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Anaerobic digestion inhibition indicators and control strategies in processes treating industrial wastewater and wastes

Indicadores de inhibición de la digestión anaerobia y estrategias de control en procesos de tratamiento de aguas residuales industriales y desechos

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Abstract

Based on a literature review, a set of inhibition indicators is presented for each stage of anaerobic digestion according to the behavior of certain parameters: the reduction of the soluble COD/total COD ratio; the low volatile fatty acids (VFA) production; the accumulation of VFA and low acetate production; and the low methane production. Moreover, we present several preventive and recovery strategies for each stage considering the detected inhibition indicator. Some of the preventive strategies are the acclimation of microorganisms to degrade organic-matter in the presence of certain inhibitors, the enrichment of the inoculum with various additives (e.g., sulfate-reducing bacteria, electron donors, mineral adsorbents or nutrients), the dilution of the influent, and the prior removal of the inhibitors. Some of the proposed recovery strategies are the reduction of the inhibitor concentration by removal strategies (e.g., precipitation, adsorption, and sulfate-reduction), intermittent feeding, and decrease of the total influent volume. Lastly, we present the challenges and future perspectives of applying the inhibition indicators and control strategies.

Keywords: Anaerobic digestion inhibition, wastewater treatment, wastes treatment, inhibition recovery, inhibition mitigation.

Resumen

Con base a una revisión bibliográfica, se presenta un conjunto de indicadores de inhibición para cada etapa de la digestión anaerobia de acuerdo al comportamiento de ciertos parámetros: la disminución de la relación de DQO soluble/DQO total; la baja producción de ácidos grasos volátiles (AGV); la acumulación de AGV y la baja producción de acetato; y la baja producción de metano. Además, se presentan estrategias de prevención y recuperación de la inhibición para cada etapa. Algunas de las estrategias preventivas son la aclimatación de microorganismos a degradar materia orgánica en presencia de inhibidores, el enriquecimiento del inóculo con aditivos (ej. bacterias sulfato-reductoras, donadores de electrones, minerales adsorbentes y nutrientes), la dilución del influente y la remoción previa del inhibidor. Las estrategias de recuperación propuestas son la reducción de la concentración de inhibidor mediante precipitación, adsorción y sulfato-reducción, la alimentación intermitente y la disminución del volumen del influente. También se presentan los desafíos y perspectivas futuras del uso de los indicadores de inhibición y las estrategias de control.

Palabras clave: inhibición de la digestión anaerobia, tratamiento del agua residual, tratamiento de residuos, recuperación de la inhibición, disminución de la inhibición.

1 Introduction

Anaerobic digestion (AD) is the biological treatment that exhibits the highest removal efficiency of organic-matter from wastewater and solid wastes (Ho *et al.*, 2017). Many industrial effluents are treated by AD on a large scale. However, AD tends to be easily inhibited by toxic and inhibitory compounds present in the treated substrate.

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Hydrolysis is inhibited by heavy metals and humic acid (Yue *et al*., 2007; Yap *et al*., 2018). The inhibition of acidogenesis has been reported in the presence of phenols and certain heavy metals. For example, fermenting microorganisms (acetogens) were observed to be completely inhibited by phenols (Chapleur *et al*., 2016). Moreover, both Cd and Cr^{3+} were reported to inhibit acidogenesis (Altaş 2009). Ammonia nitrogen and heavy metals -besides inhibiting acidogenesis- also inhibit acetogenesis. In this respect, changes were reported in the acetogenic bacteria population induced by ammonia nitrogen of a concentration of 3.3 g/L (Westerholm *et al*., 2011). Moreover, heavy metals were reported to decrease the ability of acetogens to degrade volatile fatty acids (VFA) (Mudhoo and Kumar 2013). Lastly, methanogenesis was found to be the most affected stage of the AD: Besides methanogenesis being affected by the inhibitory compounds, it is also inhibited by sulfides, fatty acids, halogenated aliphatic compounds, and nanomaterials (Chen *et al*., 2008; Puyol *et al*., 2009; Luna-del Risco *et al*., 2013, Kwietniewska and Tys 2014). Moreover, the low concentration of nanoparticles necessary to inhibit methanogenesis was further emphasized (Otero-González *et al*., 2014). Therefore, the main challenge of industrial wastewater and wastes treatments by AD remains the detection of the stage at which the inhibition occurs (hydrolysis, acidogenesis, acetogenesis, or methanogenesis), and the subsequent identification of the compound causing the inhibition. Ideally, these two steps should be achieved prior to the treatment to mitigate the problem. However, there is no available research targeting this issue. Biogas measurement is the most commonly used approach to determining the inhibition of AD owing to the ease of measuring the final gaseous products. However, by measuring only the biogas, it is challenging to determine which of the 4 stages of AD is inhibited. The low production or the lack of biogas allows the identification of inhibition. However, additional information is necessary to identify the source of the inhibition. Therefore, the objective of this work is to propose -based on the existing literature on the inhibition of anaerobic treatments- a set of parameters that could be used as indicators to identify the inhibited stage of the AD. In addition, the present work provides a guide to identifying the inhibited stage based on the behavior (change) of the selected indicators (indicators of inhibition), helps to determine the possible causes of inhibition, and presents preventive and recovery strategies for each process.

2 Inhibition indicators of AD and control strategies

The determination of the presence of certain inhibitors is challenging, owing to the complexity and cost of the respective measurement methods. Nevertheless, the continuous monitoring of several parameters allows the timely detection of the inhibition of the anaerobic processes. Observing the variations of these parameters can serve as inhibition indicators that provide the necessary information to avoid or recover from the affectation. For example, the measurement of the redox potential allows determining the presence of oxidizing chemical species that inhibit the methanogenic process of AD. Based on the available literature, inhibition of hydrolysis can be determined through monitoring the soluble chemical oxygen demand (COD)/total COD ratio in the influent and effluent of the anaerobic process. When the value of this ratio is equal in both the influent and effluent, then hydrolysis is inhibited. For determining the inhibition of acidogenesis, the measurement of volatile fatty acids (VFA) formation is a straightforward approach: Acidogenesis produces VFA; therefore, we propose that when the VFA production is low, acidogenesis is likely inhibited. During acetogenesis, the VFA produced during acidogenesis are consumed; thus, the accumulation of VFA indicates the inhibition of acetogenesis. Hence, the measurement of VFA abundance is crucial for the determination of the affectation of both acidogenesis and acetogenesis. Finally, the low or nonexistent production of methane is the main indicator of the inhibition of methanogenesis, since methane is the primary product of methanogenesis. An alternative approach to determining the inhibition of methanogenesis is the monitoring of the accumulation of acetates. The guide proposed to identifying the inhibited stage is summarized in Table 1. Furthermore, the guide is supported by the visualization of the inhibiting factors of each AD stage (Fig. 1).

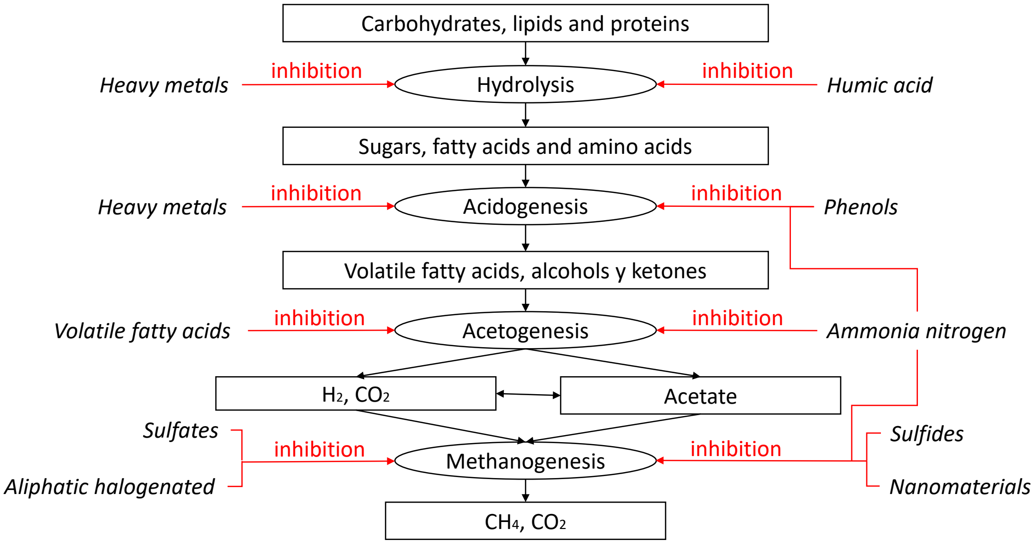

Fig. 1. Inhibitors of the individual stages of the anaerobic digestion.

2.1 *Hydrolysis*

The inhibition of hydrolysis has been observed in the presence of both heavy metals and humic acid (Yue *et al*., 2007; Yap *et al*., 2018). Moreover, if the concentration of these compounds cannot be measured, an alternative strategy can be applied to determine the inhibition of hydrolysis: The inhibited conversion of the total or particulate COD to soluble COD suggests an inhibited biotransformation rate of organic-matter (Collivignarelli *et al*., 2017). Hence, an unchanging soluble COD/total COD ratio can be used as the inhibition indicator. Consequently, since heavy metals cannot be assimilated by anaerobic metabolisms, the acclimatization of the microorganisms to hydrolyze organic-matter in the presence of heavy metals is one of the applicable strategies for the prevention of the inhibition of hydrolysis by heavy metals and humic acid. This type of acclimatization has been successfully carried out by gradually increasing the heavy metal concentration and by strengthening the metabolisms through exposure to easily digestible compounds such as ethanol and sodium acetate (Gupta *et al*., 2015). Ethanol and sodium acetate increase the metabolism rate of microorganisms, which exhibit the capacity of metal biosorption (Colussi *et al*., 2009). However, the population of bacteria that develop the enzymatic systems necessary to survive under toxic compounds increases under a gradually increasing exposure to heavy metals (Yang 2011). Hence, gradually increasing the metal concentration is a superior strategy as it allows to increase the abundance of microorganisms capable of transforming organic-matter under the presence of heavy metals and humic acid.

Alternatively, if the presence of heavy metals is confirmed prior to the AD processing, various strategies can be applied for their removal, such as anaerobic processes enriched with specific components. For example, Cu, Zn, Ni, and Cr were reported to be removed from anaerobic semi-continuous stirred tank reactors enriched with sulfate-reducing bacteria (Kieu *et al*., 2011). In addition, the removal of Cd, Cu, Ni, and Zn was reported using a UASB reactor with a polyacrylamide cryogel (Nkemka and Murto 2010). Various AD products have also been used to precipitate metals: Both anaerobically produced hydrogen sulfide (H_2S) and H_2S mixed with anaerobic effluents that contained sulfate-reducing microorganisms were used to precipitate metals (Álvarez *et al*., 2007; Jiménez-Rodríguez *et al*., 2009). The addition of layered double hydroxides is another removal strategy that allows degrading metals such as Cr. For example, the hydrotalcite-like compound (anionic clays constituted by sheets of mixed metal hydroxides) ZnGa (Zinc and Gallium) calcined and synthetized by ultrasonic irradiation, degraded 98% of Cr(IV) in a combined adsorption-photodegradation process (Zarazúa-Aguilar *et al*., 2018). Comparing these approaches, the anaerobic digestion in a semi-continuous stirred tank with sulfate-reducing bacteria seems to be a good strategy, since it presented a metal-removal efficiency of 94-100%.

Table 1. Inhibition indicators of anaerobic digestion, their possible causes and consequences, and preventive and recovery strategies for each stage of the digestion.

Anaerobic stage	**Inhibition indicator**	**Possible Causes**	**Consequences**	**Preventive strategies**	**Recovery strategies**	**References**
Hydrolysis	1) Reduction of the soluble COD/total COD ratio	a) Presence of inhibitors such as heavy metals and humic acid	I) Inhibition of hydrolytic microorganisms II) Low organic-matter degradation	i) Acclimation of microorganisms to degrade organic-matter in the presence of heavy metals. ii) Precipitation of heavy metals with sulfate-reducing bacteria and hydrogen sulfide (H_2S)	i) Reduction of heavy metal concentration using various removal strategies. ii) Adjustment of the pH and carbon-nitrogen (C:N) ratio.	Gupta et al. (2015), Kieu et al. (2011), Álvarez et al. (2007), Jiménez-Rodríguez et al. (2009), Shanmugam and Horan (2009), Wu et al. (2009), Noyan et al. (2017)
Acidogenesis	2) Low production of VFA	a) Presence of inhibitors of acidogenesis[*]	I) Inhibition of fermenting microorganisms II) Low organic-matter degradation III) Low biogas production	i) Acclimation of microorganisms to degrade phenol ii) Removal of phenol by mesophilic processes or by the use of oxidative enzymes	i) Extension of the hydraulic retention time (HRT) ii) Dilution of the influent iii) Addition of powdered activated carbon iv) Intermittent feeding	Chapleur et al. (2016), Altaş (2009), Buitrón and Moreno-Andrade (2014), Rosenkranz et al. (2013), Levén et al. (2006), Akassou et al. (2010), Gonçalves et al. (2012), Dahiya et al. (2015), Kuss et al. (2018)
	3) A high concentration of ammonia nitrogen in the effluent	a) Protein-rich wastewater	I) Affectation of acidogenic microorganisms	i) Acclimation of microorganisms to degrade organic-matter in the presence of ammonia nitrogen ii) Simultaneous nitrification and denitrification to decrease ammonia iii) Prior removal of ammonia under A/O[***] conditions	i) Addition of inorganic materials such as clay mineral, zeolite, and mordenite to adsorb ammonia ii) Use of the green algae *Scenedesmus sp* iii) Ammonia stripping	Shanmugam and Horan (2009), Nakakubo et al. (2008), Tada et al. (2005),Walker et al. (2011), Seifi and Fazaelipoor (2012), Lei et al. (2007), Fernández et al. (2008), Park et al. (2010), Nelson et al. (2003), Hernández-Fydrych et al. (2018)
Acetogenesis	4) Accumulation of VFA and low acetate production	a) High organic-matter concentration in the influent b) Lack of pH control c) Inhibition of acetogenesis	I) Acidification of the system II) Inhibition of acetogenic microorganisms and methanogenic archaea III) Diminution of biogas production	i) Dilution of the influent ii) pH control	i) Dilution with biomass ii) Addition of adsorbents iii) Prolonged exposure to the compound until acclimation is reached iv) Addition of co-substrates v) Discontinuous feeding of the system	Cysneiros et al. (2012), Palatsi et al. (2009), Kuang et al. (2006), Cavaleiro et al. (2008)
	5) High ammonia nitrogen concentration in the effluent	a) Protein-rich wastewater	I) Affectation of acetogenic microorganisms	i) Same as for acidogenesis	i) Same as for acidogenesis	
Methanogenesis	6) A low proportion of methane in the biogas composition	a) Presence of inhibitors of methanogenesis[**] b) Low C:N ratio	I) Diminution of the organic-matter degradation	i) Adjustment of the pH and C:N ratio ii) Acclimation of methanogenic archaea to the inhibitory compound iii) Prior removal of the inhibitor		Wu et al. (2009), Buitrón and Moreno-Andrade (2014), Rosenkranz et al. (2013), Orozco et al. (2010)
	7) Decrease of the specific methanogenic activity	a) Presence of inhibitors that affect methanogenic archaea[**]	I) Diminution of one or more groups of microorganisms (hydrolytic, acidogenic, acetogenic and methanogenic)	i) Acclimation of the biomass by a recirculation strategy and addition of nutrients ii) Prior removal of the nanomaterials by aerobic strategies	i) Simultaneous removal of halogenated aliphatic compounds with the anaerobic treatment. ii) Reduction of the total volume of the influent to decrease the concentration of the inhibitor	Chiavola et al. (2003), Lombi et al. (2012), Westerhoff et al. (2013), Wang et al. (2012), Choi et al. (2007), Shawaqfeh (2010)
	8) High ammonia nitrogen concentration in the effluent	a) Protein-rich wastewater	I) Affectation of methanogens II) Diminution of efficiency of the system	i) Same as for acidogenesis	i) Same as acidogenesis	
	9) Sulfide formation	a) Presence of sulfates and sulfate-reducers b) Increased pH	I) Inhibition of methanogenic archaea by competence with sulfate-reducers	i) Influent dilution ii) Prior removal of the sulfates by ion-exchange resin, Fenton oxidation, and reverse electrodialysis	i) Addition of electron donors such as a substrate for the sulfate-reducers	Chen et al. (2014), Stams et al. (2009), Liamleam et al. (2007), Dar et al. (2008), Haghsheno et al. (2009), Gutierrez et al. (2009)

[*] Inhibitors of acidogenesis: phenols and heavy metals.

[**] Inhibitors of methanogenesis: nanomaterials, halogenated aliphatic compounds, and sulfates

[***] Anoxic/Oxic

Besides, this strategy is straightforward to apply since sulfate-reducing bacteria strains can be simply conserved as these microorganisms have specific metabolic capacities that allow them to survive under various environments (Plugge *et al*., 2011).

Lastly, no strategy has been yet reported for the recovery from inhibition caused by heavy metals. However, it was suggested that the reduction of the metal concentration to non-inhibitory levels, followed by the adaptation of the microorganisms is a feasible recovery strategy for selected metals (Gupta *et al*., 2015). An option that can be explored for the reduction of metal concentration is the use of phytoremediation in situ that allowed the removal of multiple heavy metals (Cr, Ni, Cd, and Pb) (Buendía-González *et al*., 2019; Alcázar-Medina *et al*., 2020). The reduction of the metal concentration can be reinforced by adjusting the pH and the carbon-nitrogen (C:N) ratio, which is generally reported strategies for the recovery and multiplication of microorganisms (Shanmugam and Horan 2009; Wu *et al*., 2009).

2.2 Acidogenesis

Continuous measurement of VFA concentration in anaerobic systems is important because the production and accumulation of these compounds allow monitoring acidogenesis and acetogenesis rates during AD. Therefore, the inhibition indicator proposed for the acidogenesis stage is the inhibited VFA production that is induced by the presence of acidogenesis inhibitors (Table 1 and Fig. 1). The inhibition of acidogenesis by heavy metals can be controlled using the strategies presented above. The inhibition caused by phenolic compounds could also be avoided by the acclimation of the microorganisms since these compounds can be metabolized by the microorganisms present in the AD processing (Yan *et al*., 2018). There are two strategies reported for the acclimation to phenol. Namely, the gradual increase of the phenol concentration (Rosenkranz *et al*., 2013; Buitrón and Moreno-Andrade 2014), and the addition of Co-substrate that enhances the digestion of phenolic compounds (Barakat *et al*., 2012). The gradual increase of the phenol concentration during AD provided high conversion of phenol (97-100%) (Rosenkranz *et al*., 2013; Buitrón and Moreno-Andrade 2014). These phenol-conversion rates were likely achieved by allowing the microorganisms to adapt to the compound and increase their resistance and assimilation as the phenol concentration gradually increased.

An alternative strategy to avoid the inhibition of acidogenesis by phenol could be the removal of these compounds. Phenol might be anaerobically degraded through the acclimation of the proper microorganism. For example, the biodegradation of phenolic compounds in small anaerobic diluted batch cultures was performed with an initial phenol concentration of 67 mg/g (in the total solid digestate) through its AD at thermophilic and mesophilic temperatures (Levén *et al*., 2006). The authors reported a lower removal efficiency of phenol (20%) through the thermophilic process compared to the mesophilic process, which provided a complete degradation of the phenols. Similarly, decreasing the initial concentration of the target compound can increase its removal efficiency. For example, the anaerobic degradation of tyrosol with an initial concentration of 2000 mg/L was 71%, while reducing its initial concentration to 1500 mg/L lead to a 100% removal (Akassou *et al*., 2010).

Other strategies to reduce the inhibitory effects of phenol are the extension of the hydraulic retention time (HRT), dilution, and addition of powdered activated carbon. The addition of powdered activated carbon allowed the recovery of 40% of the initial (prior to the inhibition) removal efficiency of the system, consequently decreasing its recovery time from 25 to 9 days (Wang and Han 2012). Another possible recovery strategy is intermittent feeding; it was reported to promote the removal of resilient phenolic compounds and reached removal efficiencies of up to 81% (in around 20 days) (Gonçalves *et al*., 2012). From the recovery strategies studied, the addition of powdered activated carbon allowed recovering the system within shortest time period (9 days). However, powdered activated carbon did not improve the phenol removal. On the contrary, intermittent feeding (although requiring significantly more time-20 days) also increased the removal efficiency after recovery from 40 to 81%. A possible cause is that the exposition of the microorganisms to an interrupted substrate enforces the use of the inhibitor compound as a carbon source and increases the resistance of the microorganisms. Indeed, bacterial communities that were subjected to starvation periods were reported to exhibit excellent stability and resistance (Jáuregui-Jáuregui *et al*., 2014). Therefore, intermittent feeding is recommended for the recovery of inhibition by phenols as it does not require adding additional compounds, and hence, does not increase the processing costs.

However, these strategies must be validated in pilot studies and at an industrial scale as all the mentioned studies have been carried out at a laboratory scale.

If inhibition of acidogenesis is caused by a mixture of ammonia nitrogen and phenol, a pretreatment with nitrifying sludge might be applied since it nitrifies and mineralizes, respectively, the two compounds. For example, a sequencing batch reactor (SBR) with nitrifying sludge was used to remove ammonium by nitrification and phenolic compounds by mineralization, simultaneously in the same system (Suárez-García *et al*., 2019).

2.3 Acetogenesis

The VFA produced during acidogenesis are used for acetate production during the acetogenesis (Zhang *et al*., 2014). Hence, the proposed indicators of acetogenesis inhibition are VFA accumulation and low acetate production. A possible cause of VFA accumulation is an increased organic-matter concentration in the influent and/or the lack of pH control by adding buffer solutions during the operation of anaerobic systems (Table 1). Therefore, a possible strategy to reduce organic-matter concentration is the dilution of the influent. Moreover, to avoid the accumulation of VFA, it is recommended to control the pH by adding buffer solutions, with or without an automatized system, which improves the performance of the system and avoids inhibition (Cysneiros *et al*., 2012). Alternatively, the inhibition of acetogenesis can be prevented by continuously removing VFA during the anaerobic treatment. The most common systems used for VFA removal are based on upward flow, such as the up-flow fixed-bed reactor and up-flow anaerobic sludge blanket (UASB) (Saatci *et al*., 2003; Méndez-Acosta *et al*., 2011). From these two, the up-flow fixed-bed reactor provided higher removal efficiency (82%). The superior performance was likely achieved owing to better conversion rates of fixed-bed reactors through the formation of a biofilm on the supporting materials. The formed biofilm reduces the microorganisms' sensitivity to the variations in concentration and inhibitory compounds (Singh and Prerna 2009).

Nevertheless, if inhibition cannot be avoided, several strategies can be applied for system recovery, such as dilution with biomass, the addition of adsorbents, prolonged exposure to the compound until acclimation is reached (Palatsi *et al*., 2009), the addition of co-substrates (Kuang *et al*., 2006), and intermittent feeding of the system (Cavaleiro *et al*., 2008). Among these strategies, the addition of adsorbents is the most reliable option since it was reported to provide a higher production of biogas. The biogas production was improved because the adsorbents allow increasing the degradation of organic-matter such as xylan citrate, whose degradation produces biogas (Soltani *et al*., 2013). On the contrary, dilution with biomass (which inherently increases the inoculum concentration), prolonged exposure to the VFA, and intermittent feeding require greater control. Nevertheless, the latter strategies could also provide a sufficient recovery of the system. Moreover, with optimal control, they could also increase the biogas production. Namely, the addition of biomass was reported to increase biogas production by up to four times (Ho and Ho 2012).

2.4 Methanogenesis

The last stage of AD is methanogenesis, for which several inhibition indicators are proposed, such as the low methane production, high ammonia nitrogen concentration in the effluent, sulfide formation, and reduction of the specific methanogenic activity (SMA) (Table 1). Low methane production indicates the presence of inhibitory compounds of the methanogenesis. The specific methanogenic activity (SMA) indicates the capacity of the microorganisms to degrade complex substrates in methane (Sumino *et al*., 2007). This capacity is determined by measuring the potential of the microorganisms to produce methane, known as the SMA test (Souto *et al*., 2010). Since methane is the principal product of methanogenesis, measuring the SMA allows determining the inhibition of methanogenesis. Moreover, nitrogen is crucial for the formation of cells, i.e., to promote the multiplication of microorganisms. Thus, it is important to strengthen the biocenosis by adjusting the C:N ratio to avoid inhibition (Orozco *et al*., 2010). In addition, it is important to determine the inhibitor compound present. Subsequently, depending on the type of the inhibitor, an acclimation strategy of the microorganisms to the compound can be performed. Alternatively, the compound could be removed prior to the processing (Table 1).

Ammonia nitrogen is a common inhibitor of methanogenesis. Thus, the acclimation of inoculum is one of the possible strategies to avoid inhibition. The acclimation might be done by the gradual increase of the ammonia nitrogen concentration in a similar way that was proposed for the acclimation to phenol. Methanogenic archaea was adapted to high ammonia concentrations by the application of NH_4Cl pulses to slowly increase the concentration of N-NH_4^+ (Nakakubo *et al*., 2008). The microorganisms were acclimated to a N-NH_4^+ concentration of 11 g/L.

The removal of ammonia nitrogen is another strategy to avoid or to recover from inhibition of methanogenesis. The possible ammonia removal processes are presented in Table 2. Zeolite addition and ammonia stripping were performed simultaneously with methanogenic processes, allowing 88% and 98% ammonia removal, respectively (Tada *et al*., 2005; Walker *et al*., 2011). Several strategies that are applicable prior to AD are also presented: Simultaneous nitrification and denitrification can be applied if the wastewater contains a high concentration of organic matter. In this respect, Seifi and Fazaelipoor (2012) obtained 85% removal of the ammonia nitrogen concentration using a three-phase fluidized bed biofilm reactor for the treatment of sanitary wastewater. The addition of minerals, such as zeolites that are easily obtained (López *et al*., 2018), is another strategy for the removal of ammonia. Zeolites support biofilm formation and promote ion exchange in the presence of Na^+, Ca^{2+}, and Mg^{2+}. The use of zeolites allowed an efficient ammonia nitrogen removal in both a sequencing batch reactor (SBR) and directly in an ammonium-rich sludge obtained from an anaerobic digestion system, both achieving an ammonia nitrogen removal rate of 88% (Table 2).

Using green algae (e.g., *Scenedesmus* sp) and the stripping of ammonia are two additional options for ammonia removal (Table 2). Ammonia stripping provided higher removal efficiencies (98 and 95%) compared to that achieved by the addition of zeolites (88%). Nevertheless, both strategies allow the removal of ammonia nitrogen with high efficiency. However, ammonia stripping may provide higher removal efficiencies at high pH, temperature, and gas flow rate since increasing these parameters increases the saturated vapor pressure and the interaction surface area between the liquid and gaseous phases. Consequently, the reaction speed is also increased, which allows the volatilization of ammonia nitrogen (Walker *et al*., 2011). In addition, both ammonia stripping and nitrification-denitrification present an advantage over the addition of minerals or the use of algae: The former two are natural processes that allow the biogeochemical cycle of nitrogen to continue and do not affect the formation of metabolites that could influence the anaerobic digestion process.

The monitoring of sulfates and sulfides in the system also allows identifying the inhibition of methanogenesis by sulfate-reduction, since sulfate-reducing bacteria compete with the methanogenic archaea for the substrate (Kwietniewska and Tys 2014). Monitoring the sulfide formation allows determining if sulfate reduction is taking place during the anaerobic treatment which inhibits methanogenesis (Table 1). A possible intervention strategy is the dilution of the influent, which reduces the sulfate concentration, and consequently, mitigates sulfate reduction (Chen *et al*., 2014). Alternatively, the addition of electron donors reduces the inhibition of methanogenesis.

Table 2. Processes used to remove ammonia before or during anaerobic digestion.

Process	**Removal (%)**	**References**
Simultaneous nitrification-denitrification in a three-phase fluidized bed biofilm reactor	TAN** =85	Seifi and Fazaelipoor (2012)
Zeolite addition during the anaerobic digestion of an ammonium-rich sludge	88 of N-NH_4^+	Tada et al. (2005)
Zeolite addition during anaerobic digestion in an SBR*	88 of N-NH_4^+	Fernández et al. (2008)
Treatment in a cylindrical reactor with the green algae *Scenedesmus sp*	50 of N-NH_4^+	Park et al. (2010)
Ammonia stripping with biogas during anaerobic digestion	98 of N-NH_3	Walker et al. (2011)
Ammonia stripping with CO_2 during anaerobic digestion	95 of N-NH_4^+	Lei et al. (2007)
Struvite precipitation in anaerobic digestion	85 of N y P	Nelson et al. (2003)

*Sequencing batch reactor

**Total Ammonia Nitrogen

The added electron donors are used as substrates by the sulfate-reducing bacteria which mitigates their competition with the methanogens. Some examples of electron-donor compounds are citric acid (fermented to acetate and formate during AD) (Stams *et al*., 2009), hydrogen, methanol, ethanol, acetate, lactate, propionate, butyrate, sugar, and molasses (Liamleam and Annachhatre 2007). The dilution of the influent to decrease the sulfate concentration increases the total volume of the wastewater to be treated which is a major disadvantage of this approach. On the contrary, the addition of electron donors increased the methanogenic archaea population (from 0 to 10% of the total population) and decreased the population of the sulfate-reducing microorganisms (from 85 to 45% of the total population) (Dar *et al*., 2008). However, the proportion of sulfate-reducing microorganisms remained high. Therefore, additional actions are required to mitigate the competition between methanogens and sulfate-reducers. A suggested strategy is to perform the inoculum acclimation in systems where the biomass cannot be easily washed out (e.g., membrane and biofilm systems) (Chen *et al*., 2014). This strategy allows the microorganisms to be in contact with the undissociated H_2S, and hence, promotes their adaptation to the toxicity of H_2S.

The absence of sulfates eliminates sulfate-reduction. Therefore, the removal of sulfates is a promising strategy to prevent the inhibition of anaerobic digestion caused by sulfates. For the removal of sulfates prior to the anaerobic digestion, various physicochemical pretreatments have been applied, such as the use of ion-exchange resins (Haghsheno *et al*., 2009), Fenton oxidation (Wang *et al*., 2008), and reverse electrodialysis (Chao and Liang 2008). Comparing these approaches, only the use of ion-exchange resins was reported to provide a 100% removal efficiency of sulfate anions. The maximal removal of sulfate ions was obtained with the resin dosage of 1000 mg/100 mL from wastewater containing copper complexes. Another extensively studied group of methanogenesis inhibitors are halogenated aliphatic compounds. Consequently, various studies reported the removal of halogenated aliphatic compounds simultaneously with the running anaerobic treatment (examples are listed in Table 3). Various biological systems or the combination of biological and physicochemical systems have provided good removal efficiency (exceeding 90%) of halogenated aliphatic compounds. Recovery from the inhibition by halogenated aliphatic compounds has been successful through the reduction of the total volume of the influent in discontinuous systems (Chiavola *et al*., 2003). This strategy decreases the concentration of the inhibitor in the system and, consequently, the recovery of the microorganisms. To decrease the total volume and the concentration of the inhibitor in continuous systems, either the inflow must be decreased, or feeding must be repeatedly interrupted. Both approaches allow the microorganisms to remove the residual contaminant and subsequently promote microorganism recovery (Aboudi *et al*., 2015).

Table 3. Anaerobic and physicochemical processes used to remove halogenated aliphatic compounds.

Process	**Halogenated aliphatic compound**	**Type of technology**	**Removal efficiency (%)**	**References**
Treatment in an anaerobic membrane reactor	Organochlorine pesticides	Membrane	94	Xu et al. (2008)
Treatment in an anaerobic system packed with PET beads	Triadimenol	Attached biomass	96	Shawaqfeh (2010)
Anaerobic treatment in SBR*	2,4-dichlorophenoxyacetic acid	Suspended biomass	40	Celis et al. (2008)
Treatment in a nano-scale zero-valent iron Fenton system with Cu (II)	Trichloroethylene	Suspended biomass	95	Choi and Lee (2012)

*Sequential Batch Reactor

Another group of methanogenesis inhibitors is nanomaterials. However, owing to their high variability, nanomaterials have not been extensively studied. The only proposed strategy for addressing the impact of nanoparticles is their removal prior to AD. Consequently, several aerobic processes have been used for the removal of nanoparticles such as rotating biological contactors (Miao *et al*., 2016), trickling filters (Westerhoff *et al*., 2013), and sequential batch reactors (SBR) (Wang *et al*., 2012). Among these approaches, the use of trickling filters presented the highest removal efficiency (removal of 97% of nano Ti), likely due to the filtration, adsorption, and degradation mechanisms present in these systems. Moreover, trickling filters have achieved high removal efficiency of compounds that were otherwise difficult to degrade (Montes *et al*., 2010). However, more extensive research of this technology is necessary since novel trickling filters have been recently developed using various packing materials that could further increase the removal of these compounds (Garzón-Zúñiga and Buelna 2011; Vigueras-Cortés *et al*., 2013).

Lastly, a strategy available for the recovery from inhibition caused by nanomaterials is the addition of sulfates (Gonzalez-Estrella *et al*., 2015). The added sulfates lead to sulfate-reduction, and subsequently, the obtained sulfides induce the precipitation of metallic nanomaterials (e.g., zinc oxide and copper). Hence, biogenic sulfides attenuate the degree of toxicity of nanomaterials to methanogens. Adding sulfates allowed to recover the methanogenic activity to comparable levels to those observed prior to the inhibition. In addition, decreasing the inflow or repeatedly interrupting the feeding can mitigate inhibition (Aboudi *et al*., 2015). These two approaches allow reducing the concentration of the inhibitor and the recovery of the microorganisms.

3 Challenges and future perspectives (opportunities and research needs)

This work highlights the importance of monitoring various parameters (such as the VFA concentration, presence of metals-and other molecules associated with oxidizing agents-in the influent, and the specific methanogenic activity) in anaerobic digestion systems. The monitoring of these parameters allows identifying the inhibition of AD, and consequently, helps to implement control strategies and to avoid critical failures of the systems. However, the monitoring of every proposed parameter is challenging, as it requires sophisticated instrumentation and control tools. Therefore, future research is necessary that will target the development of simple methodologies that allow the use of low-cost tools and can be implemented by operators without extensive qualifications.

Methane produced in anaerobic systems during the treatment of organic-waste can be used as a renewable energy source, e.g., for electricity production. Similarly, the inhibitory compounds and the metabolites produced during AD can be reused, e.g., ammonia nitrogen and phosphate can be recovered and used as fertilizers (Vanotti *et al*., 2017). However, it is necessary to extend this technology for other inhibitors.

The development of statistical programs allows determining the optimal operating conditions of AD systems prior to the AD processing by modeling the response of the processing to the varying system and process parameters. For example, the effects of the varying concentrations of olive oil, ethanol, and phenol were modeled with controlled pH on the anaerobic treatment of wastewater originating from the food industry (Camarillo and Rincón 2012). However, more studies are necessary that apply process modeling with respect to the operating parameters related to the inhibition indicators identified in the present work. This would allow controlling the operating parameters and would help to determine the optimal operating parameters of anaerobic systems to avoid inhibition.

The adjustment of the C:N ratio is another important parameter that has been addressed in the present work. Several works proposed various compounds for the adjustment of the C:N ratio. Namely, the C:N ratio was increased to 25 by adding urea, since the low presence of nitrogen implies the inhibited multiplication of microorganisms (Zhang *et al*., 2016). The adjustment of the C:N ratio to 30 using ammonium, increased the CH_4 production and reduced the lag phase of anaerobic digestion of swine manure (Wijesinghe *et al*., 2019). Fernández-Rodríguez *et al*. (2019) observed the highest methane yield in the anaerobic co-digestion of olive mill solid waste and microalga *Scenedesmus quadricauda* when C:N ratio was adjusted to 31.9 using the microalga as a nitrogen source. However, none of the existing studies discusses the possible inhibiting effects of the added compounds. Therefore, it is necessary to study various compounds that are applicable to the C:N-ratio adjustment and to discuss their possible negative effects on anaerobic digestion.

The present work presents a complete list of inhibition indicators and provides a guide with various strategies for controlling the inhibition in systems that simultaneously carry out the four stages of AD (hydrolysis, acidogenesis, acetogenesis, and methanogenesis). However, various stages of AD (e.g., acidogenesis and acetogenesis) can also be carried out at different phases (Fuess *et al*., 2017; Zeppilli *et al*., 2017). Therefore, owing to the significant variability of the optimal operating conditions among each AD stage, constructing a guide-such as the one presented in the present work-for each AD stage is of high interest. Most available studies are limited to influents that contain a single type of inhibitor (e.g., phenols, ammonia nitrogen, sulfates, halogenated aliphatic compounds, heavy metals, or nanomaterials). Méndez-Hernández and Loera (2019) proposed the idea of applying enzymatic treatments to complex wastewater that contain more than one pollutant, but the strategy has not been performed. Consequently, the effect of influents that contain a mixture of inhibitors remains mainly unexplored. Nevertheless, a pharmaceutical wastewater-treatment plant containing a variety of inhibitory compounds was analyzed in terms of bacterial diversity: A significantly low bacterial diversity was observed, which was likely caused by the high levels of inhibition induced by the presence of the wide range of inhibitors (Zhao *et al*., 2019). Therefore, it is necessary to investigate various strategies and types of systems whose combination could be used for the removal of a wide range of compounds. The design of the next-generation of the anaerobic system that treats wastewater that contains inhibitory compounds will require certain modifications. Namely, these upgraded systems will have to include a monitoring system for each parameter that could indicate the presence of inhibition. The most significant change in the design process of anaerobic systems will be the consideration of the types of inhibitors that could be present in the wastewater to be treated. Consequently, based on the possible presence of inhibitors, it will be established whether the biomass should be suspended or attached, and the most suitable inhibition-prevention strategies will be identified. In addition, it is necessary to develop strategies to avoid the affectation of methane production in the presence of inhibitor compounds. For example, it is necessary to boost the microbiology research of methanogenic species and to identify the methane-producing microorganisms that exhibit high resistance and removal efficiency in the presence of inhibitors: A recent study identified the genus *Methanococcus* as one of the most resistant genera to high concentrations of Ni (Wang *et al*., 2019). The identification of similar microorganisms could allow improving the efficiency of biogas production in the presence of inhibitors. Consequently, AD systems could be inoculated with the proper microorganisms. Alternatively, a suitable strategy could be applied to promote the bioaugmentation of the microorganisms, e.g., the enrichment of hydrogen used as a bioaugmentation tool to control the inhibition of anaerobic digestion by ammonia nitrogen during the treatment of wastewater that contained phenols (Wu *et al*., 2019). Lastly, the implementation of cloning techniques to increase the presence of methanogens resistant to certain inhibitory compounds is another promising approach. This has been already demonstrated through the genetic manipulation of methanogens through rapid cloning techniques (Jennings 2018).

Conclusions

In order to apply the correct recovery strategy for an AD process showing inhibition, it is necessary to determine which phase of the AD is being inhibited. Therefore, it is important to monitor various parameters that we proposed as inhibition indicators for each stage of the AD process. These inhibition indicators were used to construct a guide with the aim of allowing the operators determining the possible cause of inhibition and choosing the most suitable strategy for the recovery and prevention. The guide might also be a useful instrument to the study of complex processes taking place in the anaerobic digestion process and relate them with a specific stage. However, the preventive and recovery strategies proposed must be studied at pilot and industrial scale as many of them had being studied only on laboratory scale. In addition, several future challenges were identified whose solution would facilitate the monitoring. Namely, the development of simple and low-cost technologies for the monitoring of the inhibition indicators, or the design of statistical programs that can model the response of systems to the variation of different parameters prior to experimentation. In addition, it is necessary to incorporate automatized monitoring systems for each inhibition indicator during the design process of the reactors (treatment systems).

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