Environmental Science Nano

ROYAL SOCIETY OF CHEMISTRY

PAPER

View Article Online
View Journal

Cite this: DOI: 10.1039/d4en01178c

Unraveling the effects of cerium oxide nanoparticles on the metabolism of anaerobic digestion of waste activated sludge†

Pabel Cervantes-Avilés, *[ac] Weiwei Li[bc] and Arturo A. Keller [bc]

Received 16th December 2024,
Accepted 25th May 2025

DOI: 10.1039/d4en01178c

rsc.li/es-nano

To reduce the residual solids and increase energy recovery in wastewater treatment plants, the anaerobic digestion (AD) process needs to be optimized to generate more methane from waste activated sludge (WAS). Nanomaterials (NMs) have successfully been used in anaerobic digestion to increase methane production. Focusing on NMs with high redox activity, the biochemical route for methane production can be enhanced. Here, the influence of cerium oxide nanoparticles (CeO_2 NPs) on the AD of waste sludge was evaluated in terms of metabolite production and assimilation, key enzyme activity, and organic matter transformation. The fate of CeO_2 NPs in the anaerobic reactors was also determined *via* single particle ICP-MS and TEM imaging. Results indicated that 10, 50 and 100 mg of CeO_2 NPs per g of volatile suspended solids (VSS) acted as a nanocatalyst during the anaerobic digestion of WAS, increasing the methane yield production to 8.9%, 11.3% and 14.2%, respectively. CeO_2 NPs induced a decrease in the activity of two key enzymes involved in AD, protease and F420. Thus, biogas production was enhanced *via* the redox capability of the NPs. This includes the ability to perform extracellular electron transfer (EET) to hydrolyze long-chain substrates, *e.g.* proteins into amino acids, and short-chain organic acids such as maleic acid to shorter molecules and finally to methane. At the end of the nano-enhanced AD process, the CeO_2 NPs remained in the biosolids. Therefore, potential effects of nanoceria on soil microorganisms and plants should be studied further.

Environmental significance

Th role of nanoceria in the main four phases of the anaerobic digestion process is of highest relevance since there is limited knowledge about how micro- and nanomaterials are enhancing biogas production. This results in a more sustainable and wise use of nanomaterials, including nanoceria, in anaerobic digestion of waste activated sludge. On the other hand, nanoceria is acting as a nanocatalyst in hydrolysis and methanogenesis processes, producing more methane than that in the conventional process as well as decreasing the solids conversion.

Introduction

Renewable and sustainable energy generation has become more relevant at present as fossil fuels cause environmental degradation, climate change, and air pollution. Moreover, the exploitation of fossil fuels is leading to an energy shortage crisis and new sources of energy have to be implemented.[1,2] Waste activated sludge (WAS) has been considered as both one of the most critical environmental problems of the wastewater treatment plants (WWTPs) due to its difficult management and disposal and a significant energy source.[3] WAS can be transformed into biogas which is considered a sustainable and versatile energy source, easily transformed into electricity or mechanical and thermal energy.[4,5]

Biogas is the final product in the gaseous phase of anaerobic digestion (AD), which is a microbial mediated process where organic matter is degraded under thermophilic (55 °C) or mesophilic (37 °C) conditions. The general biochemistry of AD is divided into four steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis.[6,7] Hydrolysis breaks down complex organics into monomers, which are converted into volatile fatty acids (VFAs) during acidification and further refined to acetate, hydrogen and carbon dioxide

[a] Tecnologico de Monterrey, Escuela de Ingeniería y Ciencias, Reserva Territorial Atlixcáyotl, Puebla, CP 72453, Mexico. E-mail: pabel.cervantes@tec.mx; Tel: +52 81 83 58 2000
[b] Bren School of Environmental Science and Management, University of California at Santa Barbara, CA, 93106, USA
[c] University of California, Center for Environmental Implications of Nanotechnology, Santa Barbara, CA, 93106, USA

† Electronic supplementary information (ESI) available. See DOI: https://doi.org/10.1039/d4en01178c

during acetogenesis. These last compounds serve as substrates in methanogenesis, where methanogens produce methane. The processes are sequentially connected and interdependent, with each stage conditioning the biochemical environment and substrate availability for the next. The produced biogas in these steps is mainly composed of CH_4 (50–70%) and CO_2 (30–50%),[8,9] although the composition depends on the nature of the substrate[10] and operating conditions of the AD process.[11] Biogas with high CH_4 content is desired because of its higher energy content (35.8–39.8 MJ m^{-3}), making it suitable to contribute to energy security in any region.[12] Therefore, biogas is considered a clean and renewable energy that provides energy and attenuates the effects of environmental damage when WAS is used as a substrate.[13,14]

Recently, alternatives and strategies have been tested to enhance the CH_4 production in AD. Those approaches include co-digestion with various feedstock, pretreatment of the substrate, adjustment of operating conditions, and addition of organic or inorganic additives.[15] Regarding inorganic additives, metal and metal oxide nanoparticles (NPs) have been tested during AD to improve biogas production, particularly CH_4 content.[15–17]

Some authors[9] found that nano zero-valent iron (nZVI) and Fe_3O_4 NPs enhance separately the hydrolysis–acidification process in the AD of WAS, leading to an improved biogas production.[18] The authors suggested that nZVI and Fe_3O_4 NPs provide electrons for critical microorganisms in the AD system. In addition, many bacteria develop pathways to obtain iron in the form of nutrients or electron acceptor/donor. Therefore, nZVI can not only accelerate WAS hydrolysis and acidogenesis but also change the fermentation type to increase the acetic acid concentration by donating electrons, stimulating the metabolism and growth of critical microorganisms like methanogens in the anaerobic process. The authors also demonstrated that Fe_3O_4 NPs in a two-stage AD of WAS enhanced biogas production and decreased waste volume. As well, Ali *et al.*[19] observed that 75 mg L^{-1} Fe_3O_4 NPs yielded maximum CH_4 (up to 80% more than that of the control) in AD of municipal solid waste. Abdelsalam *et al.*[20] suggested that the addition of Fe_3O_4 NPs in AD reduce the lag phase and the time to achieve the maximum production of biogas and CH_4; these authors propose that the NPs biostimulate the methanogenic activity in the entire anaerobic process, increasing the production of CH_4. Equally, Fe-based NPs can provide ferrous ions to help the growth of fermentative bacteria and methanogens[21,22] and can reduce the oxidation–reduction potential (ORP) by −0.44 V and therefore augment the conversion of organic compounds to volatile fatty acids. Biochemical routes of that conversions are unclear but evidently dependent on dissolution of Fe-based NPs.

For other NP compositions, Farghali *et al.*[23] and Cervantes-Avilés *et al.*[24] demonstrated that TiO_2 nanoparticles also increase the production of CH_4 in AD when WAS is used as substrate. In a similar way, Tian *et al.*[25] verified that proper concentration (50 mg g^{-1} VSS) of nanosized MnO_2 enhances CH_4 yield production and microbial activity in AD of WAS. Regarding the potential mechanisms for other NPs, TiO_2 NPs may assist in electron transfer during anaerobic digestion. A process called extracellular electron transfer (EET) occurs in anaerobic bacteria in the presence of TiO_2 and that process can enhance CH_4 production even in darkness.[24,26]

Conversely, some NPs have induced adverse effects in anaerobic reactors. Ünşar *et al.*[27] detected that CuO NPs are toxic for AD of WAS in the long term and that high dosage of Ag NPs decreased by 12.1% the production of CH_4. Kökdemir and Perendeci[28] reported that CH_4 production from WAS decreased 28.9% in the long-term presence of 50–500 mg Fe_2O_3 NPs per g TS, as they inhibit the methanogenic consortium. Similar effects were observed for ZnO NPs, which inhibit the degradation of macromolecular organic matter and VSS because the ZnO NPs decreased the bacterial diversity in AD.[29]

In general, some metal and metal oxide NPs have been shown to improve the CH_4 production in AD of WAS. This improvement can be attributed to an acceleration of the biochemical processes for methane production due to their redox activity and the large reactive surface of NPs, which may accelerate the reduction of organic matter performing as nanocatalysts.[15] Therefore, the ideal nanocatalysts for AD should be NPs that may reduce organic matter and present nontoxic behavior to anaerobic microorganisms.

Some NPs such as TiO_2, nZVI and Fe_3O_4 have adequate behavior as nanocatalysts in AD, increasing CH_4 production without apparent toxic effects, which suggests that other redox-active NMs could be used successfully. Therefore, the application of other NPs such as CeO_2 with closer valence and conduction bandgaps (E_g = 2.87 eV) and low solubility may increase CH_4 generation without damage to the anaerobic microorganisms.[30] In this way, it is important to clarify the differences between the biological and the physicochemical influence of NPs in AD when nanocatalysts are in the anaerobic reactors.

The aim of this work was to evaluate AD performance when incorporating CeO_2 NPs as nanocatalysts during anaerobic degradation of WAS. The evaluation consisted in monitoring the production of metabolites (amino acids, organic acids, and nucleobases/sides/tides) in the presence of CeO_2 NPs, determination of the activity of key enzymes in anaerobic digestion, measurement of the biogas production, and organic matter transformation as well as determining the fate of the CeO_2 NPs in the anaerobic reactors, including the morphological interactions of CeO_2 NPs with the microorganisms.

Materials and methods

Nanoparticle characterization

CeO_2 NPs were obtained from Meliorum Technologies (USA). Determination of primary size (8–67 nm), hydrodynamic

diameter (298.9 ± 28.8 nm), zeta potential (29.6 ± 0.4 mV) and localized surface plasmon resonance (310 nm) was performed in a suspension of (70 mg L^{-1}) CeO_2 NPs in ultrapure (UP) water. UP water was obtained from a Barnstead NANOpure water purification system (Thermo Scientific) and used throughout the work. Before characterization, the CeO_2 NP suspension was ultrasonicated for 1 h at 280 W and a frequency of 40 kHz by using an ultrasonic bath (Bransonic, Emerson), as it is recommended for ecotoxicological assessment.[31] Other main characteristics of this stock of CeO_2 were reported previously;[32,33] briefly, phase and structure are 100% ceria and cubic, shape is mainly rod (≤10% polyhedral), purity is 95.1% and the surface area is 93.8 m^2 g^{-1}.

Collection and characterization of anaerobic sludge and WAS

Samples of anaerobic sludge and WAS were collected in a WWTP in Southern California (CA, USA). The anaerobic sludge served as inoculum and was collected from an anaerobic digester (permanently treating WAS), while WAS was extracted from the bottom of the secondary clarifier in the respective pipe port. Temperature and pH were measured in both anaerobic and waste sludge at the collection time. Total, volatile, and fixed solids (TS, VS, FS) as well as total and volatile suspended solids (TSS and VSS) were characterized in the lab by standard methods.[34] In addition, both anaerobic and aerobic sludges were dried at 105 °C for 24 h, and then carbon, hydrogen, and nitrogen (CHN) composition was determined by using the Dumas combustion method (CE440 elemental analyzer). Total and soluble chemical oxygen demand (COD and CODs) and ammonium (NH_4^+) were also determined in both samples by using commercial methods (HACH) (Table S1†). Using an Agilent 7900 ICP-MS instrument (Santa Clara, CA, USA), 26 elements were analyzed in both sludges (Table S2†). Samples of sludge were digested according to standard methods prior to elemental analysis.[34]

Experimental setup

Cylindrical anaerobic reactors of 2 L were used for the experiment. All reactors contained anaerobic sludge and waste activated sludge at a ratio of 1:1 (v/v) with the characteristics reported in the ESI† (Table S1). The concentrations of CeO_2 NPs in the reactors were 10, 50 and 100 mg per g VSS. These concentrations were selected because the main values for NPs in AD are in the range of 40–800 mg L^{-1}.[35] Considering that the concentration of VSS in AD ranges from 4 to 8 g L^{-1}, the doses of NPs result in 10–100 mg g^{-1}. Control reactors did not contain added NPs and all concentrations were evaluated in triplicate. The experimental time was 96 h after spiking NPs, initial pH was 7.72, and the reactors were kept under mesophilic conditions (37 ± 1 °C) using an incubator. Selected time and temperature are common conditions at full-scale AD systems.[36] Before starting the experiments, nitrogen gas was flushed at a rate of 2 L min^{-1} for 3 min to remove the oxygen from the headspace of the reactors. The reactors were placed inside an incubator isolated from light and continuously stirred at 120 rpm. A continuous liquid replacement system measured the biogas production from the reactors. Methane content was determined in a gas chromatograph (GC-2014AT, Shimadzu) using helium as carrier gas. Samples of the liquid phase of the reactors at 0 and 96 h were collected and immediately frozen at −80 °C after collection. These samples were processed for determination of metabolites, enzyme activity (protease, acetate kinase and F420) and CeO_2 NP quantification. At the same time, samples were collected for TEM imaging and fixed according to a previous work.[37]

Metabolite determination by LC-MS/MS

The concentrations of 23 essential amino acids, 8 organic acids, and 12 nucleobases/sides/tides in the anaerobic reactors were determined before and after exposure to CeO_2 NPs. Samples at times 0 and 96 h of all experiments (control, 10, 50 and 100 mg CeO_2 per g VSS) were left to reach room temperature (21 ± 1 °C). Tubes with the samples were vortexed at 4000 rpm for 10 min, then centrifuged at 10 000*g* for 20 min. 200 μL of the supernatant of each sample were placed in vials for liquid chromatography (LC). 10 μL of isotopically labeled internal standards (ISTDs) and 790 μL of 20% water in acetonitrile were also added. LC was performed using an Agilent 1260 UHPLC binary pump coupled to an Agilent InfinityLab Poroshell 120 HILIC-Z (2.1 × 100 mm, 2.7 μm) column for separation of all analytes. The instrumental setup and procedure have been reported in a previous study.[38] Briefly, the injection volume for each sample was 1 μL, and the total cycle time was 15 min per sample. A dual eluent mobile phase with a flow rate of 500 μL min^{-1} was used for separation. Mobile phase A was prepared by diluting a stock solution (200 mM ammonium formate, pH 3) 9:1 in HPLC water. Mobile phase B consisted in the dilution of stock solution 9:1 in acetonitrile. The final ionic strength of both phases was 20 mM. Cycle time was distributed in 11.5 min for mobile phase B linearly decreasing from 100% to 70%, 0.5 min to return to the initial condition, and 3 min post-run period for column re-equilibration at 100% mobile phase B. Mass spectrometry was performed on an Agilent 6470 triple quadrupole mass spectrometer. Data acquisition and analysis were performed using the Agilent MassHunter software (version Rev. B.06.00). The precision of the method was validated by retention time (RT) and analyte response variation (ARV) of the calibration standards. RT and ARV were determined *via* 15 continuous injections of calibration standards. When the RTs were stable, the mass spectrometer was operated in dynamic multiple reaction monitoring (DMRM) mode. Continuous monitoring of RT validation and product ion ratio reduced the possibility of false positives in the method.

Enzymatic activity determination

Protease, acetate kinase (AK) and F420 enzymes were selected to be measured in all bioreactors due to the relevance of the effect of CeO_2 NPs on the hydrolysis of proteins,[39,40] acetogenesis[39,41] and methanogenesis processes,[40,41] respectively. Protease was measured according to the Folin-phenol method.[42,43] Briefly, 1 mL of anaerobic sludge was centrifuged at 3000*g* and preheated for 2 min at 40 °C. 1 mL of 0.5% azocasein was added and heated again for 10 min. Then, 10 mL of 10% trichloroacetic acid was added and samples were centrifuged at 3000*g* for 20 min. 1 mL of supernatant was separated in a colorimetric tube and mixed with 5 mL of 0.4 M Na_2CO_3 and 1 mL of Folin-phenol reagent and heated for 20 min at 40 °C. Readings were recorded at 660 nm (UV Shimadzu 1800). For AK determination, the protocol developed by Allen *et al.*[44] was followed. In summary, 2.5 mL of the anaerobic sludge was washed 3 times with NaH_2PO_4–Na_2HPO_4 buffer (0.1 M, pH 7.2). Samples were frozen overnight; next day samples were thawed and sonicated to break down the cell membrane, followed by centrifugation at 10 000*g* to remove cellular debris. Subsequently, 160 μL of supernatant was mixed with an enzyme reaction mixture (Tris-HCl 1 mM at pH 7.4, CH_3COOK 81 mM, ATP 4 mM, $MgCl_2$ 4 mM, phosphoenolpyruvate 1.6 mM, NADH 0.04 mM, 0.4 U mL^{-1}). Readings were performed at 340 nm after 0.5 and 3.5 min from initial reaction. Finally, F420 enzyme was measured according to Delafontaine *et al.*,[45] who developed a simple method based on readings at 420 nm. Sample preparation consisted in 2 mL of anaerobic sludge which was washed 3 times with NaCl 0.9% and heated for 20 min at 90 °C in a water bath. Then, samples were centrifuged at 3000*g* and the supernatant was replaced by ethanol at a ratio of 2.5 : 1 (v/v) ethanol and sludge. The tubes were vortexed for 1 min at 500 rpm and centrifuged at 3000 rpm to remove debris. Afterwards, the pH was adjusted to 13.5 with 2 M NaOH before readings at 420 nm.

Quantification of CeO_2 NPs by spICP-MS

CeO_2 NPs remaining in the mixed liquor of the reactors (non-adsorbed NPs) were quantified and sized using the spICP-MS approach. The spICP-MS analysis was performed in an Agilent 7900 ICP-MS (CA, USA) instrument. Measurements for spICP-MS were carried out in time-resolved analysis (TRA) with a dwell time of 100 μs per point. No settling time was set between measurements. The method setup, data collection and analysis were conducted using the Single Nanoparticle Application Module (method wizard) in the Agilent ICP-MS MassHunter software (Version C.01.05 Build 588.3). The instrument settings are shown in Table S3.† Au NPs (60 nm) in 2 mM sodium citrate (Nanocomposix Inc.) were used as the standard and diluted to 100 ng L^{-1} with UP water to evaluate the nebulization efficiency. Ionic standards of both Au and Ce (Agilent, USA) were diluted up to 0.1 μg L^{-1} with 1% HNO_3 (BDH Aristar® Ultra grade) and used to determine the elemental response factor. The samples were collected according to a previous work[46] and diluted with UP water to obtain final concentrations between 50 and 300 ng L^{-1} CeO_2. Before dilution, samples of the reactors were ultrasonicated for 20 min at 280 W and a frequency of 40 kHz to ensure NP dispersion.

Results and discussion

Effects of CeO_2 NPs on COD removal and CH_4 production

Addition of 10, 50 and 100 mg of CeO_2 NPs per g of VSS in the anaerobic reactors resulted in 4.6%, 7.7% and 12.9% decrease, respectively, in the COD at the end of the experiment when compared to the control (Fig. 1A). COD removals were in line with the increment in biogas production and were also reflected in the normalized methanogenic activity (NMA), which were 8.9%, 11.3% and 14.2% higher than that of the control for the same NP concentrations (Fig. 1B). This means that COD removed from WAS in bioreactors containing ceria NPs was mainly converted into biogas, increasing the CH_4 yield (Fig. 1B). CH_4 yield per gram of COD for the control group (without NPs) was higher (0.33 L CH_4 per g COD) compared to that found in similar systems treating WAS. The typical yield is between 0.25 and 0.3 L CH_4 per g COD.[47,48] Clearly, the inoculum from a full-scale anaerobic digester is adapted to WAS, but the enhancement of methane production in the presence of CeO_2 NPs is substantial, above 0.35 L CH_4 per g COD for all three nanoceria concentrations.

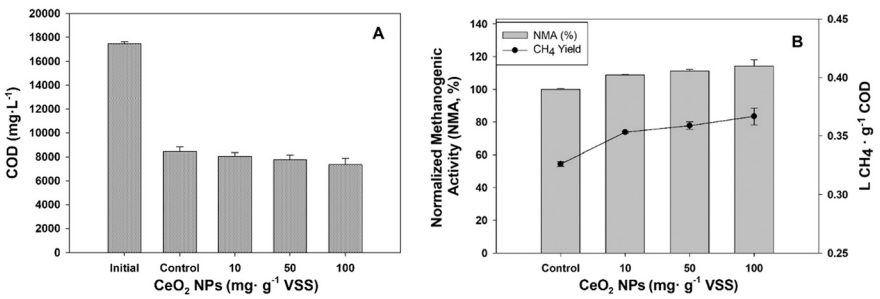

Fig. 1 COD conversion to CH_4 in the anaerobic reactors exposed to 10, 50 and 100 mg CeO_2 NPs per g of VSS. (A) shows the difference between initial (0 h) and final (96 h) COD for all treatments, while (B) represents the yield of CH_4 produced per gram of assimilated COD and the normalized methanogenic activity.

Metabolite production and assimilation: amino acids, organic acids and nucleobases/sides/tides

In the AD of WAS, protein degradation contributes up to 70% to the VS reduction,[49] and since proteins are composed of polypeptides and amino acids, the presence of these metabolites means that hydrolysis of the proteins is occurring in the anaerobic reactor. Results indicated that 20 out of 23 amino acids examined were found in the AD under all conditions (Fig. 2); only cysteine, asparagine and glutamine were not found in the reactors. These three amino acids are not common in WAS,[50,51] and that may explain their very low concentration or absence. The concentration of amino acids in the control group is in line with that previously reported for a steady anaerobic mesophilic system treating WAS as substrate.[49,50] Clearly, the presence of CeO_2 NPs increased the free amino acids between 1.1 and up to 2.2 times, indicating that the NPs catalyze protein hydrolysis in the anaerobic reactors. The effect of CeO_2 NPs was more evident in the generation of glutamic acid, aspartic acid, and lysine, which are hydrophilic amino acids, but a noticeable increase was also observed for hydrophobic amino acids such as alanine, leucine, phenylalanine and valine. This means that CeO_2 NPs can influence both hydrophilic and hydrophobic amino acids associated with the proteins in WAS. Therefore, CeO_2 NPs may have an impact on the protein and enzyme function at the cellular level[52] in AD since they are also cytoplasmatic substances.

Amino acids can be decomposed to organic acids *via* the Stickland reaction by strictly anaerobic bacteria[53] or can be fermented through hydrogen-utilizing bacteria.[54] In both routes, organic acids are also produced, influencing the acidogenesis stage of AD, and further transformed to CH_4 *via* acetogenesis and methanogenesis. In fact, an increase in malic and citric acid was observed in reactors containing CeO_2 NPs (Fig. 3). However, the reasons for both increments can differ. Malic acid is commonly produced by fermentation under anaerobic conditions, where this acid reaches its maximum yield.[55] Clearly, CeO_2 NPs enhanced up to 5-fold the production of this easily biodegradable and short-chain acid, making it available for the following stages of anaerobic digestion. In contrast, citric acid is an intermediate of the glycolytic pathway,[56] which can be performed under aerobic and anaerobic conditions. Therefore, overproduction of citric acid can be due to stimulation of glycolysis induced by the NPs. Importantly, citric acid is a substrate for malic acid production as well as an easily biodegradable substrate that reduces the inhibitory effect of humid acid during AD.[56] Therefore, the production of both citric and malic acid in the presence of CeO_2 NPs can be concatenated and thus be beneficial for AD.

Regarding ascorbic acid, the levels found in the presence of CeO_2 NPs were similar to those in the control, which means that generation of this substrate from WAS, commonly found in the organic fraction of food waste, was not altered in the presence of NPs. It is important to avoid the overproduction of ascorbic acid, since its complex degradation due to this can occur at low pH (3–6) under mesophilic conditions, retention times over 10 weeks,[57] or even by photo fermentation with the use of commercial ZVI.[58]

To the best of our knowledge, for the first time, the content of free nucleobases/sides/tides was determined to elucidate if NPs may interfere with catabolism during AD (Fig. 3). Results indicated that nucleobases did not change significatively in the presence of ceria NPs. However, some nucleotides/sides increased in concentration; for example, xanthine content increased in the presence of nanoceria, while its precursors, such as hypoxanthine (Hyp) and guanine, presented lower concentrations. This may indicate that for 10 and 50 mg of CeO_2 NPs per g of VSS, but not at 100 mg, xanthine synthesis from such Hyp and guanine could be enhanced. Since both precursors are oxidized to

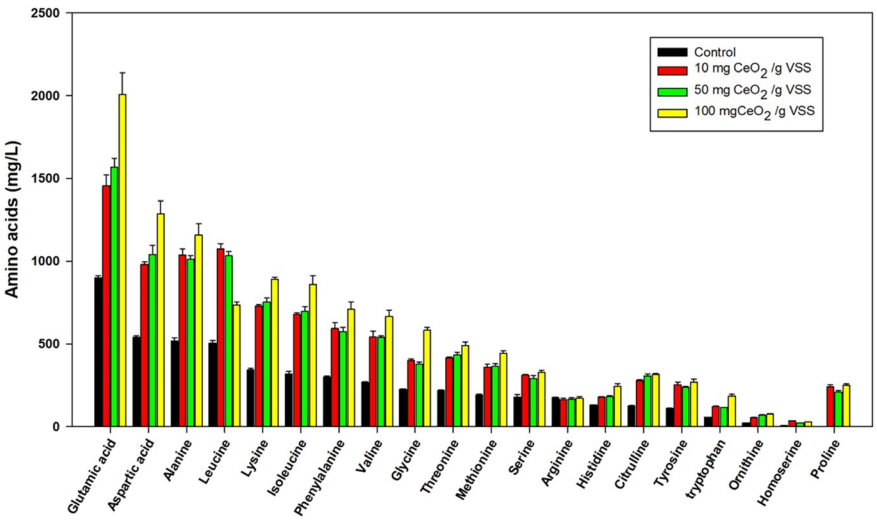

Fig. 2 Content of free amino acids in the anaerobic reactors exposed to 10, 50 and 100 mg CeO_2 NPs per g of VSS. Control stands for reactors non-exposed to added CeO_2 NPs.

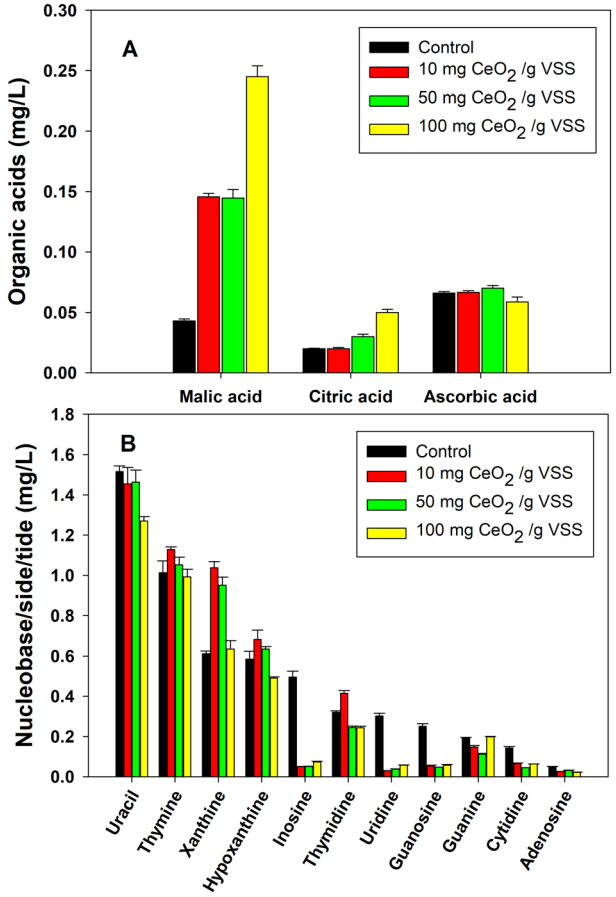

Fig. 3 Content of three free organic acids, malic, citric and ascorbic acid, (A) and main nucleobases/sides/tides (B) present in anaerobic digesters exposed to CeO_2 NPs for 96 h. Control stands for reactors non-exposed to CeO_2 NPs.

Hyp, CeO_2 NPs at 100 mg g^{-1} can affect such an oxidation process due to several reasons, such as alteration of redox balance[59] and not facilitating oxygen vacancy formation, although this hormetic-like effect for these NPs should be further evaluated, since this has been observed in AD for ZnO NPs.[60] Hyp is also a precursor of inosine, which is another nucleoside, but to accomplish this, Hyp must be linked to a ribose ring.[61] Low levels of inosine may be due to a low content of available ribose or a low rate of deprotonation of Hyp. Since AD occurs mostly at neutral to basic pH, the most probable cause for low levels of inosine can be the low availability of ribose, which is the basis of adenosine triphosphate (ATP). A low content of ribose can also affect energy-intensive processes at cellular levels. In fact, cytidine and uridine, which are nucleosides present in pyrimidine biosynthesis (uracil, cytosine and thymine), and guanosine, a nucleoside present in purine biosynthesis (adenine and guanine), exhibited a lower content than the control. This can be attributed to their downregulation due to the low availability of ribose, which would limit the ATP available for energy-intensive processes. These results may explain the overproduction of organic acids *via* catabolism and downregulation of the biosynthesis of nucleotides/sides in the presence of CeO_2 NPs. However, this should be confirmed through advanced techniques such as metabolomic analyses by monitoring of coenzymes (NAD+ and NADH) as well as some intermediate compounds.

Enzymatic activity

During the hydrolysis stage of AD, protease activity is key to break down long-chain complex organic matter and proteins to short-chain compounds and amino acids, respectively. As observed in Fig. 4, protease activity decreased between 20% and 23% in the presence of CeO_2 NPs. This means that the increase in the amino acid content in the reactors (Fig. 2) can be mainly attributed to the catalytic activity of CeO_2 NPs. Compared to the control, acetate kinase activity decreases in the presence of nanoceria except for the bioreactors containing 10 mg of CeO_2 NPs per g of VSS, where there was an overproduction. Finally, the enzymatic activity of F420, attributed to methanogens, decreased by more than 50% in the presence of CeO_2 NPs when compared to the control. Since CH_4 production increased in the presence of nanoceria, this means that this process was catalyzed by CeO_2 NPs.

The catalysis induced by CeO_2 NPs in the hydrolysis and methanogenesis during anaerobic digestion can be attributed to the intrinsic properties of the NPs, such as their redox ability through their conduction and valence bands. CeO_2 NPs can perform EET,[59] acting as an extracellular electron acceptor and using it to reduce long-chain molecules such as proteins or polysaccharides[62] as well as reduce short-chain molecules into CH_4. Liu *et al.*[59] demonstrated *via* cyclic voltammetry that EET occurs for CeO_2 NPs at 10–30 mg L^{-1} and that has a significant effect on the reducing power of AD during wheat straw degradation. EET was also reported for anammox bacteria in the presence of graphene oxide,[63] and for TiO_2 NPs in the methanogenic stage of anaerobic

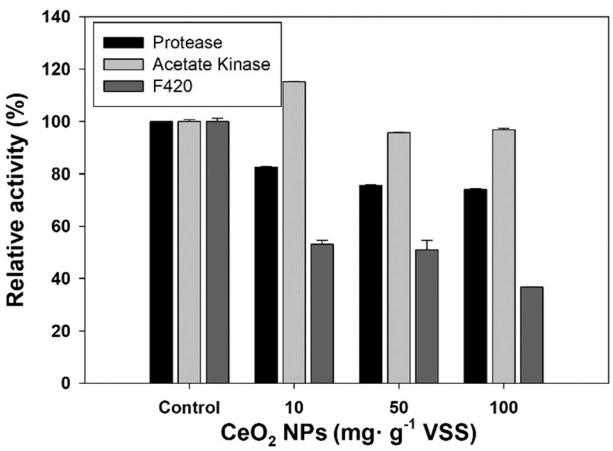

Fig. 4 Relative activity of three key enzymes in the anerobic digestion process, protease, acetate kinase and F420, in the experiments exposed to 10, 50 and 100 mg of CeO_2 NPs per g of VSS after 96 h. Asterisks "*" indicate that there are no significant differences for mean values of the same treatment ($p < 0.05$).

digestion.[24] In this experiment, it is considered that EET was promoted by CeO_2 NPs because they have a lower energy of activation (2.87 eV) than TiO_2 NPs (3.2 eV).[30,64] Therefore, nanoceria can play an important role in hydrolysis and methanogenesis simultaneously, even with low assistance of the bacteria related to such stages of anaerobic digestion, as demonstrated by the decreased enzymatic activity.

Fate of CeO_2 NPs

To perform EET, CeO_2 NPs should be in the interface of the cytoplasmatic material of microorganisms and the mixed liquor, or in the aqueous phase of the anaerobic digester. TEM imaging in bioreactor samples allowed visualization of the morphologic interactions between CeO_2 NPs and microorganisms. In Fig. 5, CeO_2 NPs can be observed as electrodense material (black dots) in the cellular membrane of microorganisms (Fig. 5C and D) as well as in the mixed liquor of the reactors (Fig. 5A and B). Similar interactions were observed for TiO_2 NPs, where NPs were mostly present in the loosely bound layer of the extracellular polymeric substances (EPS).[24] This may indicate that NPs are in in contact with proteins and polysaccharides, which are the major components of EPS.[65] NPs placed in this layer near the microorganisms and in the tightly bound EPS were observed as a cluster of NPs (Fig. 5C and D), which can also contribute to performing the redox reactions involved in the hydrolysis and methanogenesis processes. However, an analysis to determine the most suitable concentration of NPs that enhances the biogas production should be performed in a closer range of concentrations.

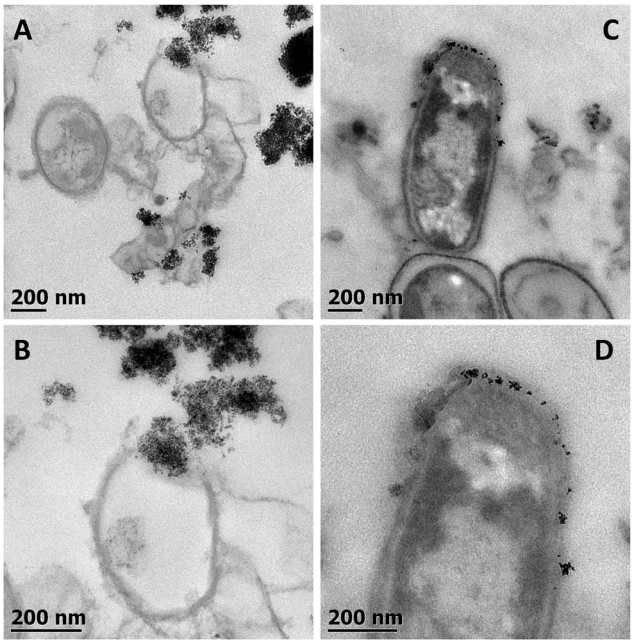

Fig. 5 Interactions between CeO_2 NPs (black electrodense material) and microorganisms present in the anaerobic reactors. Electrondense material aggregated in the mixed liquor (A and B), and nanoparticulated electrondense material in the cell membrane of microorganisms of anaerobic reactors (C and D).

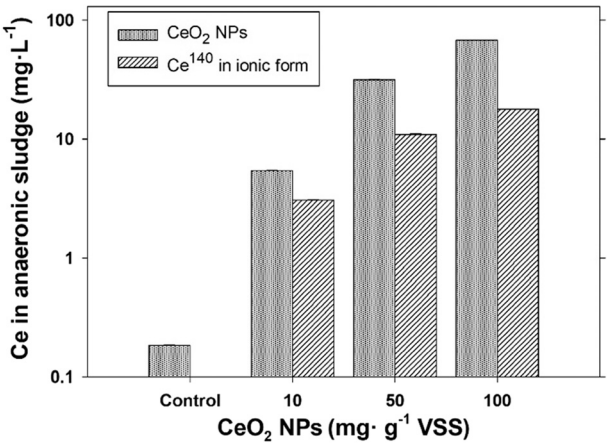

Fig. 6 Final concentration of CeO_2 NPs and Ce^{140} in ionic form in the mixed liquor of anaerobic reactors exposed to 10, 50 and 100 mg of CeO_2 NPs per g of VSS.

In order to evaluate the potential release of nanoceria to the treated effluent or biosolids, we determined the concentration of CeO_2 NPs in the mixed liquor (Fig. 6). Results indicated that the concentration of CeO_2 NPs and Ce in ionic form in the sludge is linear and increases as the spiked concentration of NPs also increases. Measured concentrations also reflect that CeO_2 NPs will mainly end in biosolids. Regarding control values, it was unexpected that the control group already contained 0.3 mg L^{-1} Ce-based NPs. This can be attributed to their incorporation in some consumer and healthcare products,[66] which end in wastewater streams. Considering that CeO_2 NPs applied as nanocatalysts to AD will end in biosolids which may be applied to agricultural soils in developing countries, consequently, their impact on soils and plants needs to be considered. Currently, it is known that CeO_2 NPs applied in the soil can translocate in the plant and transform from Ce IV to Ce III in barley (*Hordeum vulgare* L).[67] Therefore, phytoremediation can be considered to treat the biosolids containing Ce-based NPs in order to avoid the diffusion of this kind of NPs in the environment. Moreover, nanoceria also upregulated stress-response proteins;[68] however, phaseolin and lectins have been down-regulated by nanoceria in a dose-dependent manner in the common bean (*Phaseolus vulgaris*).[69] The effects of CeO_2 NPs on crops also depend on the concentration and delivery system. Therefore, more studies about the effects of CeO_2 NPs embedded in biosolids and mixed with representative soils at different stages of plants (*e.g.* vegetative or reproductive) are needed.

Conclusions

The presence of 10, 50 and 100 mg of CeO_2 NPs per gram of sludge acted as nanocatalyst during the anaerobic digestion of WAS, increasing the CH_4 production up to 14.2%. CeO_2 NPs did decrease the activity of protease and F420, two of the three enzymes monitored from the anaerobic digestion,

involved in hydrolysis and methanogenesis, respectively. Therefore, the enhanced biogas production was through the redox capability of nanoceria. This includes the ability of nanoceria to perform EET to hydrolyze long-chain substrates, *e.g.* proteins into amino acids, and short-chain organic acids such as maleic acid to shorter molecules and finally to CH_4. The fate of nanoceria in the anaerobic digester is to end up in the sludge, which may be applied to agricultural soils. Therefore, the potential effects of these NPs on soil microorganisms and plants should be further studied. On the other hand, the proper concentration of these NPs in the anaerobic digestion should be evaluated in a narrow concentration gap and for long-term experiments. In addition, a detailed life cycle assessment should be done to elucidate the impacts and potential associated cost for real applications when compared to other approaches, *e.g.* iron salt addition, to boost biogas generation.

Data availability

The data supporting this article have been included as part of the ESI.†

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

Support was provided by the University of California Center for Environmental Implications of Nanotechnology, funded by the U.S. National Science Foundation and the Environmental Protection Agency under Cooperative Agreement Number DBI-0830117. Arturo A. Keller also appreciates Agilent Technologies for their Agilent Thought Leader Award. PCA thanks SECIHTI (330129) and Instituto Tecnológico y de Estudios Superiores de Monterrey for Challenge-Based Research Funding (CHBRF-2023). The authors thank Lourdes Palma for technical support in the Microscopy Unit in the Neurobiology Institute at the National Autonomous University of Mexico (UNAM).

References

1 S. Olatunde, S. Oranusi and V. Enontiemonria, Cleaner Energy for Cleaner Production: Modeling and Optimization of Biogas Generation from Carica Papayas (Pawpaw) Fruit Peels, *J. Cleaner Prod.*, 2017, **156**, 19–29, DOI: **10.1016/j.jclepro.2017.04.042**.

2 Y. Zhang, Z. Yang, R. Xu, Y. Xiang, M. Jia, J. Hu and Y. Zheng, Enhanced Mesophilic Anaerobic Digestion of Waste Sludge with the Iron Nanoparticles Addition and Kinetic Analysis, *Sci. Total Environ.*, 2019, **683**, 124–133, DOI: **10.1016/j.scitotenv.2019.05.214**.

3 C. Vlachokostas, C. Achillas, A. V. Michailidou, G. Tsegas and N. Moussiopoulos, Externalities of Energy Sources: The Operation of a Municipal Solid Waste-to-Energy Incineration Facility in the Greater Thessaloniki Area, Greece, *Waste Manage.*, 2020, **113**, 351–358, DOI: **10.1016/j.wasman.2020.06.015**.

4 F. P. Silva, S. Nelson, M. De Souza, D. S. Kitamura, C. Eduardo, C. Nogueira and R. B. Otto, Energy e Ffi Ciency of a Micro-Generation Unit of Electricity from Biogas of Swine Manure, *Renewable Sustainable Energy Rev.*, 2017, 1–7, DOI: **10.1016/j.rser.2017.10.083**.

5 R. Hakawati, B. M. Smyth, F. Rosa and D. De Rooney, What Is the Most Energy Efficient Route for Biogas Utilization: Heat, Electricity or Transport?, *Appl. Energy*, 2017, **206**, 1076–1087, DOI: **10.1016/j.apenergy.2017.08.068**.

6 P. Wang, H. Wang, Y. Qiu, L. Ren and B. Jiang, Microbial Characteristics in Anaerobic Digestion Process of Food Waste for Methane Production-A Review, *Bioresour. Technol.*, 2018, **248**, 29–36, DOI: **10.1016/j.biortech.2017.06.152**.

7 C. M. Ajay, S. Mohan, P. Dinesha and M. A. Rosen, Review of Impact of Nanoparticle Additives on Anaerobic Digestion and Methane Generation, *Fuel*, 2020, **277**, 118234, DOI: **10.1016/j.fuel.2020.118234**.

8 P. G. Kougias and I. Angelidaki, Biogas and Its Opportunities — A Review, *Front. Environ. Sci. Eng.*, 2018, **12**(3), 14, DOI: **10.1007/s11783-018-1037-8**.

9 Y. Zhang, Z. Yang, R. Xu, Y. Xiang, M. Jia, J. Hu and Y. Zheng, Enhanced Mesophilic Anaerobic Digestion of Waste Sludge with the Iron Nanoparticles Addition and Kinetic Analysis, *Sci. Total Environ.*, 2019, **683**, 124–133, DOI: **10.1016/j.scitotenv.2019.05.214**.

10 I. Angelidaki, L. Treu, P. Tsapekos, G. Luo, S. Campanaro, H. Wenzel and P. G. Kougias, Biogas Upgrading and Utilization: Current Status and Perspectives, *Biotechnol. Adv.*, 2018, 1, DOI: **10.1016/j.biotechadv.2018.01.011**.

11 Y. Li, Y. Chen and J. Wu, Enhancement of Methane Production in Anaerobic Digestion Process: A Review, *Appl. Energy*, 2019, **240**, 120–137, DOI: **10.1016/j.apenergy.2019.01.243**.

12 V. Paolini, F. Petracchini, M. Segreto, L. Tomassetti, N. Naja, A. Cecinato, V. Paolini, F. Petracchini and M. Segreto, Environmental Impact of Biogas: A Short Review of Current Knowledge, *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.*, 2018, 1–8, DOI: **10.1080/10934529.2018.1459076**.

13 Y. Li, Y. Chen and J. Wu, Enhancement of Methane Production in Anaerobic Digestion Process: A Review, *Appl. Energy*, 2019, **240**, 120–137, DOI: **10.1016/j.apenergy.2019.01.243**.

14 I. Ullah, M. Ha, D. Othman, H. Hashim, T. Matsuura, A. F. Ismail, M. Rezaei-dashtarzhandi and I. W. Azelee, Biogas as a Renewable Energy Fuel – A Review of Biogas Upgrading, Utilisation and Storage, *Energy Convers. Manage.*, 2017, **150**, 277–294, DOI: **10.1016/j.enconman.2017.08.035**.

15 C. M. Ajay, S. Mohan, P. Dinesha and M. A. Rosen, Review of Impact of Nanoparticle Additives on Anaerobic Digestion and Methane Generation, *Fuel*, 2020, **277**, 118234, DOI: **10.1016/j.fuel.2020.118234**.

16 L. Rocha-Meneses, A. Hari, A. Inayat, A. Shanableh, M. Abdallah, C. Ghenai, S. Shanmugam and T. Kikas, Application of Nanomaterials in Anaerobic Digestion Processes: A New Strategy towards Sustainable Methane Production, *Biochem. Eng. J.*, 2022, **188**, 108694, DOI: **10.1016/j.bej.2022.108694**.

17 P. Cervantes-Avilés, J. B. D. Vargas, S. Akizuki, T. Kodera, J. Ida and G. Cuevas-Rodríguez, Cumulative Effects of Titanium Dioxide Nanoparticles in UASB Process during Wastewater Treatment, *J. Environ. Manage.*, 2021, **277**(1), 111428, DOI: **10.1016/j.jenvman.2020.111428**.

18 Z. Zhang, L. Guo, Y. Wang, Y. Zhao, Z. She, M. Gao and Y. Guo, Application of Iron Oxide (Fe3O4) Nanoparticles during the Two-Stage Anaerobic Digestion with Waste Sludge: Impact on the Biogas Production and the Substrate Metabolism, *Renewable Energy*, 2020, **146**, 2724–2735, DOI: **10.1016/j.renene.2019.08.078**.

19 A. Ali, R. B. Mahar, R. A. Soomro and S. T. H. Sherazi, Fe3O4 Nanoparticles Facilitated Anaerobic Digestion of Organic Fraction of Municipal Solid Waste for Enhancement of Methane Production, *Energy Sources, Part A*, 2017, **39**(16), 1815–1822, DOI: **10.1080/15567036.2017.1384866**.

20 E. Abdelsalam, M. Samer, Y. A. Attia, M. A. Abdel-hadi, H. E. Hassan and Y. Badr, Influence of Zero Valent Iron Nanoparticles and Magnetic Iron Oxide Nanoparticles on Biogas and Methane Production from Anaerobic Digestion of Manure, *Energy*, 2016, **120**(1), 842–853, DOI: **10.1016/j.energy.2016.11.137**.

21 Y. Lee and D. Lee, Impact of Adding Metal Nanoparticles on Anaerobic Digestion Performance – A Review, *Bioresour. Technol.*, 2019, **292**, 121926, DOI: **10.1016/j.biortech.2019.121926**.

22 P. Antwi, J. Li, P. O. Boadi, J. Meng, E. Shi, X. Chi, K. Deng and F. Ayivi, Dosing Effect of Zero Valent Iron (ZVI) on Biomethanation and Microbial Community Distribution as Revealed by 16S RRNA High-Throughput Sequencing, *Int. Biodeterior. Biodegrad.*, 2017, **123**, 191–199, DOI: **10.1016/j.ibiod.2017.06.022**.

23 M. Farghali, F. J. Andriamanohiarisoamanana, M. M. Ahmed, S. Kotb, T. Yamashiro, M. Iwasaki and K. Umetsu, Impacts of Iron Oxide and Titanium Dioxide Nanoparticles on Biogas Production: Hydrogen Sulfide Mitigation, Process Stability, and Prospective Challenges, *J. Environ. Manage.*, 2019, **240**, 160–167, DOI: **10.1016/j.jenvman.2019.03.089**.

24 P. Cervantes-Avilés, J. Ida, T. Toda and G. Cuevas-rodríguez, Effects and Fate of TiO 2 Nanoparticles in the Anaerobic Treatment of Wastewater and Waste Sludge, *J. Environ. Manage.*, 2018, **222**, 227–233, DOI: **10.1016/j.jenvman.2018.05.074**.

25 T. Tian, S. Qiao, C. Yu and J. Zhou, Effects of Nano-Sized MnO2 on Methanogenic Propionate and Butyrate Degradation in Anaerobic Digestion, *J. Hazard. Mater.*, 2019, **364**, 11–18, DOI: **10.1016/j.jhazmat.2018.09.081**.

26 G. Wang, H. Feng, A. Gao, Q. Hao, W. Jin, X. Peng, W. Li, G. Wu and P. K. Chu, Extracellular Electron Transfer from Aerobic Bacteria to Au-Loaded TiO2 Semiconductor without Light: A New Bacteria-Killing Mechanism Other than Localized Surface Plasmon Resonance or Microbial Fuel Cells, *ACS Appl. Mater. Interfaces*, 2016, **8**(37), 24509–24516, DOI: **10.1021/acsami.6b10052**.

27 E. K. Ünşar, A. S. Çığgın, A. Erdem and N. A. Perendeci, Long and Short Term Impacts of CuO, Ag and CeO 2 Nanoparticles on Anaerobic Digestion of Municipal Waste Activated Sludge, *Environ. Sci.: Processes Impacts*, 2016, **18**(2), 277–288, DOI: **10.1039/C5EM00466G**.

28 Ü. Kökdemir and N. A. Perendeci, What Kind of Effects Do Fe2O3 and Al2O3 Nanoparticles Have on Anaerobic Digestion, Inhibition or Enhancement?, *Chemosphere*, 2018, **211**, 726–735, DOI: **10.1016/j.chemosphere.2018.08.014**.

29 S. Wang, L. Chen, H. Yang and Z. Liu, Influence of Zinc Oxide Nanoparticles on Anaerobic Digestion of Waste Activated Sludge and Microbial Communities, *RSC Adv.*, 2021, **11**(10), 5580–5589, DOI: **10.1039/D0RA08671A**.

30 S. A. Ansari, M. M. Khan, M. O. Ansari, S. Kalathil, J. Lee and M. H. Cho, Band Gap Engineering of CeO2 Nanostructure Using an Electrochemically Active Biofilm for Visible Light Applications, *RSC Adv.*, 2014, **4**(32), 16782–16791, DOI: **10.1039/c4ra00861h**.

31 A. Brunelli, V. Cazzagon, E. Faraggiana, C. Bettiol, M. Picone, A. Marcomini and E. Badetti, An Overview on Dispersion Procedures and Testing Methods for the Ecotoxicity Testing of Nanomaterials in the Marine Environment, *Sci. Total Environ.*, 2024, **921**, 171132, DOI: **10.1016/j.scitotenv.2024.171132**.

32 A. A. Keller, G. Cherr, B. J. Cardinale, R. Miller and Z. Ji, Stability and Aggregation of Metal Oxide Nanoparticles in Natural Aqueous Matrices, *Environ. Sci. Technol.*, 2010, **44**(6), 1962–1967, DOI: **10.1021/es902987d**.

33 P. Cervantes-Avilés, X. Huang and A. A. Keller, Dissolution and Aggregation of Metal Oxide Nanoparticles in Root Exudates and Soil Leachate: Implications for Nanoagrochemical Application, *Environ. Sci. Technol.*, 2021, **55**(20), 13443–13451, DOI: **10.1021/acs.est.1c00767**.

34 APHA, *Standard Methods for the Examination of Water and Wastewater, Standard Methods*, 2005, p. 541, ISBN 9780875532356.

35 J. C. De Castro, E. Resende, I. Taveira, A. Enrich-Prast and F. Abreu, Nanotechnology Boosts the Production of Clean Energy via Nanoparticle Addition in Anaerobic Digestion, *Front. Nanotechnol.*, 2024, **6**, 1406344, DOI: **10.3389/fnano.2024.1406344**.

36 E. Metcalf and M. Eddy, *Wastewater Engineering: Treatment and Resource Recovery*, USA, Mic Graw-Hill, 2014, pp. 1530–1533.

37 P. Cervantes-Avilés, A. N. Saber, A. Mora, J. Mahlknecht and G. Cuevas-Rodríguez, Influence of Wastewater Type in the Effects Caused by Titanium Dioxide Nanoparticles in the Removal of Macronutrients by Activated Sludge, *Environ. Sci. Pollut. Res.*, 2022, **29**(6), 8746–8757, DOI: **10.1007/s11356-021-16221-2**.

38 Y. Huang, W. Li, A. S. Minakova, T. Anumol and A. A. Keller, Quantitative Analysis of Changes in Amino Acids Levels for Cucumber (Cucumis Sativus) Exposed to Nano Copper, *NanoImpact*, 2018, **12**, 9–17, DOI: **10.1016/j.impact.2018.08.008**.

39 Q. Guo, Y. Wang, J. Qian, B. Zhang, M. Hua, C. Liu and B. Pan, Enhanced Production of Methane in Anaerobic Water Treatment as Mediated by the Immobilized Fungi, *Water Res.*, 2021, **190**, 116761, DOI: **10.1016/j.watres.2020.116761**.

40 B. Dong, Z. Xia, J. Sun, X. Dai, X. Chen and B.-J. Ni, The Inhibitory Impacts of Nano-Graphene Oxide on Methane Production from Waste Activated Sludge in Anaerobic

Digestion, *Sci. Total Environ.*, 2019, **646**, 1376–1384, DOI: **10.1016/j.scitotenv.2018.07.424**.

41 S. Wang, L. Chen, H. Yang and Z. Liu, Influence of Zinc Oxide Nanoparticles on Anaerobic Digestion of Waste Activated Sludge and Microbial Communities, *RSC Adv.*, 2021, **11**(10), 5580–5589, DOI: **10.1039/D0RA08671A**.

42 X. Dai, C. Hu, D. Zhang, L. Dai and N. Duan, Impact of a High Ammonia-Ammonium-PH System on Methane-Producing Archaea and Sulfate-Reducing Bacteria in Mesophilic Anaerobic Digestion, *Bioresour. Technol.*, 2017, **245**, 598–605, DOI: **10.1016/j.biortech.2017.08.208**.

43 B. Dong, Z. Xia, J. Sun, X. Dai, X. Chen and B.-J. Ni, The Inhibitory Impacts of Nano-Graphene Oxide on Methane Production from Waste Activated Sludge in Anaerobic Digestion, *Sci. Total Environ.*, 2019, **646**, 1376–1384, DOI: **10.1016/j.scitotenv.2018.07.424**.

44 S. H. G. Allen, R. W. Kellermeyer, R. L. Stjernholm and H. G. Wood, Purification and Properties of Enzymes Involved in the Propionic Acid Fermentation, *J. Bacteriol.*, 1964, **87**(1), 171–187, DOI: **10.1128/jb.87.1.171-187.1964**.

45 M. J. Delafontaine, H. P. Naveau and E. J. Nyns, Fluorimetric Monitoring of Methanogenesis in Anaerobic Digestors, *Biotechnol. Lett.*, 1979, **1**(2), 71–74, DOI: **10.1007/BF01398311**.

46 Y. Huang, A. A. Keller, P. Cervantes-Avilés and J. Nelson, Fast Multielement Quantification of Nanoparticles in Wastewater and Sludge Using Single-Particle ICP-MS, *ACS ES&T Water*, 2021, **1**(1), 205–213, DOI: **10.1021/acsestwater.0c00083**.

47 H. Zhao, S. Cheng, C. Zhao, K. Ruan, J. Xu and X. Cheng, Effect of Nano-Al2O3 and Multi-Walled Carbon Nanotubes on the Anaerobic Mono-Digestion of Sludge and the Co-Digestion of Tobacco Waste and Sludge, *RSC Adv.*, 2023, **13**(50), 35621–35628, DOI: **10.1039/d3ra07170g**.

48 D. Zhang, Y. Chen, Y. Zhao and X. Zhu, New Sludge Pretreatment Method to Improve Methane Production in Waste Activated Sludge Digestion, *Environ. Sci. Technol.*, 2010, **44**(12), 4802–4808, DOI: **10.1021/es1000209**.

49 Y. Zhu, K. Xiao, Y. Zhou, W. Yu, S. Tao, C. Le, D. Lu, Z. Yu, S. Liang, J. Hu, H. Hou, B. Liu and J. Yang, Profiling of Amino Acids and Their Interactions with Proteinaceous Compounds for Sewage Sludge Dewatering by Fenton Oxidation Treatment, *Water Res.*, 2020, **175**, 115645, DOI: **10.1016/j.watres.2020.115645**.

50 S. Chen, B. Dong, X. Dai, H. Wang, N. Li and D. Yang, Effects of Thermal Hydrolysis on the Metabolism of Amino Acids in Sewage Sludge in Anaerobic Digestion, *Waste Manage.*, 2019, **88**, 309–318, DOI: **10.1016/j.wasman.2019.03.060**.

51 P. V. R. Subrahmanyam, C. A. Sastry, A. V. S. P. Rao and S. C. Pillai, Amino Acids in Sewage Sludges, *J. - Water Pollut. Control Fed.*, 1960, 344–350.

52 S. Wang, M. Gao, Z. Li, Z. She, J. Wu, D. Zheng, L. Guo, Y. Zhao, F. Gao and X. Wang, Performance Evaluation, Microbial Enzymatic Activity and Microbial Community of a Sequencing Batch Reactor under Long-Term Exposure to Cerium Dioxide Nanoparticles, *Bioresour. Technol.*, 2016, **220**, 262–270, DOI: **10.1016/j.biortech.2016.08.086**.

53 S. Chen, B. Dong, X. Dai, H. Wang, N. Li and D. Yang, Effects of Thermal Hydrolysis on the Metabolism of Amino Acids in Sewage Sludge in Anaerobic Digestion, *Waste Manage.*, 2019, **88**, 309–318, DOI: **10.1016/j.wasman.2019.03.060**.

54 M. Auffan, A. K. Kabore, A. Cuny, O. A. Ali, M. Barakat, B. Angeletti, O. Proux, J.-Y. Bottero, N. Roche and C. Santaella, Combusted-Diesel Additives Containing CeO_2 Nanomaterials Shape Methanogenic Pathways during Sludge Digestion and Enhance Biogas Production, *Environ. Sci.: Nano*, 2022, **9**(11), 4201–4213, DOI: **10.1039/D2EN00389A**.

55 Z. Wei, Y. Xu, Q. Xu, W. Cao, H. Huang and H. Liu, Microbial Biosynthesis of L-Malic Acid and Related Metabolic Engineering Strategies: Advances and Prospects, *Front. Bioeng. Biotechnol.*, 2021, **9**, 1–14, DOI: **10.3389/fbioe.2021.765685**.

56 Y. Tang, J. Sun, B. Dong and X. Dai, Citric Acid Treatment Directly on Anaerobic Digestor Sludge Alleviates the Inhibitory Effect of In-Situ Generated Humic Acids by Their Deconstruction and Redistribution, *Water Res.*, 2023, **233**, 119680, DOI: **10.1016/j.watres.2023.119680**.

57 F. E. Huelin, Studies on the Anaerobic Decomposition of Ascorbic Acid, *J. Food Sci.*, 1953, **18**(1–6), 633–639, DOI: **10.1111/j.1365-2621.1953.tb17760.x**.

58 S. Zhu, Y. Zhang, Z. Zhang, F. Ai, H. Zhang, Y. Li, Y. Wang and Q. Zhang, Ascorbic Acid-Mediated Zero-Valent Iron Enhanced Hydrogen Production Potential of Bean Dregs and Corn Stover by Photo Fermentation, *Bioresour. Technol.*, 2023, **374**, 128761, DOI: **10.1016/j.biortech.2023.128761**.

59 Y. Liu, X. Ye, Q. Xiao, C. Wang, Z. Jia, C. Cao, J. Du, X. Kong and Y. Xi, Targeted Addition of CeO2 Nanoparticles to Promote Methane Production in Anaerobic Digestion Systems of Wheat Straw, *J. Cleaner Prod.*, 2022, **377**, 134231, DOI: **10.1016/j.jclepro.2022.134231**.

60 Y.-F. Cheng, Z.-Z. Zhang, G.-F. Li, B.-Q. Zhu, Q. Zhang, Y.-Y. Liu, W.-Q. Zhu, N.-S. Fan and R.-C. Jin, Effects of ZnO Nanoparticles on High-Rate Denitrifying Granular Sludge and the Role of Phosphate in Toxicity Attenuation, *Environ. Pollut.*, 2019, **251**, 166–174, DOI: **10.1016/j.envpol.2019.04.138**.

61 I. S. Kim and E. K. Jo, Inosine: A Bioactive Metabolite with Multimodal Actions in Human Diseases, *Front. Pharmacol.*, 2022, **13**, 1043970, DOI: **10.3389/fphar.2022.1043970**.

62 L. Shi, H. Dong, G. Reguera, H. Beyenal, A. Lu, J. Liu, H.-Q. Yu and J. K. Fredrickson, Extracellular Electron Transfer Mechanisms between Microorganisms and Minerals, *Nat. Rev. Microbiol.*, 2016, **14**(10), 651–662, DOI: **10.1038/nrmicro.2016.93**.

63 D. R. Shaw, M. Ali, K. P. Katuri, J. A. Gralnick, J. Reimann, R. Mesman, L. van Niftrik, M. S. M. Jetten and P. E. Saikaly, Extracellular Electron Transfer-Dependent Anaerobic Oxidation of Ammonium by Anammox Bacteria, *Nat. Commun.*, 2020, **11**(1), 2058, DOI: **10.1038/s41467-020-16016-y**.

64 J. Huo, Y. Hu, H. Jiang and C. Li, In Situ Surface Hydrogenation Synthesis of Ti^{3+} Self-Doped TiO_2 with Enhanced Visible Light Photoactivity, *Nanoscale*, 2014, **6**(15), 9078–9084, DOI: **10.1039/C4NR00972J**.

65 B. Zhang, M. Ji, F. Wang, R. Li, K. Zhang, X. Yin and Q. Li, Damage of EPS and Cell Structures and Improvement of High-Solid Anaerobic Digestion of Sewage Sludge by Combined ($Ca(OH)_2$ + Multiple-Transducer Ultrasonic) Pretreatment, *RSC Adv.*, 2017, **7**(37), 22706–22714, DOI: **10.1039/C7RA01060E**.

66 G. Cornelis, B. Ryan, M. J. McLaughlin, J. K. Kirby, D. Beak and D. Chittleborough, Solubility and Batch Retention of CeO2 Nanoparticles in Soils, *Environ. Sci. Technol.*, 2011, **45**(7), 2777–2782, DOI: **10.1021/es103769k**.

67 C. M. Rico, M. G. Johnson and M. A. Marcus, Cerium Oxide Nanoparticles Transformation at the Root-Soil Interface of Barley (Hordeum Vulgare L.), *Environ. Sci.: Nano*, 2018, **5**(8), 1807–1812, DOI: **10.1039/c8en00316e**.

68 Y. Ma, C. Xie, X. He, B. Zhang, J. Yang, M. Sun, W. Luo, S. Feng, J. Zhang, G. Wang and Z. Zhang, Effects of Ceria Nanoparticles and CeCl3 on Plant Growth, Biological and Physiological Parameters, and Nutritional Value of Soil Grown Common Bean (Phaseolus Vulgaris), *Small*, 2020, **16**(21), 1907435, DOI: **10.1002/smll.201907435**.

69 S. Majumdar, I. C. Almeida, E. A. Arigi, H. Choi, N. C. VerBerkmoes, J. Trujillo-Reyes, J. P. Flores-Margez, J. C. White, J. R. Peralta-Videa and J. L. Gardea-Torresdey, Environmental Effects of Nanoceria on Seed Production of Common Bean (Phaseolus Vulgaris): A Proteomic Analysis, *Environ. Sci. Technol.*, 2015, **49**(22), 13283–13293, DOI: **10.1021/acs.est.5b03452**.