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IMPACT OF MICRO-AERATION ON FUNGAL COMMUNITY FROM ANAEROBIC DIGESTION

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Abstract

Anaerobic digestion (AD) is one of the most established technologies for the stabilization of wastes such as sewage sludge, generated during wastewater treatments. However, these AD processes would need to be rethought to achieve the new requirements in the removal levels of emerging pollutants according to the current water policy restrictions (Directive 2008/98/EC). Among these compounds, there is an increasing concern about pharmaceutical active compounds (PhACs) and its transferen- ce through the ecosystems by the application of sewage sludge as soil amendment, according to the circular economy policies. In the last years, micro-aeration has been proposed as a pre-treatment strategy to improve the two-stages AD process, being implemented in the first stage to enhance the hydrolysis activity without affecting the methanogens in the second stage. Applying controlled micro-aeration (~5% dissolved oxygen) could favour the proliferation of fungal communities, which are able to produce hydrolytic and oxidative exoenzymes, improving the hydrolysis capacity of the reactors in comparison with strict anaerobic conditions. These fungal enzymes might be also involved in the organic matter decomposition and in PhACs degradation, as has been demonstrated in aerobic bioremediation systems. However, there is no previous studies about the micro-aeration effect on PhACs degradation and how micro-aeration affects fungal communities during the hydrolysis. In this work, we aimed to study the effect of micro-aeration at different concentration of oxygen to evaluate the degradation of some pharmaceutical compounds.

Keywords: *anaerobic digestion; enzymes; fungi; micro-aeration; degradation*

1. Introduction

Anaerobic digestion (AD) is one of the most established technologies for the stabilization of sewage sludge generated during wastewater treatments from domestic, restaurant, hospital, industrial and other

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activities. Over the years, AD process has been efficiently optimized t satisfy the legislation for sludge treatment. However, current operational procedures do not seem to be enough for the removal of some recalcitrant compounds such as pharma- ceutically active compounds (PhAcs). Previous research has identified significant concentrations in sludge already treated by AD of some widely consumed pharmaceuticals such as acetaminophen, carbamazepine or ketoprofen [4]. These compounds are daily consumed by the global population since they are usually used in anti-inflammatory and anxiolytic treatments. In the last years, micro-aeration has been proposed as a pre-treatment strategy to improve the two-stages AD process, being implemented in the first stage to enhance the hydrolysis activity without affecting the methanogens in the second stage [3]. Applying controlled micro-aeration (~5% dissolved oxygen) could favour the proliferation of fungal communities, which are able to produce hydrolytic and oxidative exoenzymes, improving the hydrolysis capacity of the reactors in comparison with strict anaerobic conditions. These fungal enzymes might be also involved in the organic matter decomposition and in PhACs degradation, as has been demonstrated in aerobic bioremediation systems [2]. However, there is no previous studies about the micro-aeration effect on PhACs degradation and how micro-aeration affects fungal communities during the hydrolysis. In this sense, MICROFUNGI project aims to successfully remove PhACs through an improvement in the hydrolysis of a two-stage AD system promoted by microaeration. The controlled application of micro-aeration could favor the prolifera- tion of fungal population, capable of generating hydrolytic and oxidative exoenzymes, improving the hydrolytic activity of the reactors compared to strict anaerobic conditions. In addition, these fungal enzymes could also be involved in organic matter decomposition and PhACs degradation, as demonstrated in aerobic bioremediation systems.

To reach this objective, different dissolved oxygen conditions (0%; 2.5%; 5.0% y 7.5%) will be applied. The analysis of microbial communities will be determined by Illumina MiSeq sequencing and the PhACs will be quantified by UHPLC-TQ-MS/MS. The obtained results will allow to determine key operational parameters as well as relevant microbial communities involved in the removal of PhACs in AD with micro-aeration. In conclusion, this research will provide a knowledge framework that covers the field of AD with mycoremediation to the safety use of sewage sludge in soils.

2. Materials and methods

The sludge used as substrate, a mixture of primary and secondary sludge, was collected from a wastewater treatment plant in Seville, Spain. The AD test was carried out under semi-continuous and mesophilic conditions. In the system used, reactors with continuous mechanical stirring and working volumes of 1.5 L and 2.5 L were used in the first and second stages, respectively. The hydraulic retention times were set at 5 and 15 days, and the organic loading rate at 5.0 and 1.7 g VS/(L·d), respectively (Fig. 1). Methane volume was quantified using a MilliGascounter Ritter® (Ritter, Bochum, Germany) after removing CO_2 from the biogas through hermetic bubblers containing a 3N NaOH solution. The experimental conditions evaluated different dissolved oxygen dosing conditions in the hydrolytic reactor. First, two extreme points were operated, full anaerobiosis (0 L O_2/(L_R·d)) and a dissolved oxygen dosage well outside the micro-aeration range (47.7 L O_2/(L_R·d)). Then, three other stages were evaluated with different doses of dissolved oxygen in the micro-aeration range, i.e.,
2.0, 4.7, and 7.6 L O_2/(L_R·d).

Fig. 1. Bioreactors set up

For reactor monitoring, the pH, solids concentration and soluble chemical oxygen demand (sCOD) were analysed following the APHA methods [1]. Volatile fatty acids (VFAs) (C2-C5) were determined using a gas chromatography system (Auto-System PerkinElmer, Norwalk, CT, USA). The biogas composition, i.e., methane, carbon dioxide, hydrogen, nitrogen and oxygen concentration, was analysed by gas-chromatography HP 8860 (Agilent, Shangai, China). The extraction of pharmaceutical compounds was performed following the protocol of Montemuro et al. [5] with some modification and was identified and quan- tified by Agilent 6470A liquid chromatography-tandem mass spectrometry (LC-MS/MS) equipment. Ergosterol determination by liquid chromatography was also performed. Ergosterol content is a quick indicator of fungal population changes, as it is a characteristic component of the fungal cell membrane. The methods followed for this determination consisted in an extraction of methanol- KOH (10%) during 90 min at 70˚C and evaporated with N steam to be quantified by liquid chromatography [6].

3. Results and discussions

The effect of micro-aeration on the hydrolytic and methanogenic stages was evaluated. The results corresponding to each micro-aeration condition stage for methane production, sCOD and ergosterol content are shown in Table 1.

Table 1. Monitoring parameters analyzed at the end of each aeration flow stage evaluated

	Stage I	**Stage II**	**Stage III**	**Stage IV**	**Stage V**
Oxygen dossed (L O2/(LR·d))	0	47.7	2.0	4.8	7.6
Methane production (mL CH4/g VSfed)	HR:202±58 MR:69±11	HR:0±0 MR:147±60	HR:40±15 MR:217±22	HR:56±15 MR:212±34	HR:68±15 MR:178±16

sCOD (mg O2/L)	HR:708±248 MR:578±48	HR:3396±440 MR:910±82	HR:4005±532 MR:1093±46	HR:3001±252 MR:1236±63	HR:1892±394 MR:1320±65
rgoesterol (µg/g)	HR: 1.7±0.2 MR: 0.6±0.1	HR: 3.6±0.1 MR: 1.2±0.0	HR: 4.3±0.1 MR: 1.5±0.1	HR: 3.3±0.2 MR: 2.0±0.1	HR: 3.2±0.5 MR: 1.8±0.1

When the system operated under microaerobic conditions, organic matter accumulation was observed, in tandem with a drop in methane production in hydrolytic reactor (Table 1). It is observed that the condition with lower micro-aeration flow rate, i.e., 2.0 L O_2/(L_R·d) shows a higher hydrolytic activity with a solubilization percentage of approximately 62%. The ergosterol content increase corresponds to the higher organic matter solubilization (Table 1). Hence, a positive correlation between an increase in hydrolytic activity and ergosterol content is understood, which would be related to an increase in the fungal population. When the system operates with low micro-aeration flows, the observed solubilization improvement does not seem to clearly reflect an increase in methane production (Table 1). However, although micro-aeration does not impact the methane production of the complete system, it showed an effect on the degradation of recalcitrant compounds like acetaminophen and fenofibrate (Fig. 2).

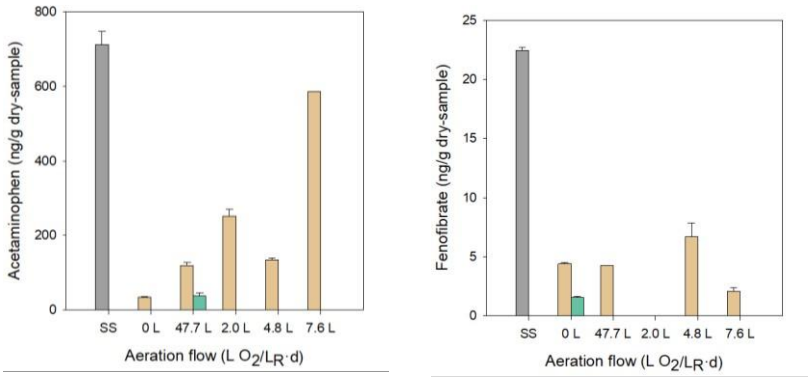

Fig. 2. Concentration of pharmaceutical compounds: acetaminophen and fenofibrate at the end of each aeration flow stage evaluated

4. Conclusions

Micro-aeration flow rate of 2.0 L O_2/(L_R·d) showed a higher hydrolytic activity. An increased hydrolytic activity was positively correlated with an increase in the ergoesterol content or fungal population. However, enhanced hydrolytic activity at low micro-aeration flows was not clearly reflected in increased methane production. Micro-aeration had a positive effect on the acethaminophen and total fenofibrate removal. Therefore, micro-aeration may represent an efficient technology for the PhACs removal from sewage sludge generated in wastewater treatment plant.

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