Understanding bioenergy production and optimisation at the nanoscale — a review

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ABSTRACT
Nanotechnology has an increasingly large impact on a wide range of biotechnological, pharmacological and pure technological applications. Its current use in bioenergy production from biomass is very limited. This paper examines the potential interrelationships between nanotechnology and bioenergy production through a comprehensive literature review and analysis of data from biomass characterisation studies. The aim of this review is to indicate how nanotechnology can be applied in biomass-to-bioenergy conversion. This study shows currently nanotechnology has been applied in the production of only two types of biomass, i.e. sludge and algae. Hence, interaction of nanomaterials with active sludge and algal cells were examined. Our extensive literature review indicates that anaerobic digestion process in sludge can potentially be enhanced by using magnetite nanoparticles, which gives higher methane yields. On the other hand, nanosilver reduces growth and causes adverse effects on the morphology of green algae. This process for bioenergy generation has already been successfully applied to sludge and algae biomass. Our study confirms that the process can also be used in the production of bioenergy from the other biomasses, such as agricultural wastes and industrial residues. Outcomes of this work will be an important tool for implementing nanotechnology in bioenergy research.

1 Introduction
This paper reviews a range of studies on nanoparticles, nanomaterials, biomass and bioenergy. It examines the potential impact of nanotechnology on microorganism in bioenergy yield. The entire approach of this work was to develop a critical understanding of nanomaterials, defining them according to the EU commissioning recommendation, biomass characterisation and evaluate the impact on bioenergy process efficiency.

1.1 Definition of nanomaterials (NMs)
There is no uniformly accepted definition of what in fact constitutes a ‘nanomaterial’. In 2008 and 2010, the International Standardization Organization (ISO) has provided overarching technical definitions for nanotechnology-related terms: ‘Nanomaterial’ is defined as a material with any external
dimension in the nanoscale or having internal or surface structure in the nanoscale, with ‘nanoscale’
defined as the size range from approximately 1 to 100 nm.[1,2] All definitions of a ‘nanomaterial’
include the size range from approximately 1–100 nm, and none of the definitions take into account
actual concerns in respect to the materials’ adverse effects on human health or the environment.
The EU definition [3] is the only definition that includes natural or accidentally occurring nanopar-
ticles, whereas all other definitions are restricted to ‘intentionally produced, manufactured, or engi-
eered NMs’. According to the EU recommendation (2011/696/EU) on the definition of a
nanomaterial is ‘A natural, incidental or manufactured material containing particles, in an unbound
state or as an aggregate or as an agglomerate and where, for 50% or more of the particles in the num-
ber size distribution, one or more external dimensions is in the size range 1 nm–100 nm’. In specific
cases and where warranted by concerns for the environment, health, safety or competitiveness, the
number size distribution threshold of 50% may be replaced by a threshold between 1% and 50%.

The different definitions are not consistent in regard to their mentioning of the state of aggregation
or agglomeration of the nanoparticles. Current level of available information on the presence of nano-
materials and products containing nanomaterials on the market is insufficient. Since the EU definition
is based on the size distribution of the constituent particles of a material expressed in number metrics,
[3] nearly every powder can be considered a nanomaterial. However, the EU has already announced
the revision of its definition that was established in 2011: the definition has been reviewed in the light
of experience and of scientific and technological developments. The review should particularly focus
on whether the number size distribution threshold of 50% should be increased or decreased.[3]

All available definitions are based on material properties. While they are conceived and applied to
found regulatory provisions for safety assessment, the definitions are not derived from toxicological
evidence of a step-change in toxicity at 100 nm or any other single overarching material property
applicable to all ‘nanomaterials’. Specific concerns that have been recognised for specific types of
NMs do not relate to their nanosize, but to their respective chemical composition or shape. There is
no evidence of a novel ‘nano-specific hazard’. Instead, there is likely to be a more gradual magnifica-
tion of the intrinsic hazard of increasingly small particles, e.g. in relation to surface area.[4]

1.2. Characteristics of nanoparticles

The characteristics of NPs depend greatly on their chemical origin, which affects their fate and
behaviour in environment.[5,6] There are four classification groups of NPs: carbon, inorganic,
organic and composites NPs. Nanoparticles have special optical, physical and chemical characteris-
tics. Properties of particles at nanoscale change in unpredictable ways that make them different with
the same substance at a bigger size. Special characteristics with high reactivity of nanoparticles make
them become ideal for variety of fields, such as energy, electronic, medical and consumer products.
Nanoparticles can contribute to produce stronger, lighter, cleaner, smarter and more efficient mate-
rials and products.[7]

Nanoparticles are interesting because their chemical and physical properties are different from
their macro counterparts (e.g. sand/sugar) (Figure 1). The sugar example is interesting — if we want
to make tea sweet faster, we use granules instead of cubes, but serves little real application. For
example, a cube of sugar reacting with water as the water dissolves the outside of the sugar. Now the
same cube of sugar cut into many little pieces — each cut makes new outer surfaces for the water to
dissolve. For smaller particles of sugar, the same volume of sugar now has much more surface area.
A particle with a high surface area has a greater number of reaction sites than a particle with low
surface area, and thus results in higher chemical reactivity.[8] Another prime example of surface
area to volume ratio at the nanoscale is gold (Au) as a nanoparticle. At the macroscale, gold is an
inert element, meaning it does not react with many chemicals, whereas at the nanoscale, gold nano-
particles become extremely reactive and can be used as catalysts to speed up reactions.[8] This
increased reactivity for surface area to volume ratio has widely taken advantage of in nature; one
biological example being the human digestive system. Having the similar microorganisms active on
both digestion (human digestion and anaerobic digestion (AD)) system, the surface area to volume ratio of biomass causes impact on AD process.

1.3. Interaction of nanomaterials with biomass

Nanoparticles can play a crucial role with liquid biomass in water purification [9] as many of them have antibacterial properties. It is now used for detection and removal of chemical and biological substances that include metals (e.g. Cd, Cu, Zn), nutrients (e.g. phosphate, ammonia, nitrate), cyanide, organics, algae (e.g. cyanobacterial toxins), viruses, bacteria, parasites and antibiotics. Basically, four classes of nanoscale materials are being evaluated as functional materials for water purification: e.g. metal-containing nanoparticles, carbonaceous nanomaterials, zeolites and dendrimers. Carbon nanotubes (CNTs) and nanofibers also show some positive result.

Nanomaterials reveal good result than other techniques used in water treatment because of its high surface area (surface/volume ratio).[10] Current and potential applications of nanotechnology in water and wastewater treatments are adsorption, membrane processes, photocatalysis, disinfection, microbial control, sensing and monitoring.[11] But knowledge on toxicity of nanomaterials is still in infancy.[12] Antibacterial activities of NPs depend upon two main factors: (1) physicochemical properties of NPs and (2) type of bacteria. It is also found that the coliform bacteria treated with ultrasonic irradiation for a short time period before Ag nanoparticles treatment at low concentrations enhanced the antibacterial effect. Many studies have also shown an important activity of silver nanoparticles against bacterial biofilms. The correlation between the bactericidal effect and AgNP concentrations is bacterial class-dependent.[13] A research finding showed that, *Pseudomonas aeruginosa* and *Vibrio cholera* were more resilient than *Escherichia coli* and *Salmonella typhi*, but at concentrations above 75 µg/mL, the bacterial growth was completely abolished.[14] In this perspective, Sweet et al. [15] studied Ag NPs’ antimicrobial activity against *E. coli* and *S. aureus* showing that *E. coli* was inhibited at low concentrations, while the inhibitory effects on the growth of *S. aureus* were less noticeable.[16] Silver nanoparticles have also significant adverse effects on growth and morphology of filamentous green algae.[17]

In order to understand the importance of the role of nanomaterials on bioenergy research, Figure 2 is given to show the pathway of biomass-to-bioenergy conversion and the interaction of functionalised nanoparticles. The biomass-to-bienergy conversion could be either a thermal, chemical or biological process. Molecular size and inorganic contaminants of organic biomass cause impact on the conversion process. Functionalised nanoparticles could come from either or both natural and synthetic (manmade) sources. Due to the existence of this multi-faceted interaction within the process, a number of issues could arise, and therefore need to be addressed appropriately.

The impacts of nanoparticles on biomass energy conversion are described in two types of biomass e.g. (1) waste sludge and (2) algae. The aim of this paper is to examine the impact of NPs on
activated sludge systems and algal biomass systems, including the inhibitory impacts, phytotoxicity and mechanisms by which bioenergy processes are enhanced or inhibited.

2. Review of this work/methodology

The paper has evaluated the potential applications of nanoparticles to enhance the efficiency of bioenergy production from organic materials.

2.1. An understanding of nanotechnology

A comprehensive review of current nanotechnology research has been undertaken to understand the key characteristics and applications and evaluate how particle size, composition and reactivity may impact on biomass-to-energy conversion.

2.2. An understanding of biomass and their characteristics

Various biomass resources were studied in terms of their source and key physical, biological and chemical composition. The impact of particle size of biomass was evaluated in relation with biomass-to-bioenergy conversion in both biochemical and bio-thermal aspects. Particle size and pre-treatment of different types of biomass were studied.

Different pre-treatment methods produce different effects on the biomass in terms of its structure and composition.[18] For example, the hydrothermal and acidic pre-treatments conceptually remove mainly the biomass hemicellulose fraction and alkaline pre-treatments remove lignin, whereas the product of a milling-based pre-treatment retains its initial biomass composition. The main objective of milling pre-treatment is to reduce particle size in order to increase the biomass-specific surface during biomass fibrillation and to reduce cellulose fibre organisation, which is measured by a decrease in crystallinity (Figure 3).
2.3. Impact on process efficiency

The potential interaction between traditional (or new/novel NPs) and biomass substrates and its impact on process efficiency and energy production (and potentially waste from the process) were evaluated.

3. Results and discussion

Nanotechnologies could enhance energy efficiency across all branches of industry and economically leverage renewable energy production. It has the potential to enhance the conversion of biomass for fuels, chemical intermediates, speciality chemicals and products. Nanotechnology is an important tool that can improve the efficiency of bioenergy. The interactions of nanomaterials were found with active sludge and a few species of algal biomass. The impact was found in the form of inhibitory,[19] adverse or enhanced yield [20] in aspect of bioenergy production. The variation in the severity of impact on the basis of particles surface area to volume ratio was also assessed. These results are described here on the basis of two biomass feedstocks which were found to give significant response to nanoparticles. These are active sludge and noble feedstock algae.

3.1. Impact of NPs on activated sludge systems

The results on the impact of nanoparticles in activated sludge are presented under a few relevant characteristics of NP and active sludge. These emphasise on concentration, size of nanoparticles and the response of microorganisms on its bioenergy yield.

3.1.1. Nanoparticles with microorganism

Microorganisms actively respond to nanoparticles and can cause a significant effect. An overview of antimicrobial properties of NPs suggests the potential adverse effect they could exert on wastewater
microorganisms (Figure 4). This has significant negative implications, although, at present, information on NPs’ effect on wastewater microorganisms during AS and AD is rather limited.[21,22] It is, therefore, difficult to make specific assertions regarding the toxic effect of NPs on wastewater microorganisms. There is a possibility that NPs in contact with a microbial community may lead to reduced efficiency of AS and AD processes, complete failure of treatment and/or environmental pollution through discharge of contaminated effluent and use of biosolids for soil amendment.[23] The silver ion has been known to be effective against a broad range of microorganisms. Nowadays, silver ions are used to control bacterial growth in a variety of medical applications, including dental work, catheters, and the healing of burn wounds.[24] The mechanism of action attributed to release of ions from Ag was demonstrated with E. coli and found to be dependent on concentration and contact time. Adverse effects included the leaking of reducing sugars and proteins, enzyme inhibition, cell disruption, and scattered vesicles which slowly dissolve thus inhibiting cellular respiration and cell growth.[25]

3.1.2. Concentration of nanoparticles
Nanoparticles could come from both natural and anthropogenic sources. It could be accumulated to a very high concentration in the waste sludge. However, impact and toxicity of NPs on sludge treatment stream is still an abandoned area of research.[19] Nguyen [19] conducted a research to determine the effects of CeO₂ and ZnO NPs on sludge anaerobic digestion process, sludge dewatering process, and toxicity of sludge to bacteria and plants. The result showed that CeO₂ and ZnO NPs could inhibit the biogas production of AD system. The exposure concentration of ZnO at 1000 mg/L caused the greatest inhibition to the biogas volume (65.3%) and the methane composition (40.7%), as compared with controlled sample. In addition, at tolerable exposure concentration of ZnO, the system could overcome the inhibition effect after 14 days of incubation. On the other hand, CeO₂ at a low concentration of 10 mg/L could increase the generated biogas volume by 11%. The positive effect of CeO₂ at low concentration was also observed on bacterial toxicity test. The ZnO NPs were more toxic to bacteria than CeO₂ NPs at the same exposure concentration.[19] However, the bacterial toxicity of both nanoparticles was reduced when they were applied rather than naturally occurring to the sludge. Moreover, at the end of AD process, the bacterial toxicity was

![Figure 4. Scanning electron microscope (SEM) images showing ENPs sorption to cells (a and b), damage to microbial cell (c and d) and aggregation to biomass (e and f) in activated sludge [36].](image-url)
again lessened. Additionally, required time to dewater the digested sludge was increased proportionally with the exposure concentration of nanoparticles.

The bacterial toxicity of nanoparticles could be greatly reduced when nanoparticles were applied in the sludge. Sludge before AD was more toxic than sludge after the digestion process. The sludge with exposure of 1000 mg/L of CeO₂ NPs before AD caused 47.5% of inhibition to bacterial viability. However, the same sample after AD just had 30.4% of inhibition toward bacteria viability. Similarly, sample with 1000 mg/L of ZnO NPs induced up to 92.3% of inhibition before AD, while after digestion process, this value was just 34.8%.[19]

The effects of metal oxide particle size on biogas and methane production during AD of cattle manure was studied by Luna del Risco.[26] In the experiment, nanoparticles of CuO showed higher influence on biogas production than the other test compounds. The concentration of 15 mg/L of CuO nanoparticles resulted in a reduction of 30% of the biogas production from the total biogas produced in the control at day 14. Biogas production in the presence of microparticles of CuO was less inhibited, whereas concentrations of 120 and 240 mg/L of bulk CuO caused a reduction by 19% and 60%, respectively. The statistical analyses have validated the differences between the two groups of particles tested (bulk and nanoparticles) of CuO ($p < 0.05$). As reported by Heinlaan [27], Neal [28] and Kasemets et al. [29], nanoparticles are toxic to bacteria due to the release of bioavailable metal ions that cause cell membrane damage, and therefore, the inhibition of biogas production can occur.

Biogas production in test samples containing nanoparticles of ZnO was compared with bulk ZnO. Concentrations of 120 and 240 mg/L of ZnO nanoparticles presented an inhibition of 43% and 74% of the biogas yield, respectively, while test bottles containing bulk ZnO presented a reduction of 18% and 72% of the total biogas produced at day 14. However, no significant difference of biogas inhibition from bulk and nanoparticles of ZnO was found.[26] From this section, it can be concluded that particle size and concentration of nano-sized CuO and ZnO affect biogas yield.

The addition of nano iron oxide (Fe₃O₄ NPs) can enhance the methane production due to the presence of the non-toxic Fe³⁺ and Fe³⁺ ions. Fe₃O₄ NPs (7 mm) were added with a concentration of 100 ppm to anaerobic waste digester at mesophilic temperature (37 °C) for 60 days and the results showed a 180% increase in biogas production and 234% increase in methane production, which could be considered the greatest improvement in biogas production using NPs.[30] The new delivery system based on Fe₃O₄ (magnetite) nanoparticles leads to enhanced AD, and consequently to higher methane production and organic matter processing (Figure 5). The improved performance is due to the presence of Fe²⁺/Fe³⁺ ions, introduced into the reactor in the form of nanoparticles in a
similar way to controlled drug delivery systems, because Fe plays an important role in transporting electron, simulating bacterial growth and increasing hydrogen and methane production rate by promoting enzyme activities.[31] \( \text{Fe}_3\text{O}_4 \) nanoparticles are the most prevalent materials because they have low toxicity and good biocompatibility.[32]

### 3.1.3. Phytotoxicity/ecotoxicity effect of NPs

The inhibition effect of nanoparticles on performance of AD needs to be investigated. Moreover, the digested sludge after AD is usually dewatered and then applied as soil conditioner, compost and other applications. However, nanoparticles accumulated in sludge can make the sludge become toxic and inappropriate to apply as biosolid. Therefore, information about phytotoxicity and bacterial toxicity of digested sludge contaminated with nanoparticles is essential to have insights about the reusability of waste sludge. In addition, the effect of nanoparticles to the dewaterability of digested sludge is still unknown. So, whether or not nanoparticles in sludge can hinder the sludge dewatering process, toxicity of nanoparticles in sludge is eliminated during AD or it causes inhibition effect on bacteria and plants — these are still questions that need to be answered.[33]

In terms of ecotoxicity, there has been significantly greater focus on aquatic rather than terrestrial species, and very little work has focused on terrestrial plants. Some studies have reported the toxic effects of nanoparticles on the germination and/or root growth of some plant species.[34] A study focuses on comparing the effects of five types of commonly used nanoparticles (multi-walled carbon nanotubes (MWCNTs)), Ag, Cu, Si, and Zn oxide) with their corresponding bulk material counterparts on germination, root elongation, and biomass of the agricultural plant *Cucurbita pepo* (zucchini). In this preliminary nanotoxicology study, initial concentrations of 1000 mg/L were chosen to ensure observation of relevant phytotoxic responses. In addition, the effect of nanoparticle or bulk Ag concentration (0–1000 mg/L) on zucchini biomass, transpiration, and Ag content was determined in a dose-response study. Assessing the impacts of nanoparticles on agricultural plants will provide insight into the risk of ecological exposure to these materials, as well as to the potential for human exposure through food chain contamination.[35]

### 3.1.4. Engineered NPs and particle size

Due to the rapid expansion of nanotechnology, engineered nanoparticles (ENPs) have been manufactured and applied widely in many industries. This fact leads to the constant discharge of ENPs to the environment, and their possible impacts to human health and environment remain a controversial topic. There are a massive amount of natural NPs in the environment, far more than the relatively small releases of CNTs, Ag nanoparticles, etc. Since the generation of natural nanoparticles is uncontrollable, many of the studies on characteristics and impacts of nanoparticles have been focused on ENPs. In most of the studies, the term ‘engineered nanoparticles’ (ENPs) is referred shortly as nanoparticles (NPs). The effect of a mixture of ENPs consisting of silver oxide (AgO, 20 nm), titanium dioxide (TiO\(_2\), 30-40 nm) and zinc oxide (ZnO, 20 nm) compared with their bulk metal salts was evaluated against unspiked activated sludge (control) using three parallel pilot-scale treatment plants.[36] The introduction of both nanoparticles and bulk metal mixtures in the wastewater treatment plants induced a two-fold increase of the microbial specific oxygen uptake rate (SOUR) compared with the control plant. The scanning electron microscopy (SEM) showed that there was selective damage on some microbial cells. Further to this, activated sludge floc size was reduced in the presence of the ENPs, while the sludge volume index (SVI) was unaffected. The fate and behaviour of nanoparticles in the environment are affected by various environmental factors (e.g. light, pH, ionic strength, natural organic matter, etc.).[37] Various influences can affect the physical, chemical or bioavailable properties of released nanoparticles in the nature. In order to assess the risks of nanoparticles and nanomaterials, we must scrutinise the possible mobility, transformation, and interaction with other materials of nanoparticles.[5]

The particle size and shape of an NP is known to impact upon its behaviour/reactivity in aquatic and terrestrial media.[38] For instance, NPs of <30 nm was cytotoxic to *E. coli* and *S. aureus* [39]
compared with 80—90-nm particle size.[40] This suggests that silver oxide (AgO) of particle size greater than 30 nm could be non-inhibitory to microbial processes. Of particular interest is the size less than 5 nm in suspension capable of inhibiting nitrification in AS.[41] Apart from particles size, their shape has been reported to play a role as shown for AgO which can exist in a triangular, spherical or rod-shaped form. Comparing the effects of the three distinct shapes, the truncated triangular form of AgO was found to exert the strongest bactericidal effect on E. coli in both agar plate and broth cultures.[42] A direct extrapolation of this observation from pure culture to complex wastewater is unclear because wastewater components can attenuate or enhance NP contact and interaction with microbial cells.

### 3.2. Impact of NPs on algal biomass systems

Algal biomass has soon been started to be widely anticipated as the next energy storehouse for meeting the world’s energy needs. Algae are also important as a potential resource for bioenergy production as well as for the extraction of high value and platform chemicals and extractives. These are low trophic-level members of aquatic systems and are critical in photosynthesis and as food sources. The results on the effect of nanoparticles on microalgae and macroalgae are presented below.

#### 3.2.1. NPs’ impact on microalgae

Silver in natural fresh water can be found in the form of silver chloride (AgCl), silver sulphide (Ag₂S) and the silver ions. The most toxic form of silver nanoparticles is the silver ion.[43] Concentration of these nanoparticles is increasing in aquatic environment and can strongly affect and damage the biota.[21,44] For instance, Ag NP concentrations above 5 g/L have already been found for groundwater, surface water and drinking water.[45] There are many possible reasons for the high toxicity of silver nanoparticles, including its high surface area/volume ratio, which greatly increases its rate of dissolution.[44] Coating of Ag NP with organic materials such as polymer-based stabiliser may also influence its toxicity.[46] Another important factor that influences nanoparticles toxicity is the bioavailability related to the aggregation behaviour.[44] Becaro [47] investigated the toxic effects of silver nanoparticles stabilised with PVA (polyvinyl alcohol) for aquatic microalgae, such as Pseudokirchneriella subcapitata algae, Artemia salina and Daphnia similis. According to dynamic light scattering measurements, the Ag NPs in solution are well dispersed, with a size range of 2–18 nm. Among the organisms studied, Ag NP showed lower toxicity to A. salina and P. subcapitata organisms and showed higher toxicity to D. similis.

Pithophora oedogonia and Chara vulgaris are predominant members of photosynthetic eukaryotic algae, which form a major component of global aquatic ecosystem. Das et al. [48] reported that nanosilver has significant adverse effects on growth and morphology of these filamentous green algae in a dose-dependent manner. Exposure of algal thalli to increasing concentrations of silver nanoparticles resulted in progressive depletion in algal chlorophyll content, chromosome instability and mitotic disturbance, associated with morphological malformations in algal filaments. SEM micrographs revealed dramatic alterations in cell wall in nanoparticle-treated algae, characterised with cell wall rupture and degradation in Pithophora.

#### 3.2.2. NPs’ impact on macroalgae/aquatic plant

Nanoparticles have a significant effect on macroalgae, e.g. sea weed and water hyacinth. Zada et al. [49] demonstrate that fermentative production of ethanol and hydrogen from water hyacinth is a commercially viable and sustainable process. Iron nanoparticles significantly affect hydrogen and ethanol production. Iron nanoparticles enhance fermentative hydrogen production. Ethanol production is also enhanced by iron nanoparticles. For fermentative hydrogen production, optimum iron nanoparticles concentration is 250 mg/L, and for ethanol production, optimum iron nanoparticles concentration is 150 mg/L. These concentrations are in addition to that already present in the
dry biomass of the plant. Maximum hydrogen yield is 57 mL/g of the plant biomass, which is 85.50% of theoretical maximum hydrogen yield. The maximum ethanol yield is 0.0232 mL/g of the plant biomass, which is 90.98% of maximum theoretical yield. This study indicates that water hyacinth accumulates different types of nanoparticles.

3.3. Mechanisms by which bioenergy processes are enhanced or inhibited

With the rapid development of nanotechnology in the last decade, the safety of manufactured nanomaterials has been studied more rigorously by scientists. Owing to its large surface area per unit volume, nanoparticles are much more active than that particle at bulk or particulate size. NPs on sludge made the digested sludge become unsuitable to be used as biosolid, since the contaminated digested sludge highly inhibited the root growth and seed germination of plants. They made digested sludge become difficult to dewater. For any types of enhanced or inhibited nature caused by nanoparticles with AS and algae biomass, there is a consistent mechanism behind it.

3.3.1. Mechanism of microbial activity

Nanoparticles possess the properties which are toxic to living organisms and even humans. Because of its nanoscale, nanoparticles are easily exposed to humans and other organisms through inhalation, ingestion and dermal contact. A number of authors have published literature on characterisation, behaviour, and toxicological information of nanomaterials.[50] Most of the research findings are focused on commercialised nanomaterials that are manufactured and applied widely, such as CNTs, fullerene and metal oxides. This is important when considering the application of these NPs to large-scale commercial plants; also, it is important in terms of fate of any NPs in the environment.

Ag NPs are able to physically interact with the cell surface of various bacteria. This is particularly important in the case of Gram-negative bacteria where numerous studies have observed the adhesion and accumulation of Ag NPs to the bacterial surface. Many studies have reported that Ag NPs can damage cell membranes, leading to structural changes, which render bacteria more permeable.[51] This effect is highly influenced by the nanoparticles’ size, shape and concentration,[52] and a study using E. coli [51] confirmed that Ag NPs accumulation on the membrane cell creates gaps in the integrity of the bilayer which predisposes it to a permeability increase and finally bacterial cell death.[53]

Metal oxide nanoparticles, such as titanium dioxide (TiO2), aluminium oxide (Al2O3), silicon dioxide (SiO2) and zinc oxide (ZnO), have received increasing interests due to their widespread industrial, medical and military applications and their intentionally or unintentionally release into the environment affecting human health, soil and aquatic organisms. Although the exact mechanism of toxicity for each nanoparticle is not fully understood, there are various characteristics that may result in damage to exposed organisms. Nanoparticles generate reactive oxygen species (ROS) such as free radicals (OH\(^-\)), singlet oxygen (\(^1\)O\(_2\)) and super oxides (O\(_2\)^\(-\)) which exert several adverse effects on microorganisms, including disruption of cell wall and damage of DNA/RNA.[38] Adverse effects included membrane leakage of sugars and proteins, enzyme inhibition, cell disruption, and scattered vesicles which slowly dissolve, thus inhibiting cellular respiration and cell growth.[25] Nano-Al\(_2\)O\(_3\), nano-SiO\(_2\) and nano-ZnO were observed to be harmful to Bacillus subtilis, E. coli and Pseudomonas fluorescens.[54] The antibacterial effects of nanoparticles on B. subtilis and E. coli increased from SiO\(_2\) to TiO\(_2\) to ZnO. Nano-ZnO was observed to cause significant toxicity to the viability of Gram-negative bacterial cells.[54]

Chen et al. [55] reviewed the toxic effect of nanomaterials on biomass and found that Ag NPs, nano-Al\(_2\)O\(_3\), nano-SiO\(_2\) and nano-TiO\(_2\) are chemically stable NPs that have no adverse effects on microbes under anaerobic conditions, while nano-Au presented no or low toxicity to anaerobic biomass, and nano-CeO\(_2\) was the most toxic to both mesophilic and thermophilic biomass. The release of metal ions caused by corrosion and dissolution of the NPs resulted toxicity in the AD process.
These toxic compounds principally obstruct the activities such as methane formation, a decrease in the methane content of biogas, or can even cause complete failure of methanogenesis.

### 3.3.2. Mechanism of inhibition of seed/plant growth

The sludge dewaterability depends on various factors. Extracellular polymeric substance (EPS), secreted by microorganisms, is a major component of sludge flocs, and is an important factor that influences the dewaterability of sludge. A high amount of EPS will increase the viscosity of the waste sludge and therefore make it difficult to dewater. Finally, the accumulation of NPs (e.g. CeO₂, ZnO) on sludge made the digested sludge become unsuitable to be used as a biosolid, since the contaminated digested sludge highly inhibited the root growth and seed germination of plants.

### 3.3.3. Effect and impact of nanomaterials on biomass

Theivasanthi and Alagar [56] found that nanoparticles synthesised using an electrolysis method show antibacterial activities against both Gram (−) and Gram (+) bacteria. Changes in surface area to volume ratio of copper enhance its antibacterial activities. Copper nanoparticles synthesised using an electrolysis method show more antibacterial activities (for *E. coli* bacteria) than copper nanoparticles synthesised using a chemical reduction method. Using electrical power while synthesising copper nanoparticles increases its antibacterial activities. The chemicals involved in the synthesis of nanoparticles are commonly available, cheap and non-toxic. The technology can be implemented with minimum infrastructure. The experiments suggest the possibility to use this material in water purification, air filtration, air quality management, antibacterial packaging, etc. Microorganisms play the key role for biochemical conversion of biomass. Therefore, the inhibition of their activity reduces the energy yield capacity of biomass. The various effects of different nanomaterials are shown in Table 1.

Ganzoury and Allam [57] reviewed the impact of three types of nano-additives on the biogas production. The categories are: (1) metal oxides, (2) zero-valent metals, and (3) nano-ash and carbon-based materials. The reviewed results are summarised in Table 2.

| Nanomaterials | Effects | Remarks | References |
|---------------|---------|---------|------------|
| CeO₂         | Inhibit biogas and CH₄ in AD | High concentration of 1000 mg/L | [19] |
|              | Increase biogas volume | Low concentration of 10 mg/L | |
|              | Digested sludge inhibits root growth and germination | | |
| ZnO          | Inhibit biogas and CH₄ in AD | High concentration of 1000 mg/L | [19] |
|              | Overcome inhibition effect | Tolerable exposure concentration | |
|              | Digested sludge inhibits root growth and germination | | |
| CuO          | Reduction of 30% of the biogas production from the total biogas produced in the control at day 14 | Low concentration 15 mg/L | [26] |
|              | Biogas production less inhibited | | |
|              | Biogas reduction by 19% and 60% | | |
| Ag⁰ at 5 nm  | Complete inhibition of growth and viability | Microparticles of CuO | |
| Ag⁰—TiO₂ at 100 nm | Complete photoactivated inhibition of growth and viability | At *E. coli* bacteria | [59] |
| 0.5 mg L⁻¹ Ag⁰ at 9—12 nm 10 and 50 μg L⁻¹ Ag⁰ | Toxic to the respiration of bacteria | Nitrifying bacteria | [41] |
|              | Inhibiting the growth of *E. coli* by 70% and 100%, respectively | *E. coli* | [60] |
| Fe₃O₄ nanoparticles | Enhanced AD, and higher CH₄ and organic matter processing | Drug delivery systems | [20] |
4. Conclusion

The performance of AD can be affected by various nanomaterials. It is very important to better understand the complex mechanisms by which these particles interact with the biomass and the process of conversion and potentially overcome adverse effects and optimise the positive effects. Particle size can influence the rate of AD as it affects the surface area for biodegradation of biomass. All nanoparticles, regardless of their chemical constituents, have surface area to volume ratios that are extremely high. This causes nanoparticles’ physical properties to be dominated by the effect of the surface atoms and capping agents on the nanoparticles surface. A high surface area to volume ratio is important for applications such as catalysis. Reactions take place at the surface of a chemical or a material; the greater the surface for the same volume, the greater is the reactivity. Therefore, the response and interaction of different nanoparticles vary with microorganisms. Although only a few studies have reported the antibacterial properties of copper nanoparticles which have a significant potential as bactericidal agent, however, other nanoparticles, such as platinum, gold, iron oxide, silica and its oxides, have not shown bactericidal effects in studies with *E. coli*. The addition of magnetite NPs (Fe₃O₄ NPs) can enhance the methane production due to the presence of the non-toxic Fe³⁺ and Fe³⁺ ions through stimulation of bacterial growth.

Nanoparticles have been popular in recent years and they have been applied widely in many fields. These nanoparticles have been used as fuel catalyst to reduce harmful emission from engine combustion. But researchers found that NPs cause inhibition effects on biodegradation, nitrification and AD process [33,61]. The adverse effect, inhibition or enhancement of energy conversion, depends upon the particle size, concentration and time. There is a potential scope to find out the effect of nanomaterials with other biomasses, e.g. agricultural, municipal solid waste (MSW). To identify the best possible use of nanoparticles in bioenergy systems is very important. The present review could be an important tool for a further research on ‘nanotechnology in bioenergy’.

Disclosure statement

No potential conflict of interest was reported by the authors.

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