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Effect of Varying Zinc Concentrations on the Biomethane Potential of Sewage Sludge

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Abstract: The anaerobic digestion of sewage sludge is highly sensitive to high zinc concentrations. Although sulfate-reducing bacteria (SRB) activity can negatively affect methanogenesis, SRB-mediated metal sulfide precipitation can alleviate zinc toxicity. A series of mesophilic anaerobic batch experiments was performed for the biomethane potential of three different sewage sludge samples for 74 days using the background sludge zinc content, alone or in combination with the external addition of 200, 300 and 400 mg Zn/L. The highest biomethane production was 165 ± 1 mL CH_4/g VS using activated sludge (AS) with a background concentration of 93 mg Zn/L. A slight decrease in the biomethane yield (i.e., 157 ± 1, 158 ± 1 and 159 ± 1 mL CH_4/g VS) was obtained in the presence of 293, 393 and 493 mg Zn/L, respectively. The potential reason for the high methanogenic activity at high inlet Zn concentrations could be that the AS used in this study was already acclimated to those conditions. Zinc was likely removed from the system by sulfide precipitation, and a removal efficiency above 99% was achieved under all zinc concentrations. A sulfate reduction efficiency of 99% was also obtained. Overall, this study details the potential utilization of biogenic sulfide as a metal detoxifying agent without detrimental effects on methane production from sewage sludge.

Keywords: anaerobic digestion; sewage sludge; biomethane production; zinc; sulfate reduction

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Citation: Kumar, M.; Matassa, S.; Bianco, F.; Oliva, A.; Papirio, S.; Pirozzi, F.; De Paola, F.; Esposito, G. Effect of Varying Zinc Concentrations on the Biomethane Potential of Sewage Sludge. *Water* **2023**, *15*, 729. https://doi.org/10.3390/w15040729

Academic Editor: Christos S. Akratos

Received: 22 December 2022
Revised: 4 February 2023
Accepted: 10 February 2023
Published: 12 February 2023

1. Introduction

Anaerobic digestion (AD) is a complex biological process where excess sewage sludge or other waste organic matter is degraded and converted into biomethane using the combined action of different microbial groups, including hydrolytic and acid-forming bacteria as well as methane-forming archaea, also known as methanogens [1–3]. Over the last decades, the AD process has shown great potential for renewable energy production from a broad range of organic wastes, and has become of technical and economic importance for wastewater treatment plants (WWTPs) [4]. As a matter of fact, the AD of the excess sewage sludge produced in WWTPs provides advantages such as the production of biomethane, the reduction in volume and pathogen load, as well as mitigation of odor and greenhouse gas emissions.

The composition of sewage sludge plays a crucial role in biomethane production [5]. Sewage sludge comprises organic carbon compounds as well as toxic organic and inorganic compounds such as heavy metals, pesticides, sulfonates, pathogens, silicates, aluminates, calcium, and magnesium [6]. The content of heavy metals is significant, both in terms of the final quality of the produced excess sewage sludge as well as the overall performances of WWTPs [7]. Indeed, metal toxicity is one of the core factors causing the partial or complete failure of biological processes during wastewater treatment [8,9].

During the AD process, heavy metal concentrations in sewage sludge can severely hamper the biological transformation and, thus, the recovery of renewable energy. Heavy metals, such as zinc (Zn), iron (Fe), copper (Cu), cadmium (Cd), lead (Pb), cobalt (Co), and nickel (Ni) can be present in substantial concentrations in municipal wastewater and sewage sludge [10,11], mainly if a discharge from nearby industrial activities occurs [1]. Indeed, the primary source of heavy metals in urban wastewater is industry, contributing to 50% of the total metal content of sewage sludge. The leaching from plumbing material, together with the use of detergent and washing powders containing Cu, Cd, and Zn, and body care products containing Zn are, instead, some of the primary domestic sources of heavy metal pollution [1].

Although trace concentrations of heavy metals can benefit the overall AD process by activating different enzymes [10,11], their excessive concentration can cause severe inhibition [1,12]. Indeed, a high concentration of heavy metal ions such as Cu, Pb and Zn can lead to enzyme inactivation and failure of AD [13]. The inhibition of AD is generally recognized by decreased biomethane production and accumulation of volatile fatty acids (VFA) [1]. In addition to their concentration, the severity of heavy metal inhibition depends on multiple factors, such as ionic form, metal speciation and the amount and distribution of methanogenic biomass in the system [14]. Previous studies showed how the methanogenic activity of anaerobic starch-degrading granules in the presence of five heavy metals (Cd, Cu, Cr, Ni, Zn) was heavily inhibited in the order of Zn > Ni > Cu > Cr > Cd [13]. Slightly different results were reported by Codina et al. [15], when analyzing the specific toxicity of six different heavy metals (i.e., Zn, Cr, Cu, Cd, Ni, Pb) to AD in activity assays. Therefore, both of these studies reported Zn toxicity for methanogens, highlighting the need for further research to shed light on the Zn effect on biomethane production.

Among the various possible solutions, metal sulfide precipitation by sulfate-reducing bacteria (SRB) is suitable to decrease or completely avoid metal toxicity to methanogens [13]. SRB utilize sulfate as an electron acceptor during the anaerobic oxidation of organic matter, and the produced hydrogen sulfide (H_2S) combines with metals to form highly insoluble metal sulfide precipitates [16]. Although the presence of sulfate can thus offer advantages during AD, including the use of sulfate as a growth element for methanogens and the decrease in redox potential, high sulfate concentrations can be disadvantageous for AD [13]. SRB can outcompete methanogens by depleting substrates such as acetate, ethanol and hydrogen in high-sulfate-rich media, resulting in an overall decreased biomethane production.

In the present study, the biomethane potential of three sewage sludge samples, i.e., aerobically stabilized, activated, and dehydrated sludge, originating from a full-scale WWTP, and characterized by a high influent zinc load, was investigated employing biochemical methane potential (BMP) tests. Three different sludge samples were investigated to evaluate the most suitable one for the subsequent tests on the impact of the different Zn concentrations. The background concentration of Zn in the sole activated sludge (AS) was then increased up to five times in order to study the effect of the increasing presence of Zn on the overall AD process. The role of SRB in Zn removal through zinc sulfide precipitation was also taken into account.

2. Materials and Methods

2.1. Sewage Sludge

Three sewage sludge samples were collected from: (1) the aeration basin of the secondary treatment (i.e., AS), (2) the aerobic stabilization basin (i.e., oxidized sludge) in the sludge treatment line, and (3) the dehydration step of AS (i.e., dehydrated sludge) of a WWTP located in Termoli (Molise region, Italy). The plant receives industrial wastewater and processes 12,500 m^3/d of wastewater per day. The background zinc concentrations found in the activated, oxidized, and dehydrated sludge of the WWTP were 92.98, 18.76, and 0.05 mg/L, respectively.

Once in the laboratory, the sludge samples were immediately analyzed in terms of total solids (TS) and volatile solids (VS) and were then stored at 4 °C in a refrigerator until further use. Table 1 presents the TS, VS, and Zn content of the three sludge samples.

Table 1. Total solids (TS), volatile solids (VS), and Zn content of the three sewage sludge samples considered in this study.

Sample	TS (g/L; %)	VS (g/L; %)	Zn (mg/L)	Zn (mg/g VS)
AS	14.98	6.41	92.98	14.22
Oxidized sludge	6.53	2.04	18.76	9.33
Dehydrated sludge	19.17%	7.95%	0.05	0.59

Note: AS—activated sludge.

2.2. Biochemical Methane Potential Tests

The BMP of the activated, oxidized, and dehydrated sludge was evaluated in batch assays. The batch experiments were performed within serum bottles placed in a water bath to maintain mesophilic conditions of 37 ± 1 °C.

A volume of 150 mL of activated and oxidized sludge was poured into 250 mL serum bottles, while 10 g of dehydrated sludge was dosed into 100 mL serum bottles. In order to exclude the influence of the low water content on biomethane production, an additional test was prepared by diluting dehydrated sludge with tap water, achieving a TS content of about 9.5%. In this case, an amount of 20 g of sludge was dosed into 100 mL serum bottles. Such TS content was employed to maintain wet conditions, as well as to avoid an excessive Zn dilution due to high water addition.

The effect of increasing Zn concentrations was evaluated exclusively on AS due to the highest background Zn concentration. The background Zn concentration was increased by 3, 4, and 5 times by adding 200, 300, and 400 mg Zn/L, respectively, dosed from a stock solution of $ZnCl_2$ (i.e., 50 g Zn/L). After dosing Zn, all serum bottles were purged with nitrogen gas for 2 min and sealed with a rubber septum and aluminum crimp to ensure anaerobic conditions. All BMP tests were conducted in triplicate without using any external anaerobic inoculum to evaluate the AD potential of the endogenous microorganisms already acclimated to high Zn concentrations. The initial pH in each bottle was adjusted to 6.8 using a 2N NaOH solution. All reagents used in this study were of analytical grade.

2.3. Analytical Methods and Sampling

To investigate the long-term effect of Zn on the AD process, methane production was monitored over a period of 74 days. Biomethane was quantified volumetrically by a liquid-displacement system consisting of a 12% NaOH trap and a vessel containing deionized water to be displaced [17]. Chemical oxygen demand (COD) in the samples was determined using standard methods [18]. Single replicates from each experimental condition involving the AD of AS were alternatively sampled on days 1, 25, 39 and 60 for the measurement of soluble chemical oxygen demand (sCOD) and immediately frozen at −20 °C. Prior to the sCOD analysis, the samples were centrifuged with a Multispin 12 mini centrifuge (Argo Lab, Carpi, Italy) at 10,000 rpm for 5 min, and the supernatant was filtered with 0.20 µm polypropylene membranes (VWR, Milan, Italy). TS and VS were determined gravimetrically after drying samples at 105 °C for 24 h (TS) and after volatilizing organic matter at 550 °C for 2 h (VS). The background Zn concentration in sewage sludge samples was determined using atomic absorption spectroscopy (Varian, AA240, Houten, The Netherlands) after digesting the samples with a MARS 5 pKo Temp microwave digestion system (CEM, Nonantola, Italy) and filtering the samples through 0.20 µm polypropylene membranes (VWR, Milan, Italy). Sulfate concentration was determined through ionic chromatography using an 883 Basic IC Plus (Metrohm, Herisau, Switzerland) as reported by Bianco et al. [19].

2.4. Statistical Analysis

All BMP tests were performed in triplicate, and the experimental data were expressed as the mean ± standard error. The statistical significance of the differences between the measured biomethane productions was assessed through a one-way analysis of variance (ANOVA). A value of $p \leq 0.05$ was considered to be statistically significant. The Microsoft

Excel (version 1908) (Office 365, Microsoft Corporation, Redmond, WA, USA) statistical package was used to perform the statistical analysis.

2.5. Calculations

The sCOD removal rate (Equation (1)) was calculated as follows:

$$\text{sCOD removal rate}\left(\frac{\text{mg sCOD}}{\text{L}\cdot\text{d}}\right) = \frac{\Delta\text{sCOD}}{\text{number of days}} \quad (1)$$

where ΔsCOD (mg/L) is the difference in sCOD concentration across each time period considered.

3. Results and Discussion

3.1. Biomethane Yield with Background Zinc Concentrations

The cumulative biomethane profiles obtained through the BMP tests carried out using activated, oxidized and dehydrated sewage sludge are reported in Figure 1. The highest BMP value of 165 ± 1 mL CH_4/g VS was observed in the AS, followed by 135 ± 2 mL and 125 ± 2 mL CH_4/g VS in the dehydrated sludge with 20% and 9.5% TS. In the case of oxidized sludge, most of the biomethane was produced in the first three days, and no significant ($p > 0.05$) biomethane production was observed afterwards, reaching a final BMP value of 86 ± 1 mL CH_4/g VS (Figure 2). The lower BMP obtained with the oxidized sludge can be reasonably explained by the fact that this sludge type had undergone aerobic stabilization, and thus the amount of residual organic matter to be converted into biomethane was lower [20]. Despite the high concentration of Zn in the AS, the high BMP value indicates that the background Zn levels of the AS did not likely affect methanogenesis (Table 1). The reason for the high BMP value in the case of the AS, despite its higher background levels of zinc, can be explained by the fact that the AS was already acclimated to a high influent Zn concentration, and by the possible activation of the different enzymes during the AD process through Zn [21].

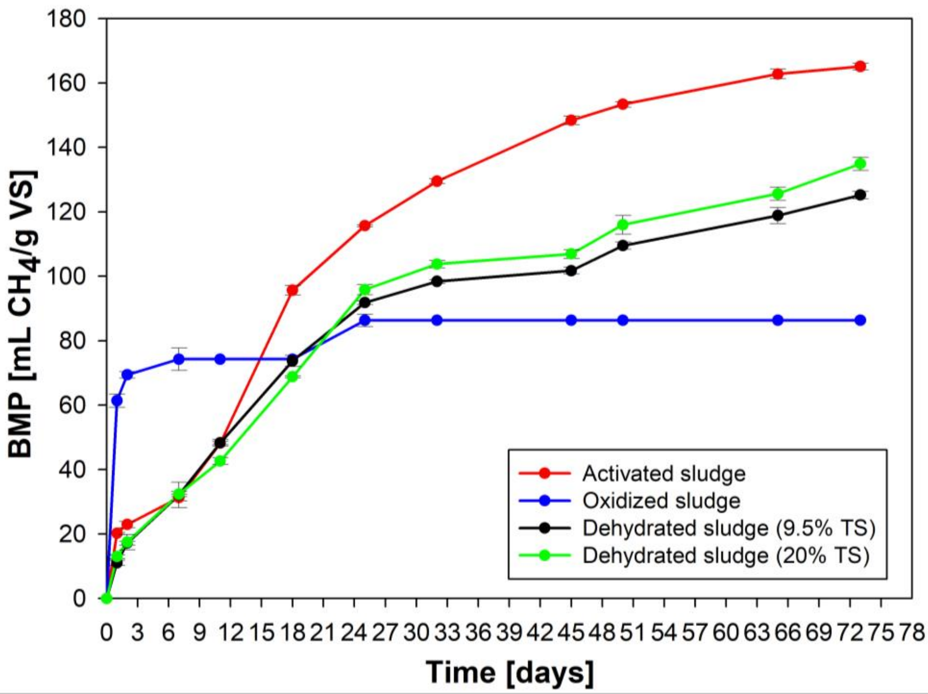

Figure 1. Cumulative biomethane profiles of activated, dehydrated and oxidized, sewage sludge obtained over 74 days of anaerobic digestion. Values and error bars represent the averages and standard deviations of triplicates for each experimental condition, respectively. TS = total solids.

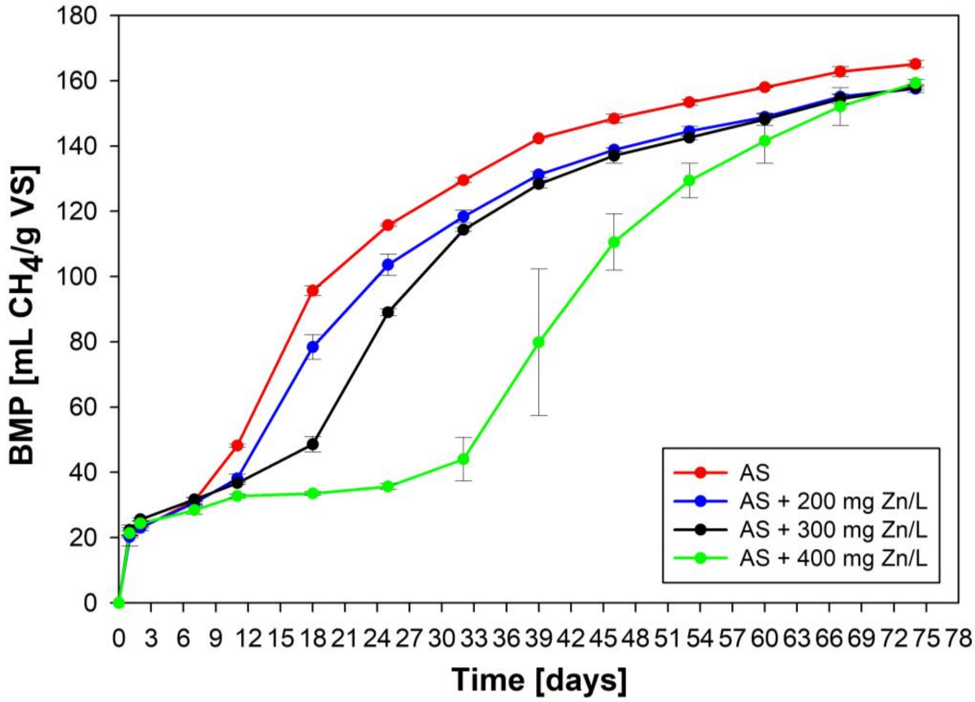

Figure 2. Cumulative biomethane profiles of the activated sludge (AS) at increasing zinc concentrations over 74 days of anaerobic digestion. Values and error bars represent the averages and standard deviations of triplicates for each experimental condition, respectively. AS = activated sludge.

In the absence of zinc, Zhen et al. [22] observed a BMP value of 126 mL CH_4/g VS using thickened waste AS with a volatile solid content of 22.4 g/L. In another study, the AD of secondary sewage sludge yielded 200 mL CH_4/g VS under mesophilic conditions [23]. When zinc was present, instead, Dokulilova et al. [1] obtained a BMP value of 93 mL CH_4/g VS using a sewage sludge containing approximately 37 mg Zn/L collected directly from the anaerobic sewage sludge stabilization tank of a full-scale WWTP. As already discussed, the presence of zinc in sewage sludge can stimulate or inhibit the AD process, and the resulting behavior is mainly associated with the different tolerances and resistances of microbial communities to this microelement, the pH and the potential combined inhibition of other intermediates (e.g., VFAs) in the AD system. The highest BMP value achieved with AS suggested that AS could be more suitable than the other sludge types present in WWTP for the AD process aimed at treating sewage sludge containing high Zn concentrations.

3.2. Effect of Increasing Zinc Concentrations on the Biomethane Yield of AS

The biomethane production profiles from AS under different feed Zn concentrations are shown in Figure 2. The biomethane production in the case of AS without the addition of any external Zn was 165 ± 1 mL CH_4/g VS. Only a slight decrease ($p > 0.05$) in methane production (4%) was observed in the case of 200 and 300 mg Zn/L added externally. In both cases, biomethane production reached about 158 ± 1 mL CH_4/g VS. Additionally, in the presence of a feed Zn concentration of 400 mg Zn/L, the final biomethane production was comparable ($p > 0.05$) to that observed with the external addition of 200 and 300 mg Zn/L, reaching approximately 159 ± 1 mL CH_4/g VS. The lower biomethane production in the initial days of the incubation period can be explained by the fact that a higher initial Zn concentration was inhibitory to methanogens, with biomethane production increasing only when Zn was likely precipitated by hydrogen sulfide. In the first 32 days of BMP tests, thus, methane production decreased with increasing zinc concentrations, confirming the possible inhibitory effect of zinc on methanogens.

In the case of the external addition of 400 mg Zn/L, the biomethane production ceased altogether after day 2 and started again only after day 32, pointing towards a recovered methanogenic activity (Figure 2). The addition of 200 and 300 mg Zn/L, resulting in a total concentration of 293 (AS + 200 mg Zn/L) and 393 (AS + 300 mg Zn/L) mg Zn/L, respectively, did not affect the final BMP as compared to the AS without any externally added Zn. In the presence of 493 mg Zn/L (AS + 400 mg Zn/L), a 70% decrease in BMP on day 32 was instead observed. A possible inhibition threshold for methanogenesis starting at values of about 200 mg/L of Zn has already been reported in the literature [12]. The BMP results with the externally added Zn likely show that enough sulfide was produced in the system, enabling the formation of zinc sulfide precipitates, which minimized zinc toxicity.

The decrease in the biomethane production rate after day 2 was common to all experimental conditions with AS, and the duration of such a lag phase increased with increasing Zn concentrations. From day 2 to day 7, the rate of methane production slowed down and was comparable for all the initial Zn concentrations, therefore indicating that Zn addition did not affect the initial BMP trend. This trend may be explained by the competition between methanogens and SRB, which likely used part of the organic matter to produce H_2S and, hence, precipitate Zn. Therefore, the biomethane yield was similar ($p > 0.05$) during this phase among the experimental conditions.

Dokulilova et al. [1] used anaerobic sewage sludge to study the effect of externally added Zn on biogas production. The results showed that no significant inhibition of methane yield was caused by the external addition of Zn concentration of 200 or 300 mg/L. A biomethane production of approximately 90 mL CH_4/g VS was obtained in both the absence of externally added Zn and in the presence of added Zn at 200 and 300 mg/L. In contrast, a significant inhibition of methane production was observed after the addition of 400 mg/L of Zn and the final methane production value dropped by approximately 14% in the same study [1]. On the contrary, in our study, the inhibition caused by the presence of 400 mg Zn/L added externally, totaling 493 mg Zn/L, was only temporary, and eventually the BMP values were comparable to those achieved with lower Zn contents. Hence, if a digester fed with sewage sludge was operated with a typical hydraulic retention time (HRT) of 15–30 days, then the high Zn concentration would lead to exploiting only a fraction of the full BMP. No significant changes ($p > 0.05$) in the final biomethane yield were caused by the external addition of Zn (i.e., 200 and 300 mg/L). However, the different biomethane production trends suggest that microorganisms need a prolonged acclimation period after adding Zn in high concentrations.

Sarioglu et al. [24] used anaerobic sewage sludge obtained from an up-flow anaerobic sludge blanket reactor treating the wastewaters of Pakmaya Yeast Factory, and the cumulative methane gas production decreased by 55 and 43%, respectively, at 500 and 1000 mg Zn/L. Lin and Chen [25] showed that 60 mg Zn/L resulted in a 50% inhibition of methanogenesis in an up-flow anaerobic sludge blanket reactor. The current study reached quite similar results, as a zinc concentration of 493 mg Zn/L (AS + 400 mg Zn/L) inhibited the methane-producing microbial community in the initial 32 days of incubation. The potential reason for the recovered methanogenic activity observed in this study at high inlet Zn concentration, which was not detected in similar studies, could be that the AS used in this study was already acclimated to high Zn concentration.

3.3. Sulfate Reduction and sCOD Removal during Anaerobic Digestion

The sulfate reduction and sCOD removal are important factors when considering metal sulfide precipitation and biomethane production. Table 2 presents the sulfate concentration in AS under different feed Zn concentrations. A sulfate reduction efficiency of more than 99% was achieved under all Zn concentrations. The high sulfate reduction efficiency implies that SRB were likely present in the AS to reduce sulfate to sulfide. The sCOD concentration profiles from AS digestion under different feed Zn concentrations are shown in Figure 3. A sCOD removal efficiency higher than 60% (Figure 3) was achieved in the case of the AS without any zinc addition as well as in the case of AS + 200, AS + 300 and AS + 400. The sCOD removal efficiency can be attributed to both methane production and sulfate reduction by SRB. From Figure 3, it is possible to observe that, as long as sulfate reduction dominated, the removal of sCOD occurred at higher rates, while this was slower once methanogenesis became the dominating process. SRB likely used sCOD for sulfate reduction at higher rates during the early phase (i.e., 25 days) compared to the rest of the experiment, thus allowing Zn precipitation. Indeed, the AS + 400 condition resulted in an average sCOD removal rate of about 22.0 mg sCOD/L·d up to 39 days, which was comparable to the rates observed in the case of the AS (19.2 mg sCOD/L·d), AS + 200 (18.4 mg sCOD/L·d) and AS + 300 (24.0 mg sCOD/L·d) during the first 25 days. The sCOD removal rates slowed down to 14.3, 11.4 and 10.0 mg sCOD/ L·d (Figure 3) from days 25 to 39 in the AS, AS + 200, and AS + 300 conditions, respectively, probably due to the earlier onset of methanogenesis compared to AS + 400 (Figure 2). A similar sCOD removal rate of about 6.7 mg sCOD/ L·d was established only after 39 days in the AS + 400 condition.

Table 2. Sulfate concentrations on days 1 and 64 in the activated sludge (AS) at increasing feed zinc concentrations.

Days	AS	AS + 200 mg Zn/L	AS + 300 mg Zn/L	AS + 400 mg Zn/L
1	408.00	514.00	500.00	520.00
64	1.72	2.82	3.00	4.50

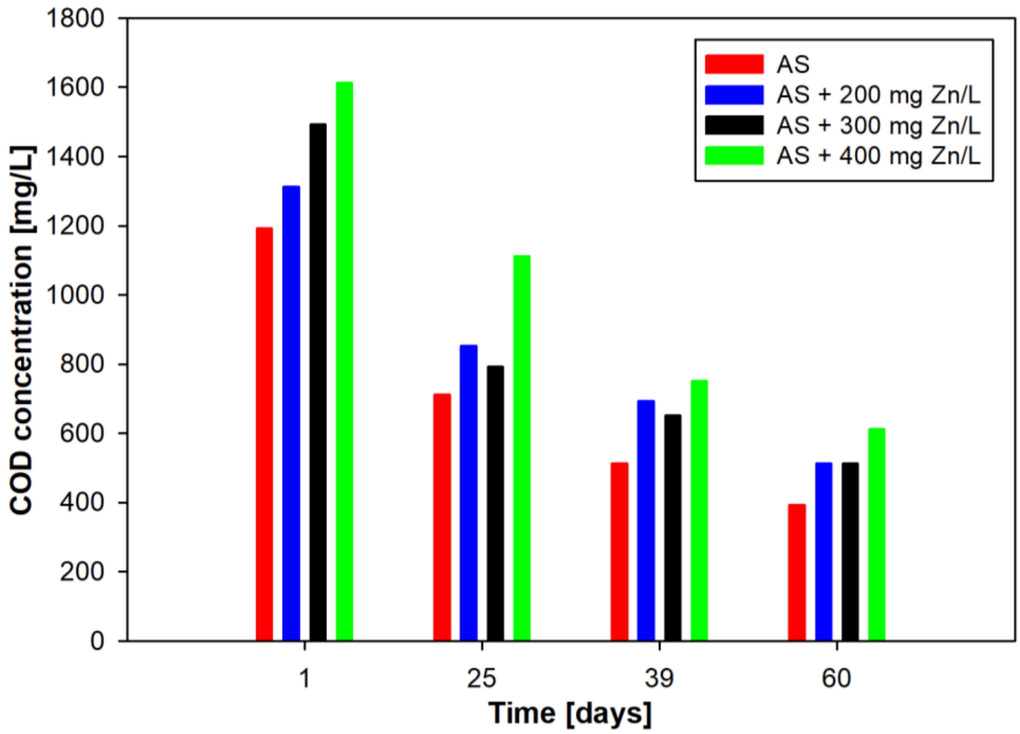

Figure 3. Soluble chemical oxygen demand (sCOD) concentration of the activated sludge (AS) at increasing Zn concentrations at different times of the BMP tests. Values represent a single replicate alternatively sampled from triplicates of each experimental condition.

The more prolonged sCOD removal rate observed in the case of AS + 400 can be explained by the fact that the high Zn concentration favored SRB activity over that of methanogens for a more extended period. Once Zn was completely precipitated by the SRB-produced sulfide (Figure 4), the methanogenic activity recovered, and the residual COD was used to produce biomethane (Figure 2).

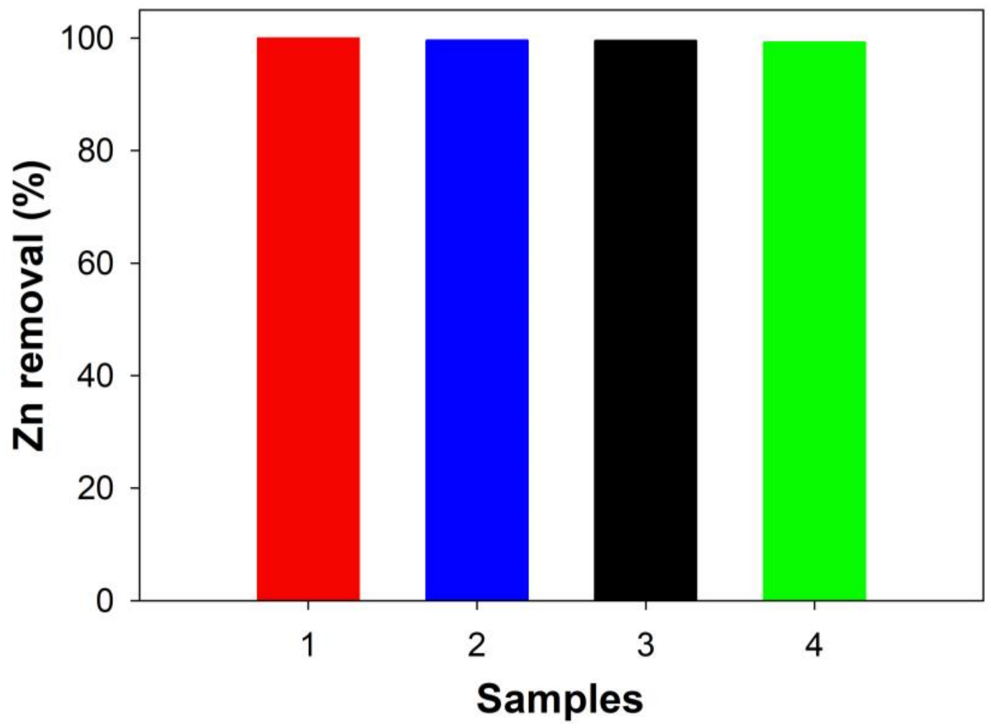

Figure 4. Overall Zn removal (%) of the activated sludge (AS) at increasing Zn concentrations: (1) AS; (2) AS + 200; (3) AS + 300; (4) AS + 400.

Different mechanisms were reported to explain the differences in competition between methanogens and SRB, namely the ratio between COD and sulfate, the capacity of microbial aggregation, and process temperature. Theoretically, organic matter can be completely degraded by SRB with a COD/sulfate ratio below 0.67 [26]. On the other hand, for a COD/sulfate ratio above 10, sulfate reduction would use only a minimal part of the available COD, and methanogenesis would not be significantly affected. However, for a COD/sulfate ratio below 1, methanogens can be outcompeted by SRB [13]. In this study, with a COD/sulfate ranging from 1 to 3, the removal of COD SRB, which likely occurred as long as high soluble Zn concentrations were present in the liquid phase, did not significantly influence the amount of organic matter available to methanogens, as also proven by the similar final BMP values (Figure 2). In addition to the COD/sulfate ratio, which is critical for the competition over organic substrate oxidation between SRB and methanogens, the overall sulfate concentration also plays a critical role by influencing the accumulation of potentially toxic sulfide levels. Although sulfate is nontoxic to anaerobic microorganisms, its reduced form (i.e., sulfide) is considered toxic to methanogens and SRB [13,27]. The diffusion of hydrogen sulfide through the cell membrane is responsible for enzyme inhibition and protein denaturation [27]. Moreover, if not removed from the system, hydrogen sulfide can cause operating problems such as corrosion and malodor. For example, a previous study observed that how even in the presence of a COD/sulfate ratio of 12, the high initial sulfate concentration resulted in a particle inhibition of methanogenesis [28]. Similarly, Kiyuna et al. [29] found that a sulfate concentration of 1800 mg/L was inhibitory to biomethane production even in the presence of COD/sulfate ratios as high as 12, 10 and 7.5.

The above-mentioned studies proved that, in the presence of high sulfate concentrations, a preliminary or concomitant sulfate removal (e.g., SRB-driven sulfate reduction) is recommended in order to obtain a satisfactory biomethane production. Metal precipitation can effectively reduce the toxic effect of hydrogen sulfide, conferring an additional advantage to the combined presence of metals and sulfate in the sewage sludge. The issue related to the excessive accumulation of sulfide can thus be mitigated by the metal sulfide precipitation, which allows the removal of toxic sulfide by forming a highly insoluble metal sulfide precipitate. Both the sulfate and metals can then be removed without considerably affecting the final biomethane yield from the sewage sludge.

3.4. Zinc Removal by Sulfide Precipitation during the AD Process

The zinc removal efficiency was calculated for the tests with AS and increasing Zn concentrations and was higher than 99% in the case of 200, 300 and 400 mg Zn/L added externally (Figure 4).

Zinc removal was most likely achieved through zinc sulfide precipitation induced by biogenic sulfide production from sulfate reduction. The high sulfate reduction efficiency indicates the presence and activity of an SRB community able to reduce sulfate to sulfide (Table 2). The mechanism of heavy metal removal can be described as the production of sulfide from sulfate by SRB followed by the formation of highly insoluble metal sulfide precipitates (Equation (2)). Sulfide produced by SRB can efficiently detoxify metals by combining and precipitating metals, thus decreasing the amount of sulfide and metal toxicity. This process can also facilitate the recovery of metals in the form of metal sulfide [30,31].

$$M^{2+} + S^{2-} \rightarrow MS \downarrow \quad (2)$$

The biological production of sulfide by means of SRB during AD can reduce the cost of additional chemicals (e.g., hydroxide and sulfide) to precipitate zinc. Moreover, the concomitant sulfate reduction during AD can reduce sulfate concentration in the effluent, improving the quality of the produced liquid digestate.

Van Houten et al. [27] studied zinc and sulfate removal using a full-scale gas-lift reactor and did not observe any interference in reactor performance due to zinc sulfide precipitation. The results of the microbial community analysis performed in the same study, showed the presence of *Methanobacterium* and *Methanospirillum*, suggesting that methanogenesis co-occurred with sulfate reduction and metal precipitation [32]. However, Zayed and Winter [33] observed that the addition of 40 mg/L Zn as zinc chloride ($ZnCl_2$) led to a 50% inhibition of methanogenesis. This inhibition was recovered entirely only after adding equimolar amounts of sulfide. These results also suggest that when sulfide was added simultaneously with Zn, methanogenesis was slightly retarded and the same amount of methane, such as in the non-inhibited control, could be achieved. Sulfide likely precipitated the heavy metals as metal sulfides and prevented or alleviated Zn toxicity. In another study, Gonzalez-Estrella et al. [34] reported that biologically produced sulfide could reduce the toxic effect of zinc oxide (ZnO) during methanogenesis. Similarly, no significant effect on the final BMP value was observed after supplementing up to 400 mg Zn/L in this study. Thus, here we demonstrate the potential of overcoming zinc inhibition on AD due to the simultaneous activity of SRB-generating sulfide. Indeed, the occurrence of a dark black color in the bottles (Figure S1) was another possible indicator of sulfide production and zinc precipitation [16]. Zinc sulfide precipitation inside a full-scale reactor did not interfere with the sulfate reduction, and methanogenesis was not suppressed [33]. Hence, metals can be easily removed by sulfide precipitation during the AD process without affecting the activity of methanogens.

4. Conclusions

Zinc is one of the most commonly found heavy metals in sewage sludge and its effect on the biomethane potential of different sludge samples, as well as on AS with increasing total Zn contents, was here evaluated. Although having the highest background Zn content, the AS produced more biomethane than the dehydrated and oxidized sludge (i.e., 165 ± 1, 135 ± 2 and 86 ± 2 mL CH_4/g VS, respectively). With regard to the AD of AS at varying Zn levels, the concentration of Zn that caused a significant, yet only temporary inhibition, was found to be 493 mg Zn/L, which induced a 63% lower biomethane production in the first 32 days. Due to Zn precipitation by means of biogenic sulfide, the biomethane yield reached a similar value in both the presence and absence of external Zn addition. The SRB present in the biological system were able to reduce sulfate, attaining a sulfate reduction efficiency of about 99% under all experimental conditions. Zn was likely removed by sulfide precipitation and removal efficiencies of 99% were achieved under all the different Zn concentrations tested. Hence, metal precipitation using biogenic sulfide during AD stands out as a promising option to overcome Zn toxicity, and strategies linked to the use of biological systems already acclimated to high Zn concentrations could offer a valid alternative in the AD of the excess sewage sludge generated during the treatment of Zn-laden wastewaters. The use of continuous or semi-continuous processes could be further investigated in the future to better evaluate the role played by zinc in more relevant industrial AD applications. In addition, future work could focus on monitoring and quantifying the SRB community inhibiting the sewage sludge, aiming at supporting more accurate control and process strategies during sludge processing.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/w15040729/s1, Figure S1: Serum bottles with dark black color likely indicating a high formation of zinc sulfide.

Author Contributions: Conceptualization, G.E., S.P. and S.M.; methodology; software; validation, G.E., S.P., S.M., A.O. and M.K.; formal analysis; investigation; resources, S.P., S.M., A.O. and G.E.; data curation; writing-original draft preparation, M.K. and S.M.; writing-review and editing, M.K., S.P., F.B., A.O., G.E., S.M., F.D.P. and F.P.; visualization; supervision, G.E., S.P., S.M., F.P. and F.D.P.; project administration; funding acquisition, F.D.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Ministry of University and Research, Italy.

Data Availability Statement: Not applicable.

Acknowledgments: The postdoctoral position of Manoj Kumar is funded on the project Watergy "L'efficientamento energetico del servizio idrico integrato". The authors thank the "Consorzio per lo Sviluppo Industriale della Valle del Biferno (COSIB)" for providing the sewage sludge samples used in this study.

Conflicts of Interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

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