

Bisphenol A on Anaerobic Digestion of Sewage Sludge

Subjects: Water Resources

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Anaerobic digestion for stabilizing sewage sludge in WWTPs, which produces biogas and stabilized biosolids, is a mature technology used worldwide. Bisphenol A (BPA) is an alkylphenol composed of acetone and phenol. It is a plastic additive that is most commonly used to produce different industrial and personal care products, e.g., polycarbonate, polysulphone, epoxy, polyacrylate, polyetherimide resins, thermal paper, beverage containers, dental sealant, and so on.

Keywords: sewage sludge ; anaerobic digestion ; endocrine disrupting compounds

1. Introduction

The increasing concern for the environment and the development of analytical techniques have led to investigations into the occurrence of pollutants in the environment, such as endocrine-disrupting compounds (EDCs), that are associated with some disorders of the reproductive system of exposed organisms. EDCs include pharmaceuticals, hormones, bisphenol A (BPA), microplastics (MPs), engineered nanoparticles (NPs), etc. Due to the common and increasing use of these compounds in industry and personal care products, and the mechanisms by which they are transported in the human body or in the environment, they enter wastewater treatment plants (WWTPs), leading to a potential environmental hazard ^[1]. Pharmaceuticals in particular are the most abundant emerging pollutants in wastewater because they are intensively used in human and veterinary medicine, as well as to promote the growth of farmed fish and livestock, and they are easily metabolized into polar and soluble forms in living organisms ^[2]. Depending on their hydrophobicity, these pollutants demonstrate medium to strong sorption onto solids. For example, about 40% of BPA and more than 50% of pharmaceuticals can adsorb to the solid fraction of sewage and are thus present in waste-activated sludge (WAS) ^{[3][4][5]}.

The growing energy demand has resulted in an urgent search for alternative and clean energy sources. A promising technology for CH₄ recovery from organic sources is anaerobic digestion. This technology is used worldwide for the utilization of sewage sludge, which contributes to global carbon neutrality objectives via the recovery of two valuable resources, biogas and stabilized biosolids. Accordingly, anaerobic digestion has received great attention in the quest to achieve a circular economy. In addition to the production of an energy source ^[6], the widely recognized advantages of this process include low sludge generation, low investment and operating costs, low energy consumption, and simple design and operation. However, the literature indicates that the effectiveness of microbiological conversions during anaerobic digestion can be disrupted by the presence of emerging pollutants or their by-products ^[7]. Another important aspect is that micropollutants and the products of their metabolism can be retained in the digestate, which affects the safe disposal of WAS.

AD comprises various steps, namely hydrolysis, acetogenesis, acidogenesis, and methanogenesis. In the hydrolysis phase, complex organic substances that cannot be directly utilized by bacteria are broken down into soluble monomers by extracellular hydrolytic enzymes. Hydrolysis is carried out by bacteria from the group of relative anaerobes belonging, e.g., to the genera *Streptococcus* and *Enterobacterium* ^[8]. During acidogenesis, acid-producing fermenting bacteria convert soluble monomers into end products and volatile fatty acids (VFAs) that can be recovered as a bioproduct. These are facultative anaerobes that utilize the oxygen accidentally introduced into the process and thus create favorable conditions for the development of obligate anaerobes such as *Pseudomonas* sp., *Bacillus* sp., or *Clostridium* sp. In the acetogenesis phase, bacterial activity leads to the formation of acetic acid and hydrogen. In the final methanogenic phase, the acidified products are converted into methane by various mesophilic bacterial species ^[9]. Anaerobic methanogens are very sensitive to changes in the operating conditions in the reactor ^[10], and their metabolism is particularly susceptible to disturbances caused by the presence of micropollutants. In general, the fermentation of the sludges reduces the concentration of emerging pollutants in the digestate. However, many studies have reported that BPA did not biodegrade during anaerobic mesophilic or thermophilic digestion ^{[11][12][13]}. Regarding pharmaceuticals, under anaerobic conditions, they are degraded from a medium to high extent ^[14]. On the other hand, MP degradation in anaerobic conditions is mainly related to the type of polymer.

Because CH₄ production from sewage sludge belongs to the most vulnerable treatment processes, and since the anaerobic microorganisms are sensitive when exposed to certain conditions, such as the presence of toxic pollutants [15], special attention should be paid to the effect of emerging pollutants on the performance of anaerobic digestion. Even though emerging pollutants are frequently detected in WAS and anaerobic treatment predominates in WWTPs, the interference of emerging pollutants with anaerobic digestion is overlooked. The fact that the influence of emerging micropollutants on the anaerobic digestion of sewage sludge in WWTPs has not been systematically reported may result from the fact that simple and rapid analytical methods for the accurate quantification of these pollutants in complex matrices such as sewage sludges were not yet developed [16]. In addition, the lack of systematic reports on the effect of emerging pollutants on anaerobic digestion results from the huge diversity of these compounds, whose structure and physicochemical properties strongly affect their behavior under environmental conditions [17]. Furthermore, their effect on the anaerobic process is often dose-dependent [18][19]. In addition, some compounds, like NPs, affect anaerobic digestion positively or negatively, depending on their composition [20][21].

2. The Effect of BPA on Anaerobic Digestion

2.1. The Presence of BPA in the Environment and Its Degradation

BPA is an alkylphenol composed of acetone and phenol. It is a plastic additive that is most commonly used to produce different industrial and personal care products, e.g., polycarbonate, polysulphone, epoxy, polyacrylate, polyetherimide resins, thermal paper, beverage containers, dental sealant, and so on [1]. This persistent organic pollutant is considered an environmental endocrine disruptor. It is one of the most common man-made pollutants with an adverse effect on humans and the environment, and it has been defined as an exogenous chemical by the US Environmental Protection Agency (EPA), listed on the Candidate List of Substances of Very High Concern (SVHC), and recommended for inclusion in the REACH authorization list by the European Chemicals Agency (ECHA) [22]. BPA has been detected in surface waters (up to 56 µg/L), sediments (up to 20 mg/kg dry weight (d.w.)), tap water (14 ng/L), effluents from WWTPs (370 µg/L), sewage sludge and biosolids (from 10 to >100,000 µg/kg d.w.), and waste landfill leachates (17 mg/L) [23][24]. It has been reported to be one of the five most commonly occurring organic micropollutants in wastewater in the United Kingdom [25]. As wastewater is considered the main source of BPA in the environment, different methods for its removal from wastewater have been reported: adsorption [1], ozonation [26], Fenton and photo-Fenton oxidation [27][28], electrolysis [29], biodegradation [3][30][31], membrane techniques [32], and membrane bioreactors [33].

Microbial degradation is the most efficient process for reducing the persistence of BPA [31][34]. However, due to its hydrophobicity, about 40% of BPA can adsorb to primary sludge and adapted and non-adapted activated sludge [3][4]. A concentration of BPA in sludge of 25.6 mg/kg d.w. has been reported [13]. If sludge containing BPA is used as fertilizer, soil pollution, and health problems may occur, as BPA can be absorbed by plants [35] and then enter the bodies of animals and humans as food. Therefore, the treatment of sludge before its application to the soil is a widespread practice. However, many studies have reported that BPA did not biodegrade during anaerobic mesophilic or thermophilic digestion [11][12][13]. Moreover, in some studies, BPA concentration increased in the mesophilic digesters because of the degradation of polycarbonate polymers, which released the BPA monomer [36]. After anaerobic digestion, sewage sludge may contain up to 36.7 mg BPA/kg d.w. [37]. As can be seen, even after anaerobic digestion, BPA will remain in the final sludge applied to agricultural fields. As an endocrine-disrupting compound, which can profoundly affect organisms at low concentrations, the tolerable daily intake of BPA was identified as 50 µg/(kg body weight-day) by the EPA and was lowered to 4 µg/(kg body weight-day) by the European Food Safety Authority (EFSA) [38].

2.2. The Effect of BPA on Methane Production from Sewage Sludge

Because sewage sludge valorization by biogas production is widely applied in the management of WWTPs, some studies have focused on the effect of BPA on biogas production. Digester sewage sludges and co-digester sewage sludges (with silage, farm manure, livestock and farming waste, food waste, and the organic fraction of municipal solid waste) have been investigated under mesophilic methanogenic conditions [16]. These sludges contained endogenous BPA at levels of up to 10,973 µg/kg d.w. (digested sludges) and up to 9069 µg/kg d.w. (co-digested sludges). The addition of BPA (228 µg/kg) did not affect biogas production and the efficiency of BPA removal reached 50%. It was concluded that BPA removal was triggered by the high endogenous BPA concentrations, which allowed for the acclimation of the microbial community, thus improving BPA degradation. This explanation seems to be correct because, in other studies, even a small amount of BPA released from polyvinyl chloride (PVC) MPs in sewage sludge (1.8–3.6 µg/L) decreased CH₄ production by approximately 24% [39]. BPA leaching caused the solubilization of extracellular polymeric substances (EPS) and the rupture of microbial cells, which released lipids and nucleic acids, thus increasing concentrations of soluble COD.

Simultaneously, the abundance of some hydrolytic bacteria decreased, and acidogenesis was negatively affected, which reduced the production of VFAs, decreasing the abundance of fermentative bacteria.

In anaerobic granular sludge spiked with 40 mg BPA/L, there were numerous indicators of the toxic effects of BPA [40]. Firstly, COD removal was substantially reduced to less than 65%, which was 30% lower than COD removal in the control variant (without BPA spiking), due to the inhibition of the hydrolysis–acidification process. Secondly, sludge stabilization was adversely affected. In the structure of EPS, the tryptophan-like proteins that protect microbes against toxicity disappeared when BPA was added, whereas they were present in the absence of BPA. BPA also affected the activity of protease, acetate kinase, and coenzyme F₄₂₀. The concentration of coenzyme F₄₂₀, which is unique to methanogens and related to CH₄ production, decreased from 0.0045 to 0.0017 μmol/L in the presence of BPA. BPA also improved the protease activity of anaerobic granular sludge, thus increasing protein hydrolysis. An increase in the activity of acetate kinase, which controls the transformation of acetyl-CoA into acetic acid, indicated that BPA stimulated the production of large concentrations of acetic acid, which inhibited methanogens' growth. This conclusion was supported by the increase in the relative abundance of Bacteroidetes (from about 13% to over 22%), which play a significant role in the acetogenic phase of anaerobic digestion. In addition, BPA was strongly toxic to *Hydrogenophaga* sp., which can utilize various organic compounds; its abundance decreased sharply (from 15.58% to 0.12%) after BPA spiking. The inhibition of sludge hydrolysis by the exposure of waste sludge to BPA has been attributed to the denaturation of α-amylase [41], which is an enzyme widely distributed in waste sludge that plays a significant role in hydrolysis. BPA changed the secondary structure of α-amylase after interacting with the enzyme via hydrophobic and hydrogen bonding.

The effect of BPA on methane fermentation was related to the production of reactive oxygen species (ROS). BPA leached from polycarbonate MPs (1.26 ± 0.18 mg/L) decreased the production of ROS, thereby increasing enzyme activity, biomass viability, and the abundance of *Methanosarcina* sp. and *Methanobacterium* sp., and thus improving CH₄ production by up to 24.7% [42]. On the other hand, when a larger amount of BPA leached (4.02 ± 0.15 mg/L), the production of ROS was stimulated, resulting in decreased biomass viability and even apoptosis, thus decreasing CH₄ production by 8.1%.

The effect of BPA on CH₄ production has been investigated in bioelectrochemical systems, which include microbial electrolysis cells and microbial fuel cells; these systems can both generate CH₄ and increase the anaerobic degradation of resistant compounds. In a bioanode single-chamber microbial electrolysis cell, organic compounds are degraded to electrons, CO₂, and H⁺ by exoelectrogenic microorganisms on the anode. The electrons are transferred to the anode by exoelectrogens. Then, electrons migrate to the cathode, where they are combined with protons to form H₂. Additionally, CO₂ and H₂ combine to form CH₄ and water. Under optimum conditions (applied voltage 0.8 V, BPA concentration 10 mg/L, hydraulic retention time (HRT) 24 h, C/N ratio 50), 95.4% BPA removal, 94.9% COD removal, a rate of CH₄ production of about 120 mL/(L·day), and a CH₄ content of over 90% were obtained [29]. These values were higher than those of the control system (a system containing electrodes without any energy input), which were 54.2%, 61.3%, 83.1 ± 2.2 mL/(L·day), and 71.1%, respectively. The removal of COD and BPA, and the CH₄ production rate and content, were increased as the C/N ratio was increased from 20 to 50. Increasing the BPA concentration from 10 to 80 mg/L decreased the CH₄ production rate from about 118 to about 97 mL/(L·day) and the CH₄ content from 93.1 to 79.8%. These decreases were explained by the fact that a substantial fraction of the electrons was consumed to reduce BPA, decreasing the amount of H⁺ evolved into H₂, which would be consumed by hydrogenotrophs.

2.3. The Effect of BPA on VFA Production from Sewage Sludge

Anaerobic digestion cannot only degrade organic substances and transform them into CH₄ but can also transform them into VFAs. VFAs can be used as raw materials for generating higher-value compounds, including polyhydroxyalkanoates (PHAs), CH₄, or alcohols [43][44][45], and as carbon sources for nutrient removal in WWTPs [46][47]. A BPA concentration of 0–200 mg/kg d.w. increased VFA accumulation; the largest increase in VFA production (2095 mg COD/L) was observed at 50 mg BPA/kg d.w. and was 1.3 times higher than the production in the control [4]. This effect was mainly due to acetic acid production, which accounted for 70% of the VFAs (3.6 times more than in the control). Large amounts of acetic acid were produced as a result of 20 and 40% increases in the activities of acetate kinase and phosphotransacetylase in the presence of BPA. The VFA content increase was attributed to the increase in protein content in the total EPS caused by BPA. Although BPA did not affect the solubilization of WAS, it increased sludge hydrolysis 1.1-fold by increasing the activities of protease and α-glucosidase. The improved bioconversion of proteins, carbohydrates, lipids, and other compounds in the presence of BPA was indicated by significantly higher relative abundances of genes coding enzymes involved in microbial metabolism. Due to the higher protein content in EPS, anaerobe cells were better protected, and more substrate was available for VFA production. The increase in acetic acid concentrations was not attributed to its production from CO₂ and H₂ by homoacetogenic bacteria because BPA did not affect the consumption of H₂. The increase

in VFA production could have resulted from the changes in the abundance of microorganisms. For example, with BPA, the abundance of Proteobacteria and Firmicutes, which can utilize VFAs, decreased from 37.2 to 30.2% and from 14.0 to 11.7%, respectively. Conversely, the abundance of *Actinobacteria* sp. and *Clostridium* sp., which can produce VFAs, increased from 6.2 to 27.0%, and from 1.4 to 1.7%, respectively. When BPA was present, the relative abundance of genes encoding enzymes involved in VFA production increased. For example, the abundance of *gltB* (involved in glutamate synthesis) increased 1.7 times; that of *asdA* (involved in transferring nitrogenous groups to L-alanine) increased 1.2 times; *aspC* and *yhdR* (encoding aspartate aminotransferase and participating in oxaloacetate synthesis) increased 1.5 and 1.8 times, respectively; AGXT (involved in pyruvate synthesis) increased 1.6 times; *dsdA* (involved in hydrolysis of D-serine to pyruvate) increased 1.2 times; *fabG* (encoding of acyl reductase and important in fatty acid synthesis) increased 1.3 times; *fas* (encoding fatty acid synthetase) increased over 4 times; and *desA1* (related to acyl reductase, which participates in the VFA formation) increased 6 times. In addition, the quorum sensing system was improved after BPA addition. The presence of BPA increased the relative abundance of *rpfC*, *crp*, and *rpfG* genes, which are responsible for the synthesis of EPS and the formation of biofilm, thus protecting cells from toxic environmental conditions.

2.4. The Effect of BPA on Hydrogen Production from Sewage Sludge

The production of H₂ from biomass and organic waste is considered a potential alternative energy source and H₂ is regarded as a clean and CO₂-free fuel with a high energy density. Digestion for H₂ production offers numerous advantages, such as low sludge production and energy requirements [48]. H₂ is considered a promising energy source; therefore, the effective transformation of biomass and organic waste into H₂ is urgently sought. However, the major disadvantage of anaerobic digestion to produce H₂ is its high sensitivity to toxins [15]. The inhibitory effect of BPA on H₂ production from the organic fraction of municipal solid waste was studied with different BPA concentrations from 0.5 to 25 mg/L [49]. The reduction in H₂ yield by 9.2–75.3% in the presence of BPA was due to the shift in the metabolic pathway from butyrate to propionate production. In the control, the cumulative H₂ production was 227.9 ± 10.5 mL which decreased to 58.9 ± 10.4 mL in the experimental batches supplemented with BPA. The decline in the H₂ yield was 37.1%. Also, the inhibitors decreased the efficiency of COD removal.

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