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Nickel spiking to improve the methane yield of sewage sludge

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Abstract

The presence of micro-nutrients can be stimulatory for the anaerobic digestion (AD) of hardly degradable wastes and thus, improve process performance. Among the essential trace elements, nickel is involved in multiple important enzymes necessary for efficient AD. The present study investigates the effect of nickel spiked sewage sludge on batch and continuous mode operation. Metal spiking was conducted in the form of nanoparticles (Ni-NPs) and salt ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$). Results from batch assays showed that 5 mgNi-Salt/kgVS in the presence of Nitrilotriacetic acid (NTA) enhanced the methane yield by ~10% compared to the untreated sample. The impact of Ni-NPs in the AD process was also positive, but slightly lower compared to the effect of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$. The stimulatory impact of Ni was also revealed in continuously fed digester boosting the methane yield by ~8%. Overall, the improved methane production indicated that methanogenic archaea were favoured by the simultaneous supplementation of Ni and NTA.

Keywords

Anaerobic digestion; Sludge; Trace elements; Nickel; Nitrilotriacetic acid (NTA)

1. Introduction

The building blocks for the microbiome of the anaerobic digestion (AD) process can be distinguished at macro- (e.g. Carbon, Oxygen, Hydrogen, Nitrogen, Sulfur) and micro-elements (e.g. Cobalt, Copper, Molybdenum, Selenium, Nickel, Zinc, Tungsten) (Roussel et al., 2018). The knowledge of macro-elements content into the feedstock is crucial in order to avoid common operational problems related to e.g.: 1) ammonia or sulfide inhibition incidents due to the increase of added nitrogen or sulfur, respectively; 2) overloading or starvation conditions due to the fed amount of organic carbon. On the other hand, the way that each

trace element (TE) affects the AD process is rather complex. The intricacy of AD renders extremely difficult to precisely understand the role of micronutrients because the effects of TEs easily shift from stimulatory to inhibitory. Unique microbial communities with alternating nutritional needs are established into dissimilar AD environments due to the variability of parameters as substrates' characteristics and/or operational conditions. Hence, the TEs do not influence in a constant pattern the multivariable and complex AD environments (Banks and Heaven, 2013). However, the role of specific TE to enhance microbial growth and activity has been previously defined to some extent in the literature (Bougrier et al., 2018; Demirel and Scherer, 2011; Romero-Güiza et al., 2016).

On this frame, nickel (Ni) has an important role for a well-performing methanogenesis and is considered to be among the most important TEs (Hu et al., 2008a; Vintiloiu et al., 2013). Specifically, Ni is involved into the expression of enzymes as CO dehydrogenase/acetyl-CoA synthase (Methanogens/Homoacetogens) and Methyl-CoM-reductase (Methanogens) (Fournier and Gogarten, 2008; Ko et al., 2018; Romero-Güiza et al., 2016). Especially for the later one, the presence of Ni is essential as it is the central atom of the complex coenzyme F430 (Prakash et al., 2014). Regarding methanogenesis, stimulatory effects were previously reported in a range from 0.03 to 27 mg-Ni/L (Romero-Güiza et al., 2016). On another perspective, a concentration of 8 to 10 mg-Ni/kgTS was found to be extremely beneficial for the microbiome (Vintiloiu et al., 2013).

Nevertheless, the optimum dosage of TE cannot be drawn through a simplified consideration, in which the sole influencing factor is the metal's concentration. For instance, the presence of chelating agents affects markedly the bioavailability of TEs for the anaerobic microbes (Serrano et al., 2017). The importance of chelating agents relies on the fact that they can replenish losses or low concentrations of TEs by avoiding the precipitation and control the limited metal availability (Flora and Pachauri, 2010). Organic agents as citric acid,

nitrilotriacetic acid (NTA) and ethylenediaminetetraacetic acid (EDTA) have been previously examined as Ni chelators in AD process increasing the methane production from 10 to 49% (Hu et al., 2008b, 2008a; Vintiloiu et al., 2013). However, the aforementioned results are reported in limiting or unadapted conditions, in which the desired effects of both Ni and Ni-chelators are relatively easily to be detected. On the contrary, the positive role of metal additives to stimulate the methanation process of an adapted AD environment operated for a long period is still challenging.

The TEs are traditionally supplemented into the digesters in their corresponding salt form. However, the sluggish dissolving rate is still a challenge and therefore a potentially limited availability of metal ions can subsequently decrease the enzymatic activity. On this topic, nanotechnology appears as a scientific advancement to potentially overcome the challenge (Abdelsalam et al., 2016). Nanoparticles (NPs) can rapidly penetrate the cell walls and speed up the TEs uptake and metabolism. The high surface area, increased adsorption, dissolution and dispersion are unique characteristics that NPs possess (Gelperina et al., 2005).

The main objective of the present study was to investigate the effect of two different nickel sources on the biogas production from waste activated sludge. The role of Ni form was assessed by examining the addition of Ni-NPs and -salts in batch assays. To validate the results in more realistic conditions, continuous mode experiments were also conducted. Emphasis was given to increase TE availability via the addition of the chelating agent. Inoculum and substrate were obtained by the same full-scale facility in order to evaluate the effect of additives in an adapted microbial community.

2. Materials and methods

2.1 Characteristics of inoculum and substrate

Inoculum and sewage sludge were collected from Lynetten Wastewater Treatment plant

(WWTP) – that operates under mesophilic conditions – in the greater Copenhagen area in Denmark. The effluent was placed in an incubator at 37 ± 1 °C for one week before usage to remove the background methanation activity. The sewage sludge consisted of 50% primary and 50% secondary sludge (v/v) and was collected from the same WWTP. The main characteristics of the inoculum and sewage sludge used in this work are given in Table S1.

2.2 AD experiments

Biochemical Methane Potential (BMP) assay was carried out to define the effect of Ni addition on the anaerobic biodegradability of sewage sludge as previously described by (Khoshnevisan et al., 2018). The extra Ni was added in the form of NPs and $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$. The effect of Ni forms was examined at two final concentrations (i.e. 5 and 10 mg-Ni/kgVS) including the already available metal and at the absence or at the presence of a chelating agent (i.e. 2 mg-NTA/L). The size of Ni-NPs was lower than 100 nm.

The lab-scale CSTR had a total and working volume of 2.3 and 2.0 L, respectively. The CSTR was operated at mesophilic conditions (37 ± 1 °C) heated with a silicone thermal jacket. The hydraulic retention time (HRT) was set at 20 days by daily supply of 100 mL sewage sludge and the organic loading rate (OLR) was 2.9 gVS/L/d. During the first period (0-20 days), the CSTR was fed with pure sewage sludge. Subsequently, at 21st day of operation, external Ni source was injected to the CSTR as a single pulse to achieve the predetermined target of TE concentration. During the second period (21-60 days), the CSTR was fed with Ni spiked sewage sludge to maintain the Ni content at the desired value. The added amount of Ni, for both CSTR and feedstock, was calculated based on the results from the batch assays. During the whole experimental period, gas and liquid samples were taken twice a week to determine methane concentration, pH and VFA. The daily biogas volume was monitored by the liquid displacement method.

2.3 Analytical Methods

Alkalinity, pH, total solids (TS), volatile solids (VS) and chemical oxygen demand (COD) based on previous study (Tsapekos et al., 2017). Volatile fatty acids (VFA) and methane content were monitored using gas chromatography GC-TRACE 1310 and GC-TRACE 1300 of Thermo Scientific, respectively, as previously described by Khoshnevisan et al. (2018). Methanogenic enzymatic activity (i.e. Coenzyme F₄₂₀) was assayed adapting the classic spectrophotometric method (Miao et al., 2013) using a Synergy Mx multimode microplate reader (BioTek Instruments, Inc., USA). The autofluorescence was measured at an excitation wavelength of 425 nm and an emission wavelength of 472 nm. The TEs in inoculum and sewage sludge were analyzed by inductively coupled plasma with optical emission spectrometry (ICP-OES). For all analysis triplicate samples were measured.

2.4 Kinetic models of methane production

The kinetics of biomethanation process was modelled using first-order kinetics and modified Gompertz model equations. Focusing on hydrolysis, first-order kinetics was used, Eq. (1):

$$M(t) = M_0 \times (1 - \exp(-Kt)) \quad (1)$$

where, $M(t)$ is the produced methane (NmLCH₄/gVS) over digestion time t (days), M_0 is the maximum methane achieved potential (NmLCH₄/gVS), K is the first-order disintegration rate constant (1/day).

In addition, the modified Gompertz equation was used to simulate the biomethanation process at the batch assays according to Eq. (2):

$$M(t) = M_0 \times \exp \left\{ -\exp \left[\frac{R_{max} \times e}{M_0} (\lambda - t) + 1 \right] \right\} \quad (2)$$

where, $M(t)$ is the accumulated methane over time (NmLCH₄/gVS), parameter M_0 is the

final methane yield ($\text{NmLCH}_4/\text{gVS}$), R_{max} is the maximum methane production rate ($\text{NmLCH}_4/\text{gVS/d}$), λ is the lag phase (days), t is the duration of the incubation period (days) and e is Euler's number (2.7183).

2.5 Statistical analysis

One-way analysis of variance (ANOVA) followed by Tukey post hoc test ($p < 0.05$) was carried out to reveal the statistically significant differences among the treatments with the software OriginPro, version 9.0.0 (Corporation, Northampton, MA). The prediction accuracy of the non-linear regression models was estimated through the coefficient of determination (R^2) and Standard Error (SE).

3. Results and Discussion

3.1 Effect of nickel spiking on pH, COD, VFA and CH_4 development in batch assays

To begin with, pH values did not differ significantly ($p > 0.05$) among all treatments on the fifth day of the incubation period (Table 1). The absence of remarkable differences was attributed to the fact that high amount of anaerobic inoculum was used and hence, the batch reactors were provided with high buffer capacity (Tsapekos et al., 2017). Similarly, negligible differences were detected through the alkalinity and COD measurements from samples taken in the middle of the experimental period. Intermediates products were produced and simultaneously, were further metabolized into methane leading to limited differences on COD degradation/accumulation. In this context, high and insignificantly different ($p > 0.05$) hydrolysis rates were observed for all the examined Ni additives' scenarios (Table S2), whose output is in accordance with the aforementioned COD measurements. While nickel is involved in the enzymatic hydrolysis of urease (Dixon et al., 1980), the solubilization of organic matter was not markedly stimulated at none of the examined scenarios. Especially for

NPs tests, despite the fact that the metabolic intermediates and key enzyme secretions are boosted by specific nanopowder characteristics (e.g high surface area), the additives did not stimulate the excretion of fully active urease to further or quicker depolymerize the sewage sludge based on the experimental observations.

Likewise, statistically insignificant ($p > 0.05$) differences were observed for the lag phases among all tests (Table S2). The result is easily explained by the fact that the microbial communities were well-adapted in the used substrate. Hence, the initially hydrolyzed monomers and the subsequently produced VFA were rapidly consumed by acidogenic and methanogenic communities, respectively. Hence, an adaptation period was not needed. The organic matter share of inoculum was higher than substrate's value (i.e. inoculum to substrate ratio of 1.5) to ensure that an enriched culture was provided in order to successfully metabolize the sludge and avoid acidification incidents. Focusing on lag phase decrease, an unadapted community or an adapted community at limiting conditions could have enlighten further the effects of Ni-forms in the initial AD steps.

In addition, accumulation of the majority of intermediates (e.g. propionic, butyric, iso-butyric, valeric, iso-valeric and hexanoic acids) was not observed, showing that a well-performing process was conducted. On the contrary, a clear difference was observed in terms of VFA accumulation. Specifically, all the Ni-treated samples had markedly lower acetate concentrations compared to the untreated experimental set. Since Ni supplementation could boost the expression of acetyl-coenzyme A synthetase (Fournier and Gogarten, 2008) –i.e. Wood–Ljungdahl pathway coupled to methanogenesis –, carbon monoxide dehydrogenase and methyl-S-CoM enzymes – i.e. methanogenesis via methanol acetate and H_2/CO_2 –, the low concentrations of accumulated acetate can be considered as an indirect indication that the methanogenic activity was stimulated by Ni supplementation.

Regarding methane production, the positive impact of Ni addition was revealed in most of the examined scenarios (Fig. 1; Table 1). The highest methane yield enhancement (~10%) was achieved adding 5 mgNi-Salt/kgVS along with 2 mg-NTA/L (Fig. 1a). Similar result was also obtained when the double concentration of Ni was added using NiCl₂ in the presence of the chelator (~8%, $p > 0.05$). Indeed, the results obtained in this work are in agreement with recent studies that examined the effects of similar Ni forms on the AD. Chen et al. (2008) reported that the AD of sludge was stimulated by the addition of 5.0 mg-Ni/L –in the form of NiCl₂·6H₂O – after 6 h and then in a significant stimulation of methane production after 24 h. In another study of Abdelsalam et al. (2017); the methane yield of livestock slurry, spiked with 1.0 mg-Ni/L using NiCl₂ as a source, resulted in ~4% higher than the obtained value using Ni-NPs. On top of this, nano-sized materials can be strongly bound with various organic and inorganic fractions limiting the availability for the methanogenic populations. Hence, the slightly limited stimulating effect of Ni-NPs compared to Ni-Salt can be attributed to a potential antagonism with ligands that led to partial precipitation of metals. As for the effect of NTA, it was concluded that the addition of a chelator has a positive effect on methane production. Due to its supplementation, most of Ni could have formed soluble organic complexes with certain amino acids and so, the bioavailability for methanogens was increased, avoiding the precipitation of sulphides, carbonates or phosphates (Hu et al., 2008a). Overall, the more distinct impacts in methanogenesis compared to the previously AD steps can be ascribed to the fact that nickel is more important for the growth of archaeal than the bacterial species; as it contributes to retain their cell membranes and boost structural stability (Vintiloiu et al., 2013). In addition to that, Ni is an essential component of the low molecular weight coenzyme F₄₃₀ which uses two coenzymes –methyl thio-ether-methyl-coenzyme M and the thiol coenzyme B –as substrates for the production of methane in all methanogens

(Chen et al., 2016). Hence, a stimulatory effect can be more easily observed when added in appropriate levels (Roussel et al., 2018).

3.2 AD of nickel spiked sludge at continuous mode operation

The most effective Ni supplementation identified by BMP assays (i.e. 5 mgNi-Salt/kgVS and 2 mg-NTA/L) was further examined in CSTR mode (Fig. 2). Initially, the CSTR was fed with sewage sludge until reaching steady conditions with no significant fluctuations ($p > 0.05$) in terms of pH, VFA, biogas and methane for ten consecutive days. At the end of the first period, the bioenergy yield was 300 ± 11 NmLCH₄/gVS (Fig. 2a), corresponding to 56% of the theoretical maximum value based on biomass chemical composition.

At the end of the first HRT, feedstock and CSTR were supplemented with the corresponding amount of Ni as revealed by BMP tests. In accordance with batch assays, pH values were relatively unchanged (7.23 ± 0.05); and on the other hand, the positive impact of Ni on methane output was also revealed in continuous mode operation. At the second steady-state, the methane yield was 8% higher compared to Period I. The achieved methane yield (324 ± 8 NmLCH₄/gVS) corresponded to 76% of the practical BMP value, which is acceptable as 70-90% of the yield obtained in BMP test is expected in CSTR mode (Tsapekos et al., 2018). Comparing the practical yield with the maximum theoretical based on COD, the nickel spiked sludge has 60% anaerobic biodegradability. It worths highlighting that process efficiency was markedly acceptable, as about 40-50% biodegradability is expected for sludge based-AD (Grosser and Neczaj, 2016).

In addition, the VFA concentrations were slightly lower than Period I (Fig. 2b). For instance, acetate concentrations were decreased to values lower than 0.3 g/L, which can be related to the improved performance of methanogenic microorganisms (Yu et al., 2016). On top of this, the highest F₄₂₀ activity was observed during the second experimental period.

Specifically, the highest relative activity was observed on the 25th day of operation, when the accumulated intermediates started to decrease and the methane production has already lifted up on the increased level compared to Period I (Fig. 2). At steady-state conditions of Period I and II the F_{420} activities were 70% and 88% of the highest, respectively. Thus, the lowest relative activity was detected at limited Ni content. Hence, the methanogenic enzymatic activities consisted perfectly with the detected experimental observations. Overall, the steady operation of CSTR fed with sewage sludge supplemented with trace amounts of Ni, advocate the positive impact of the examined micro-nutrient on an already stable and adapted AD process.

4. Conclusions

The present study demonstrated that nickel spiked sewage sludge can lead to improved anaerobic digestion. In addition, the bioavailability of trace metals for the methanogenic archaea was boosted by supplementing the nickel spiked sludge with Nitrilotriacetic acid (NTA). Spiking trace metals in the form of salt ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) had a clear stimulatory effect. Specifically, 5 mgNi-Salt/kgVS complexed with NTA can be associated with increased methane yield up to 10%. Furthermore, the improved biodegradation efficiency was similar at different reactors configurations (i.e. batch and continuously fed digesters).

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Figures

Fig. 1. Effects of spiked Ni in the form of a) NPs and b) NiCl₂·6H₂O on accumulated methane yield over time

Fig. 2. Continuous mode operation: a) methane yield and b) pH-VFA changes over time

Tables

Table 1. Physicochemical characteristics affected by different Ni addition strategies

Characteristics	pH	Alkalinity, g _{CaCO3} /L	COD, g/L	Acetate, mg/L	CH ₄ yield, NmL/gVS
Untreated	7.24	4.74 ± 0.20	23.82 ± 0.20	36.23 ± 1.81	406 ± 34
5 mgNi-NPs/kgVS	7.32	4.81 ± 0.22	20.73 ± 0.31	15.11 ± 0.76	414 ± 16
5 mgNi-NPs/kgVS & 2 mg-NTA/L	7.35	4.78 ± 0.20	23.13 ± 0.08	15.11 ± 0.76	414 ± 21
10 mgNi-NPs/kgVS	7.35	4.77 ± 0.19	22.48 ± 0.61	15.21 ± 0.76	426 ± 2
10 mgNi-NPs/kgVS & 2 mg-NTA/L	7.34	4.72 ± 0.22	23.00 ± 0.24	12.19 ± 0.61	393 ± 12
5 mgNi-Salt/kgVS	7.37	4.78 ± 0.23	23.80 ± 0.41	15.66 ± 0.78	416 ± 23
5 mgNi-Salt/kgVS & 2 mg-NTA/L	7.25	4.68 ± 0.18	21.27 ± 0.13	20.84 ± 1.04	415 ± 29
10 mgNi-Salt/kgVS	7.38	4.86 ± 0.24	21.76 ± 0.26	20.17 ± 1.01	445 ± 9
10 mgNi-Salt/kgVS & 2 mg-NTA/L	7.35	4.71 ± 0.21	25.64 ± 1.23	18.60 ± 0.93	436 ± 42