conditions, was preceded by an ultrasonic pretreatment for enhancing the hydrolysis of the particulate organic matter, leading to the accumulation of VFAs. The second methanogenic step was performed under thermophilic conditions, aimed at achieving higher methane yields and obtaining a final hygienized product (Gianico et al., 2014). To properly model this configuration, the original ADM1 was extended and modified for the thermophilic stage, replacing the acetoclastic pathway with the SAO pathway. The proposed model was calibrated, taking also into consideration the previously-reported microbial population detected using fluorescence in situ hybridization (FISH) (Gagliano et al., 2014).

2. Materials and methods

2.1. Substrates and pretreatment

Waste activated sludge (WAS) was sampled from the municipal "Roma-Nord" wastewater treatment plant, which was designed to serve a population of 700,000. The plant includes screening, primary clarification and secondary treatment of activated sludge with a high sludge retention time (20 d); the sludge was collected from the recycling stream. Anaerobic inoculum was collected from the anaerobic digester of the plant that was fed with mixed sludge; the raw WAS used as a substrate for the anaerobic tests presented a Total Solids (TS) content of 47 ± 7.5 g L^{-1} with a 66% fraction of Volatile Solids (VS).

The disintegration by ultrasound was performed using an ultrasonic processor UP400S (Dr. Hielscher, Teltow, Germany) operating at 300 W and 24 kHz. The sonication energy input was set at 0.5 kWh kg^{-1} dry solids on 500 mL of WAS (47 g TS L^{-1}) placed in a 1 L beaker with the probe located 3 cm above the beaker bottom.

2.2. Experimental set-up

Sludge digestion was performed using four anaerobic digesters operated in semi-continuous mode. Test #1, composed of one mesophilic and one thermophilic reactor in a series, was fed with raw WAS, while Test #2 was performed by feeding the same sludge after the sonication pretreatment (Fig. 1).

All jacketed reactors (V = 7 L) were completely mixed; the first mesophilic digester of the two lines was maintained at a constant temperature of 35 °C, while the thermophilic reactors were maintained at 55 °C. The duration of the semi-continuous test was 77