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Impact of Magnetite Nanoparticles Supplementation on the Anaerobic Digestion of Food Wastes: Batch and Continuous-Flow Investigations

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Anaerobic digestion is one of the most attractive technologies for the treatment of industrial, civil and agricultural organic waste because of its capacity to reduce the biodegradable matter while recovering renewable energy in the form of methane. However, this bioprocess sometime suffers of problems in the interspecies electron transfer between acetogenic bacteria and methanogenic archaea with consequent yields reduction or failure. Recently, direct interspecies electron transfer between species via solid conductive materials like nanoparticles has been observed. This study, in particular, examined the effect of magnetite nanoparticles supplementation on the methanogic conversion of organic substrates both in batch trials using pure compounds (i.e., propionate and butyrate) and continuous trials using real food waste as substrate.. Batch experiments demonstrated once again the validity of the proposed approach, whereby the conductive particles likely promoted the occurrence of direct interspecies electron transfer processes between acetogens and methanogens. Notably, continuous experiments confirmed the significance of this mechanism also for the treatment of real substrates, although the relative magnitude of the stimulatory effect was slightly lower.

1. Introduction

Anaerobic digestion is one of the most attractive technologies for the treatment of industrial wastewaters and organic wastes, because it requires less energy investments and generates less excess sludge than other treatment approaches such as the activated sludge system (Mata-Alvarez et al., 2000).
Complete conversion of organic matter to CO_2 and CH_4 via anaerobic digestion typically requires the syntrophic cooperation between acetogenic bacteria (also referred to as syntrophs) and methanogenic archaea. Indeed, catabolic reactions catalysed by acetogenic bacteria become energetically favourable only when produced reducing equivalents are efficiently scavenged by their syntrophic partners, namely the methanogenic archaea. Typically, this interspecies electron transfer (IET) process is reported to occur via diffusive transport of soluble electron carriers (e.g., hydrogen and formate) from the acetogens to the methanogens (Morris et al., 2013; Stams and Plugge, 2009). Low concentrations of electron carriers however result in slow diffusion rates, causing IET to be often the bottleneck in the methanogenic conversion of organic substrates. Recently, direct interspecies electron transfer (DIET), in which two microbial species exchange electrons via electric currents flowing through conductive solid conduits (e.g., magnetite nanoparticles) or microbial pili, has been proposed as an alternative strategy to interspecies H_2/formate transfer, through which microbial species in a community share reducing equivalents to drive the methanogenic degradation of organic substartes (Kouzuma et al., 2015; Shrestha and Rotaru, 2014).
Numerous studies reveal that DIET could be sustained and stimulated by the addition of electrically conductive materials, including granulated activated carbon (Liu et al., 2012), biochar (Chen et al., 2014), and naturally occurring iron minerals (hematite or magnetite), of nanometer to micrometer size (Zhuang et al., 2015; Cruz Viggi et al., 2014; Li et al., 2014; Zhou et al., 2014; Kato et al., 2012). These electrically conductive

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materials function as electron conduits for direct electron transfer between syntrophic, organic matter-oxidizing bacteria and CO_2-reducing methanogens.
So far, however, this interesting mechanism has been only observed in short-term batch experiments in the presence of synthetic substrates of defined composition, whereas its relevance and practical viability under conditions more closely resembling those occurring in real anaerobic digesters remains largely unknown.
In order to address this issue, in the present study we investigated the impact and practical feasibility of magnetite nanoparticles supplementation on the anaerobic digestion of “real” food wastes. To this aim, both batch and continuous-flow mesophilic anaerobic digestion experiments with or without magnetite supplementation using mixed microbial cultures were carried out.
Firstly, the influence of magnetite particles onto anaerobic digestion was assessed setting up a series of batch tests using an unacclimated methanogenic sludge as inoculum and proprionate and butyrate, chosen as model substrates to study energy-limited syntrophic communities. Then, a continuous study was set up to verify the influence of magnetite supplementation on anaerobic digestion process in stirred reactors where real food waste was the feed.

2. Materials and methods

2.1 Batch experiments with model substrates

Magnetite nanoparticles were synthesized according to a previously described protocol (Cruz Viggi et a., 2014). All batch experiments were conducted in anaerobic 120 mL serum bottles incubated statically, in the dark, at room temperature (20−25 °C). Bottles contained 57 mL of mineral medium [NH_4Cl (0.5 g/L), $MgCl_2 \cdot 6H_2O$ (0.1 g/L), K_2HPO4 (0.4 g/L), and $CaCl_2 \cdot 2H_2O$ (0.05 g/L)], 1 mL of sodium bicarbonate (10 wt%/wt), and either 2 mL of a suspension of magnetite nanoparticles, corresponding to a final concentration of 0.35 g Fe/L (magnetite-amended bottles), or 2 mL of deionized water (unamended controls). Upon preparation, all bottles were sealed with Teflon-faced butyl rubber stoppers, flushed with a 70% N_2/30% CO_2 gas mixture, inoculated with 1 mL of anaerobic methanogenic culture [corresponding to an initial volatile suspended solids (VSS) concentration of 0.20 g/L] and spiked with propionate or butyrate to a final concentration of approximately 2.5 mmol/L.
Throughout all incubations, the pH remained in the range of 7.5−7.8. During the incubations, the bottles were sampled for the determination of butyrate, propionate, acetate and methane concentrations.

2.2 Continous experiments

Two bench scale continuously stirred reactors (CSTR) operating in mesophilic (37°C) environment were used for the anaerobic digestion trials: one reactor was not supplemented with magnetite and was used as control (blank reactor, B), while the second one was added with magnetite nanoparticles (M reactor) prepared like described above. The operating volume of the two reactor was 4.5 L while organic loading rate (OLR) and hydraulic retention time (HRT) were fixed at 1.8 gTVS per litre per day and 15 days, respectively. The characteristics of the food waste used in the experimentation are are shown in table 1: this was a synthetic feed due to the mix of meat, fruit and pasta, with a considerable presence of carbohydrates.

Table 1: Feed characteristics

Parameter	Average values
Total Solids, g/kg	303
Total Volatile Solids, g/kg	297
COD, g/kg	292
TKN, gN/kg	7.8

2.3 Analytical Methods

Organic acids (acetate, propionate and butyrate) were analyzed by injecting 1 μL of filtered (0.22 μm porosity) liquid sample into a PerkinElmer Auto System gas chromatograph (2 m × 2 mm stainless steel column packed

with phase 0.3% Carbowax 20M + 0.1% H_3PO_4, matrix 60/80 Carbopack C support, Supelco; N_2 carrier gas 20 mL/min; oven temperature 120 °C; injector temperature 200 °C; flame ionization detector (FID) temperature 200 °C). Methane was analyzed by injecting 50 µL of headspace sample (with a gastight Hamilton syringe) into a Perkin-Elmer Auto System gas chromatograph (stationary phase: stainless-steel column packed with molecular sieve (Supelco, USA); carrier gas: N_2 at 20 mL/min; oven temperature: 150 °C; injector temperature: 200 °C; thermal conductivity detector (TCD) temperature: 200 °C).
Analysis of pH, total solids (TS), total volatile solids (TVS), chemical oxygen demand (COD), ammonia, total Khjeldahl nitrogen (TKN), and partial and total alkalinity were all carried out according to the *Standard Methods for Water and Wastewater Analysis* recommendations. Volatile fatty acids (VFA) from C2 (acetate) to C5 (valerate) and their normal and isoforms in the continuous anaerobic reactors were carried out using liquid chromatography as described in Raposo et al (2015).

3. Results and discussion

3.1 Batch experiments with model substrates

In order to preliminary assess the influence of magnetite nanoparticles on the kinetics of anaerobic digestion, a series of batch tests were conducted using an unacclimated methanogenic sludge as inoculum and propionate and butyrate as model substrates. In these experiments, propionate and butyrate degradation, in bottles containing magnetite nanoparticles, started after a slightly shorter lag phase and proceeded to completion more rapidly compared to the unamended control bottles.
Apparently, the pathway of propionate and butyrate degradation was not affected by the presence of magnetite; both in magnetite-supplemented and control bottles the methanogenic conversion of butyrate proceeded via the intermediate formation of propionate and acetate (and likely hydrogen although this latter compounds was not determined), whereas the methanogenic conversion of propionate proceed via the intermediate formation of acetate (and likely hydrogen). In spite of that, however, in magnetite-supplemented bottles the rate of substrates degradation and methane formation were higher than in the corresponding controls (Figure 1). Specifically, in magnetite-supplemented bottles the maximum rate of methane formation (on a molar basis) from propionate and butyrate were respectively 22% and 12% higher than in the corresponding unamended controls (Figure 1a). In agreement with that, the rates of propionate and butyrate degradation were correspondingly higher (Figure 1b).

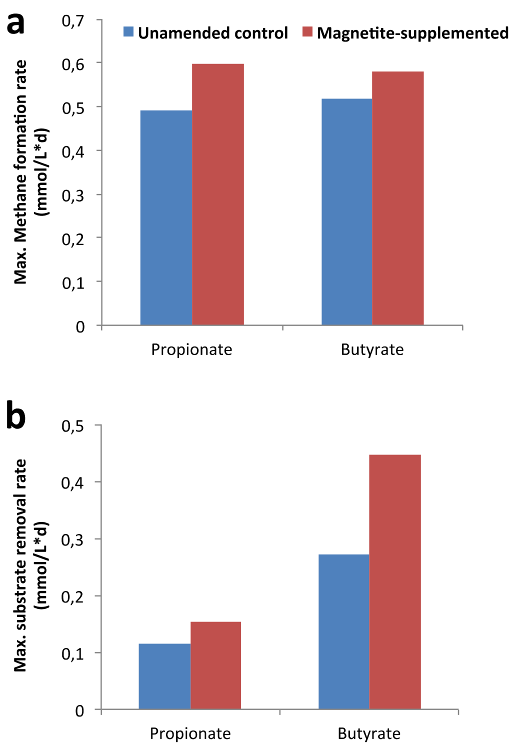

Figure 1: Effect of magnetite nanoparticles supplementation on (a) the maximum rate of methane formation and (b) the maximum rate of propionate or butyrate degradation.

Collectively, these results are in good agreement with those reported in a previous work (Cruz Viggi et al., 2014), in which we demonstrated that supplementation of micrometer-size magnetite particles to a methanogenic sludge enhanced (up to 33%) the rate of methane generation from propionate.
Taken as a whole, these findings suggest that the stimulatory effect of electrically conductive nanoparticles results from the establishment of a direct interspecies electron transfer (DIET) process, based on magnetite particles serving as electron conduits between propionate- or butyrate-oxidizing acetogens and carbon dioxide-reducing methanogens. This novel IET mechanism, schematically depicted in Figure 2, could allow overcoming some of the kinetic limitations of traditional, diffusion-based, interspecies H_2 transfer (Figure 2). .

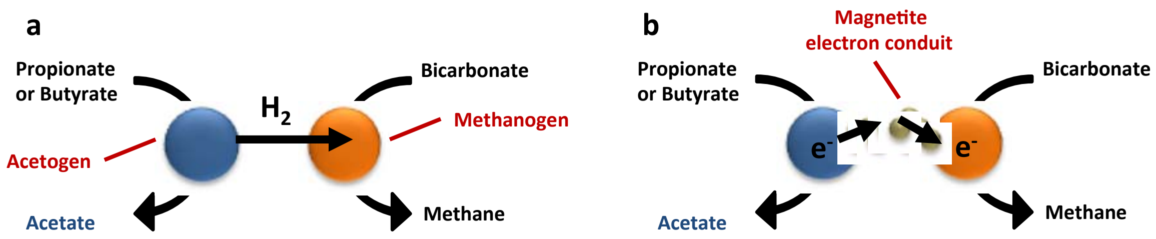

Figure ***2****: Proposed electron transfer mechanisms between an acetogen and a methanogen in magnetite supplemented cultures: interspecies H_2 transfer (A) and electronic conduction through magnetite particles (B).*

3.2 Continous experiments

Two different series of continuous trials were carried out: in the first one, the two reactors operated as continuous reactors. In these conditions the reactors operated for few HRTs and then failed because of volatile fatty accumulation. In a second series of experiments part of the solids effluent were recycled into the system so to uncouple the hydraulic and solids retention time and improve the available active biomass and buffer capacity of the system: this strategy demonstrated the possibility to operate the two continuous reactors for an indefinite time.
With specific reference to the experimental results, in the first run, without biomass recycling, the magnetite was added in the feed daily at a load of 10 mmol per day. The supplemented reactor showed higher performances in terms of biogas production but also an higher stability in typical process parameters like pH, VFA and partial and total alkalinity (data not shown).
On the other hand, the control reactor (B) operated steadily for one HRT and after that time (15 days) VFA accumulated in the systems reaching concentrations of 10-12 grams per litre. Propionic acid was the main compound found in the mixture of organic acids accumulated in the reactor.
Overall, comparing the performances of the two reactors it turned out that the supplemented reactor was more stable and was able to operate for an additional HRT with respect to the control reactor. Also biogas biogas production was slightly higher. On the other hand, surprisingly, the control reactor showed an higher methane concentration in biogas (59 vs. 56%). Table 1 resumes the main findings of the continuos operation trials.
In the second series of experiments the recirculation strategy was introduced to confer robustness to the process: in particular, the effluent of the two anaerobic digesters, both the control and the supplemented one, was centrifuged and 50% of the solid pellet was recirculated into the reactors. Clearly, in this way, also part of the magnetite was recirculated into the reactor. In order to maintain a magnetite concentration in the supplemented reactor similar to the one observed in the first series of experiments the magnetite load was lowered to 3.3 mmol per day. This load was determined on the basis of the system mass balance.
As a consequence of the partial recirculation of anaerobic biomass the systems showed stable parameters and VFA concentrations that never overpassed a level of 200 mgCOD/L. Similarly to the first series of continuous and batch tests, the supplemented reactor (M) presented higher biogas productions.
In particular, the specific biogas production in the supplemented reactor passed from 0.67 to 0.72 L/gVS with a 8% increase. Also the biogas production rate showed a similar trend, passing from 2.4 to 2.6 litre biogas per litre reactor per day. As in the first experimental run the methane percentage was slightly lower (some percentage points) in the supplemented reactor. Operational applied conditions (OLR and HRT) and yields (SGP and GPR) for the whole continuous experimentation are shown in table 2.
In general, the continuous experiments confirmed and put in major evidence that effect of the magnetite nanoparticles addition: in particular, it was emphasised how the addition of magnetic particles allowed for an increase in the biogas production also when real substrates are used and confirmed the results reported in similar studies (Hellman et al., 2012).

In general, it should be highlighted that continuous experiments confirmed results obtained in batch experiments related to the mechanism study but the improvement herein obtained was of lower magnitude, as expected for continuous reactors when compared to batch ones.

Table 2: Operative conditions without and with solid recirculation of the two digesters

	Control (*)	Supplemented (*)	Control	Supplemented
Biomass recycling	No	No	Yes	Yes
OLR, gVS/L·d	1.8	1.8	1.8	1.8
HRT, d	15	15	15	15
SRT, d	15	15	32	32
SGP, L/gVS	0.53 ± 0.12	0.66 ± 0.13	0.67 ± 0.01	0.72 ± 0.01
GPR, L/L·d	1.9 ± 0.4	2.3 ± 0.5	2.4 ± 0.3	2.6 ± 0.3
CH_4, %	59.2 ± 5.6	56.3 ± 2.9	54.3 ± 1.6	52.8 ± 2.0

(*) System failure at the second HRT

4. Conclusions

This study examined the effect of magnetite nanoparticles supplementation on the methanogic conversion of organic substrates. To this aim, both batch trials using pure compounds (i.e., propionate and butyrate) and continuous trials using real food waste as substrate were carried out. Batch experiments demonstrated once again the validity of the proposed approach, whereby the condutive particles likely promoted the occurrence of direct interspecies electron transfer processes between acetogens and methanogens. Notably, continuous experiments confirmed the significance of this mechanism also for the treatment of real substrates, although the relative magnitude of the stimulatory effect was slightly lower.

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