Implication of Microbial Biofilm in the Biodeterioration of Cementitious Materials in the specific context of Anaerobic Digestion Conditions

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Abstract. Anaerobic digestion is a renewable energy production process based on the fermentation of biodegradable biomass. The industrial digesters are usually made of cementitious materials. However, the microbial production of several aggressive compounds (CO₂, NH₄⁺ and volatile fatty acids) during the digestion leads to the deterioration of the concrete structure. The growth of microbial biofilm on the surface of concrete is suspected to generate an even more severe biodeterioration. The goal of this study is to provide a better understanding of the biofilm involvement in the biodeterioration of cementitious materials during anaerobic digestion process. More precisely, this study is focused on the biofilm heterogeneity and its development on cementitious materials in anaerobic digestion. Lab scale anaerobic bioreactors mimicking industrial anaerobic digestion medium were carried out to immerse CEM I cement pastes in this medium during 2, 3, 4 and 5 weeks. The deterioration of cement pastes was evaluated by using a scanning electron microscope to determine the deteriorated thickness and to quantify the volatile fatty acids in the medium. Biofilm attached on the surface of cement pastes was analyzed through molecular biology techniques, such as 16s rRNA gene sequencing analysis and qPCR. To assess the biofilm heterogeneity, successive stalls of the layers of the biofilm were realized using physical biofilm removal techniques. Three microbial fractions are defined: the planktonic microorganisms, the lousy attached and the strongly attached ones. Results showed that the methanogenic Archaea are found mainly in the medium while around half of the microbial population strongly attached is made of acidogenic bacteria. These results suggest that the biofilm could increase the biodétérioration of concrete since the fatty acids are massively produced at the proximity of the surface of the cementitious samples.

Keywords: Biodeterioration, Cementitious Material, Biofouling, Biofilm, Bacteria, Anaerobic Digestion.
1 Introduction

Anaerobic digestion is a renewable energy production process based on the fermentation of biomass. It consists in the succession of four microbial reactions: hydrolysis, acidogenesis, acetogenesis and methanogenesis, leading to the transformation of the biomass into two valuable interest products: the biogas and the digestate. The biogas is mainly constituted of CH₄ and CO₂ at a concentration of about 40-75% of CH₄. The digestate can be used as a fertilizer for agronomic purposes (Bharathiraja et al., 2018).

In the current period of energy transition towards non-fossil renewable energies, the anaerobic digestion process shows a growing interest (Salvador et al., 2019), especially on aspects of improving production performance and enriching CH₄ level. In this respect, the focus is on tracks that could increase its durability and thus its performance. One of those tracks could be the durability of the anaerobic digestion tanks (digesters) since it is linked to their lifetime and costs of maintenance. Industrials anaerobic digestion tanks are usually made out of concrete as it is a low-cost material, easy to use and adapted to several m³ tanks. However, anaerobic digestion media is known for being aggressive towards the cementitious materials. The microbial metabolism produces aggressive compounds (CO₂, NH₄, volatile fatty acids), which deteriorate the cementitious matrix. In addition, the formation of microbial biofilms on the concrete surface is highly suspected to increase the kinetics of this deterioration (Voegel et al., 2015, 2016). Microbial biofilms can be described as an aggregation of microorganisms on solid surfaces embedded in a self-produced extracellular matrix; called EPS (extracellular polymeric substances). This microbial organization in biofilms allows the formation of micro-environments locally within the biofilm, as a result of the heterogenic repartition of the different microbial communities, their metabolisms and the diffusion properties of biofilm. Even though the deterioration mechanisms behind the biofilm fouling effects on concrete are not yet clearly identified, there is a strong hypothesis that it is linked to the existence of hyper-aggressive conditions locally on the concrete surface, i.e. at the scale of micro-environments located at the interface between the microbial biofilm and the external surface of the concrete (Bertron, 2014; Magniont et al., 2011).

The present work aims at giving more insights in the biofilm implication in the biodeterioration process of concrete in the specific context of anaerobic digestion tanks. More precisely, this study is focused on the visualization and kinetics monitoring of the development of anaerobic digestion biofilms on cementitious materials, and the exploration of bacterial population heterogeneity in the different biofilm layers.

2 Material and Methods

2.1 Cement Paste Pellets Fabrication

Ordinary CEM I cement pastes were made with a water/cement mass ratio of 0.30. Cylindrical moulds of 2.8 cm of diameter and 6.5 cm height were used. A plastic straw was inserted inside the cement paste at the cylinder central axis, in order to leave an empty space necessary to suspend the paste in the medium during the biodeterioration assays. The cement pastes were then cured for 28 days at 20°C inside their closed moulds. After curing, cement paste pellets with a thickness of about 2.5 mm were obtained by cutting under water the cylindrical cement paste with a circular saw (Prezi, Mecatom 180). The pellets were then polished with a silicon carbide polishing wheel (ESCIL, P800-22 μm).
2.2 Microbial Inoculum and Anaerobic Digestion Culture Medium
Both microbial inoculum and culture medium were chosen to mimic industrial anaerobic digestion, according to the protocol and methods already described by (Voegel et al., 2016). The medium was a synthetic biowaste made up of different organic fractions which have been blended for 5 min at 20°C (Table 1). The inoculum used was an activated sludge sampled from a municipal wastewater treatment plant in Toulouse (France). The volumic ratio microbial inoculum/medium was 2.5/1.

Table 1. Composition of the synthetic biowaste.

<table>
<thead>
<tr>
<th>Organic fractions</th>
<th>Mass (%)</th>
</tr>
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<tbody>
<tr>
<td>Water</td>
<td>75.6</td>
</tr>
<tr>
<td>Potatoes</td>
<td>8.1</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>3.4</td>
</tr>
<tr>
<td>Minced meat</td>
<td>8.1</td>
</tr>
<tr>
<td>Milk powder</td>
<td>0.7</td>
</tr>
<tr>
<td>Crackers</td>
<td>4.1</td>
</tr>
</tbody>
</table>

2.3 Anaerobic Biodeterioration Assays
The protocol of the cement paste immersion in the culture medium is made to mimic the operating conditions of anaerobic digestion (Voegel et al., 2016). The immersion of the cements pastes pellets were tested in two types of reactors: type A and type B reactors containing respectively eight pellets and one single pellet of cement paste (Figure 1). Type A and type B reactors contained 525 and 66 mL respectively. Therefore, the ratio of the surface of solid cement paste and the volume of the liquid phase was 220 cm^2.L^(-1) in the two types of reactor. These reactors were carried out in the same conditions, at 37°C during 5 weeks for the type A reactor and 2, 3, 4 and 5 weeks for the type B reactor (triplicates for each duration tested). The gas phase (biogas) was collected continuously in the type A reactor and every day in the type B reactor with a collection bag connected to a needle.

2.4 Chemical Analysis
Samples of the medium were taken at the end of each culture and then filtered at 0.2 µm. Ammonium ions concentration was measured using Hach kit LCK 304, following manufacturer instructions. Dissolved organic carbon was determined with a TOC analyzer (TOC-SHIMADZU Combustion). Acetic, butyric and propionic acids concentrations were measured through high performance liquid chromatography (Thermo Scientific, Accela system, column Rezex ROA H^+ 8%, eluent H_2SO_4 10 mM, flow rate 170 µL.min^(-1)).
2.5 Microbial Populations Analysis
Concerning the biofilm sampling on the surface of cement paste pellets, two types of biofilm layers were defined: the “lousy attached” biomass and the “strongly attached” biomass. The “lousy attached” biomass was removed from a biocolonized cement paste pellet through an immersion of the pellet in 3 mL of phosphate buffered saline (PBS, 0.1M, pH 7.4) for 15 minutes. The “strongly attached” biomass was removed from a biocolonized cement paste pellet with a sonication treatment of 3 minutes in 5 mL of PBS. Removing treatments were not done successively, thus the “strongly attached” biomass should also contain the “lousy attached” one. 2 mL of the culture medium were as well sampled in triplicate. The samples of biocolonized cement paste originated from a type A reactor after 5 weeks of culture. Each removal protocol has been performed in triplicate, i.e. starting from three different biocolonized cement pellets. DNA extraction was performed on the three types of samples (liquid, strongly “attached and lousy attached” biomass) obtained using a DNeasy power biofilm kit, according to manufacturer instructions. Sequencing of the 16S SSU RNA was done using 515F and 806R primers targeting both bacteria and Archaea. The sequencing and its statistical analysis were performed by RTlab (USA).

2.6 Physical Analysis
Cement paste pellets from the type A reactor were taken out after 2, 3, 4 and 5 weeks. A cut of the cement paste pellets was realized with a circular saw, in order to divide the cylindrical sample into two identical parts. A part of the pellet was then impregnated under vacuum with epoxy resin (Araldite, 2020) in circular molds (diameter = 2.6 cm). After hardening of the epoxy resin, the surface was polished under water using several polishing disks having decreasing particle sizes of 200, 68, 27 and 15 µm. A thinner polishing was then done using several diamond-covered polishing disks with a decreasing particle sizes (6, 3 and 1 µm). Samples were carbon coated before observation under a scanning electron microscope (Hitachi S-4300 SE/N) coupled with a EDX detector (Thermo Scientific Ultradry) running at 15 kV.
3 Results and Discussion

3.1 Deterioration Profile of the Cement Paste
The evolution of the concentrations of volatile fatty acids (VFAs), ammonium ions and inorganic carbon from 2 to 5 weeks of anaerobic digestion process are shown on (Figure 2).

After two weeks of anaerobic digestion, the concentration of acetic and butyric acids were of 4.7 g.L\(^{-1}\) and 2.2 g.L\(^{-1}\) respectively. Their concentrations strongly decreased between week 2 and week 3 to finally stabilize around 0 g/L at end of the fourth week of anaerobic digestion. The propionic acid concentration evolved differently, since it was still increasing constantly from 1.5 to 3.0 g.L\(^{-1}\) in the period of time between the 2\(^{nd}\) and the 5\(^{th}\) week (Voegel et al., 2016). After 2 weeks, both acetic and butyric acids were consumed. This result corroborates those of Voegel et al. (2016), who showed that volatile fatty acids are accumulated during the first two weeks of the anaerobic digestion of biowaste, and finally consumed in the following weeks. More curiously, the propionic acid concentration did not drop in the weeks following the 2\(^{nd}\) week, suggesting that this VFA has not been reused or metabolized by acetogenic microbial populations or that its production rate was seriously higher than the consumption rate. The dissolved carbon dioxide production increased from 0.8 g.L\(^{-1}\), measured at the end of the week 2, to 2.5 g.L\(^{-1}\) after 5 weeks. The concentration of ammonium has remained very stable, around 0.7 g.L\(^{-1}\), from week 2 to week 5. Since ammonium ions are byproducts of amino acid fermentation (Ramsay and Pullammanappallil, 2001), it should be produced only during the acidogenesis stage occurring mainly during the first two weeks. In this way, we can make the hypothesis that the aggressive nature of the anaerobic digestion environment was at its maximum during the first two weeks of the experiment, since the concentration of aggressive compounds should be at its peak during this period.

Figure 3 allows the localization of the biodeteriorated zones in the depth of a cement paste sample exposed for 5 weeks in an anaerobic digestion medium.
Four zones can be identified (Figure 3): a first layer darker than the others, of about 100 µm. A second zone, about 20-30 µm, can be identified around the crack. The third zone has a variable thickness among the different duration tested from 150 to 400 µm. A fourth zone darker than the third one is also observed, its thickness also changes from a duration to another from 150 to 430 µm, and finally at the heart of the cement paste there is a last zone corresponding to the sound paste. Figure 3(b) shows the evolution of the relative proportion of calcium (Ca), silicium (Si) and aluminum (Al) from the outer surface to 800 µm in the depth of biodeteriorated cement paste sample. The composition in Ca, Si and Al completely changes from a zone to another. All of those zones were found in every duration of exposition tested with the same global composition in each zone. The zone 1, the closest to the cement surface, had a low Ca proportion, around 10 %, a high Si proportion, around 30 %, and a high proportion of Al, about 7 %, in regard to the other zones. The zone 2 had a particularly specific composition: the Ca proportion was significantly higher than in any other zone, up to 70-76 % in both the samples immerged 3 and 4 weeks (data not shown), and a lower proportion of Al and Si around 1 and 5 % respectively. The presence of Phosphor was also observed in the first two zones (data not shown). In the three other zones (zones 2, 4 and 5), only a very small divergence in composition was detected: between 20 and 30 % of Ca, around 10 % in average of Si and 1.5 to 2 % of Al. Those results are representative of a Ca leaching and a P enrichment in the layers close to the surface. However, chemical element proportion analysis can not precisely show every change in mineral composition, as proportion could remain the same while the quantity could change.

Analysis with other techniques, such as X-Ray Diffraction, could give more information, particularly on the residual anhydrous grains. Those results are in accordance with the observations already reported by (Voegel et al., 2016), who studied also cement paste biodeterioration after exposition to digested biowastes. These authors identified Ca leaching as part of the biodeterioration mechanisms.

Table 2 shows the evolution of the thickness of the total biodeteriorated zone for increasing time of cement paste exposition to anaerobic digestion environment. The thickness of the total biodeteriorated zone was determined by the Ca cartography obtained by SEM.

<table>
<thead>
<tr>
<th>Exposition time (weeks)</th>
<th>2</th>
<th>3</th>
<th>5</th>
</tr>
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<tbody>
<tr>
<td>Thickness of the total biodeteriorated zone (µm)</td>
<td>554</td>
<td>594</td>
<td>619</td>
</tr>
</tbody>
</table>
The thickness of the total biodeteriorated zone increases from 2 weeks to 5 weeks of exposition time, from respectively 554 µm to 619 µm. The same kind of observations on cement pastes exposed for longer period, 10 and 15 weeks, should in the future make it possible to confirm this pattern.

3.2 Microbial Populations of the Biofilm

Figure 4 shows the repartition of the different bacterial phyla within the liquid fraction and in the “lousy attached” biomass and the “strongly attached” biomass collected on the surface of a cement paste after 5 weeks in type B reactor. Planktonic and sessile (attached) bacterial populations were different. Indeed, the proportion of *Archaea* was fairly higher in the liquid fraction, *i.e.* 22.0 % against 3.4 % in the attached biomass.

Among the *Archaea* highlighted in the liquid fraction, 17.7% belonged to the *methanosarcina* genus, which are anaerobic methanogens found in a multitude of environments and capable of producing methane by the three metabolic modes of methanogenesis (hydrogenotrophs, acetotrophs, methylotrophs) (De Vrieze et al., 2012). The biofilm developed on the surface of the cement paste, and more specifically the “strongly attached” biomass, was rich in *Firmicutes*. Around 50% of the DNA sequences from the “strongly attached” biomass belonged to acidogenic populations. Among them, *Clostridium butyricum* (5.4%), is known to specifically produce butyric acid and *Megasphaera eldesnii* (29.5%), is described as able to produce acetic, propionic and butyric acids (Marounek et al., 1989). Those results suggest that the acidogenesis step of the anaerobic digestion may mainly occur in the biofilm, while the methanogenesis step would take place in the liquid fraction. Beyond these considerations based essentially on the physical organization of communities (biofilms vs. suspended/planktonic populations), would not there be a major effect of pH behind this whole phenomenon? If the pH at the surface is too alkaline for bacteria to easily grow on it, perhaps only acidogens are able to lower the pH through the acids they produce to allow a biocolonization of the surface of material.
4 Conclusion

The SEM analysis of the cement pastes exposed from 2 to 5 weeks to an anaerobic digestion medium showed a biodeterioration of the cement paste characterized by a depth up to 700 µm with calcium leaching and phosphor enrichment in the first 100 µm. Bacteria present in the biofilm colonizing the cement paste are mainly acidogens, which may confirm the hypothesis that the microbial fouling of the cement paste is caused by the local production of aggressive compounds, such as organic acids acting on reducing the surface pH of cementitious materials. The use of fluorescent dyes could help measure the pH both in the biofilm and on the surface of the cement paste through fluorescence microscopy observation techniques.

References


