

Conductive materials and their promising role in the anaerobic treatment of agro-industrial wastewaters

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Resumen

La digestión anaerobia (DA) es una tecnología ampliamente aplicada para el tratamiento de aguas residuales industriales, que permite la transformación de la materia orgánica en biogás, constituido principalmente de metano (CH₄). A pesar de las ventajas ambientales que ofrece la DA, la optimización de la producción de CH₄ y la estabilidad de los reactores siguen constituyendo un reto, particularmente durante el tratamiento de aguas residuales de composición compleja. Trabajos recientes se han enfocado en la mejora del desempeño de la DA a través de la adición de nanomateriales conductores, que incluyen materiales hechos de carbono y de hierro. Dichos materiales facilitan la transferencia de electrones entre los microorganismos, mejorando las relaciones sintróficas cruciales para la metanogénesis, pero que, además, para el desarrollo de nuevas tecnologías, como aquellas relacionadas con la oxidación anaerobia de amonio (NH₄⁺). La integración de nanomateriales conductores en los sistemas de tratamiento anaerobio ha demostrado mejorar su estabilidad y aplicabilidad. La presente revisión destaca los beneficios por los cuales los nanomateriales conductores mejoran la capacidad metabólica de los microorganismos anaerobios y discute su prometedor rol en el tratamiento anaerobio de aguas residuales agroindustriales. Los hallazgos enfatizan que al suplementar nanomateriales conductores ofrece una estrategia viable para superar las limitaciones existentes en la DA, además de promover metabolismos novedosos que puedan ayudar a reducir la contaminación del agua y mejorar de manera eficiente y sostenible el manejo de aguas residuales en el sector agroindustrial.

Palabras Claves: Digestión anaerobia, materiales conductores, aguas residuales agroindustriales, Feamox, transferencia de electrones

Abstract

Anaerobic digestion (AD) is a widely adopted technology for treating industrial wastewater, enabling the transformation of organic matter into biogas, mainly constituted of methane (CH₄). Despite its environmental advantages, optimizing CH₄ production and reactor stability remains a challenge, particularly with complex wastewater streams. Recent research has focused on enhancing AD performance through the addition of nano-sized conductive materials (nano-CMs), including carbon-based and iron-based materials. These nano-CMs facilitate electron transfer between microorganisms, thereby improving syntrophic interactions crucial for efficient methanogenesis, but also for the development of other novel technologies, such as those related to anaerobic ammonium (NH₄⁺) removal. The integration of nano-CMs in anaerobic treatment systems has demonstrated improved process stability and applicability. This review highlights the benefits by which nano-CMs enhance the metabolic capacity of anaerobic microorganisms and discusses their promising role in the anaerobic treatment of agro-industrial wastewaters. The findings emphasize that supplementing nano-CMs to AD offers a viable strategy for overcoming existing limitations, besides promoting novel metabolisms that can help to reduce water pollution and changing the way for a more efficient and sustainable wastewater management practices in agro-industrial sectors.

Key Words: Anaerobic digestion, conductive materials, agro-industrial wastewater, Feamox, electron transfer

Introduction

Agro-industrial activities are dedicated to producing, processing and commercializing agricultural and forestry products for food purposes, such as production of meat or dairy products (Rosete, 2020). Despite their economic importance, agro-industrial activities impact negatively on the environment, generating large amounts of solid wastes and wastewater (Martinez-Burgos et al., 2021). Anaerobic digestion (AD) is a consolidated biotechnology applied in wastewater treatment that allows the transformation of organic matter into biogas and other valuable products. AD is based on the metabolism of different types of microorganisms that transform organic matter into biogas, mainly composed of methane (CH₄) and carbon dioxide (CO₂), of which CH₄ produced could be used as biofuel to generate heat and electric energy when its burned (Rittmann and McCarty, 2020). Besides the production of CH₄, AD offers other important benefits, in contrast to other types of biological wastewater treatments; for example, AD generates less wasted sludge, there is no need for oxygen supplementation, microorganisms require fewer nutrient requirements, and is appropriate for high strength wastewaters (Rajeshwari et al., 2000). Because of the many benefits that AD offers, many types of wastewater and organic solid wastes are treated by this biotechnology around the world; however, the improvement in CH₄ generation and organic removal is still a challenge, especially during the treatment of complex wastewaters. Several strategies have

been developed to improve CH₄ generation, including the separation of fermentative and methanogenic steps, the use of different reactors configuration (Upflow Anaerobic Sludge Blanket reactor, Anaerobic Membrane Bioreactor, etc.) or co-digestion of different types of streams or residues (Basset et al., 2016; Bella and Rao, 2023). Recently, the use of conductive materials (CMs) has becoming popular due to the proven benefits on lab-scale AD, and many researches has conducted to tests different materials on different wastewaters under several conditions, demonstrating the great potential of adding CMs in AD process. The mechanisms in what CMs influence in AD are described below.

Electron transfer in the anaerobic digestion process

AD process occurs in four well defined steps, hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Nguyen et al., 2019) (**Figure 1**). In step 1, bacteria break the bonds of complex organic matter constituted of polymers (proteins, lipids, and carbohydrates) releasing monosaccharides, aminoacids and long volatile fatty acids. In acidogenesis (step 2) volatile fatty acids (VFAs) and alcohols are produced and then are converted into acetate and hydrogen gas (H₂) in the acetogenesis (step 3). Finally, in methanogenesis (step 4) archaea produce CH₄ by two mainly pathways, by the fermentation of acetate into CH₄ (acetolactic methanogenesis) and by the reduction of CO₂ employing H₂ as electron donor (hydrogenotrophic methanogenesis).

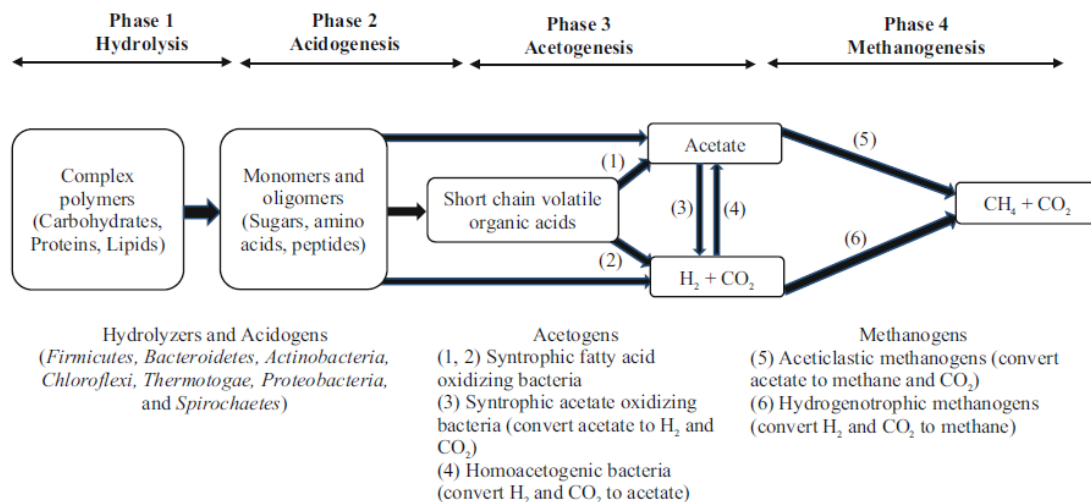


Figura 1. The four steps of anaerobic digestion process and the microbes associated to each step. Figure taken from Nguyen et al. (2019).

Despite the well knowledge of AD developed in the past years, challenges on the CH₄ production and reactors stability are still problems that need to be addressed for improving the process and applicability. Given that the AD process depends on the interaction between fermentative bacteria and methanogenic archaea, steps 3 and 4 of the AD could be considered as the limiting step that condition the CH₄ production (Wu et al., 2020). Several strategies have focused on improving the syntrophic relationship between bacteria and archaea, for example, promoting the interchange of electrons between microorganisms. In the AD process, bacteria ferment organic acids and alcohols releasing electrons in form of H₂ and formate (considered as electron carriers) that are taken by archaea to produce CH₄. This process is namely as mediated interspecies electron transfer (MIET) where electron transfer occurs through the interchange of soluble electron carriers between electron donor bacteria and electron acceptor archaea (Lovley, 2017). Despite the majority of CH₄ is produced this way, MIET requires low H₂ partial pressures that allow fermentative reactions to be thermodynamically feasible, archaea must be able to consume the H₂ produced, otherwise fermentation reactions are interrupted, resulting in VFAs accumulation thus inhibiting methanogenesis and result in digestors failure (Kamagata, 2015).

On the other hand, some microorganisms can share electrons without the need of electron carriers, stablishing direct connections between electron generation microbes and electron consumer microbes, process known as direct electron transfer (DIET) (Lovley, 2017). DIET is important in several types of metabolisms; however, a great attention has been placed on its relevance in methanogenesis given that several studies have demonstrated that DIET increase the performance of the process. Three main ways have been proposed in what DIET could occur related to the production of CH₄. The first one is that fermentative bacteria can share electrons with archaea by means of electrically conductive pilli (e-pilli). Pilli are biological structures composed of aromatic amino acids that enable the transfer of electrons microorganisms (Tan et al., 2017). The second one is that some microbes use c-type cytochromes to share electrons; however,

unlike e-pilli, the transfer of electrons only occur at a very short distance between microorganisms (McGlynn et al., 2015). Finally, the third way in what microorganisms could share electrons is through CMs, such as carbon-based materials (activated carbon, biochar), iron-based materials (magnetite, hematite or goethite) or its combination in composites. These materials serve as bridge between bacteria and archaea allowing the sharing of electrons between them without the need of complex structures (as those mentioned above). Besides, several studies have proposed that CMs are able to enrich microbes with the capacity of transfer electrons directly (Li et al., 2021; Wu et al., 2020).

Benefits of supplementing conductive nanomaterials in anaerobic digestion

Conductive nano-sized materials have been gaining popularity in their application as boosters for AD. Different materials have been tested for enhancing AD, the most important are iron-based materials, that include ferric iron oxides/hydroxides and zero-valent Fe (Fe⁰). Iron-based materials have high conductivity, are non-toxic for microorganisms, and are cheaper than other nanomaterials, such as palladium nanoparticles, that convert them into perfect boosters for AD (Wu et al., 2023). In the same way, carbon-based materials have shown their capacity to enhance AD, the most common materials used include biochar, activated carbon (granular and powder), carbon cloths, graphene, and carbon nanotubes. Carbon-based materials own high conductivity, large surface area, and they can adsorb toxic compounds. Additionally, some materials, such as biochar, are made of plant and food residues that make them a cheap option (Park et al., 2018). Recently, some research showed that combination of iron and carbon materials through different chemical methods results in modified materials, known as composites, that could resulted in a synergistic material that combines the properties of iron and carbon materials (Wu et al., 2023).

The benefits of supplementing nanomaterials in AD depend on the type of inoculum, the substrate and the operation conditions (type of reactor, hydraulic retention time/organic loading rate, temperature, etc.). In general,

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conductive nanomaterials have demonstrated clear improvements in AD performance; for example, increasing CH₄ production rate, besides COD removal and shortening the Lag

phase, among other benefits. **Table 1** shows the most important parameters improvement when nano-CMs are added to the digestors for wastewater treatment.

Tabla 1. Performance parameters improvement when conductive nanomaterials are supplemented in anaerobic digestion.

	Material	Size of materials	Parameter	Influence ¹	References
Iron-based materials	Magnetite,	10-50 nm	-CH ₄ productivity	↑	Ambuchi et al. (2017); Baek et al. (2015); Guan et al. (2023); Kato et al. (2012); Zhuang et al. (2018, 2015)
	hematite,	50-100 nm	-COD removal	↑	
	goethite,	20 nm	-Lag phase	↓	
	Fe ₂ O ₃ , Fe ⁰		-Rate of CH ₄ production	↑	
			-Micronutrient	*	
Carbon-based materials	Activated carbon,	Particle size: 0.5-2 μm	-CH ₄ production	↑	Ambuchi et al. (2017); Liu et al., (2012); Ramírez-Montoya et al. (2023); Tian et al. (2017); Yuan et al. (2018)
	biochar,	20 nm	-Lag phase	↓	
	carbon nanotubes,	Porosity: 100 nm	-Support for microbial growth	*	
	graphene	9-15 nm	-Redox mediator	*	
		200 nm - 149 μm	-Conductivity	↑	
Composites	Biochar-	149 μm	-(Pseudo) capacitance	↑	Chen et al. (2022); Dai et al. (2022); He et al. (2025); Wang et al. (2021); Zhang et al. (2017)
	Magnetite,		-Specific surface area	↑	
	Biochar-Fe ⁰		-Degradation of micropollutants	↑	

1: ↑ (Increment); ↓ (Decrement); * (Other properties)

Despite the proved benefits of adding conductive nanomaterials for AD, most of the experiments have been carried out under ideal conditions, with simple substrates (acetate, ethanol or glucose), mesophilic or thermophilic temperatures, and/or with defined co-cultures. Nanomaterials have the potential to be applied in the anaerobic treatment of complex wastewater as those produced in agro-

industrial facilities. **Table 2** shows the main characteristics of representative agro-industrial streams, such as slaughterhouse, piggery, and dairy industry wastewater. These wastewaters possess high quantities of organic matter, nutrients (ammonium and phosphorus) and other compounds, representing a great challenge for their treatment.

Tabla 2. General characteristics of representative agro-industrial wastewater.

Parameter	Slaughterhouse	Piggery	Dairy industry
	Range		
COD (mg/L)	500-15900	19100-50600	2200-10000
Total nitrogen (mg/L)	50-841	3000-5200	54-1150
Total phosphorus (mg/L)	25-200	660-920	15-283
NH ₄ ⁺ (mg/L)	200-300	1820-3300	5-277
TSS (mg/L)	270-6400	20500-46500	310-5070
Greases and oils (mg/L)	40-1385	—	70-2880
pH	4.9-8.1	6.0-6.5	4.0-12.0

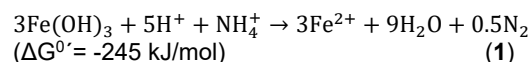
Taken and adapted from Carvalho et al., (2013); Bustillo-Lecompte & Mehrvar, (2015); Girard et al., (2015). TSS: Total suspended solids.

Potential applications of conductive nanomaterials for anaerobic treatment of agro-industrial wastewater

There is no question that CMs supplementation on the AD process offer great benefits, not only in allowing a faster transformation of organic matter to CH₄, also helping in maintain a stable process for the treatment of high strength wastewaters that are difficult to treat. Other potential applications of nano-CMs are those related to the removal of other pollutants, such as NH₄⁺. Due to the high concentration of nitrogenated compounds in agro-industrial effluents, including slaughterhouse and piggery wastewater, the AD of these effluents release NH₄⁺ because of the hydrolysis of proteins and urea, resulting in implementation of a second stage for the removal of NH₄⁺. The most popular way to remove NH₄⁺ is through the nitrification-denitrification process, that consists in oxidation of NH₄⁺ to NO₃⁻ with oxygen as electron acceptor by nitrifier bacteria, then in a second step, the NO₃⁻ produced is reduced sequentially to N₂ under anaerobic conditions (Rittmann and McCarty, 2020). Nevertheless, this process has some disadvantages, for example, in the nitrification step, high amounts of O₂ are required to allow the complete transformation of NH₄⁺ to NO₃⁻; while in the denitrification step, the quantity of COD must be appropriate for the complete reduction of NO₃⁻ to N₂, besides high amount of sludge could be generated due to the fast growth of denitrifiers and have to be assessed according to environmental legislation (Rittmann and McCarty, 2020). Another problem with this process is the generation of greenhouse gases, like Nitrous oxide (N₂O), due to the incomplete reduction of NO₃⁻ (Guo et al., 2017).

Anaerobic ammonium oxidation (Anammox) is an anaerobic biotechnology suitable for NH₄⁺ removal. The process consists of partly oxidizing the NH₄⁺ to nitrite (NO₂⁻) in a preliminary step, so that microbes are able to oxidize the NH₄⁺ to N₂ employing the NO₂⁻ as the electron acceptor in the anammox process. The advantages of this process are that requires low amount of O₂, much fewer production of sludge compared to denitrification process and no production of N₂O; however, due the low rate of growing of the microorganisms involved, enrichment of

anammox microbes is hard, besides the presence of organic matter and other pollutants could impact negatively the reactor, deteriorating its performance (Chen et al., 2022). Other anaerobic biotechnologies have the potential to be applied for wastewater treatment, for instance, the anaerobic ammonium oxidation coupled with ferric iron reduction, process named as Feammox, which occurs naturally in anoxic environments, such as sediments of rivers, wetlands, and riparian soils rich in Fe(III) minerals (Clément et al., 2005; Ding et al., 2014). Microorganisms capable of Feammox can oxidize NH₄⁺ to N₂ (**Equation 1**) in the presence of Fe(III) minerals, generating ferrous iron (Fe²⁺) (Yang et al., 2012).



Some research has been conducted to exploit the ability of Feammox microorganisms in the removal of NH₄⁺ employing different ferric iron materials; nonetheless, very few research demonstrates that nano-size Fe(III) materials can serve as electron acceptor for Feammox process. For example, nanostructured Fe oxyhydroxides were added to treat wastewater containing 100 mg/L of NH₄⁺ achieving 52 % of NH₄⁺ removal in batch conditions (Desireddy et al., 2020). Likewise, Swathi et al. (2018) demonstrated that granulated nanoscale oxyhydroxides of Fe (GNOF) with a particle size of 15 to 30 nm, achieved NH₄⁺ removal rates of 130 mg/L/d (feed NH₄⁺ = 500 mg/L and COD = 160 mg/L) in serum bottles in a semi-continuous operation with an HRT of 0.5 d.

Despite, the great potential of Fe(III) materials in the Feammox process, more research is needed to understand and demonstrate that nano-sized materials are suitable in contrast to micro and macro materials for NH₄⁺ remediation.

Conclusions and perspectives

Wastewater treatment has become an important activity with a positive impact on human populations, anaerobic treatment is gaining popularity over aerobic treatment due to the economic, environmental, and feasibility advantages that it offers. With the growing of human population, high quality water will be required and the biotechnological process for wastewater remediation will have an important

role, not just for being able to clean contaminated waters, but also for important resources that anaerobic treatment can offer, like CH₄. Also, the role of nano-CMs has shown a promising application in anaerobic treatment, enhancing the metabolic capabilities of microorganisms that allows a faster wastewater decontamination and the obtention of relevant products. In the same way nano-CMs helps to develop new processes for wastewater treatment, such as Feamox, helping to achieve environmental and economic sustainability.

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