



PONTIFICIA UNIVERSIDAD CATOLICA DE CHILE

SCHOOL OF ENGINEERING

**Removal of Volatile Organic Silicon Compounds (D4, D5) from biogas using
microaerophilic/anaerobic microbial strategies**

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Thesis submitted to the Office of Graduate Studies in partial fulfilment of the
requirements for the Degree of Doctor in Engineering Sciences

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Santiago de Chile, January 2022

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REMOVAL OF VOLATILE ORGANIC SILICON COMPOUNDS (D4, D5) FROM BIOGAS USING MICROAEROPHILIC/ANAEROBIC MICROBIAL STRATEGIES

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RESUMEN

La digestión anaeróbica (DA) se considera una tecnología rentable y sostenible para el tratamiento de lodos de depuradora (EDAR). Permite estabilizar la materia orgánica al tiempo que produce energía renovable, que puede utilizarse *in situ*. Los compuestos orgánicos volátiles de silicio (VOSiC) son omnipresentes en los elementos industriales, de cuidado personal y domésticos, por lo que están presentes en los lodos activados residuales. Estos VOSiC se consideran contaminantes emergentes debido a su afinidad con la materia orgánica. Además, disminuyen la eficiencia de generación de energía de los equipos de conversión de biogás por la precipitación de óxidos de silicato en los elementos de calentamiento y la consiguiente reducción de la transferencia de calor. Este trabajo evaluó los cambios en la estructura de la ecología microbiana y la biodegradación de los siloxanos, centrándose en la eliminación biológica de los principales siloxanos presentes en los lodos de DA. El enfoque utilizado promovió la evolución dirigida de la microbiota nativa de la DA para aumentar los organismos capaces de resistir las concentraciones de oxígeno y biodegradar eficazmente los siloxanos asignados en la materia orgánica. Se logró la aclimatación del sustrato utilizando el polímero de siloxano polidimetilsiloxano (PDMS) para seguir utilizando estos organismos en la estabilización microbiana de los oligómeros de siloxano -VOSiC- Octametilciclotetrasiloxano (D4) y Decametilciclopentasiloxano (D5). Nuestros resultados muestran que el PDMS es una fuente de VOSiCs en los digestores anaeróbicos; este es un descubrimiento crucial ya que esta fuente no se había tenido en cuenta anteriormente. Por último, proponemos una vía de biodegradación para los siloxanos D4 en condiciones de microaireación que muestra cómo el oxígeno y los organismos dentro de los lodos de DA superan la naturaleza recalcitrante del impedimento estérico del siloxano para transformarlo en silanoles que no poseen una amenaza para el medio ambiente o las energías basadas en el biogás.

ABSTRACT

Anaerobic digestion (AD) is considered a cost-effective and sustainable technology for sewage sludge treatment in wastewater treatment plants (WWTP). It allows organic matter stabilisation while producing renewable energy, which can be used on site. Volatile organic silicon compounds (VOSiC) are ubiquitous in industrial, personal care, and household elements and therefore are present in waste activated sludge. VOSiCs are considered emergent contaminants because of their affinity with organic matter. In addition, they decrease the energy generation efficiency of biogas conversion equipment by precipitation of silicate oxides on heating elements and consequential reduction of heat transfer. This work evaluated the changes in microbial ecology structure, and siloxanes biodegradation focused on biological removal of main siloxanes present in AD sludge. The used approach promoted the directed evolution of the AD native microbiota to enhance the organisms capable of resisting oxygen concentrations and efficiently biodegrade siloxanes allocated in the organic matter. Substrate acclimatisation using siloxane polymer polydimethylsiloxane (PDMS) was accomplished to further use these organisms in the microbial stabilisation of siloxane oligomers -VOSiC- Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5). We evidenced a change in the microbial community structure in the presence of oxygen and siloxanes that allowed the biological system to use PDMS and VOSiCs in the methane synthesis efficiently. Our results show that PDMS is a source of VOSiCs in anaerobic digesters; this is a crucial discovery since this source was not previously considered. Also, we found that microbial siloxane stabilisation is possible using the same inocula for polymers and oligomers, opening the possibility to develop *in situ* systems that handle siloxanes within the AD digester. Finally, we propose a biodegradation pathway for D4 siloxanes under microaerated conditions that show how microaeration and organisms within the AD sludge overcome the recalcitrant nature of siloxane steric hindrance to transform it into silanols that do not possess a threat to the environment or the biogas-based energies.

LIST OF PAPERS

1. The impacts of siloxanes and what conventional wastewater treatment plants can do to remove these pollutants– Review paper – Reviewed draft
2. Microaerobic conditions in bio-sludge promote changes to bacterial composition which favour the biodegradation of polymeric siloxanes – Original paper – Published
3. Microaerated anaerobic digestion enables biological degradation of D4 and D5 siloxanes in batch cultures – Original paper – Reviewed draft

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INTRODUCTION AND OBJECTIVES

More than ten million tons of siloxanes are produced every year in the world¹. These prevalent chemical compounds have affected every aspect of human life since the '90s, becoming practically irreplaceable in today's society². However, siloxanes pose a serious threat to the environment by disrupting ecological cycles and trophic chains. They also have become a matter of special concern by reducing the energy conversion efficiency of combined heat and power (CHP) systems combusting biogas from anaerobic digestion (AD). Biogas is composed of methane ($\text{CH}_4 \approx 60\%$), carbon dioxide ($\text{CO}_2 \approx 30\%$), water vapour ($\text{H}_2\text{O} \approx 5\%$), traces of hydrogen sulphide ($\text{H}_2\text{S} \approx <2\%$), ammonia ($\text{NH}_3 \approx <1\%$), and volatile organic silicon compounds ($\text{VOSiC} \approx 1\%$)^{3,4}. The most common and stable VOSiCs are Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5)^{5,6}. The combustion of Biogas-VOSiCs, when present in concentrations ranging from 2 to 317 $\text{mg}\cdot\text{m}^{-3}$, forms silicate deposits in the internal machinery, irreversibly damaging the moving components of the system⁷. Moreover, silica (SiO_2) from VOSiCs destroys the steam reform catalyst and releases microparticles into the atmosphere that, among other effects⁸, cause silicosis disease in humans^{4,9}.

PDMS, and particularly VOSiCs, were introduced in the '90s to replace chlorofluorocarbons (CFC), hydrochlorofluorocarbons (HCFC), and parachlorobenzotrifluoride (PCBTF), due to – what appeared at the time – their "environmentally friendly" characteristics^{10–13}. Despite that the industrial use of siloxanes could be dated to 1940¹, they still are a critical component of personal care products. Siloxanes begin their journey to the WWTP after being rinsed down the drain by the consumer. Once there, siloxanes can be released to the atmosphere by different physicochemical and biological processes that transform non-volatile to volatile siloxanes^{2,14}. Also, siloxane-related compounds have been linked to various health impacts on different animal models and possibly also in human populations^{1,15,16}. For example, exposure to D4 is classified as human hazard class reproductive toxicity two by the European Chemicals Agency¹⁷ after several studies on animal models. D5 also cause health issues to the nervous system, cancer, and hormonal disorders, according to reports from the US Office of Environmental Health Hazard Assessment (OEHHA)¹⁸. The widespread use of siloxanes in many industries (e.g., cosmetic, automotive, medical, and food processing) has led to an estimated VOSiC emission between 1.4 - 4.2 $\text{g}\cdot\text{year}^{-1}$ *per capita* in the UK and 0.4 - 85 $\text{g}\cdot\text{year}^{-1}$ *per capita* in the US, solely from personal care products¹⁹. VOSiCs have been recently classified as substances of very high concern (SVHC) for the environment by the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) organisation^{17,20}, and countries such as the US²¹, Japan²², Canada^{23,24}, and the UK²⁵. These countries have framed D4 and D5 siloxanes as substances with high production volume, persistence, bioaccumulation, and ecological concern.

Because VOSiCs are present in the personal care products reaching the WWTP influent stream¹⁴, it has been traditionally assumed that only this input is responsible for the damage to the combustion engines. Therefore, most of the publications mainly address D4 and D5^{5,19,26} as substances of concern, without considering siloxane polymers as potential precursors of biogas contamination. PDMS are known to be abiotically degradable in soils at low moisture conditions by acidic clay minerals with Lewis acid sites (Al^{+3} or Fe^{+3})^{27,28}.

However, it is not known if PDMS could be abiotically cleaved or biodegraded in the AD sludge. If PDMS could be microbially degraded in the anaerobic sludge, they could provide an additional source of VOSiC within the AD reactor itself. However, their susceptibility to biodegradation is still uncertain, and researchers still question whether these compounds can be mineralized biologically and, if so, whether aerobic or anaerobic conditions would be more effective^{29,30}. Since the siloxanes problem was first recognized³¹, several studies have summarized non-biological siloxane removal techniques or the general effects of VOSiC on AD. However, there is still a lack of knowledge on the PDMS presence and its effects on AD biogas. There is also a critical need to test and develop new biological approaches to tackle the impacts of siloxanes both on the environment and the economy of WWTPs .

Problem Statement

PDMS and VOSiCs start their journey to the WWTP after being rinsed down the drain by the consumer. This input to the treatment systems creates severe damage to the biomass-based energy conversion systems due to their presence in AD biogas. Biogas-VOSiCs cause significant economic losses by the machinery clogging, engine damaging, loss of thermal efficiency and the consequent reduction of anaerobic digestion benefits. Also, it is known that siloxanes can be released to the atmosphere by different physicochemical and biological processes that transform non-volatile to volatile siloxanes³² within the wastewater process. The non-volatile forms that are not transformed within the water recovery process or in the solids treatment (anaerobic digestion) remain attached to the organic solids. These polymers and oligomers also pose a threat to the environment by the post digestion sludge pollution, usually used as an organic fertilizer to food crops or as a soil amendment.

Given the hazards posed by siloxanes, both during and after wastewater treatment, it is important to understand their sources, transformation, and degradation. This work focuses on the research and development of microbial strategies to degrade polymeric and oligomeric siloxanes (*i.e.*, D4, D5), and to develop efficient control and mitigation strategies for siloxane-related issues. Biostimulation approaches were performed to obtain a microbial consortium that biodegrades siloxane polymer and oligomers. Microbial changes were elucidated to identify the ecological changes derived from the developed strategies tested. Chemical characterisation of the molecules derived from PDMS and VOSiC catabolism was performed using mass spectrometry. Possible siloxane biodegradation mechanisms were proposed, integrating the chemical characterisation and microbial identification of most abundant organisms that might be responsible for siloxane removal.

Hypotheses

- I. To enhance the enzyme production and reach the Si-O and Si-C bond dissociation energy, oxygen as the final electron acceptor stimulates the bioaugmented microbial consortia. PDMS, D4 and D5 conversion into semi-volatile and non-volatile forms are enhanced, treating siloxanes in the AD sludge.
- II. The use of oxygen as a more energy-producing final electron acceptor increases methane production, driving substantial microbial ecology changes within the AD sludge and enabling the catabolism of recalcitrant compounds such as siloxanes. PDMS is degraded by microbial means, serving as a carbon source for methane production.

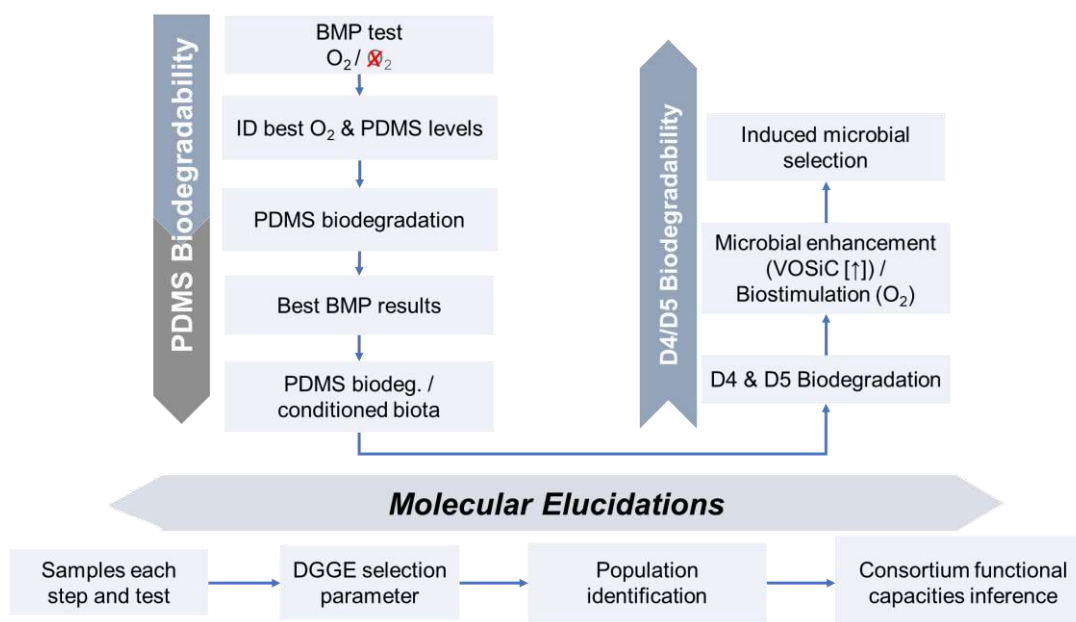
Objectives

Determine microbial conversion of polymeric siloxanes (PDMS) to D4-D5 volatile siloxanes and their stabilization using microaeration with oxygen as final electron acceptor, evaluating their microbial degradation from anaerobic digestion sludge, assessing chemical and microbiological changes within the sludge.

Specific objectives

- I.** *Assess the biodegradability of polydimethylsiloxanes (PDMS) as main siloxane polymers by a selective enhancement and biostimulation of WWTP microbial siloxane mineralizer consortia under anaerobic or microaerophilic digestion.*
- II.** *Evaluate the hydrolysis of D4 and D5 oligomers (volatile siloxanes) by a PDMS-degrading microbial consortia using oxygen as a final electron acceptor.*
- III.** *Elucidate the possible chemical changes on the polymeric (PDMS) and volatile (VOSiC) siloxanes microbial metabolism under microaerated conditions.*
- IV.** *Characterize diversity changes in the native microbial communities of the WWTP sludge under siloxanes concentrations and oxygen presence, by using 16S rRNA gene fingerprinting DGGE technique.*

General experimental development



FIRST CHAPTER

The impacts of siloxanes and what conventional wastewater treatment plants can do to remove these pollutants

Abstract

Since siloxanes first synthesis (1940), they have been crucial in personal care, household, construction, pharmacy, and food industries. Just recently, with the significant increase in siloxanes world production (more than 2 million per year), scientific research is understanding and testing siloxanes safety/impact on the ecosystem. Different reports, government policies, and scientific investigations agree that volatile organic silicon compounds (VOSiCs) are the most noxious compounds in siloxanes chemistry. VOSiCs, specially Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5), are currently considered by several governments as emergent contaminants due to their recalcitrant nature and their impact on different ecosystems. Polymeric and volatile siloxanes from human elements (*i.e.*, personal care products, food, pharmacy) go to the drainage systems by usual hygiene practices ending in the wastewater treatment plants (WWTPs). Further, these siloxanes enter the environment by the WWTPs post-treatment products (*i.e.*, water, sludge) being a critical source and confluence point. This migration behaviour was recognised due to the VOSiCs pollution and mediated damage in biogas-based conversion systems. The substantial damage on the energy co-generation machinery has raised awareness on siloxanes risks and drove the track to understand their impact on green energy sources such as anaerobic digestion. This review intends to give an updated look at the siloxanes history, impacts, environmental importance, and methods to treat them from the main release font, anaerobic digestion biogas. We also expect to raise awareness on the siloxanes-mediated problems by understanding the effects and current solutions reported in the available literature.

Introduction – Siloxanes in biogas and the current flaws on its removal

More than ten million tons of siloxanes are produced every year in the world¹. These prevalent chemical compounds have been present in every aspect of human life, becoming practically irreplaceable in our society. Since the 1990s, siloxane applications have ranged from paint additives, antifoams, pharmaceutical products, personal care products, and food stabilizers². Until recently, siloxanes were considered innocuous to humans and the environment; nonetheless, the oligomeric forms known as volatile organic silicon compounds (VOSiC) are now considered emergent pollutants to the biota and natural ecosystems. In addition, VOSiCs have become a matter of particular concern to municipalities due to the damage caused to the energy conversion systems that use anaerobic digestion (AD) biogas in the wastewater treatment plants (WWTPs). Biogas is composed of methane ($\text{CH}_4 \approx 60\%$), carbon dioxide ($\text{CO}_2 \approx 30\%$), water vapour ($\text{H}_2\text{O} \approx 5\%$), traces of hydrogen sulphide ($\text{H}_2\text{S} \approx <2\%$), ammonia ($\text{NH}_3 \approx <1\%$), and volatile organic silicon compounds ($\text{VOSiC} \approx 1\%$)^{3,4}. The most common and stable forms of VOSiCs in biogas are

octamethylcyclotetrasiloxane (D4) and decamethylcyclopentasiloxane (D5)^{5,6}, which can be present in concentrations ranging from 2 to 317 mg·m⁻³. Then, when VOSiCs polluted biogas is burnt, D4 and D5 siloxanes develop silicate deposits in the internal machinery, irreversibly damaging the moving components of the system⁷. Moreover, silica (SiO₂) from VOSiCs destroys the steam reform catalyst and releases microparticles into the atmosphere that, among other effects⁸, causes silicosis disease in humans^{4,9}.

As a critical component of personal care products, siloxanes begin their journey to the WWTP after being rinsed down the drain by the consumer. Once there, siloxanes can be released to the atmosphere by different physicochemical and biological processes that transform non-volatile siloxanes into volatile forms (*e.g.*, D4, D5)¹⁰. Non-transformed siloxanes remain attached to the organic solids in the post-digestion sludge and find their way to the environment by the sludge usage as crop fertilizer (**Infographic 1**). It is commonly believed that volatile siloxanes only arrive at the WWTPs through the wastewater distribution lines. However, recent reports suggest that they also form endogenously *via* microbial siloxane polymer (PDMS) catabolism during treatment^{2,11,12}. Accordingly, elucidating the siloxane sources, transformation, and degradation through wastewater treatment will help to develop efficient control or mitigation strategies preventing their environmental release. Considerable research has been conducted in developing methods to remove siloxanes from biogas. Current biogas upgrading technologies are adequate to remove pollutants such as H₂S, CO₂, halogens, and other volatile organic compounds. Nonetheless, these methods are often costly and usually cause adverse environmental impacts, such as the production of ecotoxic by-products or residues generation (*e.g.*, spent cartridges, solvents, solid sorbents)¹³. Activated carbon, silica, and zeolites adsorbents are widely used and relatively inexpensive¹⁴, but in siloxanes removal, they require high amounts of energy for regeneration or are expendable¹⁵. Moreover, some adsorbent materials may become saturated and less cost-effective to regenerate, requiring total replacement. Overall, these techniques usually increase the biogas treatment expenses and generate new complex wastes that require further cleaning or special disposal methods¹⁶.

In contrast, biological processes do not accumulate and concentrate contaminants in or on a different phase, becoming naturally more cost-effective and sustainable than physicochemical approaches. Siloxanes biological degradation can form simpler, more biodegradable, and less harmful compounds^{17,18}. However, researchers still question whether siloxanes can be mineralized biologically and, if so, whether aerobic or anaerobic conditions would be more appropriate^{19,20}. Since siloxane-related problems were initially described²¹, several reviews have summarized the non-biological siloxane removal techniques and the general effects of VOSiCs on AD. This review presents a thorough description and analysis of the harmful effects of siloxanes on biogas and the environment. Also, we offer a critical comparison of the physicochemical and biological techniques used thus far to remove them from the wastewater and sludge streams.

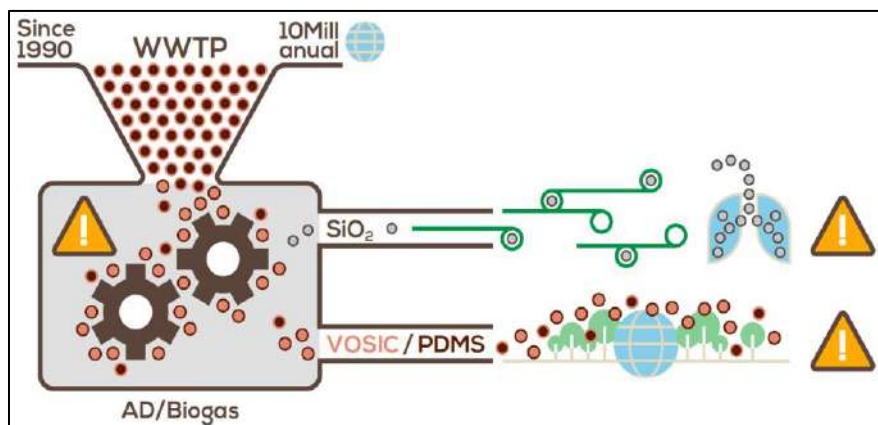


Figure 1. Flow of siloxanes through the WWTP and the potential risks to the environment and human health.

The extent and origin of siloxanes impacts

1. Environmental relevance

VOSiCs were synthesized around 1940 by Dow Corning and used mainly in haircare foams and shampoos until the 1980s¹. Later, they were used to replace ozone harming solvents, such as chlorofluorocarbons (CFC), hydrochlorofluorocarbons (HCFC), and tetrachloroethylene due to – what appeared at the time – their environmentally friendly and non-toxic characteristics²². Not surprisingly, VOSiC emissions have increased alongside siloxane production; for example, the U.S. emits *ca.* 10,000 tons per year. Moreover, several studies have expressed concern regarding the persistence, global spread, and VOSiCs environmental toxicity^{23,24}. These siloxanes summon along riversides and sea sediments, where they get in close contact with marine organisms that bioaccumulate VOSiCs in fat tissues and internal organs^{1,11}. The bioaccumulation is then magnified by trophic processes along the food chain, ultimately disrupting the entire ecosystem^{1,11,14,22}.

Consequently, the U.S. Environmental Protection Agency (U.S. EPA) classified D4 and D5 as harmful substances to water and soil environments²⁵. In Germany, D4 and D5 are substances of very high concern (SVHC) by the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulatory authority^{26,27}. In general, VOSiCs (*i.e.*, D4, D5) are labelled as emergent contaminants by similar regulatory entities in other countries such as Japan²⁸, Canada²⁹ and the UK³⁰.

2. Physicochemical properties

Siloxanes are chemical compounds with silyl ether bonds, as monomers, dimers, trimers, oligomers, or polymers². A typical siloxane molecule arrangement can be divided into an organosilane unit (Si-C bonds) and a silicon-oxygen (Si-O) *bridge* between organosilanes³¹. The bond energies of Si-O, C-H, and Si-C bonds are 103 kcal·mole⁻¹ (Δ EN 1.7 – polarized covalent), 100 kcal·mole⁻¹ (Δ EN 0.4 –covalent), and 69 kcal·mole⁻¹ (Δ EN 0.7 – covalent), respectively¹⁴, demonstrating the high energy required to cleave a siloxane molecule. Furthermore, Si-O bridges are shielded by a steric hindrance produced by the methyl groups

in the organosilane part of the molecule providing additional chemical stability and hydrolysis resistance.

PDMS are siloxane compounds synthesized from silicon, hydrocarbon (methyl, ethyl, phenyl), and oxygen with more than ten monomeric units bonded by Si-O-Si links. On the other hand, VOSiCs are siloxane oligomers derived from PDMS hydrolyzation formed *via* chemical synthesis, biochemical degradation¹², or as products of ring-chain equilibrium from oligomeric rearrangements³². PDMS and VOSiC have unique physicochemical properties. They are insoluble in polar solvents, highly compressible, hydrophobic, and bind strongly to organic matter on sludges, soils, and sediments³³. Additionally, they are generally non-reactive exhibiting high thermo-chemical stability³⁴. Specifically, VOSiCs have low viscosities and high volatility. They are classified as aroma-free solvents, primarily used in personal care and industrial dry-cleaning applications^{5,23,34}.

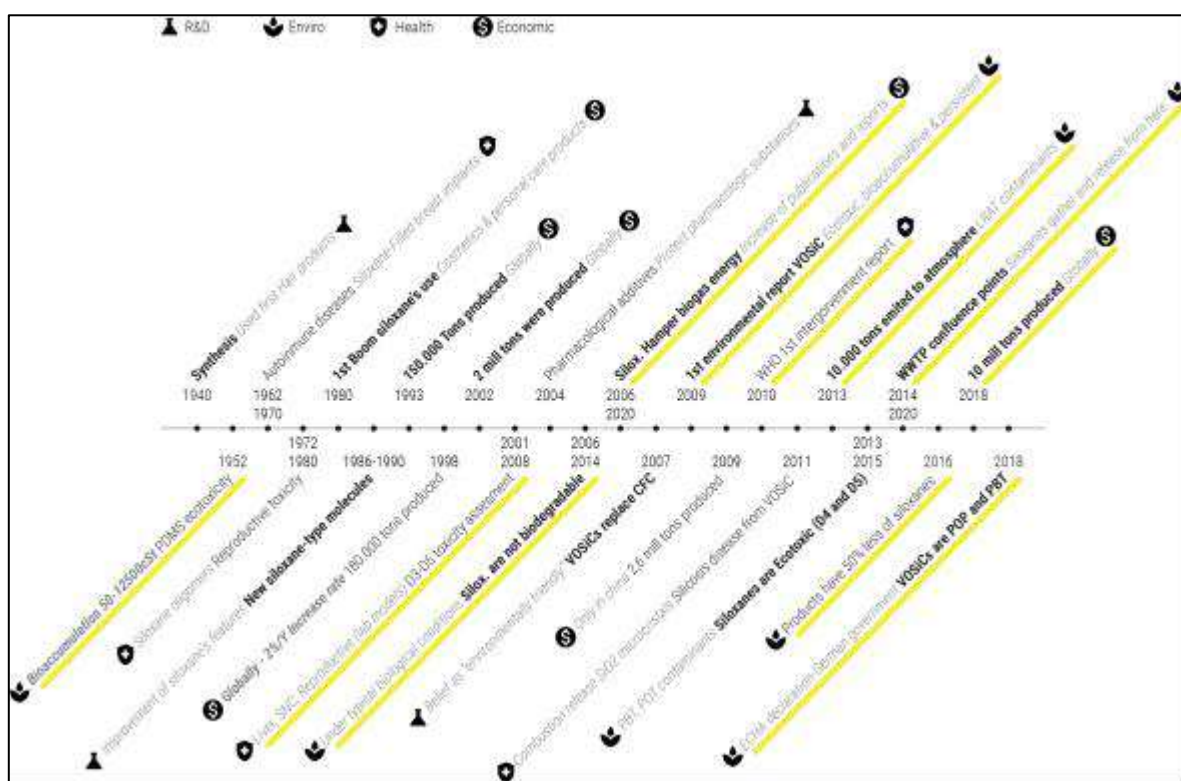


Figure 2. Highlights in siloxanes development. Events underlined in yellow denote key impacts on environmental and human aspects.

3. Industrial production and applications

China leads the world siloxane production, especially for the synthesis of high-density siloxanes (*i.e.*, silicones). As a result, it is common to find 10 - 40 times more VOSiCs on surface sediments in Asian countries compared to European countries³⁵. Global annual production of siloxanes was approximately 150 thousand tons in 1993; yet, around 1998, it increased at a 2% annual rate, resulting in 2 million tons of siloxanes in 2002³⁶⁻³⁸. As of 2006, Europe, the USA, and China produced around 1.5 million tons^{22,39}, while in 2009, the global production of siloxanes was *ca.* 2.6 million tons^{2,22,38}. Siloxane production has been

increasing an extra million tons every year⁴⁰. Current estimates calculate that 10 million tons are yearly synthesized¹, where the leading producer and consumer is China, followed by Western Europe and North America. Furthermore, the siloxane market in Europe, America, and Asia equalled 7.3 million USD in 2002, almost 11.5 billion USD in 2009, and 19 billion USD in 2017, which proves a substantial and sustained growth over the years². Based on these numbers, the annual rate of increase for 2022 is estimated to be 6.5%^{2,39}, which is a number that may continue increasing as in 2002.

Industrial applications of siloxanes are extensive. PDMS – also known as dimethicone or simethicone – are often used in various fluids, resins, and elastomers present in many industrial and home products^{41,42}. Since the methyl substituents in the siloxanes structure are easy to replace with other functional groups, siloxane-containing formulations can be customized to provide new physicochemical characteristics in a wide range of applications¹³. Therefore, PDMS compounds and VOSiCs are present in many personal care product formulations (>80%), where antiperspirants are the most common. For example, almost 50% of solid (*i.e.*, roll-on) antiperspirants produced in the last ten years have VOSiCs in their formulation¹. Finally, siloxanes are also often used in cookware additives, cosmetics, pharmaceuticals, water repellents, and as an oil substitute in the food industry^{2,43}.

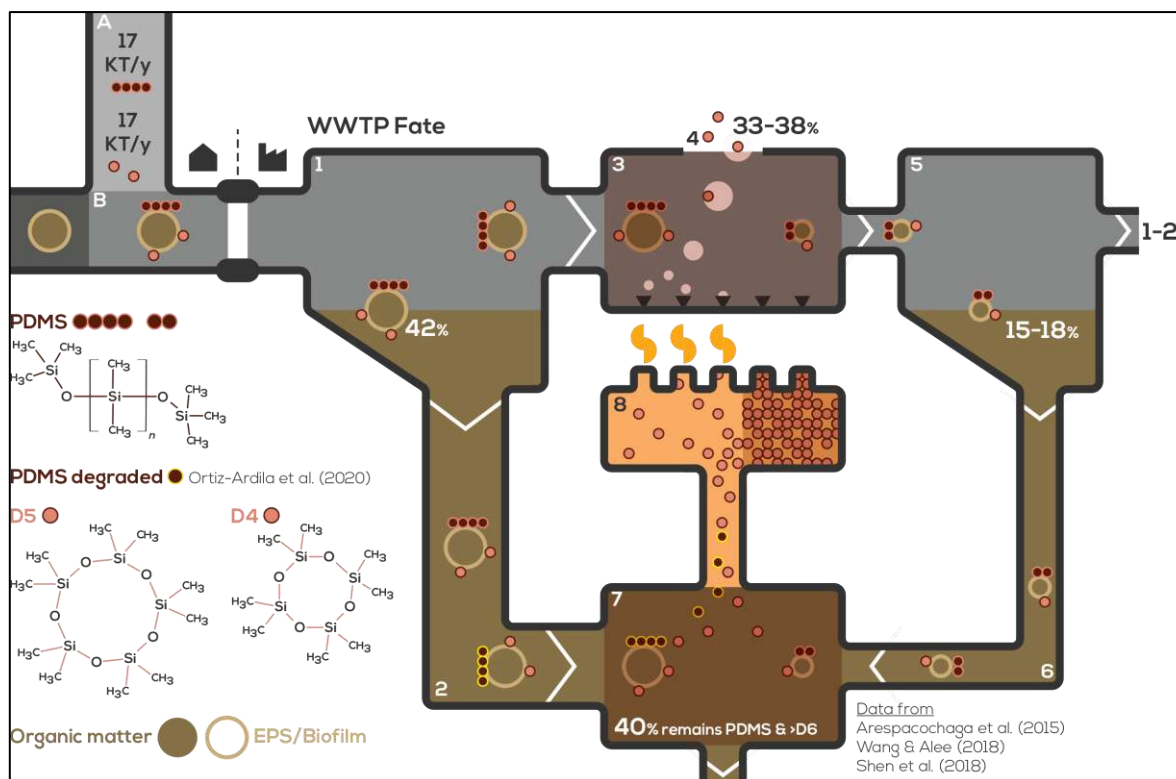
Siloxanes are critical to the personal care and pharmaceutical industries. They act as carrier/emulsifying agents, ensuring the delivery of the active ingredients of the formulation. Indeed, due to their high volatility, VOSiCs are the first choice as fragrance adjuvants in aerosol products³⁸. Consequently, VOSiCs are directly released into the atmosphere through aerosols, being a critical siloxane pollution source, but not the primary one^{22,25}. The main route of siloxanes contamination is through shower, laundry, and kitchen wastewaters (D/T personal care products, soaps, and softeners formulations)^{15,44}. Once in the WWTP, siloxanes migrate in different chemical forms to the atmosphere throughout the wastewater treatment. Then, the widespread use of siloxanes has facilitated their migration to the environment, and its increased production has worsened this problem.

4. Fate along WWTPs

Once siloxanes are in the wastewater, they bind to the microbial extracellular polymeric substances (EPS) and the organic matter in the pipe lines^{23,38} (**Fig. 3, Steps A, B**). On average, 17 thousand tons of PDMS and between 1.6 – 3.3 tons of D4-D5 siloxanes go to the WWTPs per year^{5,14,23}; there, siloxanes have different fates, depending on the WWTP treatment stages. In a conventional WWTP, around 42% of the siloxanes settle in the primary clarifier (*i.e.*, 50 mg·kg DW⁻¹ on average), while an additional 38% desorb and volatilize in the aeration of the activated sludge process (**Fig. 3, Steps 5,6**)^{15,23,38}. After this process, approximately 15 - 18% of the initial siloxanes load remain in the mixed sludge as polymeric and non-volatile siloxanes, while 1-2% is discharged from the WWTP as linear siloxanes in the treated effluent (**Fig. 3, Steps 3,4,7,8**)^{5,10}.

From the initial siloxane load, approximately 60% of the siloxanes reach the AD stage. Here, the increased temperatures in the reactor (*e.g.*, 37°C) weaken the physical forces between the siloxane molecules and the organic matter, allowing their desorption to the biogas (**Fig. 3, Steps 9,10**)^{5,23,45}. Several studies claim that only the VOSiCs from the initial load on the

WWTP stream are responsible for the biogas combustion engine damage^{7,21,46,47}. However, some hypotheses refer that VOSiCs are microbially produced from the PDMS hydrolysis in the anaerobic digester^{23,38,39}. Ortiz-Ardila *et al.* (¹²) demonstrated that the endogenous AD microbiota could produce VOSiCs from PDMS, revealing an additional source of D4-D5 within the digestion process itself. Very little is known about the microbial metabolism of polydimethylsiloxanes, particularly the active microbes, enzymes present, the operative conditions, and microbes metabolic pathways. This critical knowledge gap undercuts our ability to track, monitor, and control siloxanes in the WWTP process, and subsequently in the environment⁴⁸.



5. Effects of siloxanes on biogas-based energy conversion systems

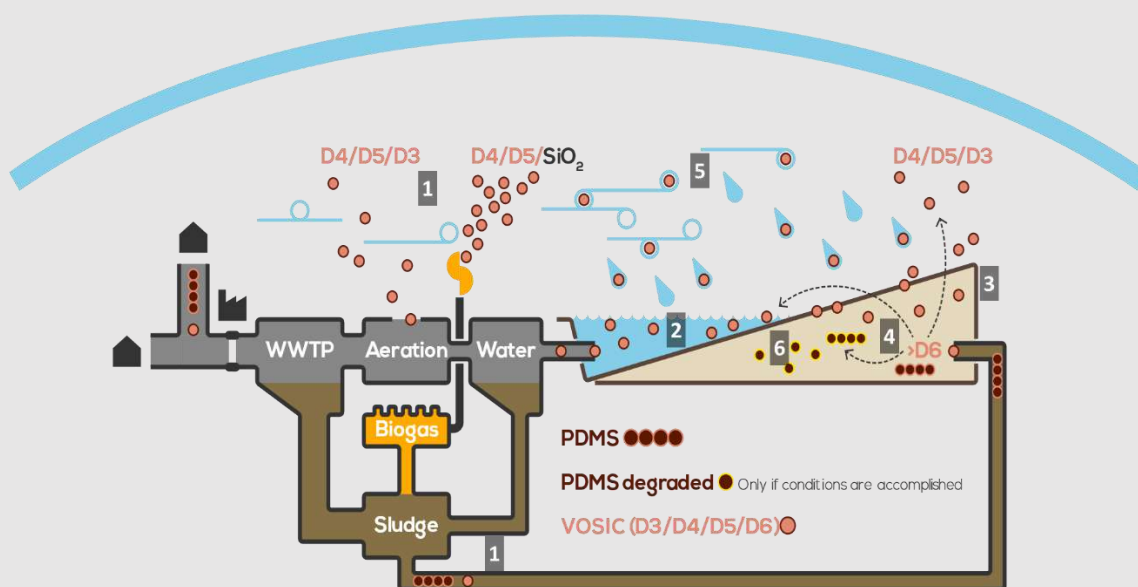
The presence of volatile siloxanes negatively impacts Biogas-based energy conversion systems^{5,6}. From the combustion of VOSiC-polluted biogas, silicates are produced and deposited on the internal machinery surfaces. These silicates decrease the thermal conductivity, modify the geometry of the combustion chambers, and clog the pistons and the

exhaust lines^{23,49}. Consequently, the general efficiency, compression, and lubrication of the system are reduced. In addition, the silica deposits lead to incomplete combustion increasing the CO₂, CO, NO_x, and SO_x emissions and interfering with the post-combustion pollutant control process (*i.e.*, catalytic removal using Pd-Pt/Al₂O₃)^{50,51}. Finally, when nanocrystalline SiO₂ particles are released into the atmosphere, they represent a serious health risk to humans due to their carcinogenic, mutagenic, asthmogenic, and reproductively toxic (CMAR) characteristics²³.

A typically WWTP spends *ca.* 12 USD on 100 m³ biogas cleaning; however, if siloxanes are present, these costs can increase up to 60 USD per 100 m³-biogas (*n.b.*, *via* AC adsorption). Also, biogas cleaning from siloxanes could require up to 60,000 USD per year for equipment maintenance and sorbent matrix replacement⁷. Although, differences in total costs would be expected depending on the size of the plant (*i.e.*, population served), the amount of biogas treated, and the VOSiC load^{52,53}. Alternatively, if no measures are taken, the damage from siloxanes could exceed 70,000 USD per year in repair expenses, not accounting for the replacement of mechanical parts or the repair of the engines^{19,41}. Considering this, manufacturers of CHP systems have imposed stricter limits for maximum allowable VOSiCs or excluded siloxanes from the warranty altogether. The acceptable limit for siloxanes is 5 mg·m⁻³ of biogas in standard internal combustion engines; 0.5 mg·m⁻³ for gas grid injection; and 3 - 10 ppb for microturbine engines in the European Union^{11,15,33,54}. These limits are trending downward, where some manufacturers even require that the output siloxane concentration must be nearly zero to preserve the systems^{13,55}.

VOSiCs and PDMS output from water treatment and environmental degradation (Infographic 1)

Siloxane discharges from WWTPs reach the atmosphere (1), water (2), and soil (3), negatively impacting the biota and the ecological dynamics in each environmental compartment^{5,15,56}. The volatile characteristic of VOSiCs favours their partitioning from water or sludge to the atmosphere (4), promoting their dispersion in the environment^{38,57}. VOSiCs are well known for their atmospheric persistence and long-range atmospheric transport (LRAT)^{24,58,59} (5), with estimated migration rates of 5,284 km, 3,447 km, and 2,966 km for D4, D5, and D6, respectively^{54,55}. Consequently, D4 and D5 have been classified as persistent organic pollutants (POP) and persistent bioaccumulative and toxic (PBT) contaminants^{26,27}.



Amended soils with post digestion AD sludge may be contaminated with organosilicon pollutants (*n.b.*, 36-50 g of organosilicon pollutant per 100 g of soil). If soil conditions are favourable (low soil humidity and low pH), siloxanes transform abiotically into more biodegradable dimethylsilanediol (DMSD) within 2 weeks^{25,37,60} by processes that include hydrolysis, volatilisation, and Lewis acid-metal catalysis^{18,61} (6). However, in moist soils, siloxanes are slowly degraded at a rate of 6% (w/w) per year (*e.g.*, agricultural land, compost)^{61,62}. When VOSiCs and siloxane analogues (*i.e.*, small linear oligomers, cyclic non-volatile oligomers, among others) are present in high-moisture soils, they tend to polymerise (4) into mixed oligomers composed of cyclic and linear compounds⁶². Siloxane polymerisation makes them harder to cleave and biodegrade, representing a potential risk to siloxane-sensitive organisms and, in the case of plant uptake, may contaminate the human food supply^{22,60}. Finally, particle-bound siloxanes transfer toward water bodies *via* runoff or percolation (4), where they bioaccumulate in the aquatic ecosystem². Thus, understanding siloxanes transformations and tracking their fate in each phase (*i.e.*, water, sludge, gas) and treatment step of the WWTP is crucial to mitigate their detrimental effects on the environment and potential hazards to human health.

Current strategies to cope with siloxanes

The concentrations of siloxanes and other pollutants in biogas depend on the waste/feedstock and the operating variables of the anaerobic digestion process. WWTPs usually divide the siloxane removal processes into pre-treatment and post-purification stages. The pre-treatment stage usually uses air (volatilisation) or heat (desorption) to remove volatile contaminants from the sludge *prior* to AD. Opposite, the second stage is a post-purification process that cleans only the biogas, and it is meant to increase its calorific Wobbe index (MJ per m^3)². However, the specific cleaning process must be selected on a case-by-case basis, considering its limitations, drawbacks, efficiency, sustainability, and associated costs. Here, we review and discuss the most common siloxane-removal methods used nowadays.

A. Physical methods

Classical methods to enhance the CH_4 content have been inherited from the natural gas industry⁶³. These methods are based on physical processes (*i.e.*, adsorption, absorption) that remove other constituents from the biogas stream, increasing the methane content. Sorbent matrixes used in these methods must attain the rules of quick and high pollutant retention, strong selectiveness, and almost complete regeneration after saturation³⁹. Nonetheless, complex wastewaters and sludges have introduced new, non-desirable compounds in biogas that limit the efficiency and hamper the economic feasibility of physical methods³⁶. For example, the presence of siloxanes in biogas has been challenging since 1996⁶⁴, requiring the coupling of different physical-based technologies to clean the biogas^{13,65}. Here, we present the current most used physical methods to remove siloxanes.

1. Adsorption

Activated carbon, silica gel, zeolites, and alumina are the most used and investigated adsorption materials to remove siloxanes in biogas^{66,67}. Physical adsorption relies on the attraction of the biogas pollutants (*e.g.*, siloxanes, sulphides) to a porous solid material by their Van der Waals interactions³⁴. Adsorption is the first choice to remove gaseous pollutants from biogas. Among other features, it is efficient, easy to operate, easy to couple with other technologies and cheap in terms of Capital and Operative expenses) CAPEX/OPEX^{68,69}. AC adsorption has become the first choice for VOSiCs treatment^{13,51,70} due to its proven overall removal efficiency (*i.e.*, 84 - 98% removal rate) at low concentrations ($<10 \text{ mg}\cdot\text{Nm}^3$ of biogas). Then, carbonaceous materials such as activated carbon (AC) are frequently used for biogas cleaning due to their proven efficiency and extensive knowledge of their chemistry (*e.g.*, pore size, surface area, active sites availability, and spatial distribution)⁶⁸. This extensive knowledge in AC chemistry and its chemical interactions with siloxanes (*e.g.*, polarity, dipole-dipole, Debbie) is a critical factor that reassures its capacity to retain siloxanes in industrial and technical applications^{14,16,71,72}. Nonetheless, this knowledge also helps understand the AC limitations in siloxane treatment. In adsorbents, parameters such as surface area (*i.e.*, Brunauer–Emmett–Teller-BET- area) or pore size are used to infer the pollutant removal efficiency. Usually, the higher is the surface area, the better is the pollutant removal. In siloxanes treatment, this rule is despicable; nano porous materials ($<0.7 \text{ nm}$,

higher BET surface) are the least efficient ones because siloxane molecules do not fit inside the pore (D4 size: 1.08 - 1.03 nm)^{15,73}.

Consequently, the correct choice of the sorbent material becomes more challenging due to the necessity of accurately characterizing several physicochemical parameters that are not considered on a routine industrial basis. In general, AC can achieve good siloxane removal from complex matrices (*i.e.*, biogas), and it is more efficient than typical non-carbonaceous materials⁶⁹. Nonetheless, physical adsorption cannot eliminate the biogas siloxanes completely, requiring further cleaning steps (*e.g.*, scrubbing, cooling, cryogenic separation)⁷⁴. Thus, siloxane removal using AC is reserved for the final treatment stages where its single-use results in a VOSiC reduction of more than 98% because all the other biogas contaminants have been previously removed^{53,67}. Then other sorbents (*e.g.*, silica gel, zeolites, and alumina) are used for raw biogas due to their affinity with non-siloxane pollutants (*i.e.*, sulphur, water, dust)^{66,67}. However, with adsorption, efficiency is not everything. The more efficient the process is, the more challenging the regeneration of the sorbent is^{16,75}. This is a critical dilemma faced by WWTPs, considering that the saturated matrices recovery is necessary to decrease the operating costs of biogas cleaning^{76,77}. Accordingly, further treatment of the adsorbed contaminants is another critical aspect that has not been addressed in the physicochemical methods. Usually, the saturated matrices that cannot be recovered and the matrix recovering waste are landfills discarded, generating new complex toxic products to the environment. Since this is a critical matter, current research is devoted to balancing sorption efficiency, regeneration feasibility, waste management, and sustainability⁷⁷.

Physical methods matrixes are easily saturated with siloxanes, and usually, their complete regeneration is extremely difficult. Adsorbed VOSiCs can polymerise themselves with the sorbent, leading to an irreversible bonding between siloxane molecules and the sorbent matrix⁷⁸. Usually, D3 and D4 VOSiCs have a polymerisation ratio between 20-80% when they are trapped on physical adsorbents^{77,79}. This phenomenon forces the use of expensive desorbing techniques such as advanced oxidation processes (O₃, H₂O₂) or very high temperatures coupled with strong acids to ensure sorbent regeneration. Thus, most siloxane-saturated sorbents cannot be easily regenerated once used; these techniques permanently damage the sorbent matrix^{14,17,41,80}. These low regeneration rates lead to a constant filter replacement, adding new waste and complex materials to be discarded or incinerated. In general, siloxane treatment waste could require equal or higher associated costs and clean as the siloxanes on their own.

Among the currently available methods for siloxane removal at an industrial scale (*e.g.*, membranes, deep chilling, absorption), adsorption requires the least CAPEX and OPEX⁵³. Adsorption methods have lower CAPEX than other technologies that require complex machinery, energy requirements and installations (*e.g.*, cryogenic separation, membranes)^{53,63}. However, they still represent 70-90% of the annual OPEX in a typical WWTP configuration¹⁷, which increases if siloxanes are present¹⁹. Siloxanes presence increases the CAPEX of adsorption technologies between 6-7%¹³ (*e.g.*, 200 euros are needed to replace an AC saturated filter²¹), meaning a final potential increase from 81 to 500 € per Kg VOSiC treated¹³. Thus, adsorption becomes a clear non-profitable cleaning option for any WWTP, despite the scale¹⁷. For example, in a large scale WWTP (2.5 million

inhabitants), AC contactors are used to remove siloxanes⁴⁹. However, when matrixes are fully saturated with siloxanes, the cost for replacing AC media are around 2000 € per contactor. In general, if siloxane concentrations are high ($>80 \text{ mg}\cdot\text{Nm}^{-3}$ of biogas), the sorbent media should be replaced *ca.* 14 times per year⁴⁹. These prospects and expenses force the small-scale WWTPs to burn the biogas since its cleaning costs are not affordable⁸⁰.

Furthermore, molecular repulsion, active site occupation, competitive adsorption, pore saturation, adduct formation by siloxane polymerization⁷⁸, among others, cause low-efficiency issues and incomplete sorbent regeneration^{13,81}, increasing the treatment expenses. For example, common biogas pollutants (*e.g.*, water moisture, H_2S , dust) can saturate and clog the AC mesopores, reducing the overall efficiency of the contactor and decreasing the siloxane removal^{41,79}. On the other hand, in pre-cleaned biogas (upgrade stage), sorbents cannot selectively retain only siloxanes, reducing the removal efficiency by the competitive adsorption between other volatile organic contaminants (VOCs) and VOSiCs^{82,83}. VOCs are not the only ones having dynamic competition for the sorbent catalytic sites. Several authors^{72,84,85} explained that D5 siloxanes could displace the molecules by their higher electrostatic interactions and size. This displacement results in a high D4 and D3 post-cleaning concentration that is impossible to prevent due to the physical methods no specificity.

Finally, as physical adsorption is the predominant method used to clean biogas-siloxanes², current developments pursue new sorbents and ways to deal with siloxanes. Several studies have focused on developing new materials to fulfil the minimum siloxane concentration required to protect the biogas energy systems. Materials such as perlite⁸⁶, halloysite, recycled silica⁸⁷, graphene-oxide aerogels⁸⁸ and silica-based aerogels⁸⁹ have shown good siloxane removal performance, fast regeneration, and low retention times, making them promising options to clean biogas. Nevertheless, the early stages of these new developments make their fast implementation in the WWTP systems difficult. Then, further research on cost-effective, environmentally sustainable, and easy to scale up sorbents is needed.

2. Refrigeration and Freezing Cryogenic condensation

Considering that biogas is a mixture of compounds with different eutectic points, refrigeration and cryogenic condensation can separate non-siloxane pollutants, such as dust, water, aromatics, and other substances^{23,90}. In general, when the temperature decreases and pressure increases, non-methane gas components begin to liquefy or solidify, obtaining methane as a final product⁶³. Cryogenic condensation is especially efficient for biogas cleaning and upgrading due to the significant differences between the methane liquefaction point (-185°C) and other biogas components (*e.g.*, general pollutants: $<-25^\circ\text{C}$, CO_2 : -55°C), at the typical 10 bars of operating pressure^{41,90,91}. Refrigeration temperatures ($4 - 5^\circ\text{C}$) remove siloxanes partially (15 - 20%) since small molecules such as D4, D3, L3 require specific conditions (-65°C to -72°C at 10 bar). Then, cryogenic condensation is the most efficient method for siloxane removal at biogas upgrading stages (99%)^{41,92}. Typical cryogenic systems can only work at $-25^\circ\text{C} / 1 \text{ bar}^7$. Thus, plants are required to perform several treatment cycles or increase pressure treatment (10 - 15 bar) to obtain the desired biogas quality⁹³. In some cases, this is not enough and, when VOSiCs concentration is higher than 50 mg per m^3 , siloxane solids can clog treatment pipelines and machinery. Inevitably,

due to the very low temperature, methane clathrates (CH_4 hydrates in crystal form) can clog the system, especially the cooling units^{55,94}. For this reason, new materials and designs have to be implemented in the cryogenic units to prevent clogging issues and machinery damage from siloxane solids⁹¹.

Rough estimations assure that 0.4 € per Nm^3 is the standard cost using a typical biogas-upgrading cryogenic unit⁹⁵. However, in the actual context, this is not accurate when siloxane concentrations are usually higher than $50 \text{ mg} \cdot \text{m}^{-3}$. More accurate estimations^{34,63} clarify that 0.42 € per Nm^3 is only for the cooling units energetic requirements. The mentioned value excludes the cost of the pumps, liquid nitrogen (used in direct cryogenic condensation), operators, clogging-preventing mechanisms, heat exchangers (indirect cryogenic condensation), and maintenance costs, among others^{91,93}. Nonetheless, from all the physicochemical methods for siloxanes removal, cryogenic separation is the one that produces less waste, is solvent-free, does not use sorbents or cartridges and can remove several pollutants in one cycle^{34,57}. Nonetheless, due to the high economic investment, this technology is usually reserved to produce high purity methane when high concentrations of siloxanes are present²³.

Los Angeles County district biogas plant is an applied example of this technology use; the plant treats and removes siloxanes in an industrial facility developed by Pioneer Air Systems^{14,96}. This plant uses a temperature of -23°C on different condensers to fulfil the thermal difference between the optimum -70°C and the affordable temperature (-23°C)⁹⁶. The arrangement of sequential coolers enhances siloxane removal, decreases energy use, and cleans biogas. Nonetheless, cryogenic condensation is still considered an emergent technology^{31,34}, mainly due to the high CAPEX and OPEX. Several reports have developed cryogenic hybrid systems at laboratory and semi-pilot scales, which are now gaining particular interest in the scientific community^{91,97}. These hybrid systems are mainly based on coupling physical adsorption, chemical absorption, and membranes to overcome cryogenic drawbacks and be used as future biogas-upgrading tools^{91,97}. However, much is still needed for its full implementation. Finally, it is commonly believed that biological methods will replace cryogenic ones due to the high costs and complexity⁹⁸.

3. Membranes

Specific polymeric materials (membranes) could physically retain methane as the most important and invariable part of biogas, while pollutants such as CO_2 , H_2S and siloxanes pass through a thin polymeric membrane ($0.2 - 2 \text{ nm}$)^{34,53}. Membrane separation is usually meant as a biogas upgrade method^{41,99} that uses less physical volume than biogas cleaning methods^{57,100}. These systems are usually composed of a compressor/vacuum, power unit, moisture removal, a recirculation unit, and the membrane unit³⁴. The membrane unit is usually made from polymeric, inorganic, and mixed materials with specific porosity, permeability, resistance, and physicochemical characteristics to ensure the biogas cleaning requirements. Among these materials, the polymeric membranes are the most used ones; usually, these membranes contain imidines, sulphones, carbonates, even siloxanes polymers as adsorption agents¹⁰¹. In general, membrane separation is only used as a polishing step on WWTP plants that require biomethane, or when CO_2 concentration is too high, that needs an extra step after physical adsorbents treatment.

Since 1998, membranes have been applied for siloxane removal. Initial efforts showed between 80% to 90% of total siloxane removal using dense polymeric membrane materials¹⁰² or ceramic membranes¹⁰³. Compounds such as hydrogen sulphide, oil vapours and particles can damage membrane selectivity and physically tear it. Then, reports agreed that the design cost of a specific membrane resistant to harmful biogas compounds¹⁴ (*i.e.*, H₂S, dust, VOCs) was too costly to be applied outside the laboratory setting. Accordingly, further efforts were focused on maintaining the membrane selectivity and ensuring a high chemical resistance. For example, in the 2000s, a selective membrane was developed that retained almost 98% of D4 and D5 siloxanes¹⁰⁰. Despite that, this membrane had some methane losses, and it was not tested outside the lab; it was fully permeable to water and general biogas pollutants. Then, methane losses, selectivity, and biogas management became critical factors in the design and use of biogas cleaning membranes.

Biogas compression prior to membrane treatment is a common approach to control these factors. This extra step releases the membrane from the burden of the constant flow and assures that the separation only depends on the membrane specificity and capacity⁴¹. Using compressed biogas and elastomeric membranes, studies^{41,104} report good siloxanes permeability and methane selectivity. Nonetheless, the working pressure increase does not significantly affect the other pollutants present in the biogas. Trace substances such as BTEX, halogens, hydrocarbons and VOSiCs still damage the membranes and reduce the cleaning efficiency. These molecules remain on the membrane surface, blocking the internal pores, producing fouling effects, and irreversibly damaging the membrane⁷. Although some membranes could deal with BTEX substances, H₂S, water, and other contaminants, most are privatively expensive and cannot remove siloxanes efficiently¹⁰⁴. These special membranes are so expensive to use outside the lab that their scale-up and industrial application are theoretical. Then, despite the cost, biogas cleaning employs membranes only to fulfil particular requirements or as ultimate measure^{23,55}.

Membrane physical adsorption also requires compressors, vacuum pumps, electric power, and specialised personnel, increasing the treatment costs^{15,55}. In general, membrane adsorption as siloxanes abatement technique have higher CAPEX and same OPEX than the usual AC adsorption⁵³; however, it has less removal efficiency (60%). Membrane adsorption as a cleaning method in a typical WWTP (100 to 400 Nm³ h⁻¹) represent CAPEX around 2500 € per Nm³ h⁻¹, which can be increased to 6000 € per Nm³ h⁻¹ when siloxanes are present³⁴. In contrast, OPEX is highly variable due to the membrane replacement, maintenance of the units, energy used (0.2 - 0.38 kWh per Nm³) and operation man-hours^{34,63}. Finally, membrane separation has higher maintenance expenses (3-4% more) than the usual physicochemical methods⁶³ due to usual fouled membranes replacement and machinery maintenance.

Due to the costs and membranes limitations, multistage systems (different membranes in a serial arrangement) are currently used to treat biogas and remove siloxane. In theory, this approach gives a better cleaning efficiency without the membrane selectivity issues and more affordable CAPEX and OPEX than traditional membrane methods³¹. Nonetheless, R&D behind membrane creation is complex and, in many cases, is only applicable to laboratory scales because the membrane saturation, methane losses, and membrane clogging problems usually appear only in the scale-up stages^{15,23}. For example, mixed matrix membranes have

been developed as an alternative for biogas upgrading. Gas-liquid adsorption membranes are mixed systems where the membrane is regenerated by a counter-current liquid phase maintaining the pore availability and ensuring a good membrane selectivity^{105,106}. This approach is still in the early stages; however, initial efforts have shown a VOSiCs removal of 92.5% from synthetic biogas using a condensing-liquid membrane in lab conditions¹⁰⁷. In conclusion, the physical adsorption with membranes as a biogas cleaning method is only promising if the scale-up costs are affordable and commercially available.

Membrane separation is a promising technique that uses the high molecular differences between VOSiCs and methane to achieve a full biogas upgrade^{11,13,23,53}. Further development and research could derive into creating a membrane that can upgrade biogas to a high-quality biomethane in a single step, as it was planned at the beginning^{2,41}. Research areas such as nanotechnology⁶³ and multi-stage membrane systems¹⁰⁸ seem to present the most promising upgrading results, especially for siloxane removal^{109,110} at more affordable costs¹¹¹. Still, the current state of the membrane separation technology is focused on solving the technical issues with the existing methods¹¹¹. Therefore, full-scale siloxane removal membrane systems seem far from the current industrial scope.

B. Chemical methods

Techniques based on chemical phenomena constitute the second major technology used for siloxane removal. Chemical methods are usually understood as chemical reactions between the biogas pollutants (*i.e.*, siloxanes, H₂S, VOCs) with known reactants in a scrubbing arrangement. However, these methods also include technologies that use reagents where the pollutants are taken up into the matrix without changing their chemical form (chemical absorption). One of the main differences from physical methods is that siloxanes cannot be easily retrieved into initial molecular forms due to the reactive behaviour of these methods; therefore, the regeneration of the matrix or the reagent is usually unfeasible. Finally, chemical methods are usually coupled with other technologies (*e.g.*, physical, biological) and used for biogas cleaning or upgrading, according to the pollutant concentrations and final biogas/biomethane requirements.

1. Chemical adsorption

During chemical absorption, the target compound (sorbate) undergoes a chemical interaction with a liquid medium (sorbent), where it is selectively retained. Chemical adsorption is driven by electrostatic interactions such as hydrogen bonding, ionic bonding, and dipole-dipole interactions between the sorbent and sorbate. The most referenced chemical absorption approach is biogas scrubbing, where water or other chemicals (*e.g.*, amine, organic solvent, mineral oil) are used to perform an initial biogas cleaning. In water scrubbing, the hydrophilic compounds (*e.g.*, H₂S, CO₂, polar compounds) are retained when the biogas is bubbled in a counter-current flow^{112,113}. Water scrubbing is used extensively by biogas upgrading facilities (around 41% of all biogas installations use it¹¹⁴). Nonetheless, due to their non-polarity, siloxanes are not effectively removed by water or other polar solvents (*e.g.*, methanol, ethanol)^{7,23,41}. For example, L2, D3, and D4 siloxanes removal was tested using high-pressure water scrubbing, achieving less than 52% removal in the best-case scenario¹¹⁵.

Also, several non-polar or slightly polar solvents (*e.g.*, hexane, acetone, iso-octane, hexadecane, amines) have been tested without robust results^{116,117}.

Siloxanes chemical adsorption goes beyond the mere polarity. Mineral^{102,118} and PDMS¹¹⁹ oils did not show good siloxane recovery from biogas; instead, they yielded methane losses and combustion engine damage due to the oil aerosols formation. If well, water scrubbing is less efficient than chemical scrubbing, it produces less recalcitrant waste, it is easy to implement and cheaper than chemical options⁹². For example, biogas water scrubbing reduces around 10%, 15%, and 15% of CO₂, H₂S, and NH₃, respectively, while amines can reduce H₂S and NH₃ up to 30% at atmospheric pressure^{31,113,115}. Chemical scrubbing is a good option to remove general siloxanes ($\approx 95\%$ reduction)^{7,14,23}; nonetheless, regeneration requirements are more time consuming, expensive and demand a significant energetic input to recover the matrix. Here, acid or alkali solutions are used to cleave the siloxane bonds, thereby generating secondary compounds that are toxic or more recalcitrant.

In general, chemical absorption techniques involve destructive removal, which may be advantageous because they transform VOSiCs into innocuous forms by cleaving the Si-C and Si-O bonds. However, the nucleophilic reaction that attacks the siloxane bond is highly endergonic; therefore, high temperatures are needed during treatment. These additional energy input results in higher processing costs than conventional chemical scrubbing approaches¹²⁰. Moreover, despite their efficiency, alkali solutions (*i.e.*, sodium hydroxide) have been banned for biogas cleaning because they react with methyl groups yielding carbonate precipitates that physically damage the reaction units and other machinery^{7,64,121}. Then, acid-based absorption may be more feasible. Strong acids, such as sulfuric acid (H₂SO₄), achieves 90% siloxane destruction at 60°C⁶⁴, while nitric and phosphoric acids have achieved removal efficiencies as high as 95% at 60°C¹²⁰. By comparison, H₂SO₄ is more effective for siloxane removal; however, acid traces can reach the engine and cause corrosion^{7,13}. Nitric acid is also corrosive; however, it is a more suitable option since iron vessels are protected from corrosion by the chemical passivation^{7,13,120}.

Despite their effectiveness, these destructive acid/base approaches have been framed as non-viable options¹²² because they produce toxic wastes^{2,55} that require further treatment and related expenses for the WWTP. These elements represent a safety concern to the plant personnel and system operators that must have special training to handle these reactants and wastes⁷. Accordingly, acid treatment has few real-world applications due to high reagent costs, time and energy requirements, and the possibility of causing damage to the machinery, among others^{7,123}. Also, the general requirement of elevated temperatures and pressures is another drawback of chemical absorption techniques. These high temperature/pressure conditions are needed to promote the dissolution of VOSiCs into the sorbent and assure their partitioning^{31,90}. Usually, the necessary conditions only can be achieved by using specialized heaters, diffusers, and pressurization equipment at the gas stripping column (*i.e.*, scrubbers)^{14,41}. Then, all together, the treatment of the residues (*e.g.*, saturated non-regenerable reactants, solvents, water)^{34,55} and the pressure/heating requirements decrease the economic viability by increasing the CAPEX/OPEX⁵⁷.

The CAPEX/OPEX of chemical absorption depends on the chemical compound, scrubbers, electrical consumption, and regeneration requirements. For example, CAPEX is 1400€ to

3400€ per $\text{Nm}^{-3} \text{ h}^{-1}$ for amines, 1200€ to 4800€ per $\text{Nm}^{-3} \text{ h}^{-1}$ for organic solvents, and 1200€ to 2500€ per $\text{Nm}^{-3} \text{ h}^{-1}$ for water scrubbers^{13,63}. OPEX could be high as 20% of the initial CAPEX depending on the load and characteristics of the pollutant^{13,63}. Also, chemical adsorption cannot guarantee sufficient removal of all the biogas contaminants; instead, it must be coupled with physical adsorption, cryogenic separation, or membrane separation to assure the optimum biogas cleaning^{7,64}. Consequently, chemical absorption will most likely remain restricted to high purity biomethane applications or be used as a rough cleaning side method.

Several examples of siloxane removal using chemical adsorption at the industrial level can be found in the literature, most of them using it as a polishing method or initial pre-cleaning step (*e.g.*, water scrubbing). Köhler and Ziegler Anlagentechnik (Germany) company uses a gas scrubber as an intermediate treatment to remove siloxanes¹⁴. The raw biogas is refrigerated then delivered to the exchange column in a counter-current flow with hydrocarbon mid-polar and non-polar solutions. Then, the partially siloxane-free biogas is further dried and passed through activated carbon contactors to finish the cleaning¹²⁴. Another example of industrial-scale chemical scrubbing is the Selexol™ application on adsorption columns^{7,13,34,57,125}. This polyethylene glycol mixture retains 99% of all volatile siloxanes, CO_2 , and H_2S as a whole at lab- and pilot-scale using it as polishing step^{7,57}. Selexol™ is being used currently by biogas plants such as the Mountain Gate Landfill in Los Angeles (USA) or the Dortmund-Huckarde plant in Germany^{14,96}. Yet, the system has several drawbacks, including 1) the inability to deal with high siloxane concentrations, 2) the inability to handle high temperatures^{66,92}, 3) diminished efficiency over time and with repeated regeneration cycles, and 4) the production of environmentally toxic waste during regeneration³³. Accordingly, Selexol™ users have reverted to the less expensive and well-known physical adsorbents^{7,92} or coupled its use with other cleaning technologies to compensate for its deficiencies⁵⁷. In conclusion, chemical absorbents have several drawbacks in siloxanes removal that must be overcome to qualify for broader application despite some initial positive results.

Current chemical absorption research is focused on: liquid-reagent regeneration, environmental impact reduction, performance enhancement, and recycling the expended reagents^{2,34}. Among the general approaches, developing new highly efficient synthetic solvents that are easier to regenerate is the most promising nowadays; however, further research is still needed to scale up^{126,127}. Recent investigations have focused on developing ionic liquids (*e.g.*, eutectic solvents) that exhibit better efficiencies, siloxane removal rates, stream-lined regeneration cycles, high CO_2/VOC affinities, and increased biodegradabilities^{128,129}. For example, ionic eutectic solvents have been tested to remove the most common biogas VOSiCs (*i.e.*, L3-L5, D3-D5)^{130,131}. Experimental reports showed that tetrapropylammonium bromide - tetraethylene glycol mixture (TPABr:TEG) removes 99% of biogas-VOSiCs at more affordable CAPEX than now¹³¹. Finally, despite the new investigations and interest in siloxane removal by chemical absorption, this technology only represents between 10 and 30% of the total biogas market⁶³, mainly because there is a strong possibility of producing environmental damages related to bad practices and improper waste disposal. Then, as with most physicochemical methods, the environmental toxicity should be mandatorily addressed in further investigations.

2. Catalysis

Catalysis is another recent chemical approach to remove biogas-VOSiCs^{7,13,23}. Catalysts were originally designed to reduce H₂S concentration in biogas, known as doped activated carbon or catalytic carbon¹³². The complexity of integrating physical adsorption with a controlled-chemical reaction makes it difficult to implement for siloxanes. VOSiC conversion requires high activation energy at high temperatures to cleave the Si-O and Si-C bonds, damaging the active sites and catalyst matrices. Since the 1999S, with the description of the PDMS abiotic degradation in soils^{133–135}, there has been an exhaustive search for materials that contain natural acid and alkali active sites capable of cleaving the Si-O bonds. Catalyst developers took this as a model, using acid phases such as alumina, zeolites, or basic oxides (*i.e.*, CaO, MgO) to assure the conversion of siloxanes¹³⁶. However, reports show that the catalytic process suffers from these main limitations 1) the reactions are highly endergonic (>400°C), 2) mass transfer (gas-to-catalyst) during acid catalysis is inefficient, 3) catalysts can be poisoned by hydrogen sulphur (H₂S) presence in biogas¹³⁷, and 4) carbonate is formed in the presence of CO₂ during alkali catalysis¹³⁶. Given these challenges, expenses associated with catalysis will be generally higher than traditional technologies. In terms of costs, catalysis, in general, is an expensive approach; carbon-based heterogeneous catalyst OPEX ranges from 3 to 120€ per m³ h⁻¹¹³², being higher than physical or chemical absorption.

Alumina and zeolite catalysts are the most developed in siloxane removal; they have natural Lewis acid (*e.g.*, Al⁺³) active sites that can open the VOSiCs heterocyclic ring and allow the Si-O/Si-C bonds at endothermic conditions^{66,123}. Also, some zeolites can withstand catalyst poisoning with small amounts of H₂S¹³⁸. These materials can partially catalyse H₂S oxidation while electrochemical interactions adsorb the VOSiCs¹³⁹, showing an essential advantage against other materials⁶⁶. On mass-transfer issues, current research has developed AC-supported heterogeneous catalysts with chromium (VI) and copper (II) as the active catalytic phase that seem to have better results than traditional catalysis⁷⁴. Despite promising results, environmental impacts associated with the disposal of the expended catalysts⁷⁴ is a critical drawback. Therefore, research involving molten carbonate cells¹²³ has been developed, yet this approach found that siloxanes remain affixed in the active sites, poisoning the catalyst and decreasing its efficiency.

More recently, synthetic zeolites have been tested in the catalytic conversion and removal of D4 from biogas⁶⁶. Despite the promising results, limitations such as lack of specificity, limited loading capacity, demanding regeneration requirements, and siloxane polymerisation reactions with the sorbent, make this approach unprofitable during long-term operation⁷⁸. New research has focused on siloxane polymerisation and its irreversible attachment to the support phase using novel approaches^{2,31,66}. Among these approaches, photocatalysis tests showed 98% degradation of D4 siloxane using woven paper coated with TiO₂ PC-500¹⁴⁰. Nonetheless, after the first 72h of operation, siloxane polymerisation decreased 30% the efficiency due to an irreversible siloxane/silica polymers deposition on the matrix¹⁴⁰. Catalysis is not feasible for VOSiC treatment in biogas nowadays. Siloxanes polymerisation, economic expenses, catalysis poisoning, among others, refrain its industrial use. Since this is a novel approach, research is looking for new support materials and compartmentalising the process to protect the catalyst, using it only as a polishing step after initial biogas cleaning¹²³.

C. Other physicochemical methods

Due to the limitations of the classical physicochemical methods, the lack of specificity, regeneration issues, non-sustainability, and environmental impacts of the produced wastes, siloxanes research has focused on developing other techniques. These approaches have focused on preventing VOSiC release during the AD process or applied chemical treatment while the VOSiCs are still attached to the biosolids. However, these approaches have not been scale-up or tested outside laboratory conditions.

1. Pre-oxidation

There is only one report on chemical pre-oxidation on siloxane treatment; using peroxidation (*i.e.*, peroxymonosulphate, dimethyldioxirane), the degradation of the biofilm exopolysaccharides (EPS) from sludge was tested¹⁴¹. It is known that siloxanes are retained in the EPS by electrostatic interactions, then results showed that this approach transforms volatile siloxanes into silica and lower molecular silanes with 40% to 80% of efficiency¹⁴¹. Process efficiency was directly correlated with the oxidation agent, reactant concentration, and process temperature. Lab results show that using Fenton oxidation with dimethyldioxirane; siloxanes decreased by more than 80% in the sludge with proportional reductions in the biogas. Despite this initial success in the laboratory, applying the same treatment to an industrial scale sludge is very challenging due to the reagents cost and the extra infrastructure required for this.

2. Pre-aeration on AD sludge

Physicochemical methods drawbacks are mainly related to matrix regeneration or disposal. Therefore, a method that does not require regeneration would be ideal for removing VOSiCs from AD sludge. Considering that siloxanes are attached to the organic matter by electrostatic forces and are highly volatile, promoting their desorption *prior* to digestion could be a suitable option to prevent siloxane biogas contamination. Pre-digestion techniques have also been tested for methane upgrading, including gas purging, thermal treatment, hydrothermal liquefaction, and enzyme addition. Gas purging can detach the volatile siloxane molecules from the AD sludge with relatively low costs³⁸. During lab tests, 40% less D5 was found in AD biogas using an airflow of 0.15 L m^{-1} and a retention time of 96h¹⁴². Moreover, given the electrostatic interactions of VOSiC with sludge, combining thermal treatment with gas stripping, the siloxane desorption is enhanced^{20,143}.

Accordingly, reports show that the conditions of $\sim 80^\circ\text{C}$, 0.5 L m^{-1} of air, and 48h of retention time, are sufficient to decrease the biogas-VOSiC concentration by 90%, without regenerating the matrices or using expensive treatment units^{143,144}. Then sludge pre-treatment prevents VOSiC contamination of biogas, enhances AD hydrolysis rates^{145,146}, and decreases the concentration of inhibitors such as ammonium or sulphate⁵⁷. Despite the effectiveness of gas stripping, this approach has critical drawbacks; desorbed VOSiCs must be trapped and treated before being released into the atmosphere. If these siloxanes are not treated, they would severely impact human health and the environment³⁸. Additionally, while gas stripping is relatively effective in removing VOSiCs due to their volatility, other siloxanes such as linear oligomers, non-volatile cyclic siloxanes, and PDMS are not effectively

removed by this technique and remain attached to the AD sludge. Siloxane polymers in the sludge could be transformed to VOSiCs by microbial metabolism¹², contaminating the AD biogas and other WWTP stages. In conclusion, despite the positive lab results, high efficiency, easy application, and low costs, the environmental impacts caused by releasing siloxanes into the atmosphere are too high.

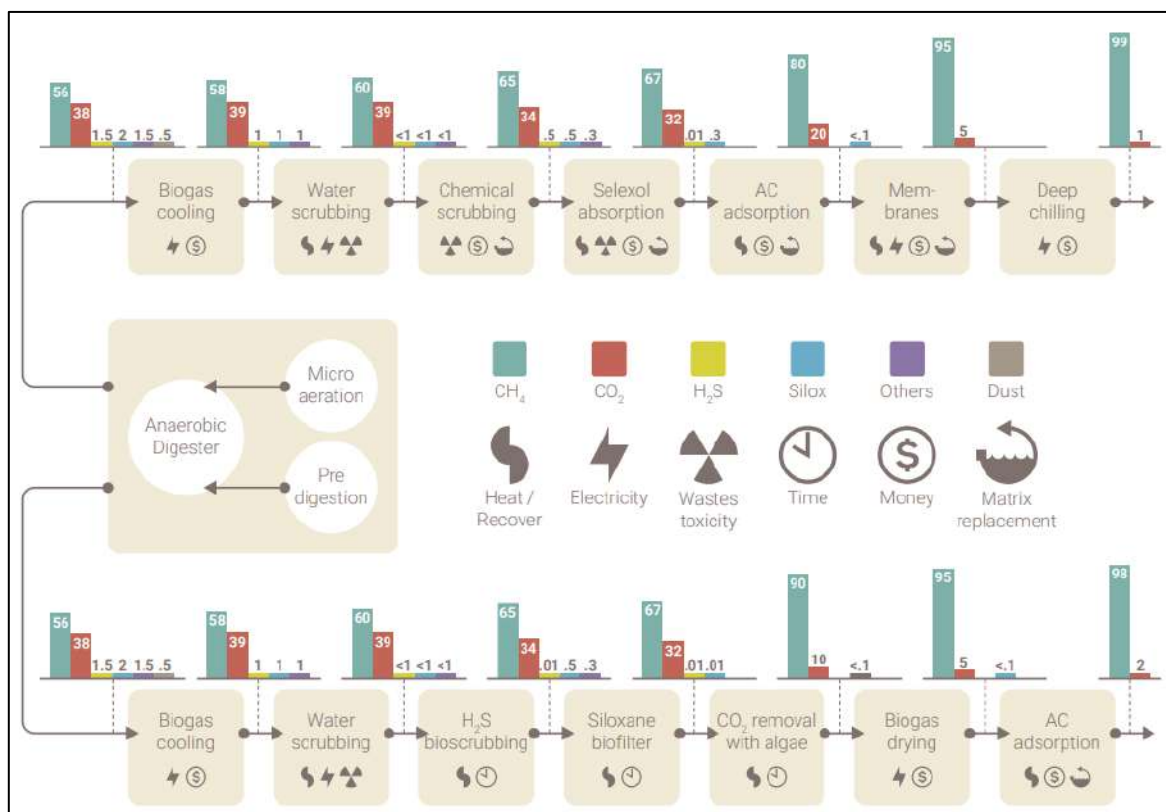


Figure 4. Theoretical comparison of biogas treatment units using either, physicochemical methods alone (above) or a combination of physicochemical and biologically based methods (below). Theoretical comparison of biogas treatment units using either, physicochemical methods alone (above) or a combination of physicochemical and biologically based methods (below). Icons represent the main drawbacks of each technology. Bar chart stands for the biogas quality pre and post each treatment unit. Main biogas components are represented by colours on the bar chart.

D. Biological

PDMS and VOSiCs biodegradation is a matter of discussion in current research because of the traditional belief that siloxanes are not biodegradable^{2,7,63,132}. In general, literature involving biological treatment has focused on VOSiC removal (*e.g.*, D3, D4, D5)¹⁴⁷ identifying major obstacles such as the recalcitrant nature of VOSiCs (stemming from their steric hindrances)⁴⁶, the high energy required for bond cleavage¹⁴, and mass transfer issues¹⁴⁸ resulting from their volatility. Considering that microbes are the agents of VOSiC biodegradation, biological technologies require a suitable microbial consortium capable of catabolising these kinds of compounds. Such capabilities of microbiomes have been tested

under aerobic, microaerated, anaerobic, and anoxic conditions to determine the optimal electron acceptor and operating conditions for siloxane degradation.

The microbiomes found in anaerobic digesters are well known for their resilience, microbial diversity, and metabolic flexibility (*e.g.*, mutualism, syntrophy). Accordingly, most biological treatment approaches use anaerobic sludge as inoculum¹⁴⁹ due to its potential to catabolise recalcitrant compounds such as siloxanes. Then, several studies have tried directed evolution to promote the proliferation of siloxane-degrading microbes selectively in combination with different electron acceptors¹⁴. Directed evolution uses siloxanes as the primary carbon source, ensuring that only microbes capable of breaking Si-O and Si-C bonds are present in the media¹⁵⁰. However, despite multiple attempts, no biologically based techniques have been successful in the VOSiCs complete removal. In general, the lack of knowledge on the microbial species and their specific metabolisms, together with siloxane volatility and unidentifiable siloxane structural conformations, has hindered the progress of biological treatment.

1. Aerobic conditions

Biological approaches can be compared with a heterogeneous catalyst because microbes (active phase) are supported on a biofilm or physical structure (supported phase) that behaves similarly to a catalyst. Therefore, scientists have used this model to verify the existence of an active phase (microbe) capable of catalysing siloxanes conversion into innocuous products. Early biodegradation studies used siloxanes (*e.g.*, oligomers, polymers, VOSiCs) as a co-substrate or dosed cultures with known amounts of siloxanes to assess their conversion or disappearance. These efforts used conventional conditions such as mesophilic temperatures, pure cultures, and aerobic regimes^{150,151}, yielding the identification of different microbes allegedly responsible for siloxane conversion. For example, *Pseudomonas* sp. strains (*P. putida* & *P. fluorescens*) from silicone-polluted soils were isolated and fed with siloxane polymers (*i.e.*, polydimethylsiloxane, oligoethosylsiloxane, and α - ω -polydimethylsiloxanediol), finding minor biodegradation¹⁵¹. Also, eukaryotic fungi *Phanerochaete chrysosporium*, *P. sordida*, *Aspergillus sydowii* were tested for siloxane polymer degradation as a co-substrate of a glucose/sucrose mixture¹⁵⁰. In both cases, biodegradation of siloxane polymers was suggested as plausible but not conclusively verified.

These two examples used siloxane polymers as co-substrates or in a mixture. This approach¹⁵¹ makes it difficult to correctly identify which one of the silicone oils within the mixture was biodegraded. Unfortunately, this knowledge is crucial since the involved oils have different chemical conformations, steric hindrances, and terminal functional groups, where the diol terminals present less steric hindrances and better nucleophilic attack points for the microbial enzymes. On the other hand, the use of eukaryotic fungi¹⁵⁰ may not be applicable outside the lab due to the engineering challenges associated with growing filamentous fungi at full-scale, such as the complex rheology¹⁵², enzyme production¹⁵³, and the difficulties of feeding siloxane-polluted matrixes (*e.g.*, WWTP sludge, soils, biogas) to the system. In sum, these lacks, and challenges may explain why any of these reports were not further explored or upscaled. Despite the initial reports uncertainties, they justified further exploring the siloxane biological treatment as possible^{150,154–158}.

Other studies went further by applying biotrickling filters to remove VOSiCs. This approach offered several clear advantages, including the direct treatment of siloxanes in biogas streams, the immobilization of the microbial biomass and the reduction of the treatment residence time. An initial comparison between aerobic and anaerobic biotrickling filters showed that biodegradation of VOSiCs was 30% more effective in aerobic conditions than anaerobic conditions¹⁴⁸. Then crucial upscaling factors were identified, including establishing a resilient/flexible microbial population, ensuring sufficient enzyme availability, and augmenting siloxane mass transfer (from the gas phase to microbial phase)¹⁴⁸. Also, biotrickling filters make easier the extraction and identification of the key microbes in the siloxanes biodegradation. The first report on microbial identification revealed that *Rhodanobacter* sp., *Zooglea* sp., *Mesorhizobium* sp., *Xanthomodacea* sp., and *Pseudomonas* sp. derived from the AD sludge inoculum might be responsible for D4 biodegradation (20% in 79d - batch) in biotrickling conditions¹⁵⁹. This report showed the intricate microbial network behind siloxane biodegradation, directing future research towards identifying the active phase (same as with catalyst) and its enzymatic potential. Consequently, further studies have looked for an efficient microbial inoculum microbially diverse and where the supported phase (*i.e.*, biofilm) resolves the VOSiCs mass transfer issue between the gas and microbial phase.

Siloxane biodegradation studies that include microbial identification are scarce. Most recognised and referenced reports^{50,160} developed aerobic biotrickling filters with pure culture microbes for D4 biodegradation. From those studies, we know that biofilm formation seems crucial to overcome the VOSiCs gas/liquid mass transfer limitations. The exopolysaccharides (*e.g.*, rhamnolipids) produced during the microbial (*i.e.*, *Pseudomonas aeruginosa* S240⁵⁰, *Phyllobacterium myrsinacearum*¹⁶⁰) growth can retain siloxanes and make them available for biodegradation. Nonetheless, operational factors such as pH, inlet VOSiC concentration, micronutrients, residence time, and liquid flow rate influence the VOSiCs microbial catabolism¹⁶⁰. Once the microbes have access to siloxanes, the VOSiCs biodegradation takes place under aerobic conditions. It is proposed that D4 siloxanes produce methanol from their aerobic biodegradation after microbes perform a direct cleavage of the Si-O bonds in the siloxane ring⁵⁰. Other studies suggest a stepwise mechanism for the same molecule biodegradation, beginning with the initial oxidation of the siloxane methyl substituents followed by siloxane ring cleavage to overcome the Si-CH₃ steric hindrance¹⁶⁰.

A deeper analysis of the proposed D4 biodegradation mechanisms^{50,160} makes it clear that both theories are mechanistically different. It was previously reported that the main products of VOSiC metabolism are dimethylsilanediols (DMSD), silicic acid, water, and CO₂ generated by the progressive oxidation of the siloxane molecule¹⁴. Also, it is known that siloxanes have a steric hindrance produced by the methyl groups attached to the silicon molecule. These methyl groups create a shielding effect on the Si-O bond, thereby protecting it from enzymatic catalysis^{46,161,162}. Consequently, it seems that proposing a direct cleavage of the siloxane ring⁵⁰ passes the D4 steric hindrances over and do not consider the formation of the usually reported metabolic products. Therefore, a more accurate biodegradation pathway should include the initial oxidation of the methyl substituents to overcome the steric hindrance. In such a case, microbes may oxidise the outer methyl groups⁴⁶, thereby enabling silicase-type enzymes to reach the heterocyclic ring, which cleaves it and oxidises the

molecule to silanol-type compounds^{14,160}. Then VOSiC biodegradation results in less volatile molecules (*e.g.*, silanols, silanediols, DMSD)¹⁶³, promoting VOSiCs removal from biogas.

One of the main advantages of the biological methods to clean biogas is the possibility to remove more than one pollutant from it. There are opposite positions for siloxanes and other pollutants removal on this behalf. For example, D5 and H₂S removal by biotrickling filters were tested using activated sludge as inocula¹⁸. Results suggest that protons released by *Acidothiobacillus* sp. during H₂S oxidation are used by siloxane-degrading microbes (not identified) to achieve the D5 conversion to dimethyl-D3 siloxane in an apparent syntrophic relationship¹⁸. Although such a syntrophic relationship was not confirmed, the report concludes that chemical adsorption is the governing mechanism for D5 removal since the siloxane biodegradation is negligible (20%).

On the contrary, using the same approach to remove L2, L3, D4, and D5 siloxanes in a different study¹⁶⁴, results prove the possibility to metabolise siloxanes and H₂S at the same time. Biotrickling filters initially inoculated with AD sludge showed a microbial community change after several cycles, becoming more enriched with *Acidothiobacillaceae*-related species, *Reynarella* sp., and *Chitinophaga* sp.; which might be responsible for the siloxane and H₂S catabolism¹⁶⁴. Also, silicone oil as an organic phase partially resolved the mass transfer issues between the gas and microbial phases, showing an improvement of 70% more siloxanes removed from the biogas¹⁶⁴. Both examples show that complex microbiomes such as those present in AD sludge can mineralise siloxanes and other molecules. Therefore, it is critical to identify the microbes involved in the degradation processes, delineate their ecological relationships, and determine the optimal growth conditions.

Finally, biological approaches do not produce other recalcitrant wastes during treatment, representing a significant advantage over physicochemical methods. Microbes produce organic metabolites, such as silanols and silanes, from VOSiC catalysis, which are environmentally innocuous. For example, silanols are hydrophilic and non-volatile, making them easy to remove from the stabilized sludge using solvents or adsorbents¹⁶⁵. Even if the silanols are not treated, they do not represent a potential risk to aquatic or benthic biota¹⁴. Although biological siloxane removal is an up-and-coming field, VOSiCs are only biodegradable under the specific conditions, where it is apparent that having oxygen as a final electron acceptor makes the difference in efficiency terms²³. Nonetheless, precursors such as PDMS or linear oligomers have not been adequately studied, and there are no reports or efforts to scale-up biological siloxane removal technologies for biogas applications.

2. Anaerobic conditions

The first investigations that tested siloxane degradation under anaerobic conditions found that siloxane polymers were not biodegradable¹⁶⁶; however, VOSiCs (*i.e.*, D4) could be microbially catalysed after long retention times¹⁶⁷. These long retention times in VOSiCs biodegradation are expected; under anaerobic conditions, microbes need to overcome the lack of free energy (energetic final electron acceptor) by ecological means such as syntrophy, mutualism, and other ecological relationships¹⁶⁸. Then, even though AD microbiota is highly adaptable, resilient, and metabolically diverse, the extra energy is still required to overcome the high bond energies of Si-O and Si-C bonds to yield VOSiCs biodegradation¹⁴. Since AD

microbiomes typically lack strong electron acceptors, studies have tested the effects of supplementing fumarate, succinate, iron ions, nitrate, and sulphate compounds to enhance D4 metabolism¹⁶⁹. Among these alternative electron acceptors, 5% m/v (from total culture volume) nitrate promoted a 16.3% reduction in D4 after 11 days proving that long retention times could be avoided under the right operative conditions¹⁶⁹.

AD is a critical stage for siloxane removal from water lines during secondary wastewater treatment, achieving removal rates of 93% by the siloxane adsorption onto the AD sludge³⁶. Real-world siloxanes in the WWTP are not only VOSiCs; siloxane polymers are also present in the wastewater treatment trial. Nonetheless, initial reports pointed out that PDMS cannot be biodegraded¹⁶⁷. This assumption was partially rebutted using a biochemical methane potential (BMP) assay testing the PDMS biodegradability with Luria broth medium as a co-substrate¹⁵⁵. Results showed that PDMS enhances methane production on biogas due to its surfactant activity¹⁵⁵; however, the lack of final metabolites identification on the BMP post-digestion sludge strained the study findings. Then the contribution of PDMS biodegradation in the overall scheme of siloxane degradation (from polymers to oligomers) was discarded, leading to a lack of interest in this topic due to early misconceptions and ambiguous results about PDMS biodegradability. Nevertheless, the AD stage not only can remove siloxanes from water lines. It also gathers and traps all kinds of siloxanes serving as an environmental release barrier and, accordingly, the point where treatments should be targeted.

In 2018, D4 and D5 were recognised as emergent environmental contaminants^{26,170}; this incentivised the VOSiCs removal research and led to an increase of publications from 2018. These reports helped curate most of the previous knowledge, challenges, and experiences; then, the biological treatment techniques were slowly improved^{6,50,159,169,171}. For example, the combination of physical and biological methods yielded better removal rates in tested biogas. Dual-stage systems such as biotrickling filters followed by activated carbon cartridges increased VOSiCs removal compared to each system operating alone¹⁷¹. Despite the apparent efficiency, the complexity of these systems opens the question of whether siloxanes were degraded biologically or just trapped by the physical adsorbent. These questions are crucial for assessing the industrial feasibility of dual-stage systems and evaluating critical operating parameters such as waste production, regeneration, and maintenance. Considering the uncertainties of these complex systems, recently, anaerobic VOSiC treatment research has focused on developing single-stage strategies. Pioneer approaches such as using nitrate as a final electron acceptor⁴⁸, microbial co-cultures to promote biofilm development⁸³, and microbes supported on activated carbon to improve VOSiC mass transfer⁶ obtained promising removal results. These works exploited the lessons learned from previous investigations^{50,159,169} proving that the combination of several techniques is required to overcome the siloxanes removal particular challenges and ensure their sustainable treatment.

Among the recent investigations, the use of adsorbents as microbes support matrices has reached particular interest due to the possibility of enhancing removal rates, extending adsorbent lifetime, and treating siloxanes sustainably. The previously reported¹⁷¹ idea of a biotrickling filter prior to AC treatment in a dual-stage system was recently tested again to remove siloxanes and VOCs (*i.e.*, limonene, toluene, hexane) in synthetic biogas streams⁸³. Results showed that D4-D5 removal efficiency was not improved due to the VOCs

competitive adsorption in the AC matrix. Nonetheless, it seems that the biotrickling filter confers a protective effect for the AC filters, increasing their lifetime, reducing their CAPEX/OPEX, and improving the removal efficiency of more generic pollutants (*i.e.*, limonene, toluene, hexane)⁸³. Other recent approaches have tested anoxic conditions in biofilters to remove biogas VOSiCs. For example, a microbial inoculum from a D4-saturated biofilter was tested using nitrate as a final electron acceptor⁴⁷. This study identified new microbial species capable of moderate siloxane biodegradation (53% removal) and concluded that *Methylibium* sp. and *Pseudomonas* sp. used separately instead of co-cultured yield better D4 biodegradation rates⁴⁷. Despite the moderate results, this study exemplifies that basic science, microbial screening, and microbial enzymatic capacities should be studied deeper towards improving the VOSiCs biodegradation.

Biological approaches in VOSiC removal from biogas have vast future potential. Among different advantages, the possibility for simultaneous removal of other pollutants (*e.g.*, H₂S) could make biogas upgrading easier, more cost-effective, and sustainable^{15,23,132}. Then, unlike physicochemical methods, biological methods do not generate recalcitrant by-products, nor do they pose safety problems to plant machinery, personnel, and the environment^{7,172}. Also, the lower CAPEX/OPEX costs, fewer energetic requirements, and less waste production make biological approaches a more attractive topic for further research and development⁵³. Nonetheless, biological cleaning methods still must overcome the problems of mass transfer limitations¹⁴ and general less efficiency ($\pm 40\%$ lower) compared to physicochemical approaches. Still, promising efforts have been developed on this behalf; microbes have the tools to partially overcome mass transfer issues, using biofilms whose extensive exopolysaccharide matrix serves to trap biogas-VOSiCs^{50,160}. Accordingly, if all these issues can be addressed, the future of biological siloxane treatment looks promising^{23,173}. In conclusion, biological approaches fall somewhere between the most economical, the most sustainable, and robust approaches.

It is important to assess the coupling of aerobic treatments with anaerobic treatment to enhance microbial siloxane biodegradation, especially *via* the supplementation of more powerful electron acceptors. Controlled oxygen addition could be a viable option; however, it has not been thoroughly evaluated. Instead, nitrate, sulphate, and other co-substrates have been tested with unremarkable results. Noting the wealth of knowledge associated with classical approaches such as aerobic and anaerobic treatment, there is a relative dearth of knowledge regarding hybrid approaches. For instance, very little is known about the metabolic pathways, enzymes, and mechanisms involved in VOSiCs microbial catabolism in the presence of alternative electron acceptors. These topics should be the *foci* of further investigations since more powerful electron acceptors could yield better results. Finally, future technologies should not generate additional waste that could be toxic to humans or the environment, nor pose risks to biogas conversion systems. Therefore, the treatment of emergent contaminants, such as siloxanes, must be performed considering the environmental sustainability and ecological impact.

3. Microaerated conditions

Microaeration is a novel method gaining momentum in the last ten years. This technique was not widely studied for siloxane removal due to the long-held belief that even small amounts

of oxygen would be toxic to anaerobic microbiomes. Until 2017 microaeration was applied for the first time to enhance biological siloxane removal, without significant results on siloxane removal⁵⁵. In conventional microaeration, small doses of oxygen are added to the anaerobic system to promote the degradation of H₂S and other recalcitrant compounds *in situ* by providing oxygen as an electron acceptor for aero-tolerant and facultative microbes. A pioneer investigation recently used a hollow-fibre membrane bioreactor (HF-MBR) with anaerobic sludge to remove VOSiCs in biogas⁴⁸. In this report, nitrate was used initially as the final electron acceptor under anoxic conditions, but mid-way during the experiment, the authors switched to microaerobic conditions. Final siloxane removal was less than 21% without significant differences between anoxic or microaerobic conditions⁴⁸.

Despite the marginal difference, the sudden change from anoxic to microaerobic conditions could explain the low siloxane removal efficiency during microaeration. This abrupt change may have adversely affected the structure and stability of the microbial community, which in turn may have caused the diminished siloxane catabolism observed in the study. Reports and results in microaerated biodegradation of siloxanes are scarce and sometimes contradictory. PDMS biodegradation was recently tested under microaerobic conditions throughout a long-term incubation period¹². Results showed that oxygen changes the microbial ecology within the AD sludge, increasing the abundance of *Thauera*- and *Rhodococcus*-related phylotypes during microaeration treatments¹². Also, microaerated treatments showed additional methane production and PDMS biodegradation proved by the siloxane-related by-products identified by GC-MS. This report strongly suggests that PDMS is biodegradable under microaerobic conditions, contradicting past reports claiming PDMS is non-biodegradable. Finally, it re-opens the debate regarding applying oxygen within AD systems to enhance siloxane microbial metabolism.

Coping with siloxanes without targeted treatment

Despite the many treatment options, most small-scale WWTP cannot afford the CAPEX/OPEX of specialised siloxane-removal operations^{23,34}. Part of the problem is that small biogas plants experience greater waste heterogeneity than larger plants, resulting in more variable and infrequent VOSiC loads². Therefore, small biogas plants cannot rely on a single, generalised strategy; instead, they apply reactive strategies alongside diligent monitoring of siloxane-related damage⁷. Although this procedure involves a higher OPEX cost due to the more frequent oil changes, endoscopic inspections, valve examinations, combustion engine inspections, and general maintenance expenses, it is generally less expensive than the CAPEX associated with building a complete siloxane removal system^{7,41}. Still, this incident-oriented approach is more suitable for small biogas plants due to the infrequency of VOSiC exposures; however, it is not environmentally sustainable because it does not prevent VOSiCs release into the environment^{1,7}.

Siloxane treatment is a complex process that will always bear a relatively high cost; accordingly, another option could be developing and adopting more sustainable alternatives by the siloxanes production industry. Nonetheless, the industry devoted minimal effort to seek replacements for siloxanes^{1,2}. One of the few examples of seeking replacements is the current politics from the Danish Environmental Protection Agency, which is attempting to promote siloxane replacement by qualifying their specific applications as necessary or

unnecessary¹⁷⁴. Here, cosmetics applications were deemed necessary (mandatory siloxane use), while others, such as painting additives, cleaning agents, polish agents, and antifoams, were deemed unnecessary (non-mandatory siloxane use). Accordingly, it seems that siloxanes are still irreplaceable in personal care products where they are used as chemical stabilizers, emulsifying agents, anti-soiling agents, and solvents¹⁷⁴.

Sustainable alternatives to siloxanes do exist. PDMS used as an antifoam agent in washing, cleaning, and polishing products could be replaced with mineral oils (in washing and polishing products), paraffin/vegetable oils (in cleaning products), lipophilic tensides (in polishing products), and polyethylene glycol or polypropylene glycol (in cleaning and polishing products)¹⁷⁴. Many of these replacements are less expensive than polymeric siloxanes; however, at the same time, many suffer slight deficiencies and do not provide the same aesthetic qualities of siloxanes. Mineral oils and lipophilic tensides, for example, cannot produce the same level of dirt removal and floor shining as PDMS-based cleaning products¹⁷⁴. Then, this is a challenging path that requires further research in new materials and governmental or industrial cooperation. There may never be a perfect replacement for VOSiCs; however, this is a relatively underexplored field that deserves greater attention and effort before being written off.

Outlook

Exponential siloxane production and consumption growth indisputably impact daily human life, the economy, and the environment. The boom of siloxane production and its widespread use has led to a new type of environmental pollutant that is emitted faster than naturally degraded. This phenomenon has led to a worldwide dispersion of these emergent pollutants^{24,58}, impacting nearly every ecosystem on the planet. Moreover, the WWTP has been identified as the primary nexus between human siloxane use and environmental dispersal. At the same time, the accumulation and slow release of siloxanes at the wastewater treatment plant presents a unique opportunity for targeted treatment and environmental damage prevention developing in situ removal strategies. Nonetheless, knowledge regarding the transformation and fate of siloxanes throughout the WWTP would facilitate their control and treatment in the near future. Then, in such as case, WWTPs would no longer act as an agent of siloxane dispersal but would constitute a bulwark in protecting the environment.

Since 2006, siloxanes have been considered the most adverse components in biogas^{14,15,21}. Among all the various approaches developed for siloxane removal, only physical adsorption with activated carbon seems to fulfil most of the requirements (*i.e.*, economic, efficiency, and technical) to be used at an industrial scale. However, this approach is not without its disadvantages, especially regarding waste production from spent cartridges or saturated sorbents. Consequently, the development of new techniques or the improvement of the current physicochemical approaches is needed. Because biological methods are deemed more sustainable and less expensive, biologically based technologies for biogas cleaning have been gaining momentum in the anaerobic digestion field. Biological siloxane removal reached its peak attention in 2014 and only recently regained its interest among scientists due to its environmental advantages. Yet, the slow or poor biodegradation of siloxanes, the lack of knowledge on the involved microbes or their biochemical interactions, and the immaturity of

the developed studies have discouraged the advancement of biological treatment methods as a siloxane removal approach.

Although the devoted research efforts, the recalcitrance, environmental mobility, and massive production of siloxanes present a major challenge to developing any sustainable treatment solution. Siloxane and biogas pollutant removal are critical topics for most WWTPs worldwide; therefore, they must be assessed holistically as an integrated process, evaluating system inputs, outputs, energy consumption, requirements, waste production, and environmental performance. These future methods should not be expensive or require high amounts of energy, nor should they produce more waste and environmental impacts than the siloxanes themselves.

Conclusions and outlook first chapter

- Siloxanes possess a serious threat to the general ecological dynamics in the aquatic and soil environments due to their recalcitrant behaviour and bioaccumulation capacity.
- Most of the reports on siloxane biodegradation assure that siloxane polymers (PDMS) are not biodegradable and VOSiCs in the WWTP mainly come from direct discharges from human sources.
- Despite that microaeration has been used previously to treat recalcitrant compounds, it has not been thoroughly studied to improve siloxanes biodegradation.
- Siloxanes from human sources gather in the WWTPs where they are released into the atmosphere. Consequently, the strategies developed should be *in situ* applicable towards reducing their emission and environmental impact.
- Currently, the strategies to remove siloxanes are reactive and not preventive. This behaviour has led to the use of cleaning approaches that produce new wastes and do not solve finally the siloxanes environmental threat.

SECOND CHAPTER

Microaerobic conditions in bio-sludge promote changes to bacterial composition which favour the biodegradation of polymeric siloxanes

Abstract

Volatile organic silicon compounds (VOSiC) are one of the most adverse pollutants to the biota and ecological dynamics as well as biogas-based energy conversion systems. However, there is a lack of understanding regarding the source of VOSiCs in biogas, especially arising from the biochemical conversion of siloxane polymers such as polydimethylsiloxanes (PDMS). The biodegradation of PDMS was evaluated under anaerobic/microaerobic conditions (P_{O_2} = 0, 1, 3, 5 %), using wastewater treatment plant (WWTP) sludge as an inoculum and PDMS as a co-substrate (0, 50, 100, 500 ppm). On average, strictly anaerobic treatments produced significantly less methane than the 3 and 5% microaerated ones, which show PDMS biodegradation at 50 ppm. *Thauera* sp. and *Rhodococcus* sp. related phylotypes were identified as the most abundant bacterial groups in microaerated treatments, and siloxane-related molecules were identified as remnants of PDMS catabolism. Our study suggests that the presence of VOSiC (e.g., D4 – D6) in biogas is not only due to its direct input in wastewaters, but also to the PDMS microbial catabolism using the oxygen traces that enter the system when the anaerobic digesters are fed. Microaerobic conditions may enhance the VOSiC degradation within the digester and therefore reduce its biogas concentration. Also, an effective VOSiC treatment might allow WWTPs to become barriers for the emission of these ecotoxic contaminants to the environment.

Keywords: Siloxanes, Microaerated, Anaerobic digestion, biogas, biodegradation, volatile

Introduction

Anaerobic digestion (AD) is probably the most environmentally sustainable and cost-effective technology to manage sewage sludge in wastewater treatment plants (WWTP). It allows efficient organic matter stabilisation and energy production, which can be used on-site for the internal operations of the facility. AD takes place in the absence of oxygen, where organic matter is converted into biogas – a gas composed of methane, carbon dioxide, and water vapour. Depending on the characteristics of the influent waste stream, AD biogas can also contain trace concentrations of pollutants, notably hydrogen sulphide (H_2S) and volatile organic silicon compounds (VOSiC). Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5) cyclic siloxanes are the most common VOSiC present in the anaerobic digester's biogas^{1,2}. VOSiCs cause severe, usually irreversible damage to the internal components of the energy generation systems which convert biogas into secondary forms of energy^{3,4}. Specifically, silicate deposits, formed inside combustion engines, decrease the thermal conductivity and lubrication of components, clog the pistons and lines, reduce compression efficiency, and cause overheating issues⁵⁻⁷. For WWTPs, this results in millions of dollars in annual losses due to the need to invest in filters and technologies to remove VOSiCs from biogas. Siloxanes can increase operating costs up to €6 per 1,000 m³ of wastewater treated and add an extra €50,000 of annual expenses⁵.

PDMS, and particularly VOSiCs, were especially used in the '90s to replace or clean chlorofluorocarbons (CFC), hydrochlorofluorocarbons (HCFC), and parachlorobenzotrifluoride (PCBTF), due to – what appeared at the time – their "environmentally friendly" characteristics^{8–11}. Despite that the siloxane industrial use could be dated to 1940¹², they still are a critical component of personal care products. Siloxanes begin their journey to the WWTP after being rinsed down the drain by the consumer. Once there, siloxanes (as emergent contaminants) can be released to the atmosphere by different physicochemical and biological processes that transform non-volatile to volatile siloxanes^{13,14}. As well, siloxane-related compounds have been linked to a variety of health impacts on different animal models and possibly also in human populations^{12,15,16}. For example, exposure to D4 is classified as human hazard class reproductive toxicity 2 by the European Chemicals Agency¹⁷ after several investigations and reports on animal models. D5 also can cause health issues to the nervous system, cancer, and hormonal disorders according was reported by the US Office of Environmental Health Hazard Assessment (OEHHA)¹⁸. The widespread use of siloxanes in many industries (*e.g.*, cosmetic, automotive, medical, and food processing) has led to an estimated VOSiC emission between 1.4–4.2 g·year⁻¹ *per capita* in the UK and 0.4–85 g·year⁻¹ *per capita* in the US, solely from personal care products¹⁹. VOSiCs have been recently classified as substances of very high concern (SVHC) for the environment by the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) organisation^{17,20}, as well as by countries such as the US²¹, Japan²², Canada^{23,24}, and the UK²⁵. These countries have framed D4 and D5 siloxanes as substances with high production volume, persistence, bioaccumulation, and ecological concern.

Through the WWTP processes, VOSiCs could be desorbed and volatilised by the aeration process (**Fig. 5 Steps 5-6**) or remain adsorbed onto the bio-sludge (**Fig. 5 Steps 3, 4, 7, 8**). Usually, siloxanes, and specially PDMS, remain attached to the organic matter (OM) due to their high affinity with the solid matrix^{1,26}. In the AD stage, the increased temperature of the process weakens the physical forces that maintain the siloxane molecules attached to the OM, releasing them into the biogas (**Fig. 5 Steps 9-10**)^{1,26}. As VOSiCs are present in the personal care products reaching the WWTP influent stream¹⁴, it has been assumed that only this input is the responsible for the damage to the combustion engines. Therefore, most of the publications refer mainly to D4 and D5^{19,27,28} without really take into account the siloxane polymers effect on biogas contamination. If well, PDMS is known to be abiotically degradable in soils at low moisture conditions by clay acidic minerals with Lewis acid sites (Al⁺³ or Fe⁺³)^{2,29}. It is not clear if PDMS could be biodegraded or if is abiotically cleaved in the AD sludge. Therefore, in case that PDMS could be microbially degraded in the anaerobic sludge, it can provide an additional source of VOSiC within the AD reactor itself. We consider that the PDMS microbial hydrolysis and further chemical transformation represent an extra VOSiC input to the biogas and to the environment. In consequence we tested this hypothesis in the present work to understand better the siloxanes transformations and elucidate the PDMS biodegradability.

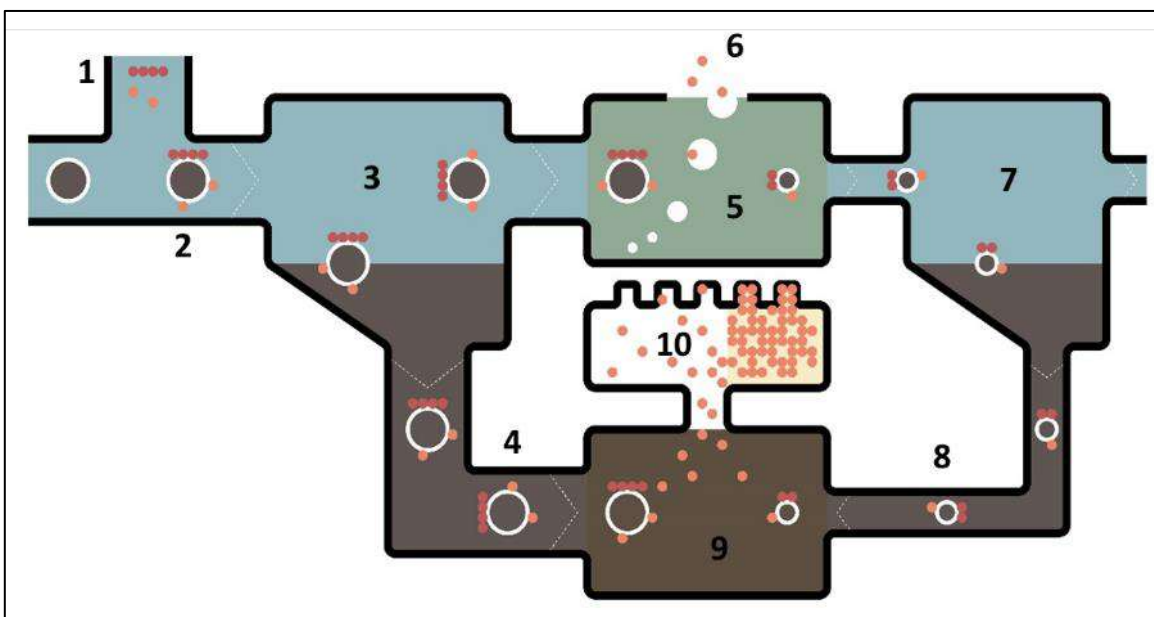


Figure 5. Possible fate of siloxanes in a typical WWT process. **1.** PDMS and non-volatile siloxanes (red dots), VOSiC (orange dots) are released into wastewater streams, **2.** PDMS and VOSiC attach to the organic matter, OM (brown circles), **3.** A fraction of the OM settles with siloxanes in the primary sludge, **4.** Primary sludge to AD, **5.** OM to the aerobic treatment where PDMS can be partially cleaved and VOSiC molecules released, or retained on OM, **6.** VOSiCs released to the atmosphere by the aeration process, **7.** OM, along with siloxanes, settle in the secondary sludge, **8.** Secondary sludge to AD, **9.** PDMS are cleaved to VOSiCs, which along with existing VOSiC are released to the biogas stream, **10.** SiO₂ molecules depositing on combustion engine components.

Currently, granular activated carbon (GAC) is the most common technology used by WWTPs to remove siloxanes³⁰. However, critical disadvantages have been reported, mainly related to its inherent unspecific adsorption characteristics and the number of competitive adsorption reactions among the pollutants present in biogas streams^{31,32}. Moreover, physicochemical processes, such as GAC adsorption, are costly, and only separate pollutants, concentrating them onto a different phase. Other technologies, namely membrane separation, chemical absorption (with strong acid or alkali solutions), catalysis, and deep chilling, have severe drawbacks, which make these processes not affordable for most WWTPs^{13,14,33}.

On the other hand, biologically based methods certainly constitute the most sustainable and economical alternatives to use. However, studies on the biological degradation of siloxanes are scarce, mainly focused on VOSiCs, and particularly under anaerobic environments¹⁹. Precisely, the high dissociation energy of the Si-O and Si-C bonds³⁴ making the biodegradation of the siloxane bonds difficult to achieve², especially in siloxane polymers and absence of higher energy electron acceptors in the anaerobic digestion trophic web. Siloxane biodegradation studies have been focused mostly on D4 or D5 removal; however, no information on the microbial groups or the biochemical interactions involved in siloxanes degradation has been provided. These efforts have been focused on using oxygen as a final electron acceptor mainly by filamentous fungi and aerobic bacteria (i.e., *Pseudomonas* sp.)^{35–37}. Aerobic conditions seem to be suitable for a more heterogeneous and diverse group of

bacteria³⁸, which may produce the required enzymatic machinery to hydrolyse siloxanes^{36,39}. However, the aerobic regime has drawbacks such as the increased biomass yield and the deficient syntrophic microbial relationships due to the ecological competitions for space and resources³⁸. In addition, a strict aerobic environment may be insufficient to ensure complete degradation of organic matter in general⁴⁰, recalcitrant compounds⁴¹ or siloxanes. Alternatively, the controlled addition of small amounts of oxygen to the anaerobic environment (*i.e.*, microaeration) can promote the required microbial changes and fulfil the thermodynamic requirements to allow for the catabolism of more recalcitrant compounds in general^{42–44}. A microaerobic environment may help overcome the low energy production, low microbial diversity, and weak biofilm formation issues of strictly anaerobic environments^{44–46}, while increasing hydrolysis rates and produce more diverse enzymes^{41,47}. Consequently, microaeration arises as a useful strategy to extract the positive aspects of both aerobic and anaerobic conditions without their drawbacks. This strategy could lead to the biodegradation of complex polymers such as PDMS.

This study aimed to evaluate the biodegradability of siloxane polymers as potential D4/D5 source using an anaerobic/microaerobic hybrid approach. Anaerobic digestion performance was evaluated under increasing oxygen partial pressures and PDMS concentrations using the biochemical methane potential (BMP) assay. Microbial ecology changes and the presence of PDMS catabolic by-products were characterised for each experimental condition.

Materials and Methods

Biochemical methane potential (BMP) assay

The BMP protocol was based on Labatut et al.⁴⁸, using 250mL Schott bottles as reaction units with 50mL of effective sample volume. BMP bottles were initially loaded with an organic substrate mixture (OS)⁴⁹, PDMS, micronutrient solution, and WWTP sludge as microbial inoculum. The OS used was based on the equal mixture (COD basis) of glucose, sodium acetate, sodium casein, cellulose (technical grade-Merck), and coconut oil (industrial grade). 200 ppm of OS were added to each BMP, to promote initial microbial growth and reduce the lag phase, while pushing the native microbiota to use PDMS as an alternative carbon source. PDMS (100cSt - Texas Inc.) was added to each BMP bottle at the following concentrations: 50, 100, and 500 ppm in a mass basis. Selected micronutrients, considered critical for the AD microbiome^{49,50}, were also added to each BMP bottle (**Table S1 – Supporting Information**). The microbial inoculum was obtained from the effluent of an active anaerobic CSTR reactor, treating secondary sludge from "La Farfana" municipal WWTP (Santiago, Chile). The inoculum, which volatile solids concentration was 15.2 g·L⁻¹, was added to each BMP bottle at an inoculum-to-substrate (I/S) ratio of 2. Once loaded, the bottles were gassed with pure nitrogen, sealed, and placed in a shaker incubator (90 RPM - 37°C±1). 12 days after the BMP experimental set up started, pure oxygen (99.8%) was added to each bottle by internal atmospheric volume substitution, using gas tight syringes, to reach the following target partial pressures: 0%, 1%, 3% and 5%*v/v*. Oxygen was re-placed weekly on a biogas day measurement (when necessary) to maintain the selected microaerated conditions. Tested oxygen partial pressures were chosen based on other reports of microaerophile biota growth (*i.e.*, 1-10% PO₂)^{51,52} and reported methanogenic archaea tolerance (*i.e.*, <5% PO₂)⁵³.

Additional bottles only containing inoculum but no organic substrate, micronutrients, or added oxygen were incubated to account for background methane production from the sludge itself, which was then subtracted from the other treatments at the end of the assay. As well, treatments with organic substrate, micronutrients, oxygen and not PDMS were performed (*i.e.*, blanks). The BMP assay was ended when the cumulative biomethane production curve reached a *plateau* phase, which was on average, 55 days. An infographic with the experimental design, including treatments and analyses, is shown in **Fig. S1 – Supporting Information**. Biogas volumetric production in each BMP bottle was measured using a glass syringe using the volume displacement method. For every biogas production measurement, methane content and volume were determined by pumping the biogas through a two-step system based on Standard Methods 2720B, where a first vessel containing NaOH (20% ^{m/v}) removes carbon dioxide and a second vessel, containing MgSO₄, retains water, obtaining methane volume by difference. These measurements were confirmed weekly using a gas chromatography thermal conductivity detector (GC-TCD), which in addition to methane and carbon dioxide, determine oxygen and nitrogen in the bottles' gas phase according to the Standard Methods 2720C.

Theoretical biochemical methane potential and biodegraded fraction

The theoretical biochemical methane potential of the organic substrate (OS) and the different PDMS concentrations was estimated using The Buswell Formula⁵⁴, which assumes that all the organic content is converted to methane and carbon dioxide. Buswell estimations were performed with the molecular formula of each compound in the OS (*i.e.*, glucose, sodium acetate, sodium casein, cellulose, and coconut oil). For coconut oil, the calculation was based on a mixture of capric, lauric, myristic, oleic, and palmitic acid, according to the characterization reported by Otamiri et al.⁵⁵ characterisation. Finally, the molecular formula CH₃(C₂H₆SiO)_nSi(CH₃)₃ was used for the PDMS calculation, assuming 20 siloxane units (*n* = 20) in a 100cSt-siloxane oil. The biodegraded fraction (*f_D*), which defines the maximum extent of substrate converted to methane, was determined as follows:

$$f_D = \frac{B_o}{B_u} \quad (1)$$

Where, *f_D* is the substrate biodegraded fraction (decimal, %), *B_o* and *B_u* correspond to the observed and theoretical methane potential (mL CH₄ per g VS added or g COD added), respectively. *B_o* was determined directly from the BMP assay, whereas *B_u* was calculated using The Buswell Formula, as described above.

Characterisation of bacterial community dynamics

Changes in the bacterial communities were characterised using the Denaturant Gradient Gel Electrophoresis (DGGE) fingerprinting technique and 16S rRNA gene sequencing. The Operative Taxonomic Unit (OTU) was defined per phylotype, assuming each one of the different bands elucidated on the DGGE gel corresponded to one of the most dominant bacterial populations present under the tested conditions⁵⁶, here in particular for the different PDMS concentrations and oxygen partial pressures. From the BMP initial sludge and final digestate (from the combined triplicates), total DNA extraction was performed) using a

DNeasy power soil kit (Quiagen Inc) extraction kit. Extracted DNA integrity was assessed by 1% agarose electrophoresis Gel-Red stained, according to Chen et al.⁵⁷ and Cheng et al.⁵⁸ methodologies. 16S rRNA gene amplification from bacterial populations was accomplished by Polymerase Chain Reaction (PCR), using universal bacterial primers 358F-GC and 907R. For DGGE analyses, a 40 bp GC clamp was added to the 5' extreme of the forward primer⁵⁶. Bacterial community profiles in DGGE were conducted for amplicons of ± 500 bp in length.

DGGE electrophoresis was performed using a 0.75-mm thick polyacrylamide gel (acrylamide:bisacrylamide - 37.5:1) submerged in a TAE buffer (40 mM Tris, 40 mM acetic acid, 1 mM EDTA; pH 7.4) at 60°C⁵⁶. Denaturant gradient was carried out in urea-formamide at differential concentrations between 40% to 80%^{56,59}. The DGGE was run at 100V for 16h and later stained with SYBr Gold (Molecular Probes) at 0.01% for 30 min to then be revealed using UV transilluminator equipment (BioRad Technologies). Most-intensive, visible profile bands were excised from the DGGE gel, considering the assumption of each band represented a different bacterial population. The 16S rRNA gene from DNA in each band was amplified using the same previously described primers without the 40bp GC clamp⁵⁶. PCR products were confirmed by 1% electrophoresis on an agarose gel prior shotgun sequencing (Macrogen Inc.). DGGE band sequences were compared to the 16S ribosomal RNA sequences (*Archaea* and *Bacteria*) database (NCBI) using BLASTN (Megablast program, default parameters)⁶⁰. The first 50 sequences were downloaded from NCBI and clustered to 97% identity using cd-hit-est⁶¹. The representative 16S rRNA sequences were aligned using MAFFT (auto mode, L-INS-i strategy)⁶². The 16S rRNA phylogenetic tree was inferred by maximum likelihood (ML) with IQ-TREE v1.6.8 (-m TESTNEW -bb 1000 -alrt 1000)^{63,64}. The DGGE bands were phylogenetically positioned using the 16S rRNA ML tree as a reference using EPA-ng v0.3.6⁶⁵, to then be visualised and edited with iTOL⁶⁶.

Analytical and statistical methods

Biogas composition was measured using an Agilent 7820A GC-TCD with a Carbosphere 1010 capillary column with helium as a carrier gas, according to ASTM method D3612⁶⁷. The BMP anaerobic sludge was characterised pre- and post-digestion for the following physicochemical parameters: total solids (APHA- TS2540B), chemical oxygen demand (APHA- COD5220D-), fixed and volatile solids (APHA-TVS-2540E-) in accordance to Standard Methods⁶⁸. The lipophilic fraction of siloxanes was recovered from the post-digestion anaerobic sludge by solvent-assisted extraction^{69,70} (from both the solid and liquid fractions) using a n-hexane-acetone mixture (1:1-v/v-). Extracted cyclic siloxanes, D4 and D5, were determined using an Agilent 7820A GC equipped with a flame ionisation detector (FID) and an HP-5 capillary column, as described in Popat and Deshusses⁷¹. Chemical structure identification of extracted compounds was performed using a Shimadzu 8050 gas chromatography-mass spectrometer (GC-MS) triple quadrupole. Samples were injected in a *splitless* mode, and separation was carried out using a capillary column-Rtx-5MS (30m x 0.25 mm x 0.1 μ m) with a helium flow of 1 mL min⁻¹ as a gas carrier, according to Sanchís et al.⁷². Selected Ion Monitoring was used to elucidate siloxanes presence, and molecules were identified by comparing the NIST14.L mass-spectra library based on an 85% similarity for the cut-off. As phenyl-siloxanes were identified using this method, column bleeding was tested running samples with the solvent extraction mixture alone under the same protocol as real samples, assuring no column bleeding.

All treatments and determinations were performed in triplicate. First, parametric statistical assumptions were tested using the Levene (homoscedasticity), Kolmogorov-Smirnov (normality), and residual regression model (graphical normality) tests. Then, all the data were analysed under a General Linear Model (GLM) under the test for factorial analysis of variance (2-way ANOVA) to elucidate the interactions of the tested factors. Finally, the HSD Tukey and Scheffé tests were performed as post hoc analysis. For all the tests, the α error assumed was 0.05 under a significance of 95%, using the IBM SPSS statistics 22 software (IBM).

Results and Discussion

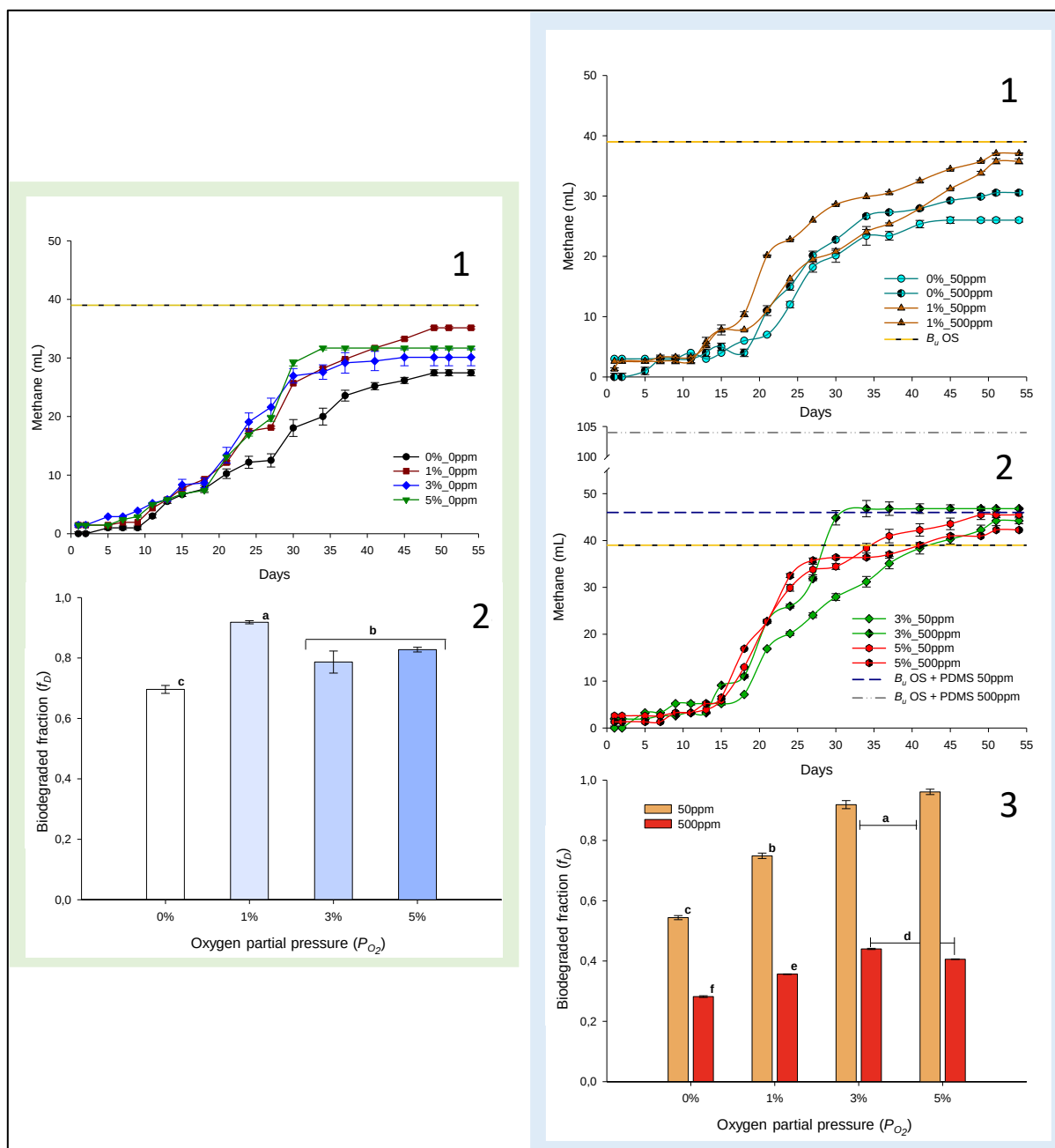


Figure 6. Results BMP experiments with PDMS. **GREEN BACKGROUND (GB):** Cumulative methane production (1) and biodegraded fraction (2) of the organic substrate (OS) alone under anaerobic and microaerated conditions (i.e., blanks). **BLUE BACKGROUND (BB):** Cumulative methane production (1, 2) and biodegraded fraction (3) of the organic substrate (OS) supplemented with increasing PDMS concentrations under anaerobic and microaerated conditions. Methane production is normalised by the substrate's chemical oxygen demand (COD), including PDMS, when applicable, and it is expressed at STP conditions. The yellow, blue, and grey segmented lines in both backgrounds denote the theoretical biochemical methane potential (B_u) of the OS,

OS+PDMS 50 ppm, and OS+PDMS 500 ppm, respectively. Letters a,b,c,d,e,f denote HSD Tukey groups with significant differences ($\alpha 0.05$).

Results based on the biochemical methane potential (BMP) assay show that microorganisms under either anaerobic or microaerated conditions could use the organic substrate (OS). Furthermore, strictly anaerobic conditions produced 11%, 17%, and 25% less methane than the microaerobic conditions, respectively under 3%, 5%, and 1% oxygen partial pressures (P_{O_2}), without PDMS added (**Fig. 6-Green Background**). The calculated theoretical biochemical methane potential for OS, OS+PDMS 50 ppm, OS+PDMS 100 ppm, and OS+PDMS 500 ppm, were 39.45 mL, 45.99 mL, 52.53 mL, and 104.83 mL, respectively (**Fig. 6**). The 100-ppm treatment results were omitted for clarity in the graphs, given that they were almost identical to the 50-ppm treatment (**Fig. S2 –Supporting Information**).

Microaerated conditions favour the overall methane production of simple and recalcitrant compounds

Results show that methane production was not negatively affected by the change from anaerobic to microaerobic conditions (**Fig. 6**). In fact, methane production was improved on a volumetric basis. As expected, no treatments reached the theoretical methane production due to the overestimation from the Buswell formula value that does not consider the fraction of substrate allocated to microbial synthesis⁷³. The anaerobic treatments (*i.e.*, without oxygen) reached 70% of the theoretical methane production of the OS ($f_D = 0.7$), whereas the microaerated treatments (*i.e.*, $P_{O_2} = 1, 3$, and 5%) reached between *ca.* 80 and 90% (**Fig. 6 GB**). This is in agreement with the results of previous studies, where the addition of small amounts of oxygen has increased methane production and organic matter removal^{74,75} – a result that previous studies have attributed to an improvement in the hydrolytic capabilities of the microbial consortium^{76,77}. As for the OS supplemented with PDMS, higher methane yields were observed both, for increasing PDMS concentrations and for increasing oxygen partial pressures (P_{O_2}) up to 3% (**Fig. 6 BB 1, 2**). Therefore, comparing the results with the supplemented PDMS treatments with the blanks suggest that extra methane production comes from the PDMS catabolism. As well it is clear that treatments with PDMS exceed the theoretical methane production calculated from the organic substrate alone (**Fig. 6 BB yellow line**). This indicates that PDMS may be used as a carbon source for methane production despite being only partly biodegradable under these conditions.

The biodegraded fraction decreases along with by PDMS concentration increment, particularly under strictly anaerobic conditions. As well, biodegraded fraction increases with the oxygen partial pressures showing a better performance in the general biodegradation of the used substrate (**Fig. 6 BB 3**). For example, the biodegraded fraction of the OS supplemented with 50 ppm PDMS increased from 0.54 to 0.96 under oxygen partial pressures of 0% and 5%, respectively. However, the biodegraded fraction of the OS supplemented with 500 ppm PDMS was only 0.44 and 0.41 under oxygen partial pressures of 3% and 5% and decreased even further under anaerobic conditions ($f_D = 0.28$). This suggests that high concentrations of PDMS, or the products of its hydrolysis, may be inhibitory to the anaerobic and/or aerobic microbiota, but its effects may become less significant to the trophic web under microaerobic conditions ($1 \leq P_{O_2} \leq 3\%$). Indeed, a myriad of alcohols such as phenol and

aliphatic alcohols, which are known to be toxic to microorganisms^{78–82}, were found at the end of the BMP trials in the liquid phase characterized by the GC-MS analyses (**Fig. S3–Supporting Information**). Alcohols like methanol are one of the products from the oxidation and subsequent hydrolysis of VOSiCs (*i.e.*, D4 and D5)³⁶, therefore it is apparent that from PDMS hydrolysis also alcohols were produced as demonstrated in this study with antimicrobial compounds such as phenol and aliphatic alcohols.

We saw that oxygen addition not only can improve the biodegradability of simple substrates (*i.e.*, OS), and apparently also of more complex, recalcitrant compounds (*i.e.*, PDMS). This is not surprising, considering the high energy required to cleave the siloxane (Si-O-Si) and methyl-siloxane (Si-CH₃) bonds (*i.e.*, 103 and 69 kcal/mole, respectively³⁴). This type of energy requirements cannot be easily reached by the conventional anaerobic microbiota, which harvests considerably less energy than its aerobic counterpart⁸³. Based on the energy premise, other studies have used nitrate as a final electron acceptor to overcome the energy requirements for the cleavage of siloxane bonds⁸⁴. However, nitrate use creates an anoxic environment where the AD microbial diversity only could be narrowed and become unstable⁸⁵, instead of microaeration were general diversity (*i.e.*, enzymatic, microbial) and resilience is greatly increased⁵¹. On the other hand, controlled oxygen additions appear as a more suitable option, due to its easy management, low concentration required, and more regulated redox potential. Furthermore, the anaerobic digestion (AD) microbiome has a lower diversity of microorganisms than its microaerobic counterpart^{43,45,46}, impacting both the production and variety of hydrolytic enzymes. This is why hydrolysis usually becomes the rate-limiting step for the anaerobic digestion of influent streams composed of particulate and/or recalcitrant compounds^{46,86}.

It is apparent that, in this study microaeration improved methane production which must come from a better enzymatic hydrolysis and therefore a PDMS oxidation and use (as the methane production exceed the theoretical calculations of OS alone) (**Fig. 6**). For treatments containing OS alone, exponential methane production started after day 10, but for microaerated treatments supplemented with PDMS it started on, or shortly after, day 12, coinciding with the first addition of oxygen to the reactors. Such an effect is apparent when observing the methane production of the strictly anaerobic treatments, which exponential phase took between 18 to 20 days to start (**Fig. 6 BB 1**). In general, microaerated treatments showed a shorter lag phase, not only coinciding with the beginning of oxygen addition but also with its consumption, as discussed below. Jenicek et al.⁷⁴ and Cirne et al.⁸⁵ demonstrated that microaeration decreases the concentration of inhibitory compounds, such as lactic acid, sulphide, and ethanol, resulting in an improved and faster COD conversion. Thus, methane production from siloxane-containing substrates may be faster under microaerobic conditions due to the improved process kinetics resulting from the presence of low dissolved oxygen concentrations.

In summary, the presence of oxygen in trace concentrations are likely to enhance the thermodynamics of the anaerobic digestion due to an increased production and diversity of hydrolytic enzymes; thus, improving process kinetics and biodegradability of both, simple substrates, and siloxane polymers.

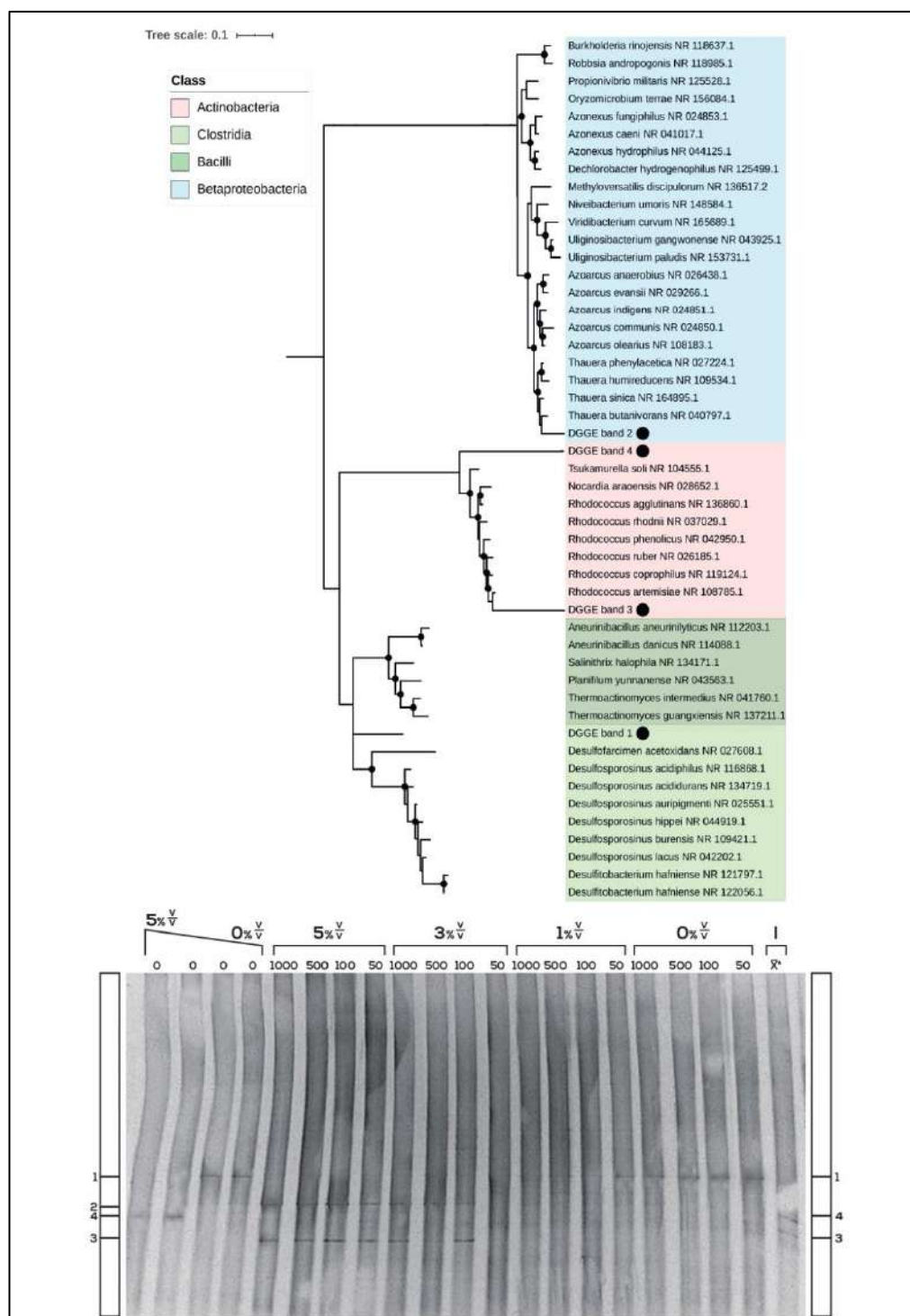


Figure 7. Molecular elucidations in microbial dynamics **A.** Maximum likelihood inferred 16S rRNA phylogenetic reconstruction from excised DGGE bands. Black circles correspond to a 1000 bootstrap, SH-aLRT $\geq 80\%$ and Ultra-fast bootstrap $\geq 95\%$. **B.** DGGE fingerprinting image from the BMP sludge with anaerobic treatment ($0\% \text{ v/v}$), microaerobic $1\% \text{ v/v}$ O_2 , $3\% \text{ v/v}$ O_2 , $5\% \text{ v/v}$ O_2 and inoculum (I). Numbers on the top of each column (0, 50, 100, 500 and 1000 ppm) correspond to PDMS concentrations per treatment, $\bar{X}^*$

corresponds to the PDMS concentration inferred from literature. Dominant DGGE bands correspond to the chart at the bottom and the phylogenetic reconstruction.

Coupled effects of oxygen and PDMS: a shift driver of bacterial community structure and sludge ecology

Comparisons of the 16S rRNA bacterial populations under anaerobic and microaerobic conditions revealed that oxygen dosage may have driven significant changes of the native anaerobic sludge community (**Fig. 7**). 16S rRNA-DGGE fingerprinting profiles and dominant DGGE bands (here defined as bacterial populations) sequenced, evidenced that the abundance of members closely related to *Desulfofarcimen* sp. decrease as oxygen partial pressures increase (**Fig. 7B**, Band 1). This shift can be explained by the presence of reactive oxygen species (ROS), produced in redox reactions (triggered by oxygen)⁴⁴, which are toxic to strict anaerobes⁸⁷ such *Desulfofarcimen* sp.. These organisms do not have the enzymatic machinery to reduce ROS (*i.e.*, aerobic respiration), consequently causing a decrease in its viability and abundance⁸⁸, as evidenced by the disappearance of DGGE band 1. However, in treatments where these organisms were not part of the dominant population ($P_{O_2} = 1\%$ treatments) (**Fig. 7B**, Band 1), methane production did not seem to be compromised. In fact, methane production almost reached the theoretical maximum in the treatments with organic substrate alone (**Fig. 6**). This suggests that other, non-dominant populations of facultative fermentative organisms can efficiently contribute to sustain the AD trophic chain, maintaining its balance, and showing an apparent community replacement with the same functional capabilities as is suggested by Wu et al (⁸⁷) on synergetic systems.

Additionally, under microaerobic conditions, the abundance of strict anaerobes decreased, while other, better-adapted populations seemed to have thrived, changing the structure of the native anaerobic sludge microbiota. Consequently, in the 3% and 5% P_{O_2} treatments, aerobic/facultative populations, such as those closely related to *Thauera* sp. (β -Proteobacteria) and *Rhodococcus* sp. (Actinobacteria), increased their abundance due to the presence of oxygen as final electron acceptor (**Fig. 7B**, Bands 2 & 3) - a bacterial replacement that has been shown in previous studies^{47,88}. The increase in abundance of aerobic bacteria, stimulated by the presence of oxygen, could be the result of cooperative relationships between aerobic and anaerobic organisms^{89,90}. Aerobic bacteria consume oxygen and may use the extra energy available from respiration to cleave siloxane molecules, thus depleting oxygen concentrations and maintaining ORP levels low enough to allow methanogenesis⁸³. Indeed, our results show that oxygen is rapidly consumed after its addition, requiring subsequent re-additions to re-establish the target partial pressures of each treatment (*i.e.*, $P_{O_2} = 1\%, 3\%, 5\%$) (**Fig. 6** and **Fig. 8**).

As **Fig. 4** shows, the oxygen consumption decreases along the experiment time, meaning that oxygen is mainly consumed in the logarithmic methane production stage (hydrolysis stage) and is not consumed at the same rate when methane production arrives to a steady state (acetogenesis-methanogenesis) (**Fig. 6**). As well, oxygen consumption was significantly higher at 1 and 3% oxygen partial pressures as compared to $P_{O_2} = 5\%$, for both 50 and 500 ppm PDMS concentrations (**Fig. 8**), suggesting that the “extra” available oxygen may have not been used by the aerobic bacteria, and/or, may have been inhibitory to strictly anaerobes. This is supported by the fact that, when the oxygen partial pressure was changed from 3% to

5%, the biodegraded fraction only increased from 0.92 to 0.96 for the 50 ppm PDMS treatments, and even decreased from 0.44 to 0.41 for the 500 ppm PDMS treatments (**Fig. 6**).

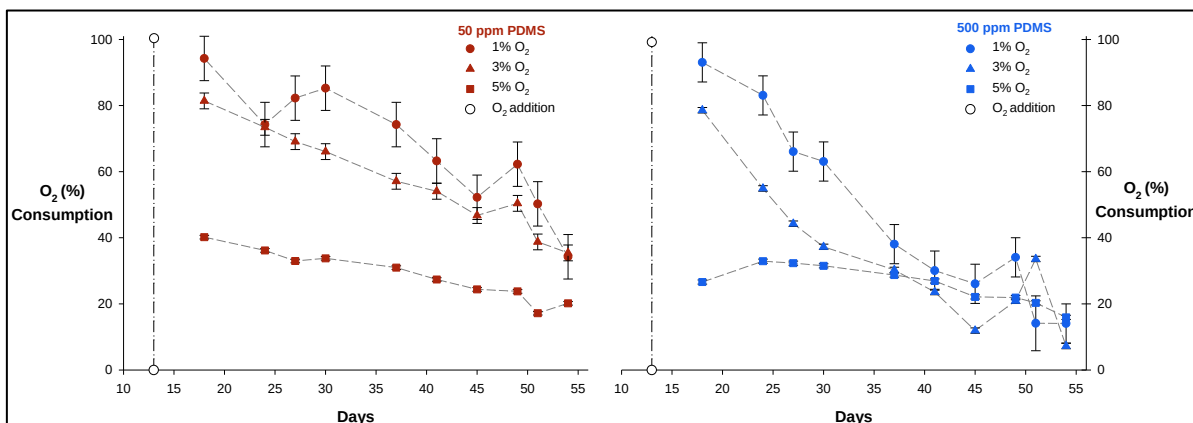


Figure 8. Oxygen consumption (%) relative to the amount added during the BMP in the PDMS treatments. Vertical segmented lines represent the first addition of oxygen (subsequent additions after each P_{O_2} measurement is omitted for clarity).

The correlation between oxygen consumption and methane production proves that aerobic/facultative bacteria reduce dissolved oxygen concentrations (and thus oxygen partial pressures), protecting strictly anaerobic methane-producing organisms. This particular mutualistic relationship, studied previously using non-siloxane substrates^{45,75,87}, supports the hypothesis that the presence of oxygen leads to a change in the bacterial community structure. This change could favour the production of new enzymes and the possibility of harvesting more energy from substrates, which may explain why PDMS is more biodegradable under microaerated conditions. This phenomenon may be the basis that allows the cleavage of the high energy covalent bonds formed by silicon, resulting in the biodegradation of recalcitrant compounds such as siloxanes (*i.e.*, PDMS). Our results show that microaeration coupled with PDMS presence led to an evident change in the bacterial structure and then in the system ecology, evidenced by the differences in population dynamics shown on the DGGE fingerprinting profiles. We could infer that the PDMA cleavage (and biodegradation), requires the oxygen stimulation of the whole anaerobic sludge to bring about specific groups of aerobic/facultative bacteria, which can collaborate and develop mutualistic relationships with the existing microorganisms of the native sludge^{87–89}.

Finally, it should be stressed that the presence of oxygen alone may not be the only driver for the microbial ecology changes observed in the AD microcosm. Microaerated conditions together with the presence of siloxanes, may have been responsible for the increase of *Thauera* sp. and *Rhodococcus* sp., as suggested by the DGGE profiles (**Fig. 7B**). First, because *Desulfofarcimen* sp. are shown to be dominant under anaerobic conditions and 1% oxygen partial pressures without PDMS addition, as well as under anaerobic conditions with PDMS addition (**Fig. 7B**, Band 1); demonstrating that PDMS by itself is not capable of driving a change in the ecological composition. Second, because when the oxygen partial pressures are $\geq 3\%$ in treatments without PDMS, the aerobic bacteria *Tsukamurella* sp. increase its dominance (**Fig. 7B**, Band 4), but they are absent under the same oxygen partial pressures in the PDMS-supplemented treatments, where *Thauera* sp. and *Rhodococcus* sp.

are dominants. These differences demonstrate that PDMS and oxygen interactions drive the AD sludge ecological changes, enabling the siloxanes biodegradation without hindering methane production (**Fig. 6**).

Oxygen drives the PDMS metabolism to volatile and non-volatile siloxanes

Proof that the bio stimulation of siloxane-hydrolytic microorganisms is driven by the presence of oxygen is apparent when we analyse the bacterial community structure (**Fig. 7**) and the metabolic products resulting from the PDMS degradation, as revealed by GC-MS analyses (**Fig. 9**). The presence of cyclic siloxanes and its relative concentrations (%) per treatment, not only demonstrate that the microbial metabolism of PDMS is feasible, but also that produce cyclic siloxanes such as D7, D8, and D9, as they were not present in the initial, native anaerobic sludge. **Fig. 9** shows that initial concentration of VOSiCs (*i.e.*, D4, D5, and D6) in inoculum sludge increased from 0.60% up to 10% m/m in treatments with oxygen partial pressures of 1% and 3%, evidencing a contribution in VOSiC concentration from the above explained PDMS hydrolysis.

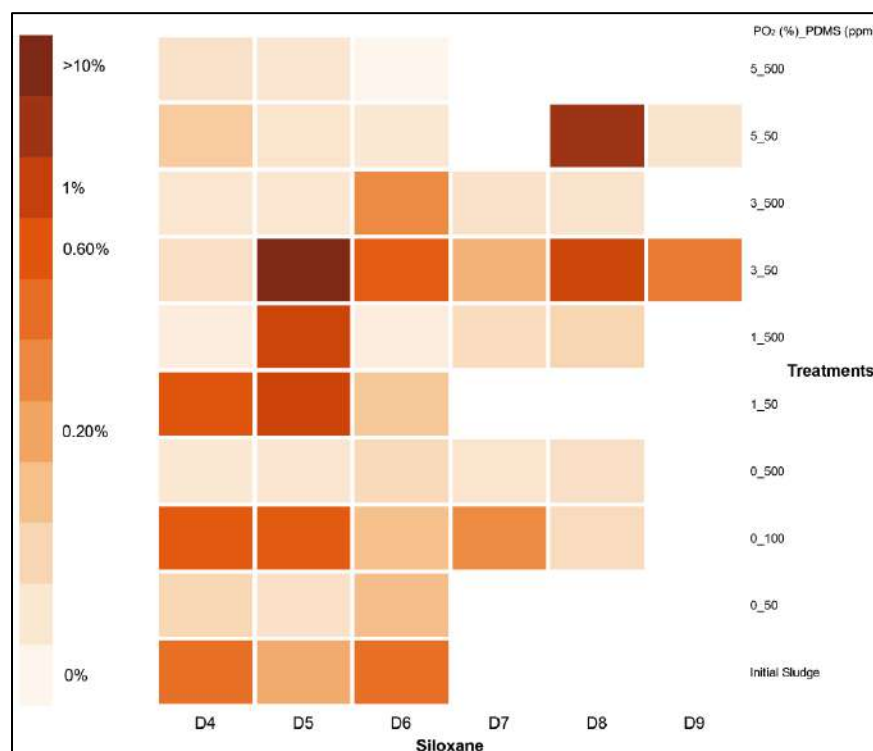


Figure 9. Heat map showing relative concentrations (% m/m) of cyclic siloxanes identified by GC-MS (QQQ) for the treatments supplemented with PDMS (right). Outside bar (left) shows the colour key according to the concentration found for each sample. Cyclic siloxanes were recovered with n-hexane: acetone 1:1 solvent extraction.

In most anaerobic treatments, short-chain, volatile siloxanes (*i.e.*, D4 to D6) maintained concentrations between 0.03% and 1% (**Fig. 9**), suggesting that under anaerobic conditions cyclic siloxanes could also be metabolised to silanes or other non-cyclic or volatile metabolic products. Under anaerobic conditions, the presence of long-chain, non-volatile siloxanes (*i.e.*,

D7 and D8) was only observed when PDMS concentrations were higher than 100ppm (**Fig. 5**). This result could be due to the increase in volatile siloxanes that can be re-polymerised up to D7 and D8 via α - ω -silanediols, as Cabrera-Codony et al.^{32,91} and Soreanu et al.³⁴ proposed. However, it is also possible that PDMS was only partly catabolised due to the lack of a more energetic final electron acceptor. In this way, remaining products from PDMS cleavage could have undergone a chemical rearrangement into larger cyclic siloxanes, as previously described for soil and anaerobic sludge matrices^{2,29}. Previous studies report that PDMS is hardly to non-biodegradable under aerobic or anaerobic conditions^{2,15,37,92,93}. It is suggested that D4 and D5 siloxanes came in wastewaters attached to the AD sludge biosolids (due to their hydrophobicity), and then released to the biogas by volatilisation under mesophilic temperatures^{1,19,28}. Other studies hypothesised that D4 and D5 present in the biogas also could come from the resulting products of PDMS hydrolysis as compounds that looked for more stable, less energy-repulsive cyclic structures^{34,37,94}. In our study, when oxygen was present, higher concentrations of volatile cyclic siloxanes (D4 to D6) and non-volatile siloxanes (D7 to D9) were found (**Fig. 9** and **Fig. S4 – Supporting Information**). This demonstrates that metabolic products from PDMS degradation might contribute to the pool of volatile siloxanes found in WWTP-derived biogas.

Also, the presence of intermediates, such as phenyl-siloxanes, linear siloxanes, tripropylsilanes, and other siloxane-type molecules with fragmentation patterns in mass/charge peaks (m/z) 73 m/z , 355 m/z , and 429 m/z (**Fig. S4 – Supporting Information**), strongly suggests that the bacteria present under microaerobic conditions could catabolise the PDMS molecules, despite the characteristic steric hindrance of methyl-siloxane molecules^{26,95,96}. Furthermore, these metabolic intermediates are susceptible to being re-polymerised into cyclic siloxanes or stabilised to silanols, at which point could be used as a carbon source within the AD trophic chain and produce biogas (**Fig. 6**). This may explain the presence of higher cyclic siloxanes in the microaerated treatments (**Fig. 9**) and the resulting increased methane production. These results demonstrate that microaeration may be a viable alternative to oxidize siloxane molecules, decreasing VOSiCs emissions to both, the atmosphere and biogas streams if it is applied in longer retention times or continuous systems. This will, in turn, decrease their concentration in the environment, and reduce subsequent biogas cleaning efforts required for its use in WWTPs.

Our results demonstrate that the microbial metabolism of PDMS is possible under both, microaerobic and (partially) anaerobic conditions, and that the resulting products are transformed into cyclic molecules, namely D4 to D9. This conclusion is critical to understand the fate and production of cyclic siloxane molecules in the AD biogas from PDMS cleavage, which up to now most of the studies refer as slow or negligible^{34,37,94}.

Biochemical considerations in the PDMS biodegradation under a microaerated environment

The diversity of the cyclic siloxanes (different from the ones in the initial sludge **Fig. 9**) found in this study may be due to the use of oxygen and the presence of siloxanes, which drive the microbial community composition changes in the anaerobic sludge. Oxygen reduction and substrate oxidation likely lead to higher energy yields than those obtained from fermentation. This enables enzymes to harvest the necessary energy to degrade the Si-C

bonds in siloxane molecules, yielding additional carbon to produce additional methane, as suggested in this study (**Fig. 6**). Indeed, it is expected that the new dominant bacterial groups will produce additional and/or new enzymes that may be responsible for the PDMS degradation in the BMP sludge. But, overcoming the steric hindrance of siloxanes and the unfavourable thermodynamics of degradation, requires microbial associations, such as the ones between anaerobic and aerobic bacteria that allowed the extra methane production under oxygen presence. Other studies have evidenced this cooperative behaviour by observing associations between *Pseudomonas* sp. strains and other α and β -Proteobacteria^{35,37}. This phenomenon could not be demonstrated in our study due to the limitations of using 16S rRNA-DGGE analyses; however, it is strongly suggested that *Thauera* sp. and *Rhodococcus* sp. (β -Proteobacteria and Actinobacteria, respectively) members in this study, might be working together to catabolise PDMS molecules. These organisms are known to have robust enzymatic capabilities to metabolise recalcitrant pollutants, possibly explaining the formation of metabolic products and cyclic siloxanes in our study (**Fig. S4 – Supporting Information**).

Several studies have reported that *Thauera* sp. are the main bacterial group responsible for the decomposition of cyclic hydrocarbons in anaerobic digesters, when nitrate is supplemented as a final electron acceptor⁹⁷. However, when oxygen is present, *Thauera* sp. could yield more energy via aerobic respiration, consequently enhancing metabolic diversity. Under these conditions, the metabolism of aromatic recalcitrant compounds (*i.e.*, Phenylsiloxanes in **Fig. S4 – Supporting Information**) is possible, mainly through the Benzoyl-CoA pathway⁹⁸. These natural capacities, suggest that the presence of oxygen enabled *Thauera* sp. to degrade cyclic siloxane metabolic intermediaries into simpler molecules that can be used by other members of the AD trophic web for biogas production. On the other hand, *Rhodococcus* sp., a member of the Actinobacteria phylum, is a well-known metabolically diverse organism that could degrade recalcitrant compounds such as naphthalene, aromatic substrates, herbicides, among others⁹⁹. One of the reported features of this organism is its capacity to deal with steric hindrances of organic compounds¹⁰⁰. This feature may be key for the degradation and conversion of PDMS and its metabolic intermediates to biogas.

Conclusions

The results of this study show that trace amounts of oxygen in an otherwise strictly anaerobic environment, drive the ecological, and biochemical, changes needed to biodegrade PDMS producing additional methane than the theoretically calculated. Microaeration may decrease VOSiC production from PDMS catabolism, by enhancing the use of oligomeric siloxane molecules as an energy source for microbial growth. Our study shows that the presence of VOSiC in biogas may not be the sole result of volatile siloxanes coming in wastewaters, but also a consequence of PDMS cleavage during anaerobic digestion that produced new VOSiC compounds as proved by GC-MS elucidations. The current results should also be tested under continuous bioreactor conditions to assess their further scalability. Although, microaeration should be further tested and studied, its application not only may prevent high concentrations of VOSiCs in biogas, but also allow wastewater treatment plants to become barriers for mitigating the emission of these ecotoxic contaminants to the environment.

Conclusions and outlook second chapter

- Siloxane polymers (PDMS) are biodegradable under microaerated conditions, producing siloxane-related metabolites that are more biodegradable and probably less toxic.
- Siloxane biodegradation under microaerated conditions produces more methane that might come from the anaerobic metabolism of the siloxane-related metabolites elucidated.
- Microaerated environments promote a significant microbial change in terms of ecological composition. These changes might be the key to the biodegradation of recalcitrant compounds such as siloxanes.
- VOSiC can be produced from microbial PDMS metabolism, especially under microaerated conditions.
- Since under normal anaerobic conditions, it is normal to have oxygen inputs that come with the reactor feeding. It is highly possible that VOSiCs in the WWTPs AD reactors also come from the microbial depolymerization of the PDMS, a non-previously considered source.

THIRD CHAPTER

Microaerated anaerobic digestion enables biological degradation of D4 and D5 siloxanes in batch cultures

Abstract

Siloxanes, usually believed as non-biodegradable compounds are one of the current most problematic pollutants to biogas-based energy technologies. Microbial biodegradation of volatile organic silicon compounds (VOSiC) has been previously reported using anaerobic and aerobic conditions, being the last ones the most promising in terms of actual biodegradability and possible scale-up. Since aerobic digestion generates significantly more biomass and does not produce biogas from waste stabilization, anaerobic approaches are preferred because they do not have these drawbacks. Considering microaeration as the middle point between both approaches, it is a novel and useful concept for treating recalcitrant compounds such as VOSiCs and producing energy from their conversion. VOSiCs biodegradation was evaluated under anaerobic/microaerobic conditions ($P_{O_2} = 0, 1, 3\%$), using wastewater treatment plant (WWTP) sludge as an inoculum and VOSiC as a co-substrate (0, 50, 500 ppm). On average, strictly anaerobic treatments produced significantly less methane than the 3% microaerated ones, which show VOSiCs biodegradation at 50 ppm and 500ppm. From chemical elucidations, siloxane-related molecules were identified as part of the VOSiCs (*i.e.*, D4-D5) catabolism. Our results support the biodegradation of D4 and D5 siloxanes under microaerated conditions and methane production from its metabolic conversion. A biodegradation pathway is proposed considering the metabolic products chemically identified in the post-digestion sludge; our results strongly suggest that microbial ecological adaptation is important to assure good siloxane biodegradation and handle the oxygen concentrations dosed in the tested treatments. Microaerobic conditions may enhance the VOSiC degradation increasing the system enzymatic diversity to overcome the siloxanes steric hindrance and catalyse the VOSiCs ring-opening to further stepwise biodegradation. Our results support the hypothesis that under microaerated conditions, the anaerobic digesters in the WWTPs could become a barrier for the emission of these ecotoxic (*i.e.*, D4-D5) contaminants to the environment.

Keywords: Siloxanes, Microaerated, Anaerobic digestion, biogas, biodegradation

Introduction

Anaerobic digestion (AD) is a syntrophic biological process carried out at low oxidoreduction potentials ($ORP < 200\text{mV}$) wherein electron acceptors other than oxygen are used. This closed-cycle biological treatment adds value to wastes and offers a partial solution to organic waste management and fossil-based energy dependence^{1,2}. Raw AD biogas is composed of methane, carbon dioxide, water vapour, traces of hydrogen sulphide, ammonia, and volatile organic silicon compounds^{3,4}. The presence of VOSiCs causes irreversible damage to the energy conversion systems by forming silicate deposits in the internal machinery when biogas is combusted. Octamethylcyclotetrasiloxane (D4) and Decamethylcyclopentasiloxane (D5) are the most common and stable forms present in

biogas, with concentrations ranging from 2 to 317 mg/m³^{5,6}. VOSiCs are primarily derived from domestic wastewaters, but may also form endogenously ^{via} microbial siloxane polymer (PDMS) catabolism during wastewater treatment⁷⁻⁹. These VOSiCs are released into the atmosphere during multiple stages of the treatment process, which threatens human health and the environment due to their persistent bioaccumulative and toxic (PBT) characteristics¹⁰.

Usually, VOSiCs in biogas are treated using physicochemical methods like activated carbon adsorption, chemical scrubbing, cryogenic separation, and selective membranes^{11,12}. Nonetheless, these methods also introduce new forms of wastes that may require complex and expensive treatments before disposal. Biological treatment has emerged as a potentially more sustainable and cost-effective alternative to these physicochemical methods^{11,13}. In addition, unlike physico-chemical approaches, which require multiple treatment units, biological treatment can be achieved in a single unit that removes more than one pollutant¹⁴. Despite several reports demonstrating the feasibility for the biological degradation of VOSiCs, many researchers maintain the traditional belief that polymeric siloxanes and VOSiCs are not biodegradable^{7,15-17}. Since 2008, however, biological siloxane treatment has become a hot topic in current research, with a particular interest in D4 and D5 siloxanes. VOSiC biodegradation has been evaluated under aerobic, microaerated, anaerobic, and anoxic conditions, in an attempt to identify optimal conditions for VOSiC stabilization and removal. Yet, few studies have so far achieved meaningful levels of siloxane removal and biodegradation within biogas matrices. These short-comings can be attributed to issues such as biological recalcitrance, mass transfer limitations, or just simply lack of knowledge regarding microbial dynamics in VOSiCs catabolism.

Among the more promising studies involving VOSiCs biodegradation, Li et al.¹⁸ and Wang et al.¹⁹ developed aerobic biotrickling filters for D4 biodegradation. Both studies applied biological aerobic approaches to investigate the mechanisms of VOSiC biodegradation using pure microbial cultures. In brief, Li et al.¹⁸ claimed that aerobic organisms produce hydrolases to perform a direct ring-cleavage on the Si-O bond opening the D4 ring, however, they did not consider the steric hindrance, acting as an enzymatic shield, produced by the methyl groups attached to the silicon molecule. On the other hand, Wang et al.¹⁹ suggested a gradual oxidation process, beginning with the replacement of one methyl substituent in the silicon atom, with a nucleophilic compound such as the OH radical, surpassing the aforementioned steric hindrance. Despite that both approaches are currently under discussion and serve as a starting point to understand the mechanisms of microbial VOSiC metabolism, the latter seems to be more likely because it provides a more plausible pathway for siloxane biological catalysis. Following the aforementioned studies, subsequent efforts have primarily entailed, either using oxygen as the final electron acceptor¹⁹⁻²¹ to promote Si-C and Si-O cleavage, or shaping the ecology of anaerobic microbiomes to increase the VOSiC degradation^{6,22-24}. Other studies have also tried using different electron acceptors, such as nitrate, sulphate, and co-substrates^{9,21}, but results have shown low efficiency, long retention times, poor microbial diversity, and a poor metabolic activity that may not be necessarily correlated with the observed siloxane degradation products.

Thus, a viable option may be to couple the high degradation efficiency of aerobic treatments with the diversity of the anaerobic microbiota. For example, H₂S abatement in anaerobic systems via controlled oxygen dosing has been already proven to be successful^{25–27}. However, the first study using microaeration²⁸ showed little effect as an approach to enhance microbial VOSiC degradation due to the inherent volatility and recalcitrance of these compounds. Santos-Clotas et al. (²⁸) used a hollow-fiber membrane bioreactor (HF-MBR) inoculated with anaerobic sludge initially supplemented with nitrate as the final electron acceptor. Later, they replaced nitrate with oxygen, but did not observe any significant changes in the microbial ecology or reactor performance; therefore, they concluded that microaeration was not a suitable approach for D4 biodegradation. On the contrary, PDMS biodegradation using microaeration was recently demonstrated by Ortiz-Ardila et al.²⁹ despite initial reports suggesting that siloxane polymers and oligomers cannot be biodegraded under microaerated conditions²⁸. The common belief that microaerated conditions hinder the methane production in anaerobic digesters was proved that is not applicable in the case of PDMS biological degradation, where methane may be produced from siloxane metabolic products²⁹.

Polydimethylsiloxanes (PDMS), commonly known as silicones, are polymeric organometallic materials synthesized from silicon, hydrocarbon (methyl, ethyl, phenyl), and oxygen groups organized as a backbone of Si-O-Si units^{30,31}. On the other hand, VOSiCs or volatile methyl siloxanes (VMS) are non-polymeric siloxane oligomers composed of the same silicone-oxygen monomers as in PDMS and sharing some of their physicochemical characteristics, but with a strong volatile character due to their cyclic configuration. In general, both PDMS and VOSiCs (hereafter referred as siloxanes) have a series of unique or special properties, including low surface tension, non-volatility (polymers), immiscibility in polar solvents, and resistance to: chemical changes, oxidations, and thermal attacks (low flammability)^{32–34}. The increase in siloxane usage in personal care formulations and household products due to the above-mentioned characteristics, have made siloxanes essential and (so far) irreplaceable for human society. The non-polar characteristic of siloxanes favours their attachment to particulate organic matter, and notably to biological sludge (*e.g.*, soils, sludge, sediments) transforming WWTPs into siloxane concentrating points. Considering the chemical stability and physicochemical characteristics of PDMS, siloxane polymers have been considered non-biodegradable, and therefore only few research efforts have been aimed to develop biological-based methods for the treatment of PDMS^{32,35}. Also, it is critical in this effort to study and understand the degradation mechanisms of siloxanes and its metabolic pathways from its polymeric to its oligomeric forms. Ortiz-Ardila et al.²⁹ observed that PDMS could be degraded biologically under microaerobic conditions, while increasing methane production beyond the amount expected (thermodynamically) from polymer catabolism of PDMS alone. Siloxane metabolic intermediates (*i.e.*, VOSiCs) were also detected, which suggests that PDMS was degraded biologically. Indeed, the hypothesis that VOSiCs can be produced from PDMS catabolism^{7,9} seems theoretically plausible. VOSiCs are cyclic oligomers that come from PDMS hydrolyzation under chemical synthesis, or as products of ring-chain equilibrium in the small polymers rearrangement^{34,36,37}. VOSiC production from PDMS can also occur from abiotic soil degradation and subsequent reorganization of the ionized oligomers^{35,38}. Briefly, Lewis acidic (Al⁺³ or Fe⁺³) active sites in clay soils break PDMS polymer into small, ionized

oligomers that seek chemical stability by cycling themselves into siloxane-ring compounds such as VOSiCs^{38–41}. Microaeration-assisted PDMS metabolism apparently produces the same ionized oligomers that require chemical stability by re-arranging into siloxane-ring structures producing VOSiCs. This chemical behaviour was observed in the experiments performed by Ortiz-Ardila et al.²⁹. The authors showed that supplying small amounts of oxygen in an otherwise anaerobic environment increased PDMS biodegradation with respect to the strictly anaerobic controls. Their results open new possibilities in siloxanes biological control and help to explain the biochemical transformations of siloxanes within the WWTP. Indeed, previous studies have shown that endogenous AD microbiota can be ecologically adapted to use oxygen as a final electron acceptor^{42,43}, thereby increasing its metabolic diversity^{44,45} and possibly their siloxane-degrading capabilities²⁹. Thus, we pose the question: can this enhanced microbiota also degrade VOSiCs and transform them into non-volatile, innocuous compounds?

In this study, we evaluated the biodegradation of D4 and D5 VOSiCs using an anaerobic/microaerobic hybrid approach. Accordingly, we tested anaerobic and microaerobic conditions in batch experimental units under increasing oxygen partial pressures and D4/D5 concentrations using the biochemical methane potential (BMP) assay. We seek to compare and partially elucidate the oxygen role in the change of the anaerobic microbial ecology and in the presence of VOSiC catabolic by-products from its biological conversion. Finally, we aim to propose a biochemical biodegradation pathway of D4 siloxanes under microaerobic environments comparing it with previous elucidations in fully aerobic conditions.

Materials and methods

Biochemical methane potential (BMP) assay

The BMP protocol was based on Labatut et al.⁴⁶, using 250mL Schott bottles as reaction units with 50mL of effective sample volume. BMP bottles were initially loaded with an organic substrate mixture (OS)⁴⁷, a D4/D5 mixture, micronutrient solution, and WWTP sludge as microbial inoculum. The OS used was based on an equal mixture (COD basis) of glucose, sodium acetate, sodium casein, cellulose (technical grade-Merck), and coconut oil (industrial grade). 200ppm of OS were added to each BMP, to promote initial microbial growth and reduce the lag phase, forcing the native microbiota to acclimate and eventually use VOSiCs as an alternative carbon source. D4 and D5 (technical grade – Sigma-Aldrich) VOSiCs were added to each BMP bottle at the following final concentrations: 100 (50ppm D4 + 50ppm D5) and 1000 (500ppm D4 + 500ppm D5) ppm on a mass basis. Selected micronutrients, considered critical for the AD microbiome^{47,48}, were also added to each BMP (Supplementary Information -SI- 1).

Two microbial inocula were used and compared in this study. One inoculum, hereafter referred to as “acclimated sludge”, which was obtained from the effluent of an active anaerobic CSTR reactor, treating secondary sludge from “La Farfana” municipal WWTP (Santiago, Chile). This inoculum was maintained for 6 months under microaerated conditions ($PO_2 = 1\% O_2$) and fed with a mixture of OS and 50ppm PDMS. A second inoculum, hereafter referred to as “fresh sludge”, was obtained from the same source but it was used as

is. Each sludge was added to each BMP bottle at an inoculum-to-substrate (I/S) ratio of 2^{49–51}. Once loaded, the bottles were purged with nitrogen gas, sealed, and placed in a shaker incubator (90 RPM - 37±1°C). 12 days after the BMP experimental set up was started, pure oxygen (99.8%) was added to each bottle by internal atmospheric volume substitution, using gas tight syringes, to reach the following target partial pressures: 0%, 1%, and 3%^{v/v}. Oxygen was re-placed weekly on a biogas day measurement (when necessary) to maintain the selected microaerated conditions. Tested oxygen partial pressures were chosen based on previous studies on microaerophile biota growth (*i.e.*, 1-10% PO₂)^{52,53} and reported methanogenic archaea tolerance (*i.e.*, <5% PO₂)⁵⁴.

Additional bottles containing only inoculum but no organic substrate (OS) or added oxygen, were incubated to account for background methane production from the sludge itself, which was then subtracted from the other treatments at the end of the assay. Also, treatments with organic substrate, micronutrients, oxygen and not added VOSiCs were evaluated (*i.e.*, blanks). The BMP assay was ended when the cumulative biomethane production curve reached a *plateau* phase, which was on average 55 days. Biogas volumetric production in each BMP bottle was measured using a glass syringe using the volume displacement method for detailed information refer to Ortiz-Ardila et al.²⁹ supplementary information. For every biogas production measurement, methane, CO₂, O₂, and nitrogen concentrations were measured using a gas chromatograph equipped with a thermal conductivity detector (GC-TCD), according to the Standard Methods 2720C⁵⁵. Oxygen concentration in the BMP headspace was measured twice per week using Planar Trace O₂-Sensitive Spots (PSt6) with an oxygen sensitivity of 0-15% air saturation (PreSens-Germany).

Theoretical biochemical methane potential and biodegraded fraction

The theoretical biochemical methane potential of the OS and the different D4/D5 concentrations was estimated using the Buswell Formula⁵⁶, which assumes that all the organic content is converted to methane and carbon dioxide. Buswell estimations were performed with the molecular formula of each compound in the OS (*i.e.*, glucose, sodium acetate, sodium casein, cellulose, and coconut oil). For coconut oil, the calculation was based on a mixture of capric, lauric, myristic, oleic, and palmitic acid, according to the characterization reported by Otamiri et al.⁵⁷. Finally, the molecular formulae C₈H₂₄O₄Si₄ for D4 and C₁₀H₃₀O₅Si₅ for D5 were used for the calculation of VOSiC. The biodegraded fraction (f_D), which defines the maximum extent of substrate converted to methane, was determined as follows:

$$f_D = \frac{B_o}{B_u} \quad (1)$$

Where, f_D is the substrate biodegraded fraction (decimal, %), B_o and B_u correspond to the observed and theoretical methane potential (mL CH₄ per g COD added), respectively. B_o was determined directly from the BMP assay, whereas B_u was calculated using the Buswell Formula, as described above.

Analytical and statistical methods

Biogas composition was measured using an Agilent 7820A GC-TCD with a Carbosphere 1010 capillary column with helium as a carrier gas, according to ASTM method D3612⁵⁸. The BMP anaerobic sludge was characterised pre- and post-digestion for the following physicochemical parameters: total solids (APHA- TS2540B), chemical oxygen demand (APHA- COD5220D-), fixed and volatile solids (APHA-TVS-2540E-), in accordance to Standard Methods⁵⁹. The lipophilic fraction of siloxanes was recovered by solvent-assisted extraction^{60,61} from the solid and liquid fractions of the post-digestion anaerobic sludge) using a n-hexane-acetone mixture (1:1-^{v/v}-). Also a Tetrahydrofuran extract was obtained according to the methodology reported by Zhang et al.²⁰ and further derivatized using the Bis(trimethylsilyl)trifluoroacetamide (BSTFA) trimethylsilylating agent, according to Zhang et al.²⁰ and Wang et al.¹⁹. Chemical structure identification of extracted compounds was performed using a Shimadzu 8050 gas chromatography-mass spectrometer (GC-MS) triple quadrupole. For non-polar compounds, samples, both derivatized and underivatized, were injected in a *splitless* mode, and separation was carried out using a capillary column HP-5MS (60m x 0.25 mm x 0.25 μ m) with a helium flow of 1 mL min⁻¹ as a carrier gas, according to Sanchís et al.⁶². For polar compounds, same samples were injected in the same way but using a HP-Innowax column (60m x 0.25 mm x 0.25 μ m) based on the methodology reported by Zhang et al.²⁰. Selected Ion Monitoring was used to elucidate siloxanes presence, and molecules were identified by comparing the NIST14.L mass-spectra library based on an 85% similarity cut-off. As phenyl-siloxanes were identified using this method, column bleeding was tested by running samples with the solvent extraction mixture alone using the same protocol as real samples.

All treatments and analyses were performed in triplicate. First, parametric statistical assumptions were tested using the Levene (homoscedasticity), Kolmogorov-Smirnov (normality), and residual regression model (graphical normality) tests. Second, all the data were analysed under a General Linear Model (GLM) by using the test for factorial analysis of variance (2-way ANOVA) to elucidate the interactions of the tested factors. Finally, the HDS Tukey and Scheffé tests were performed as post hoc analysis. For all the tests, the α error assumed was 0.05 under a significance of 95%, using the IBM SPSS statistics 22 software (IBM).

Results and discussion

Our results show that microaeration favours both the rate and extent of methane production (**Fig. 1**). On average, microaerated treatments produced 10% more methane than anaerobic ones from the degradation of OS and the VOSiC mixture. Also, the acclimated sludge showed better performance than the fresh one, suggesting adaptation of the native sludge microorganisms to the substrate and to the microaerobic conditions (**Fig. 1**-Green line). Methane production curves showed that the acclimated sludge began to produce methane 2 to 4 days earlier than fresh one and showed a more stable methane production during the experiment. Finally, our results show that in high VOSiC concentrations (500ppm) total methane production is almost the same despite the oxygen presence (**Fig. 1-Charts 3, 6, 9**),

suggesting that microaeration improves the general process stability but not the VOSiC conversion to methane.

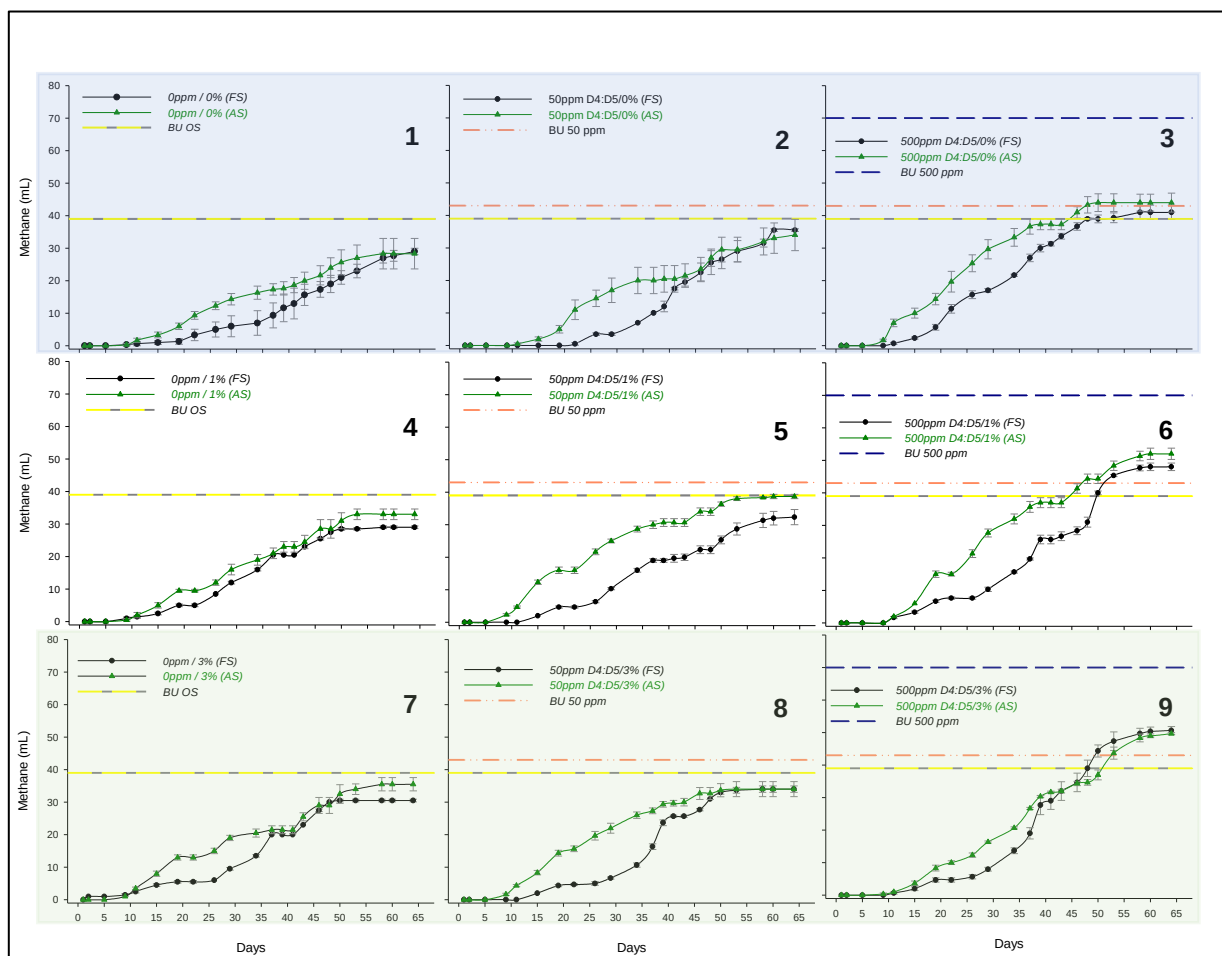


Figure 10. BMP results methane production with D4:D5 mixtures. **BLUE BACKGROUND (BB):** Cumulative methane production of anaerobic treatments: **1)** Organic substrate (OS). **2)** OS + 50ppm D4:D5 **3)** OS + 500ppm D4:D5. **WHITE BACKGROUND (WB):** Cumulative methane production of PO₂ 1% v/v microaerated treatments: **4)** Organic substrate (OS) **without VOSiCs**. **5)** OS + 50ppm D4:D5. **6)** OS + 500ppm D4:D5. **GREEN BACKGROUND (GB):** Cumulative methane production of PO₂ 3% v/v microaerated treatments: **7)** Organic substrate (OS) **without VOSiCs**. **8)** OS + 50ppm D4:D5 **9)** OS + 500ppm. Methane production is expressed as volume in STP conditions. The yellow, orange, and blue segmented lines in all backgrounds denote the theoretical biochemical methane potential (^{Bu}) of the OS, OS+ 50 ppm D4:D5 VOSiC mixture, and OS+ D4:D5 500 ppm VOSiC mixture, respectively. Black lines (FS) correspond to fresh sludge and green lines (AS) to acclimated sludge.

Microaeration improves the rate and extent of methane production regardless of the substrate

In general, microaeration increased the volumetric methane production compared to the strictly anaerobic treatments, suggesting that small oxygen concentrations are beneficial to anaerobic systems. Among the treatments not supplemented with VOSiC (*i.e.*, blanks) (**Fig. 10**-Charts **1,4,7**), microaerated 1% and 3% PO₂ yielded 17% to 25% more methane when the acclimated sludge was used. Surprisingly, treatments using fresh sludge were not negatively impacted by oxygen presence, yielding up to 5% more methane when 3% PO₂ was used. An improvement in methane production under microaerobic conditions has been previously reported not only for organic substrates in general^{27,43,63}, but also for polymeric siloxanes²⁹ and other recalcitrant compounds^{52,53}. Previous reports attribute this extra methane production to a shift in the AD microbial ecology that leads to an improved hydrolysis during the initial stages of the AD process^{64,65}. Accordingly, it is apparent that the controlled addition of oxygen triggers the ecological microbial change that favours facultative anaerobes (*i.e.*, heterotrophs) and strengthens the enzymatic diversity of the sludge, resulting in increased methane production^{26,29,66,67} from the better OS and VOSiC metabolism. These results are also correlated with the degradation products found in the post-digestion sludge of D4, suggest a metabolic oxidation of the D4 molecules according to the mechanism described by Wang et al.¹⁹ producing metabolic intermediates that can be used for the extra methane production.

This enhanced methane production, which must come from enhanced enzymatic hydrolysis leaded by the VOSiC oxidation and use is noticeable when the theoretical methane calculation (*i.e.*, Buswell formula) and actual production rates are compared. Our microaerated treatment results show that methane production exceeded the theoretical maximum of OS + VOSiC mixture at 50ppm (**Fig. 10** – Charts **5,6,9**). For example, microaerated 50ppm D4:D5 treatments, in general, produced 15% more methane than the treatments without siloxanes (*i.e.*, blanks), showing an apparent VOSiC biodegradation and further conversion to methane, despite if the sludge was previously adapted or not (*i.e.*, old, or new sludge). In general, treatments with VOSiCs supplementation and microaerated conditions began to produce methane almost a week earlier than the anaerobic counterparts (**Fig. 10**). This behaviour was previously reported in siloxane polymers biodegradation under microaerated regimes²⁹, where methane production started around the first addition (and consumption) of oxygen in the reactors (**Fig. 11**). Fast oxygen consumption is expected due to the highly reducing environment within the anaerobic environment. This, in turn, supports redox processes^{26,52}, decrease the concentration of mostly of inhibitory compounds^{27,68}, and promote the syntrophic microbial relations⁶⁹, leading to an improved and faster COD conversion²⁹.

Results showed that previous microbial adaptation to oxygen addition might be the reason of the simple substrates improved biodegradability (*i.e.*, OS) in the microaerated treatments when adapted sludge is used. This behaviour has been reported in anaerobic systems where oxygen presence drive changes in the microbial ecological composition^{42,67} and consequently increases the enzymatic catabolism of the tested substrates. Our results show that adapted

sludge produce more methane in volumetric basis when oxygen is present, probably due to a better transformation of the OS components by the methanogenic organisms present in the sludge (**Fig. 10-Charts 4,7**). Since microbial adaptation seems to be a key factor on the organic substrate conversion to methane, a valid hypothesis could be that adapted sludge has a better performance when VOSiCs are supplemented in the microaerated treatments. Previously reported results^{43,52,70} show the validity of this hypothesis for other recalcitrant molecules, our results using VOSiCs as substrate also support the aforementioned hypothesis. When D4:D5 mixture is used in different concentrations, the increase in the methane production is noticeable by comparison with the non-siloxane blank treatments for anaerobic and microaerated treatments (**Fig. 10-Charts 3,6,9**). For example, 500ppm VOSiC supplemented treatments with fresh sludge had 33% (anaerobic), 58% (PO₂ 1%) and 40% (PO₂ 3%) more methane than the blanks, while adapted sludge had 41% (anaerobic), 65% (PO₂ 1%) and 66% (PO₂ 3%) comparing with their respective blanks. This comparison clearly shows that previous adaptation may be important in recalcitrant compound biodegradation, especially for volatile siloxanes.

The difference between well adapted microbial populations in microaerated conditions are noticeable in the 50ppm D4:D5 VOSiC treatments where the adapted sludge produced 13% more methane than fresh one (**Fig. 10-Chart 5**). Previous adaptation again proofs to be significantly important for the process stability and methane production since fresh inoculum produced 5% less methane in average than treatments using adapted sludge. In our case, it seems that directed evolution and microbial adaptation allowed the selection of the enhanced populations with the desired functional traits to biodegrade recalcitrant compounds such as VOSiCs. This phenomenon has been thoroughly reported on different experimental settings^{71,72}, where the approach is based on the restriction of the environmental and substrate conditions to improve microbial diversity and the methane conversion rates. Despite from the benefits of previous adaptation to substrate, our results show some sort of microbial inhibition when high VOSiC concentrations are used especially in the acclimated sludge. From our post-digestion chemical elucidations in the digestate, we found a high number of antimicrobial compounds (*i.e.*, phenol, aliphatic alcohols) on the acclimated sludge treatments when 500ppm of the VOSiC mixture is used. This issue was previously reported in our microaerated PDMS biodegradation results²⁹, where alcohols presence tend to inhibit microorganisms to produce more methane and consequently hinder the full siloxanes biodegradation.

Inhibition due to antimicrobial alcohol presence could explain the differences between acclimated and fresh sludge 500ppm treatments. Despite the oxygen presence, in average inocula produced similar amount of methane at the end of the experiment (**Fig. 10-Charts 3,6,9**). Nonetheless, comparing volumetric methane production of 500ppm microaerated treatments with the anaerobic ones, fresh sludge has better production rates (17% and 23% of CH₄ for 1% - 3% PO₂) than the adapted counterpart (18% and 12% of CH₄ for 1% - 3% PO₂). Results suggest that the biochemical degradation capabilities of the native AD microorganisms can be significantly improved under microaerated conditions, selecting for more fit populations to degrade VOSiCs, deal with antimicrobial compounds and produce slightly more methane from its products. In summary, our results strongly suggest that despite that it is important to use a previously acclimated inoculum to reduce the lag phase⁷², this is

not mandatory to obtain VOSiCs microbial biodegradation. Our data shows shorter lag phases, lower variability among replicates, and sustained methane production during the time of the experiments when acclimated sludge is used but not a significantly bigger final methane production (**Fig. 10**-Charts 5,6,8,9). As it was stated by other reports it seems that oxygen has the capability of shaping the microbial population even if it was not previously acclimated, improving PDMS²⁹ and organic compounds biodegradation^{44,53}. In summary, VOSiC can be degraded under microaerated conditions, despite the inocula preconditioning or previous adaptation to siloxane substrates. As in previous reports, oxygen presence can enhance the thermodynamics and kinetics of the anaerobic digestion, probably due to the change in the sludge microbial ecology and enhanced VOSiC hydrolysis. Still, microaeration should be tested in each specific case since its effect depends on the microbial populations present in the anaerobic sludge and the operative conditions of each siloxane treatment system.

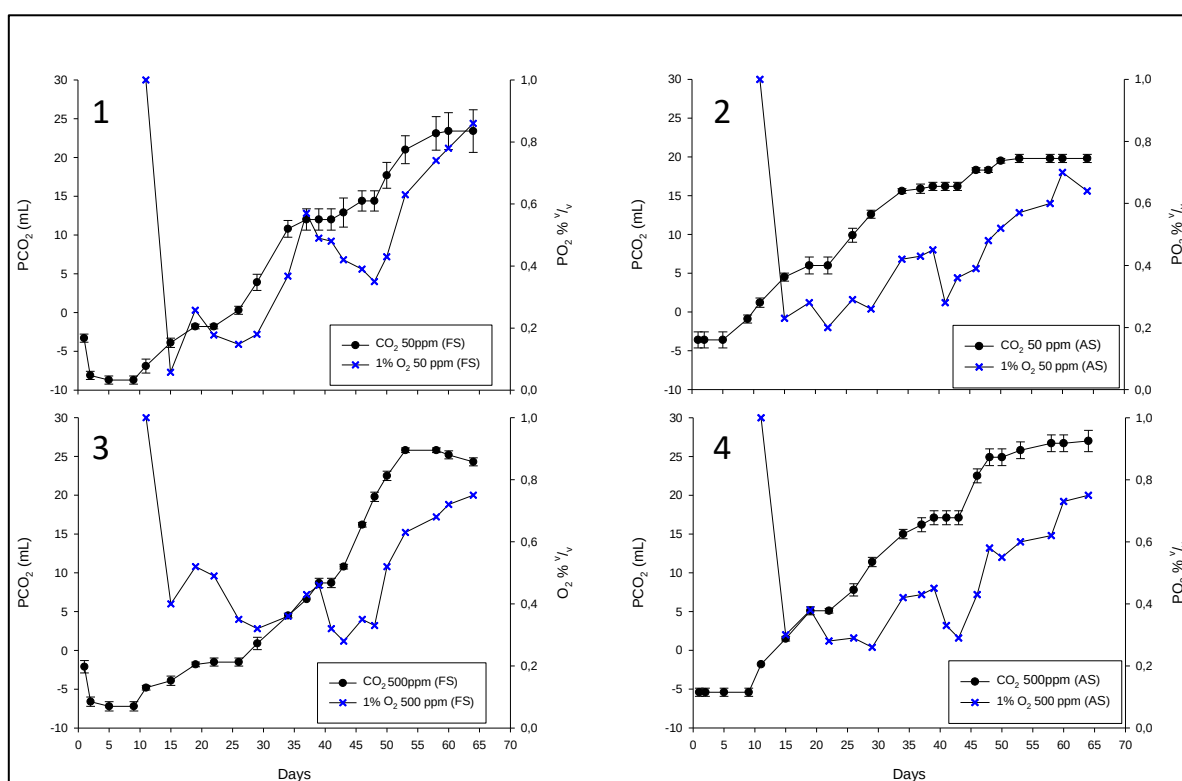


Figure 11. Carbon dioxide (CO₂) production and oxygen consumption (%) comparing the used inocula and relative to the O₂ amount added during the BMP experiments. Since oxygen was added every time after measurement, its addition is omitted in the graphs for clarity. The letters FS stands for fresh sludge and letters AS for acclimated sludge.

O₂ / CO₂ equilibrium under microaerobic conditions

Our results show that oxygen is rapidly consumed after its dosage, requiring subsequent re-addition to re-establish the target microaerated regimes (*i.e.*, PO₂ = 1%, 3%) (**Fig. 11**). This behaviour seems particularly active during the exponential methane production stage of the BMP, which has the highest oxygen consumption rates (**Fig. 10** and **Fig. 11**), and it is when

facultative hydrolytic/acidogenic bacteria are expected to be more active. Consequently, it might be that facultative anaerobic organisms can deplete the added oxygen, increase the enzymatic diversity, and use the extra energy available from respiration to oxidise the VOSiC molecules; this mechanism might maintain the appropriate oxygen reduction potential (ORP) levels to not hinder methanogens⁶⁷. Also, data shows that the acclimated sludge has a continuous oxygen usage without sudden consumption peaks (day 25 to 40) during the experimental run as in the fresh one (**Fig. 11, Charts 1,2**). This behaviour might be due to the previous acclimation of the used sludge that shaped the microbial ecology in the inoculum to withstand dissolved oxygen in the media and used it on the siloxanes metabolism. Nonetheless, when VOSiC concentration increased to 500ppm, the oxygen consumption profiles between both inocula seem to be similar (**Fig. 11-Chart 3,4**). These results suggest that high siloxane concentrations seem to stabilise microbial response to oxygen in the media, which is especially clear at high O₂ partial pressures (3%) (- **Supplementary information Fig. S1**).

Accordingly, we theorised that VOSiCs at high concentration have an oxygen protection effect to the fresh inoculum microorganisms. This is apparent comparing the 50 ppm 3% PO₂ treatments with the 500 ppm in terms of oxygen consumption and CO₂ production (**Fig. S1**). In the 50ppm treatments, the fresh sludge showed a lower CO₂ production between days 20 to 50 than the acclimated inoculum, which is directly related with a less oxygen usage (**Fig. S1- Chart 1,2**). On the contrary, oxygen consumption and CO₂ production profiles are similar for both inocula when 500ppm of the VOSiC mixture is applied (**Fig. S1- Chart 3,4**). Consequently, it might be possible that VOSiCs in the liquid medium decrease the oxygen diffusion and protect the strict anaerobes in the fresh inoculum microbial from O₂ toxicity. This theory has a theoretical fundament on the siloxanes capacity to increase the viscosity in aqueous solutions⁷³ and their low water solubility⁷⁴, with the increased viscosity, dissolved oxygen on aqueous phase cannot easily reach the microbial cells and consequently they will be protected from it. Due to siloxanes coating behaviour³⁵, we theorise that VOSiC molecules could be hydrophobically attracted to bacterial membranes conferring a protective layer that could delay oxygen diffusion to the microbial cell. Also, since microbial populations in the fresh sludge are not used to biodegrade siloxanes as the same rate as acclimated inocula, this protection effect is more noticeable and shows the main difference between a previously adapted inocula and a fresh one.

Finally, comparing oxygen consumption profiles among adapted and fresh sludge, it seems clear that microaerated conditions and VOSiC concentration have a positive interaction in the microbial ecology and performance within the BMP units. Despite that the above-mentioned differences in oxygen consumption between both inoculums, the methane production is not so different between the treatments (**Fig. 10**). The possible reason of this result is that despite the differences in the oxygen consumption profiles (**Fig. 11, Fig. S1**) show a clear previous adaptation of the microbiota to all microaerated conditions, the fresh sludge bacteria seem to be positively enhanced by the more energetic electron acceptor in an ongoing adaptation process. Oxygen has been previously reported in other experiments such as BTEX (benzene, toluene, ethylbenzene, xylenes) degradation⁷⁵, carbon deprivation⁷⁶, and composting processes⁷⁷ as a key factor to shift microbial populations allowing or enhancing these processes. In conclusion, the correlation between O₂/CO₂ balance and methane

production demonstrates that oxygen allows the facultative bacteria to reduce dissolved oxygen concentrations so that methane-producing organisms are not compromised by O₂ toxicity. This particular mutualistic relationship, studied previously using non-siloxane substrates^{42,63,78}, supports our hypothesis that microaeration drives changes in the ecological structure of the microbiota. Accordingly, the new ecological structure might enhance the production of new enzymes and the possibility of harvesting more energy from substrates, which may explain why VOSiCs are more biodegradable under microaerated conditions.

To summarize, we could infer that VOSiC microbial catalysis requires oxygen stimulation and a stable O₂ consumption profile to assure process stability. Also, despite that, adapted inoculum seems to have enough enzymatic strength to begin CO₂ production before the oxygen addition (*ca.* 12 days), and it is not sternly affected by the high oxygen concentrations (3% O₂-**Fig. S1**- Chart 2,4). A previous adaptation of the inoculum seems no to be mandatory to assure VOSiC conversion and increased methane production under microaerated regimes. Oxygen-driven ecological microbial change seems to bring about specific bacterial groups that develop mutualistic relationships among them and native sludge organisms^{42,43,79} enhancing organic substrate (OS) and VOSiCs conversion to methane.

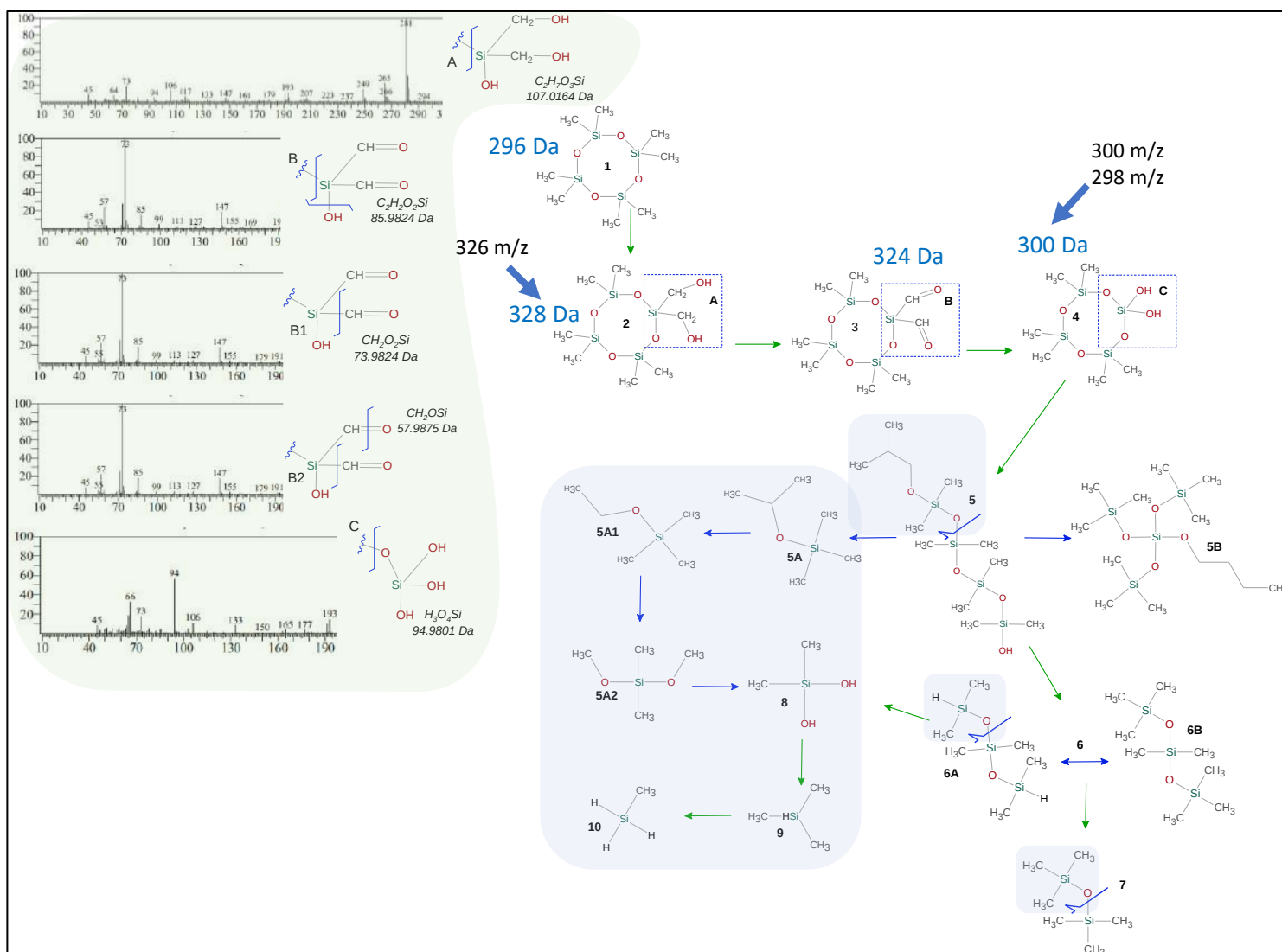


Figure 12. Possible pathway of D4 biodegradation under microaerated conditions in the BMP assays. Numbers in blue refer to the molecule weight in Daltons (Da). Dotted squares zoom to focus the part of the oxidised molecule and frames the mass spectra peak fragment that confirms the structure. Numbers in black with the m/z symbol correspond to the mass peak found that confirms the molecule existence. Green arrows show the general biodegradation pathway. Blue arrows show alternative pathways from the marked precursors (Blue background).

Microaeration allows microbial VOSiCs catalytic oxidation.

From the post-digested samples, we perform a liquid-liquid solvent assisted extraction finding siloxane-related molecules chemically identified by mass spectrometry (GC-MS). Samples were tested for polar compounds (HP Innowax Column), non-polar compounds (HP—5MS column) and further derivatised to be re-tested with the same columns and conditions. We found molecules **1**, **2**, and **3** (**Fig. 12.**) in the non-polar GC-MS tests from the

microaerated treatments (1% and 3% PO₂). Molecules 4 and 5 were found in the polar fraction of the GC-MS, same as 6 and 7 from the derivatised fractions (**Fig. 12.**). Our results suggest a stepwise pathway that begins with the overcoming of the steric hindrance produced by the methyl groups attached to the silicon part in the siloxane bond^{80–82} that hinders the opening of the siloxane ring. Our proposed pathway recognizes the enzymatic difficulty to cleave the Si-O bond and considers an initial oxidation of the methyl terminal up to primary alcohol (**Fig. 12 – 2A**). Nonetheless, previous reports¹⁸ proposed a D4 biodegradation pathway based on the microbial siloxane-ring opening by the initial catalysis of the Si-O bond without considering siloxanes steric hindrance. Siloxanes steric hindrance has been known to confer stability to the VOSiC molecule⁸⁰, be partially responsible for its most important physicochemical characteristics (*i.e.*, resistance to chemical and physical changes)^{80–82} and siloxanes recalcitrant nature.

Mass-spectra elucidations show that molecule 2 is present, especially in the microaerated treatments. As shown in **Fig. 12**, the proposed pathway is based on the initial oxidation of the methyl groups attached to the silicon molecule up to primary alcohols. This oxidation is identified by the change in the mass of the D4 molecule from 296 Da to 328 Da by substituting two H⁺ by the same number of OH- groups. The finding of a siloxane molecule with 326 ^{m/z} as a molecular peak and D4 fragment pattern demonstrates the presence of the methyl-oxidised molecule in the microaerated treatments only. This methyl oxidation is also confirmed in the mass-fragmentation pattern of that molecule, where fragmentation peak 107 ^{m/z} is related with the fraction Si-CH₂-OH in molecule 2 (**Fig. 12– Molecule 2A**), demonstrating that the D4 siloxane methyl substituents were microbially oxidised under microaerated conditions. Our results and elucidations prove that facultative anaerobes must remove the steric hindrance on siloxane molecules to begin their oxidation and further biodegradation. Wang et al. (¹⁹) previously suggested this microbial method to biodegrade siloxanes under aerobic conditions. Since the above mentioned molecules were found exclusively in the microaerated treatments Wang et al. (¹⁹) report support our results in terms of final electron acceptor used. Consequently, we consider that siloxane Si-CH₃ oxidation is only possible when a more energetic final electron acceptor is present (*e.g.*, nitrate, oxygen). Nonetheless, as this is the first time that microaerated conditions are successfully applied in VOSiCs biological degradation, the suggested pathway in this area requires further study and confirmation by radioisotope methods.

The proposed biodegradation pathway continues with the conversion of primary alcohols present in molecule 2 to aldehydes (**Fig. 12 – Molecule 3) following organic oxidation reactions. We confirmed the identification of molecule 3 by the mass peak at 324 Da and mass spectra fractions 57 ^{m/z}, 73 ^{m/z} and 85 ^{m/z}, showing the oxidation from the Si-CH₂-OH to the aldehyde substituent Si-CH-O. This gradual reaction, clearly observed in the 1% and 3% PO₂ treatments, was not previously described in siloxane molecules. Reaction products suggest the presence of dehydrogenases enzymes that use NAD⁺ aerobic system to catalyse the alcohol oxidation. Consequently, the found chemical products imply an aerobic metabolism in a methane production microaerated system indicating a balance between aerobic and anaerobic biochemical reactions. Subsequent reaction steps suggest a nucleophilic attack on the aldehydes carbonyl centre that derives in the removal of the carbonyl group and substitution with the -OH radical (**Fig. 12 – Molecule 4**). This reaction**

product was corroborated by the mass peaks 300 m/z and 298 m/z and the mass fraction 94 m/z that confirms the existence of the Si-OH bond, validating the microaerated biological oxidation of the D4 VOSiC molecule. Finally, terminal alcohol attached to the Si atom allows the steric hindrance overcoming using the molecular repulsion between the -OH free electrons and the oxygen free electron pairs in the Si-O-Si cyclic structure. As it was previously suggested by Wang et al. (¹⁹) proposed mechanism.

Since the siloxanes recalcitrant nature is mainly due to their steric hindrance, previous studies¹⁸ proposed direct oxidation and substitution of the Si-CH₃ molecule with the alcohol OH terminal (Si-OH). Nonetheless, other reports^{19,83} agree that substitution is not possible even in more thermodynamically ideal conditions (*i.e.*, complete aerobic regime), considering the energetic and enzymatic requirements that microorganisms must fulfil to achieve such reaction. Accordingly, our proposed biodegradation pathway only can be possible after a microbial ecology adaptation through the directed evolution approach using microaerated systems and the substrate acclimation used in our study. Siloxane degradation studies agree that once the siloxane ring can be opened, the catalysis process of the linear molecules occurs stepwise^{20,84} breaking the molecule one or two siloxane monomer at time¹⁸. Our chemical elucidations concur with this statement and suggest that once the D4 molecule is open the OH terminals are susceptible to react with other metabolic product present in the system, for example, the isobutyl substituent in the molecule 5 (**Fig. 12 – Molecule 5) which might be the result of an ether formation between the siloxane molecule and isobutanol present in the system. Also, previous reports confirm that alkyl siloxanes or siloxane molecules with long organic substituents such as molecule 5B are more prone to be biodegraded by microbial means than the ones that do not have organic substituents⁸⁵.**

As previous studies suggest, linear siloxane molecules begin to be catalysed one or two monomers at the time¹⁸, our mass spectra elucidations also support this statement finding the cleavage product of the elucidated octamethyltetrasiloxanol (**Fig. 12 – Molecule 5). It seems that the trimethylsiloxane 5A is released after the Si-O bond break in molecule 5 and is further catalysed by the demethylation of the isobutyl substituent (**Fig. 12 – Molecule 5A1). Isobutyl demethylation is a well-known process performed by several aerobic organisms, such as *Pseudomonas* sp.⁸⁶, *Nocardia* sp.⁸⁷ and *Rhodococcus* sp.⁸⁸ where oxygen-dependent demethylase enzymes could transform alkyl products into alcohols that will be further metabolised⁸⁹. Correlating The proposed biodegradation pathway reactions correlated with O₂/CO₂ balance results and the increase in methane production, supports the idea of a microbial ecological change that allows the microorganisms in the acclimated sludge to catabolise recalcitrant organic compounds. Also, the demethylation reactions in biogas systems has been previously reported, for example, Boada et al.²⁴ refers that *Methylibium* sp. organisms perform demethylation reactions (*i.e.*, methylotrophic activity) from siloxane molecules to obtain energy and sustain their development. Accordingly, after having found these molecules in the post-digested samples, we confirm that VOSiCs are being biodegraded by a mixed microbial community that oxidise the alkylic substituents (*i.e.*, butyl, methyl, ethyl) to obtain energy from their transformation up to CO₂ or methane production. We also found the methyl ether produced by the possible demethylation and further oxidation of the 5A1 molecule and subsequent formation of the Dimethylsilanediol DMSD (molecule 8). Dimethylsilanediol has been widely reported as siloxane biodegradation product^{15,20,33,84} due****

to its chemical nature as siloxane monomer with OH- substituents which reduces completely its volatileness and allow microorganisms to degrade it up to silanes.

Finally, the proposed biodegradation pathway suggest from our chemical identifications that remanent trisiloxanes (molecules 6A, 6B) are cleaved releasing a siloxane monomer that is transformed into DMSD. From our GC-MS elucidations we found that trisiloxane molecules with methyl substituents in the hydrogen terminations (molecule 6A) are less common in the analysed samples. Considering that L3 molecules (molecules 6A, 6B) were not added to the BMPs in the experimental set up, they must come from the D4 biodegradation, and subsequently they should have an OH- termination where the H- and CH₃- substituents are in molecules 6A and 6B. We theorise that it is possible that mass-spectra identification by NIST library could mixed the OH- (17 ^{m/z}) terminal with a CH₃- (15 ^{m/z}) one, since they only have a difference of 2 ^{m/z}. Nonetheless, we decide to leave the molecules as we found and identify in the GC-MS analysis for result traceability. Our results also show that trisiloxane catabolism follows the previous process of cleaving one monomer at time, releasing a siloxane molecule that is further oxidised to DMSD and a disiloxane remanent (molecule 7) that will also be transformed into Dimethylsilanediol (molecule 8). These stepwise reactions after the siloxane ring opening were previously explained by Li et al ¹⁸ suggesting that the biodegradation of siloxane molecules is easier when the methyl substituents are oxidised to Si-O-CH₃ ethers that could be further transformed into methanol and be metabolised up to CO₂ by C1 metabolic cycle pathway.

In summary, the GC-MS elucidations demonstrate that microaerated conditions favour the VOSiC siloxanes oxidation by process of subsequent oxidation steps that first remove the methyl substituents shielding effect (*i.e.*, steric hindrance). Accordingly, it seems that oxygen presence boosts the enzymatic diversity allowing microorganisms to biodegrade VOSiCs present in the BMP sludge, reducing their volatility and assuring their retention in the AD sludge. This proposed pathway under microaerated conditions shares several similarities with the D4 aerobic biodegradation previously proposed one¹⁹. Also, we found that linear siloxanes are catabolised one monomer at time, as previous reports state¹⁸. We conclude that the siloxane biodegradation pathway under microaerated conditions is highly dependent on the previous adaptation of the used microbiota behaving similar to a complete aerobic culture but with the feature of transforming oxidised siloxanes into methane as our results suggest.

Conclusions and outlook

The current study proposed and confirmed the hypothesis that D4 and D5 VOSiCs could be microbially biodegraded using microaerated conditions that favour the molecules oxidation and further stabilisation by their loss of volatile character. We found that VOSiC catalysis is microbially-mediated when oxygen is used as the final electron acceptor, producing metabolic intermediates that are less recalcitrant and serve as electron donors for methane production. Our results have shown that inoculum previous acclimatation is important for the process kinetics, however, it is not mandatory since the presence of oxygen and siloxanes might interact to develop the required ecological changes for siloxane biodegradation and improve the volumetric methane production. The additional methane evidence the VOSiC microbial conversion to that resulting from the degradation of the organic substrate alone and the presence of siloxane oxidised molecules in the digested sludge. Data shows that oxygen

drives complex microbial adaptation processes that could be inferred by the oxygen consumption and CO₂ production profiles, both relating to the efficiency in methane production, VOSiC biodegradation, and AD process.

Finally, we proposed a microaerated microbial D4 biodegradation pathway that considers also previous data on aerobic and abiotic VOSiC oxidation mechanisms. From the elucidated molecules we could partially infer the biochemical reactions that were happening in the BMP units and support the theory of a balance ecosystem between facultative anaerobe microorganisms and strict anaerobes. We also verify previous siloxane-oxidation studies about the importance of overcome the methyl substituents steric hindrance to ensure the VOSiC siloxane ring-opening and further stepwise stabilisation. Despite that, for the best of the authors knowledge, this is the first time that a VOSiC biodegradation pathway under microaerated conditions is proposed, it must be studied and confirmed by further studies.

Considering that in the WWTP oligomeric and polymeric siloxanes arrive through the wastewater, understanding their chemical behaviour, transformations, and fate within the process will help to develop suitable treatment strategies to reduce siloxane effects on biogas-based energy systems, environmental emissions, and harmful ecological effects. This study suggests that microaeration of the anaerobic sludge will significantly decrease the concentration of VOSiCs in the gas phase by decreasing their volatile character through the increase in polarity achieved by the microbially catalysed oxidation, esterification, and cleavage reactions. Nonetheless, for microaeration to benefit WWTPs, and for WWTPs to become barriers to the emission of these ecotoxic contaminants to the environment, such a strategy must be coupled with efficient biodegradation for the VOSiCs that goes to biogas.

Supplementary information

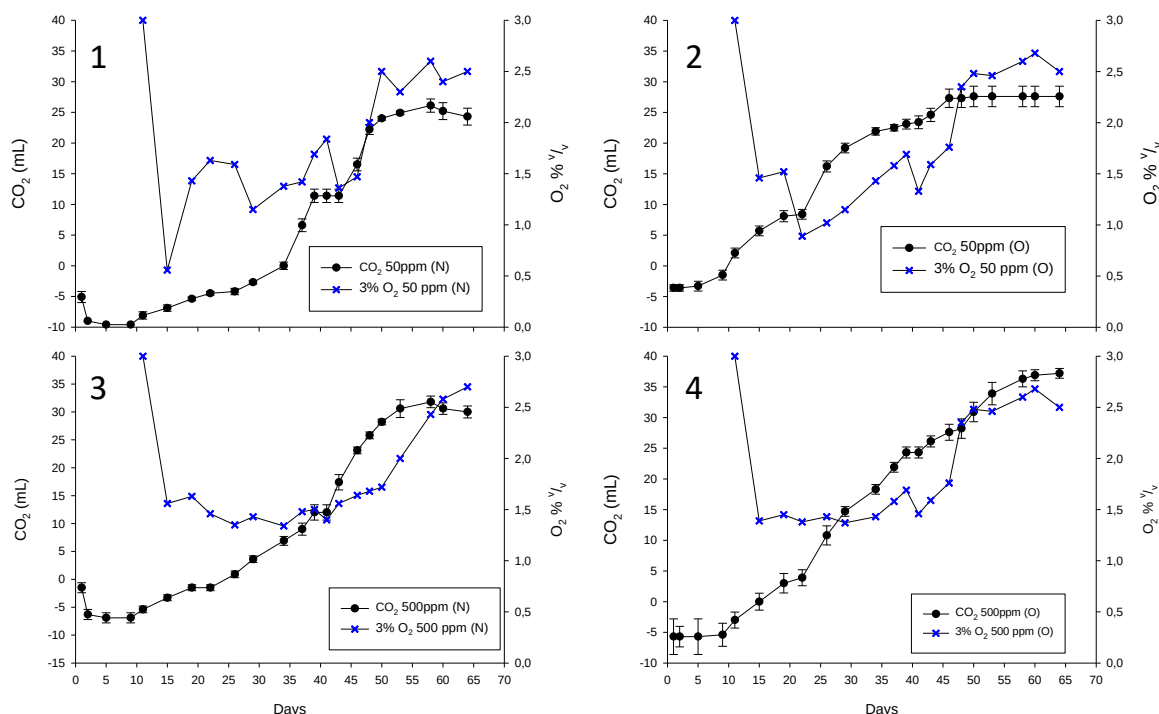


Fig. S1

Conclusions and outlook third chapter

- D4 and D5 VOSiC siloxanes are biodegradable under microaerated conditions.
- Previous microbial adaptation to microaerated conditions and siloxane substrate does not seem to be a mandatory step for D4 and D5 biodegradation. Nonetheless, treatments with acclimated inocula seemed to have a better performance in siloxane biodegradation terms than fresh sludge.
- The overcoming of the siloxanes steric hindrance seems to be mandatory to assure the subsequent mineralization of D4 and D5 volatile siloxanes.
- The siloxane biodegradation pathway might be by subsequent stepwise stages that first open the siloxane ring and then breaks the linear molecule releasing one or two siloxane monomers per time.
- D4 and D5 biodegradation under microaerated conditions yield more methane from the anaerobic metabolism, suggesting a siloxane stabilization up to methane.

OVERALL CONCLUSIONS

Siloxanes are a new type of environmental pollutant that is emitted faster than they can be naturally degraded. These compounds are present in almost every industry but especially in personal care products, which residues are the primary source of siloxane contaminants to the WWTPs and the environment. WWTP has been identified as the primary nexus of their environmental dispersal, polymeric (PDMS) and volatile (VOSiC) siloxanes arrive at the wastewater treatment facilities attached to the organic matter present in the black waters. Here siloxanes are concentrated in the solid portion and slowly released into the atmosphere by the different stages of the water treatment process. Nonetheless, siloxane accumulation and slow release at the wastewater treatment plant presents a unique opportunity for targeted treatment and prevention of environmental damage. Consequently, approaches that could enhance the siloxane metabolism *in situ* are especially relevant for a better future scale-up and application.

Among all the various approaches developed for siloxane removal, only physical adsorption seems to fulfil every requirement (*i.e.*, economic, environmental, and technical). However, this approach is not without its disadvantages, especially regarding waste production from spent cartridges or saturated sorbent. Biological siloxane removal is a relatively new research topic with a greater potential for future advancement on siloxane treatment, mainly because it could be easily adapted to the current AD facilities or could be implemented within the AD treatment. Yet, the slow or poor biodegradation of siloxanes, the lack of knowledge on the involved microorganisms or their biochemical interactions, and the immaturity of the developed studies have discouraged the advancement of biological treatment methods as a siloxane removal approach. We also considered that the lack of a multidisciplinary approach regarding the siloxane problem is a critical point that had to restrain the development of good and reliable approaches in VOSiC prevention and treatment. Consequently, we decided to use a microaerated approach and a directed evolution method for microbial development and siloxanes biodegradation improvement. According to our results, trace amounts of oxygen in an otherwise strictly anaerobic environment drive the ecological and biochemical changes needed to biodegrade siloxanes (PDMS and VOSiC), producing additional methane than the theoretically calculated.

Our study shows that the presence of VOSiC in biogas may not be the sole result of volatile siloxanes coming in wastewater but also a consequence of PDMS cleavage during anaerobic digestion that produced new VOSiC compounds, as proved by GC-MS elucidations. We found that VOSiC catalysis is microbially-mediated when oxygen is used as the final electron acceptor, producing metabolic intermediates that are less recalcitrant and serve as electron donors for methane production. This metabolism could close the cycle of siloxane pollution in the AD sludge. Our results show that it is possible to biodegrade siloxane polymers and oligomers using the same approach and inocula. Accordingly, using the same microorganisms to stabilize siloxane molecules and prevent their environmental release completely is possible.

Finally, we proposed a microaerated microbial D4 biodegradation pathway that considers previous data on aerobic and abiotic VOSiC oxidation mechanisms. From the elucidated molecules, we could partially infer the biochemical reactions in the BMP units and support the theory of a balanced ecosystem between facultative anaerobe microorganisms and strict anaerobes. We also verify previous siloxane-oxidation studies about the importance of overcoming the methyl substituents steric hindrance to ensure the VOSiC siloxane ring-opening and further stepwise stabilization. To the best of the authors' knowledge, this is the first time that a VOSiC biodegradation pathway under microaerated conditions is proposed; it must be studied and confirmed by further studies.

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