Impact of trace element additives on anaerobic digestion of sewage sludge with in-situ carbon dioxide sequestration

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Abstract

Anaerobic digestion (AD) of sludge at wastewater treatment plants can benefit from addition of essential trace metals such as iron, nickel and cobalt to increase biogas production for utilization in combined heat and power systems, fed into natural gas pipelines or as a vehicle fuel. This study evaluated the impact and benefits of Ni/Co and olivine addition to the digester at mesophilic temperatures. These additions supplement previously reported research in which iron-rich olivine (MgSiO_4) was added to sequester CO_2 in-situ during batch AD of sludge. Trace element addition has been shown to stimulate and stabilize biogas production and have a synergistic effect on the mineral carbonation process. AD with 5% w/v olivine and 1.5 mg/L Ni/Co addition had a 17.3% increase in methane volume, a 6% increase in initial exponential methane production rate and a 56% increase in methane yield ($\text{mL CH}_4/\text{g COD}_{\text{degraded}}$) compared to the control due to synergistic trace element and olivine addition while maintaining 17.7% CO_2 sequestration from olivine addition. Both first-order kinetic modeling and response surface methodology modeling confirmed the combined benefit of the trace elements and olivine

24 addition. These results were significantly higher than previously reported results with olivine
25 addition alone [1].

26

27 **Keywords:** anaerobic digestion, trace elements, carbon dioxide sequestration, olivine, renewable
28 methane production

30 **Introduction**

31 Trace metals are essential in the biochemistry of methane production during anaerobic
32 digestion (AD) of sludge. Trace metals, such as cobalt (Co), nickel (Ni), selenium (Se), iron (Fe),
33 molybdenum (Mo) and tungsten (W) are vital constituents of cofactors and enzymes in biogenic
34 methane production and their addition to AD has been shown to stimulate and stabilize biogas
35 process performance [2-5]. Combinations of trace elements can exhibit significant synergistic
36 effects [5]. Increased concentrations of Ni and Co can accelerate initial exponential rates,
37 increase total volume of methane produced, and increase cell densities of methanogens [3, 6]. An
38 increase in methane production is probably due to enhancement of *mcrA* (the gene coding for the
39 alpha subunit of methyl-coenzyme M reductase) transcription level shifting methanogenic
40 consortium dynamics, which increases methanogenic activity [6]. Addition of Ni and Co also
41 improves process stability and efficiency, and substrate utilization, hence enables higher organic
42 loading rates (ORLs) [2, 4]. A study on the feasibility of iron-rich activated sludge as a
43 stabilizing agent for AD of food waste confirmed the positive effects of the Fe addition on the
44 stability of the AD process [5]. Other metals, such as Se, Mo, and W, which may increase AD
45 performance are required 10-times lower concentration than Fe, Ni, and Co [7].

46 Recently, there has been more focus on biogas production at wastewater treatment plants
47 (WWTPs) [8] due to the largely untapped potential to add renewable methane production [9].
48 The largest barrier to biogas utilization is biogas upgrading and treatment including removal of
49 CO₂ and trace contaminants [10]. Pipeline gas specifications typically require the biomethane to
50 have a content quality similar to natural gas with >96% methane content and a minimum heating
51 value of 37 MJ/m³ [11]. Utilization of biogas clean-up technologies increases biogas production

costs by 20-72% because of high operating pressure, electricity demand, chemical and water requirements as well methane loss (0.1-8%) [12].

Mineral carbonation, an accelerated natural weathering process, utilizes calcium- and/or magnesium-rich natural ores for in-situ CO₂ sequestration. Previous research evaluating the kinetics of the carbonation reaction of aqueous CO₂ with minerals include olivine [1, 13], wollastonite [14], serpentine [15] and forsterite [16]. Other research has investigated the effects of natural mineral addition on AD [17-21]. Previous studies revealed that 17.5% of the CO₂ was sequestered at mesophilic temperature in an anaerobic digester supplemented with 5% w/v fine olivine [1]. One challenge is that grinding the minerals to suitable size for introduction to the AD can increase cost by 60% [22]. The cost of the olivine addition may be offset with the benefits of trace metal addition to AD by increasing methane production volume while olivine addition increases methane content in the biogas. The synergistic effect of olivine and trace element addition was determined by evaluating a combination of these factors to achieve a highly efficient and sustainable treatment process. First-order reaction kinetics are often used to describe complex biological reactions in batch AD systems [22-24] while response surface methodology (RSM) has been applied for evaluation and optimization of multiple variables affecting the studied parameters. Previous studies have applied RSM for optimization of parameters during various types co-digestion [23, 25-27].

This study presents the results of the synergistic effects of the addition of both trace elements and mineral carbonation (olivine) into the digester environment during AD of sludge. To the best of authors' knowledge, a study of the synergistic effects of the addition of both trace elements and mineral carbonation during AD to increase methane content and production volume has not been

previously reported. First-order reaction kinetics and RSM were used to evaluate the impact of the addition on the AD performance parameters.

2 Methods

2.1 Sludge from Municipal Wastewater Treatment Plant

The sludge samples were obtained from Stickney Water Reclamation Plant (WRP) of the Metropolitan Water Reclamation District of Greater Chicago (MWRDGC) located in Stickney, IL, where the digesters are operated at mesophilic temperature ($\sim 37^{\circ}\text{C}$). The raw sludge (substrate) and digested sludge (inoculum) were obtained from the inlet and outlet stream of the digester, respectively.

2.2 Experimental Design and Set-up

The AD experiments were carried out at mesophilic temperature (37°C) in 650 mL Wheaton bottles with a working volume of 550 mL as described previously [1]. In brief, each experimental condition was carried out in duplicate/triplicate, with one vessel placed in a Challenge Technology's MPA-200 Biomethane Potential Analyzer system (Springdale, AR) and the remaining vessels placed in a chest incubator. Both the MPA-200 and chest incubator were operated with the same conditions: batch mode and at 50 rpm agitation, and were otherwise identical continuously stirred digesters. The MPA-200 (Figure 1A) utilizes an eight-position water bath providing temperature control and agitation, an eight-channel respirometry-based unit for online measurement. The gas production volume was adjusted to dry ambient temperature and pressure (20°C , 1 atm) during recording [28]. The gas was produced at 37°C and measured at ambient temperature which caused water condensation, therefore calculations included the

saturated vapor pressure of water (47 mmHg or 6.2 kPa) [28]. Gas volume measurement could not be performed on the incubator system digesters.

Periodically the gas outlet (i.e. exhaust line) from the digester was closed and 10 mL of biogas sample was withdrawn from the headspace of each digester and stored in a glass vial (Agilent Technologies, Santa Clara, CA) using a 10-mL gastight syringe (Hamilton, Reno, NV). Each glass vial was pre-flushed with helium under standard conditions. Biogas was analyzed for methane and carbon dioxide concentrations using a gas chromatograph (GC) as described in section 2.4. The gas outlet from the digester remained closed until the digester had replaced the biogas sample as determined by the current biogas production rate. A total of seven samples were taken from the digester over the course of the experiment and each time the sample volume was included in the calculations for gas production volumes.

The initial substrate sludge to inoculum sludge ratio was a 1:2 total solids (TS) and equaled approximately 1.4% (w/v) of the digester. The corresponding food to microorganisms (F/M) ratio calculated on a volatile solids (VS) basis of substrate and inoculum was 1:1.8 (0.54). A mineral media solution was also added to the reactor at a final concentration of 0.5 g/L each sodium sulfide (Na_2S) and L-cystiene ($\text{C}_3\text{H}_7\text{NO}_2\text{S}$), and 0.625 g/L each potassium phosphate (K_2HPO_4), ammonium chloride (NH_4Cl), sodium carbonate (Na_2CO_3), and yeast extract. For the digesters containing Ni and Co, the metals were added as nickel (II) chloride hexahydrate ($\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$) and cobalt (II) chloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$). All chemicals were analytical grade from Sigma Aldrich (St. Louis, MO) or Fischer Scientific (Waltham, MA). The digesters were initially flushed with helium gas (99.999% purity, Airgas, IL) to obtain anaerobic conditions.

2.3 Digester Operation

The olivine rock was obtained from Ward's Natural Science (Rochester, NY). The total solids concentration was adjusted for digesters that contained olivine and was used to determine TS reduction in the final samples. The fine ground olivine had a mesh size >120 and diameter <0.125mm). The three main components of the olivine in descending order are magnesium (307 mg/g), iron (56 mg/g) and manganese (0.76 mg/g) [1]. The olivine was placed in a suspended bag in the reactor medium. The bag is made from 304 stainless steel screen with a 230 mesh size and suspended in the reactor media by a stainless steel wire (Figure 1B).

The trace elements (TE), Ni and Co were added at equal concentrations. The experimental matrix includes: positive control digesters which simulate conventional digester operation in the lab, positive control digesters plus 0.75 mg/L TE, 5% w/v fine olivine digesters plus 0.75 mg/L TE, and 5% w/v fine olivine digesters with 1.5 mg/L TE (Table 1).

2.4 Analytical Methods

A Shimadzu GC-2014 gas chromatograph equipped with a thermal conductivity detector (TCD) and a Supelco 80/100 Porapak Q packed column (5 m × 1/8 inch × 2.1 mm) (Sigma-Aldrich, St. Louis, MO) was used for analysis of gas samples. Helium (99.999% purity, Airgas, IL) was used as the carrier gas. The column temperature was set at 100 °C isothermally and the TCD temperature was set at 170 °C.

The total and volatile solids concentration were determined per Standard Methods 2540B and 2540E, respectively [29]. The total chemical oxygen demand, total organic carbon, total phosphorous, total alkalinity, total nitrogen, and ammonia nitrogen concentrations were determined by methods associated with the HACH test kits (Loveland, CO). The mineral content of anaerobic digester samples were determined by standard Total Recoverable Metals analysis by ICP using EPA 200.7/8 Method [30, 31].

All statistical comparisons were conducted using the student's t-test with a 95% confidence interval. The statistical analysis was conducted with GraphPad Software's QuickCalcs online toolbox (www.graphpad.com/quickcalcs) (La Jolla, CA). Time course data were analyzed with a paired t-test and averaged initial or final liquid digestate samples were analyzed with an unpaired t-test.

2.5 RSM Model Development

The full model methodology was described by Linville et al. (2016) [1]. In brief, the model was determined using the software program Design Expert 9 [32]. The program allows data fitting of model equations containing the unknown constants. The coefficient parameters of the models were estimated through a multiple regression analysis including an ANOVA to evaluate the interaction between process variables and the response [25, 32]. Only significant terms were included in the model ($p\text{-value} < 0.05$) except when required to maintain the hierarchical structure. The quality of the fit of the model equation was determined via the coefficient of determination, R^2 , and its statistical significance was checked through an F-test [32, 33].

Using the proposed model equations, optimization analysis were performed to find pairings of parameter inputs that maximize methane volume and content. With two response variables (content and volume) that are dependent on the same inputs (olivine dosage, trace elements dosage and digestion time), a multi-objective optimization (MO) was performed to find the maximum content and volume using Matlab® version 7.4.0 (R2015b) Optimization Toolbox [23]. The model equations and boundary conditions produce the Pareto front.

3. Results and Discussion

Experiments were conducted with 5% w/v fine olivine in an elevated bag system, the optimal conditions from previous work [1]. Previously, it was determined that placing the olivine in the digester media the elevated bag system (Figure 1B) reduces silica buildup in the digester, and slows and controls release of divalent cations into the digester medium. Controlled cation delivery enhances alkalinity buffering, improves process stability, and increases contact time between the divalent cations on the particle surface and carbonic acid (bicarbonate) in the media as well as and reduces the need for the replenishment of the olivine. Interaction between surface divalent cations and solubilized carbonic acid is the primary mechanism for removing CO₂ [1]. The sludge samples contain a mixture of highly diverse and complex microbial consortium from the WWTP. In general, the major phyla of bacteria found in WWTP sludge were *Firmicutes*, *Bacteroidetes*, *Proteobacteria* and *Spirochaetes* and the major archaea were *Methanosarcina*, *Methanosaeta* and *Methanoculleus* [27, 34-38]. Bacterial profiles can exhibit very low variation within replicates and low variation in time [34] leading to low variations between experimental conditions. The AD experiments lasted 28 days and were terminated when the daily biogas production volume was less than 1% of the total biogas volume [39, 40]. The control experiments were conducted in order to simulate conventional AD operations in the field and as basis to compare the improvement in gas production volume and methane content of digesters with olivine (Table 1).

The PC+0.75TE digesters (Table 1) had an average methane content of 76.3% over the course of the experiment (Table 2). The PC digester had a statistically lower average methane content of 75.9% (p-value 0.0008). The TE addition increased the final cumulative methane production volume (mL) by 4%; not significantly different by the paired t-test (p-value 0.5390, Figure 2A). The 5O+0.75TE digesters had an average methane content of 79.5% (p-value

<0.0001) compared to the PC digesters due to the 17.7% CO₂ sequestration from the olivine addition. However, there was only a 2.5% increase in the final methane production volume, which was not considered statistically significant (p-value 0.4574, Table 2). The most significant improvement in AD performance occurred with the 5O+1.5TE digesters, which exhibited both an increase in final methane production volume and CO₂ sequestration. The 5O+1.5TE digesters had an average methane content of 79.7% (p-value <0.0001) compared to the PC digesters resulting from an 18.7% CO₂ sequestration. The 5O+1.5TE digesters also had a 17.3% increase in the final methane production volume which was considered very significant statistically (p-value 0.0044). These results indicated that methane production volume from AD of sludge benefited from the higher concentration of Ni and Co addition. Trace elements addition has been reported to increase methane production volume by 40-65% for AD of food waste [7], methanol conversion by methanogenic sludge [3], AD of sulfur-rich stillage [2], and AD of agricultural residues [4]. Furthermore, both 5% w/v olivine digesters exhibited similar increases in methane content from olivine addition. As a comparison, in the previous experiment the 5% w/v olivine digesters without the addition of Ni/Co saw a similar 17.5% CO₂ sequestration but only a 1.2% increase in methane production volume [1].

Methane production rates were evaluated to further determine the impact of trace element addition on AD performance. First-order reaction kinetics were assumed [22, 24, 41] for fitting the methane production rate curves (Figure 2B) because sewage sludge is a heterogeneous substrate. Methane production volume data fitted appropriately into a simple exponential equation ($R^2 > 0.88$ for all conditions). The results show that the trace element addition combined with olivine addition enhanced the reaction rate constant (k -value) compared to the PC. The k -value for the PC was $0.302 \pm 0.002 \text{ day}^{-1}$ and was $0.310 \pm 0.002 \text{ day}^{-1}$ for 5O+0.75TE

and $0.320 \pm 0.002 \text{ day}^{-1}$ for 5O+1.5TE (p-value 0.024 and 0.002 respectively, Table 2); however, the trace element addition alone did not have an effect on the rate constant as shown by PC+0.75TE (k-value of $0.300 \pm 0.002 \text{ day}^{-1}$ and p-value 0.316). These results contrast with Gonzalez-Gil et al. (1999), which reported an increase in the initial exponential rate of biogas production during methanol conversion by methanogenic sludge due to a 1.3 mg/L Ni and Co trace element addition; however, this may be due to the difference in substrate [3]. The maximum potential methane production rate ($P_{\text{CH}_4, \text{max}}$) increased for the 5O+1.5TE digesters ($292.7 \pm 3.1 \text{ mL CH}_4/\text{day}$) compared to the PC ($246.7 \pm 1.3 \text{ mL CH}_4/\text{day}$) (p-value 0.0003, Table 2). This also indicates the synergetic impact of trace elements and olivine on AD of sludge.

Improved substrate utilization, indirectly measured by the increase in methane yield due to the increased efficiency is presented in Table 2. Statistical analysis shows that the methane yield based on Y_{CH_4} ($\text{mL CH}_4/\text{g COD}_{\text{degraded}}$) value is statistically larger for 5O+1.5TE ($481.6 \pm 11.7 \text{ mL CH}_4/\text{g COD}_{\text{degraded}}$, p-value 0.0041) compared to the PC ($309.4 \pm 7.1 \text{ mL CH}_4/\text{g COD}_{\text{degraded}}$) (Table 2). There is no statistical difference in the remaining comparisons, which may be due to the single replicate for gas production volume. The increased methane yield for 5O+1.5TE indicates that the combined effect of Ni/Co supplement and Fe addition from the olivine has a positive effect on the methanogen activity. Similarly, Zhang et al. (2015) reported that addition of trace elements in the combination of Fe (100 mg/L) + Co (1 mg/L) + Ni (5 mg/L) increased the methane yield from AD of food waste by 25% [5].

Optimization of the studied parameters (trace element addition, olivine addition, and digestion time) was elucidated using RSM. This statistical technique is a useful tool for optimization of AD when the response (increased methane production volume or content) may

be influenced by several variables. Previous research utilizing RSM includes parameters such as ratios of co-digestion feedstocks, change in volatile solid concentrations or change in specific influent concentrations such as ammonium or alkalinity [25-27, 42]. The RSM results indicated that the 2nd or 3rd order equations did not fit well with the experimental data because of the limits of response surface methodology as described in different applications. The limitation of RSM was explained by comparing quadratic, cubic and quartic models [43, 44]. The 4th order equations matched experimental data with a high degree of fit. Based on the ANOVA results, the following reduced quartic model was selected to describe the process:

Methane Gas Volume

$$mL = 24.26 + 165.78 * T - 24.21 * TE - 2.85 * O + 2.44 * T * TE + 3.37 * TE * O - 16.85 * T^2 + 0.72 * T^3 - 0.011 * T^4$$

Methane Content

$$\% = 73.79 + 1.07 * T + 0.79 * TE + 0.12 * O - 0.034 * T * TE - 0.13 * T^2 + 6.32 * 10^{-3} * T^3 - 9.90 * 10^{-5} * T^4$$

Where T represents digestion time (days), TE represents the trace elements addition (mg/L), and O represents olivine addition (g). According to the coefficient of each factor, digestion time had the greatest influence on the response for methane gas volume with trace elements addition and olivine addition having a negative impact; however, this is offset by the positive coefficient for the synergistic T*TE and TE*O terms. For methane content, digestion time, trace element addition and olivine addition all had a positive effect based on the coefficient of each term; however, the T*TE term indicates a negative impact on methane content indicating a consumption of the trace elements over time. The higher order operational parameters such as T², T³ and T⁴ in both equations indicated the limits of the RSM to model the experimental data over the length of the experiment. Furthermore, the p-value determined that all of the model

terms were significant ($p\text{-value} < 0.05$) except for the O term in the methane gas volume equation ($p\text{-value} 0.2493$) and the TE term in the methane content equation ($p\text{-value} 0.1230$); however, these terms are required to support the hierarchical structure of the model.

The coefficient of determination (R^2) was calculated as 0.9919 for methane gas volume (Figure 3A) and 0.9765 for methane content (Figure 3B), which indicates that only 0.81% and 2.35% of the total variation could not be elucidated from the regression model. Similar results ($0.93 < R^2 < 0.99$) were obtained from other RSM analyses [25-27, 32]. The adjusted coefficient of determination ($R^2_{\text{adj}} = 0.9885$ for methane gas volume and $R^2_{\text{adj}} = 0.9683$ for methane content) closely matches the determination coefficient (R^2) indicating the models ability to adequately estimate the experimental data with a high degree of fit. This further indicates the fine adjustment considering this size sample and model component number [27]. The F-value was 292.34 for methane gas volume and 118.92 for methane content. Previously reported RSM models gave F-values ranging from 5.3 to 153.7 [25, 27, 32] indicating an acceptable model accuracy and high degree of fit. It should be also noted that verification experiments need to be conducted under optimal conditions to compare predicted values and actual values of dependent variables. Future studies include the verification of optimal conditions.

The interactive effects of the independent variables on methane gas volume and methane content from the MO optimization were illustrated by four-dimensional plots (Figure 3C and 3D, respectively). The three variables are indicated on the three axes of the plot and the response is indicated by the colored dot. The results show that trace element addition, digestion time and olivine dosage all has a positive effect on methane gas volume. It should be noted optimum olivine dose determined by Linville et al. 2016 [1] was used in the experiments. According to the MO optimization modeling results, the maximum methane production volume (802 mL, Figure

3C) can be obtained with 25.5 days digestion time (x-axis), 1.5 mg/L trace elements addition (y-axis) and 27.5 g olivine addition (5% w/v)(z-axis) which had a corresponding methane content of 79.6%. Similarly, the maximum methane content (80.7%, Figure 3D) can be obtained with 6.0 days digestion time, 1.5 mg/L trace elements addition and 27.5 g olivine addition (5% w/v); however, the corresponding methane volume was only 601 mL due to the early digestion time. The maximum methane content occurred during the early phase of the digestion due to magnesium carbonate crystal growth on the surface of the olivine blocking the active site [1]. The results from the MO optimization indicate that the optimum was not in the designated boundaries of this study for trace element addition and olivine addition which is similar to the study by Gonzalez – Fernandez et al. (2011) [25]. These results corroborate the hypothesis that the increase in methane production volume is a function of trace element addition and olivine addition and the increase in methane content is a function of CO₂ sequestration from the olivine addition. In other words, the results indicates that the olivine addition is responsible for physical/chemical biogas upgrading, while the trace element addition and olivine addition is associated with a synergistic increased microbial activity of the methanogens presumably due to the enhancement of *mcrA* transcripts level [6].

The previously determined benefits of the olivine elevated bag design were also observed in this experiment. The metal concentrations for iron, magnesium, and silicon for the 5O+0.75TE and 5O+1.5TE digesters were statistically higher compared to the PC digesters (Table 3). Manganese was also statistically greater in the 5O+1.5TE digester compared to the PC digesters (Table 3). Olivine addition also provides Fe to support metabolic activities of the methanogens, which may have further enhanced the synergistic effect of Ni and Co [5]. It should be noted that Fe concentration in the digesters increased by approximately 140 mg/L after addition of olivine

(Table 3) and was the same in all olivine supplemented digesters. Furthermore, there is no statistical difference in the Fe concentration in the PC or PC+0.75 digesters compared to the initial samples. It is also important to note that the total alkalinity of the initial samples was 2.1g/L which is in the reported desirable range (2.0-5.0 g/L as CaCO_3) for digester operations [45], and therefore, minimizes the benefit of the olivine addition. The impact of the treatment process would be more significant if the alkalinity in the control digesters was limiting. The metabolism-generated alkalinity from the microbes also contributed to the increase in total alkalinity [45]. As the microbes degrade nitrogenous organics (proteins) into ammonium, ammonium bicarbonate alkalinity is formed inside the cell which in turn maintains a pH close to neutral inside the cells [46, 47]. There was no statistical difference for any of the test conditions compared to the PC digester for final ammonia nitrogen concentrations, the final alkalinity concentration was statistically higher in the 5O+1.5 digesters compared to the PC digester. Although, the VFA concentration was not measured during the experiment, the results may indicate that some portions of the metabolic alkalinity was used for neutralizing the increase in VFA concentration during the AD of sludge [48].

The optimal concentrations of trace elements were only in partial agreement with previous researches [2, 3, 5, 7] due to the differences in characteristics of substrates and inoculums and digester operating conditions. The results of its study clearly showed the positive synergistic effects of trace element and olivine addition on methane production volume and content from sewage sludge.

Conclusion

The addition of Ni and Co alone did not have an impact on the methane production volume, initial exponential methane production rate and substrate utilization during AD of

sludge. However, a synergistic effect between the trace element addition and the olivine addition to increase the methane production volume and methane content by CO₂ sequestration was observed. The best results occurred in the 5% w/v olivine + 1.5 mg/L Ni/Co digester at approximately 25 days digestion time as indicated by the RSM results. The 5% w/v olivine + 1.5 mg/L Ni/Co digesters had a 17.3% increase in total methane production volume, a 6% increase in initial exponential rate and a 56% increase in methane yield (mL CH₄/g COD_{degraded}). There was also a 5.1% increase in methane content resulting from a 17.7% CO₂ sequestration compared to the control. The increased methane production volume and rate due to addition of trace elements could amplify the benefits of the CO₂ sequestration from olivine at WWTPs making the process more economical and efficient.

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353 **References**

- 354 [1] J.L. Linville, Y. Shen, M. Urgan-Demirtas, S.W. Snyder, Effect of particle size and doses of
 355 olivine addition on carbon dioxide sequestration during anaerobic digestion of sewage sludge at
 356 ambient and mesophilic temperatures, *Process Biochemistry* 51 (2016) 59-72.
- 357 [2] J. Gustavsson, S.S. Yekta, C. Sundberg, A. Karlsson, J. Ejlertsson, U. Skjellberg, B.H.
 358 Svensson, Bioavailability of cobalt and nickel during anaerobic digestion of sulfur-rich stillage for
 359 biogas formation, *Applied Energy* 112 (2013) 473-477.
- 360 [3] G. Gonzalez-Gil, R. Kleerebezem, G. Lettinga, Effects of nickel and cobalt on kinetics of
 361 methanol conversion by methanogenic sludge as assessed by on-line CH₄ monitoring, *Applied*
 362 *and Environmental Microbiology* 65(4) (1999) 1789-1793.
- 363 [4] B. Demirel, P. Scherer, Trace element requirements of agricultural biogas digesters during
 364 biological conversion of renewable biomass to methane, *Biomass & Bioenergy* 35(3) (2011)
 365 992-998.
- 366 [5] W. Zhang, L. Zhang, A. Li, Enhanced anaerobic digestion of food waste by trace metal
 367 elements supplementation and reduced metals dosage by green chelating agent [S, S]-EDDS
 368 via improving metals bioavailability, *Water Research*, 2015, pp. 266-277.
- 369 [6] B. Uenal, V.R. Perry, M. Sheth, V. Gomez-Alvarez, K.-J. Chin, K. Nusslein, Trace elements
 370 affect methanogenic activity and diversity in enrichments from subsurface coal bed produced
 371 water, *Frontiers in Microbiology* 3 (2012).
- 372 [7] V. Facchin, C. Cavinato, P. Pavan, D. Bolzonella, Batch and Continuous Mesophilic
 373 Anaerobic Digestion of Food Waste: Effect of Trace Elements Supplementation, *Icheap-11: 11th*
 374 *International Conference on Chemical and Process Engineering*, Pts 1-4 32 (2013) 457-462.
- 375 [8] USEPA, RFS Renewable Identification Number (RIN) Quality Assurance Program; Final
 376 Rule, 40 CFR Part 80, United States Environmental Protection Agency, Washington, DC, 2014.
- 377 [9] US Environmental Protection Agency, Clean Watersheds Needs Survey 2008 - Report to
 378 Congress, US Environmental Protection Agency, Washington, DC, 2010.
- 379 [10] L. Lombardi, E. Carnevale, Economic evaluations of an innovative biogas upgrading
 380 method with CO₂ storage, *Energy* 62 (2013) 88-94.
- 381 [11] Y. Shen, J.L. Linville, M. Urgan-Demirtas, M.M. Mintz, S.W. Snyder, An overview of biogas
 382 production and utilization at full-scale wastewater treatment plants (WWTPs) in the United
 383 States: challenges and opportunities towards energy-neutral WWTPs, *Renewable &*
 384 *Sustainable Energy Reviews* 50 (2015) 346-362.
- 385 [12] M. Beil, W. Beyrich, U. Holzhammer, T. Krause, Biomethane, Agency for Renewable
 386 Resources, Gultow-Prutzen, Germany, 2013.
- 387 [13] S. Kerisit, E.J. Bylaska, A. Felmy, Water and carbon dioxide adsorption at olivine surfaces,
 388 *Chemical Geology* 359 (2013) 81-89.
- 389 [14] D. Daval, I. Martinez, J. Corvisier, N. Findling, B. Goffe, F. Guyot, Carbonation of Ca-
 390 bearing silicates, the case of wollastonite: Experimental investigations and kinetic modeling,
 391 *Chemical Geology* 265(1-2) (2009) 63-78.
- 392 [15] A.H.A. Park, R. Jadhav, L.S. Fan, CO₂ mineral sequestration: Chemically enhanced
 393 aqueous carbonation of serpentine, *Canadian Journal of Chemical Engineering* 81(3-4) (2003)
 394 885-890.
- 395 [16] O.S. Pokrovsky, J. Schott, Kinetics and mechanism of forsterite dissolution at 25 degrees C
 396 and pH from 1 to 12, *Geochimica Et Cosmochimica Acta* 64(19) (2000) 3313-3325.
- 397 [17] J.H. Ahn, T.H. Do, S.D. Kim, S. Hwang, The effect of calcium on the anaerobic digestion
 398 treating swine wastewater, *Biochemical Engineering Journal* 30(1) (2006) 33-38.

- [18] E.P.A. Van Langerak, H. Ramaekers, J. Wiechers, A.H.M. Veeken, H.V.M. Hamelers, G. Lettinga, Impact of location of CaCO₃ precipitation on the development of intact anaerobic sludge, *Water Research* 34(2) (2000) 437-446.
- [19] R.E.F. Lindeboom, I. Ferrer, J. Weijma, J.B. van Lier, Silicate minerals for CO₂ scavenging from biogas in Autogenerative High Pressure Digestion, *Water Research* 47(11) (2013) 3742-3751.
- [20] C. Tada, Y. Yang, T. Hanaoka, A. Sonoda, K. Ooi, S. Sawayama, Effect of natural zeolite on methane production for anaerobic digestion of ammonium rich organic sludge, *Bioresource Technology* 96(4) (2005) 459-464.
- [21] I.M. Power, S.A. Wilson, D.P. Small, G.M. Dipple, W. Wan, G. Southam, Microbially Mediated Mineral Carbonation: Roles of Phototrophy and Heterotrophy, *Environmental Science & Technology* 45(20) (2011) 9061-9068.
- [22] H.N. Gavala, I. Angelidaki, B.K. Ahring, Kinetics and modeling of anaerobic digestion process, *Adv Biochem Eng Biotechnol* 81 (2003) 57-93.
- [23] M. Brule, H. Oechsner, T. Jungbluth, Exponential model describing methane production kinetics in batch anaerobic digestion: a tool for evaluation of biochemical methane potential assays, *Bioprocess and Biosystems Engineering* 37(9) (2014) 1759-1770.
- [24] H. Siegrist, D. Vogt, J.L. Garcia-Heras, W. Gujer, Mathematical model for meso- and thermophilic anaerobic sewage sludge digestion, *Environmental science & technology* 36(5) (2002) 1113-1123.
- [25] C. Gonzalez-Fernandez, B. Molinuevo-Salces, M. Cruz Garcia-Gonzalez, Evaluation of anaerobic codigestion of microalgal biomass and swine manure via response surface methodology, *Applied Energy* 88(10) (2011) 3448-3453.
- [26] A. Ahmad, S.M.U. Shah, A. Buang, M.F. Othman, M.A. Abdullah, Evaluation of aerobic and anaerobic co-digestion of *Tetraselmis suecica* and Oil Palm Empty Fruit Bunches by Response Surface Methodology, *Micro/Nano Science and Engineering* 925 (2014) 243-247.
- [27] J. Jimenez, Y. Guardia-Puebla, O. Romero-Romero, M.E. Cisneros-Ortiz, G. Guerra, J.M. Morgan-Sagastume, A. Noyola, Methanogenic activity optimization using the response surface methodology, during the anaerobic co-digestion of agriculture and industrial wastes. *Microbial community diversity, Biomass & Bioenergy* 71 (2014) 84-97.
- [28] M. Walker, Y. Zhang, S. Heaven, C. Banks, Potential errors in the quantitative evaluation of biogas production in anaerobic digestion processes, *Bioresource Technology* 100(24) (2009) 6339-6346.
- [29] A.P.H.A. (APHA), A.W.W.A. (AWWA), W.E.F. (WEF), Standard methods for the examination of water and wastewater, 20th ed., American Public Health Association, Washington, D.C., 1998.
- [30] US Environmental Protection Agency, Method 200.7, Determination of Metals and Trace Elements in Water and Wastes by Inductively Coupled Plasma-Atomic Emission Spectrometry (Revision 4.4 EMMC Version), US Environmental Protection Agency, Cincinnati, OH, 2007.
- [31] US Environmental Protection Agency, Method 200.8, Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma - Mass Spectrometry (Revision 5.4 EMMC Version), US Environmental Protection Agency, Cincinnati, OH, 2007.
- [32] R.C. Jin, B.S. Xing, W.M. Ni, Optimization of partial nitrification in a continuous flow internal loop airlift reactor, *Bioresource Technology* 147 (2013) 516-524.
- [33] H.C. Causton, B. Ren, S.S. Koh, C.T. Harbison, E. Kanin, E.G. Jennings, T.I. Lee, H.L. True, E.S. Lander, R.A. Young, Remodeling of yeast genome expression in response to environmental changes, *Molecular Biology of the Cell* 12(2) (2001) 323-337.
- [34] C. Abendroth, C. Vilanova, T. Guenther, O. Luschig, M. Porcar, Eubacteria and archaea communities in seven mesophile anaerobic digester plants in Germany, *Biotechnology for Biofuels* 8 (2015).

- [35] Y. Yang, K. Yu, Y. Xia, F.T.K. Lau, D.T.W. Tang, W.C. Fung, H.H.P. Fang, T. Zhang, Metagenomic analysis of sludge from full-scale anaerobic digesters operated in municipal wastewater treatment plants, *Applied Microbiology and Biotechnology* 98(12) (2014) 5709-5718.
- [36] S.G. Shin, S. Lee, C. Lee, K. Hwang, S. Hwang, Qualitative and quantitative assessment of microbial community in batch anaerobic digestion of secondary sludge, *Bioresource Technology* 101(24) (2010) 9461-9470.
- [37] C. Sundberg, W.A. Al-Soud, M. Larsson, E. Alm, S.S. Yekta, B.H. Svensson, S.J. Sorensen, A. Karlsson, 454 pyrosequencing analyses of bacterial and archaeal richness in 21 full-scale biogas digesters, *Fems Microbiology Ecology* 85(3) (2013) 612-626.
- [38] J. Guo, Y. Peng, B.-J. Ni, X. Han, L. Fan, Z. Yuan, Dissecting microbial community structure and methane-producing pathways of a full-scale anaerobic reactor digesting activated sludge from wastewater treatment by metagenomic sequencing, *Microbial Cell Factories* 14 (2015).
- [39] VDI, VDI 4630: Fermentation of organic materials - Characterisation of the substrate, sampling, collection of material data, fermentation tests, Verein Deutscher Ingenieure (Association of German Engineers), Verein Deutscher Ingenieure, Düsseldorf, Germany, 2006.
- [40] K. Koch, J.E. Drewes, Alternative approach to estimate the hydrolysis rate constant of particulate material from batch data, *Applied Energy* 120 (2014) 11-15.
- [41] M. Brule, H. Oechsner, T. Jungbluth, Exponential model describing methane production kinetics in batch anaerobic digestion: a tool for evaluation of biochemical methane potential assays, *Bioprocess and Biosystems Engineering* 37 (2014) 1759-1770.
- [42] D. Montgomery, Design and analysis of experiments, John Wiley, US, 2005.
- [43] L. Casettari, R. Mosca, R. Revetria, F. Rolando, Effectiveness and limits of response surface methodology in application to discrete and stochastic simulation of manufacturing plants, *Appl Math Sci* 7 (2013) 4137-4172.
- [44] B. Zhao, S.X. Wang, J. Xing, K. Fu, J.S. Fu, C. Jang, Y. Zhu, X.Y. Dong, Y. Gao, W.J. Wu, J.D. Wang, J.M. Hao, Assessing the nonlinear response of fine particles to precursor emissions: development and application of an extended response surface modeling technique v1.0, *Geosci Model Dev* 8(1) (2015) 115-128.
- [45] Metcalf, Eddy, Wastewater Engineering: Treatment and Reuse, 4th ed., McGraw-Hill, Boston, 2003.
- [46] R. Speece, Anaerobic Biotechnology for Industrial Wastewater, Archae Press, Nashville, TN, 1996.
- [47] O.N. Agdag, D.T. Sponza, Effect of alkalinity on the performance of a simulated landfill bioreactor digesting organic solid wastes, *Chemosphere* 59(6) (2005) 871-879.
- [48] P. Zhang, Y. Chen, Q. Zhou, X. Zheng, X. Zhu, Y. Zhao, Understanding short-chain fatty acids accumulation enhanced in waste activated sludge alkaline fermentation: Kinetics and microbiology, *Environmental science & technology* 44(24) (2010) 9343-9348.

Figure 1. Schematic of A) challenge system digesters, B) improved flow pattern with the stainless steel bag.

Figure 2. Time-course profiles of anaerobic digestion experiments: (A) cumulative methane production; (B) methane production rate (mL/day). Data are means of replicates and error bars show standard deviations.

Figure 3. Actual vs. predicted values for reduced cubic models of (A) methane gas production (mL) and (B) methane content (%), and four-dimensional (4D) response surface showing the effects of interactions on AD under mesophilic conditions (37 °C) for three factors; x-axis represents digestion time (days), y-axis represents trace element addition (mg/L), z-axis represents olivine addition (g), and the color represents the response of (C) methane gas production (mL) and (D) methane content (%).

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510 **Table 1. Experimental Design Matrix**

Experimental Design (number of replicates)	Acronym ^a
Positive Control (2)	PC
Positive Control with 0.75 mg/L Ni/Co (3)	PC+0.75TE
5% w/v fine olivine with 0.75 mg/L Ni/Co (3)	5O+0.75TE
5% w/v fine olivine with 1.5 mg/L Ni/Co (3)	5O+1.5TE

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512 ^aPC = positive control, 5O = 5% olivine, TE = trace elements, the combination of Ni+Co.

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Table 2. Averaged methane content over the course of the experiment (%), final methane production volume (mL), maximum methane production rate (PCH_{max}), reaction rate constant (k) and methane yield (YCH) for different AD conditions

	Averaged methane content	Final methane production volume	PCH_{max}	k	YCH
AD condition	(%)	(mL)	(mL CH ₄ /day)	(day ⁻¹)	(mL CH ₄ /g COD _{degraded})
PC	75.9 ± 0.7	661.6	246.7 ± 1.3	0.302 ± 0.002	309.4 ± 7.1
PC+0.75TE	76.3 ± 0.8	688.3	243.1 ± 1.8	0.300 ± 0.002	306.8 ± 6.9
5O+0.75TE	79.5 ± 0.7	678.2	244.3 ± 1.4	0.310 ± 0.002	282.5 ± 5.4
5O+1.5TE	79.7 ± 0.5	775.8	292.7 ± 3.1	0.320 ± 0.002	481.6 ± 11.7

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526 **Table 3. Initial (Time = 0 days) and final sample analysis of digester content.**

	Initial Value	PC	PC+0.75	5FO+0.75	5FO+1.5
TS (%)	1.43%	1.20%	1.22%	1.51%	1.61%
VS (%)	0.75%	0.56%	0.57%	0.57%	0.63%
Chemical Oxygen Demand (g/L COD)	13.6	9.7	9.5	9.2	10.7
Total Alkalinity (g/L as CaCO ₃)	2.1	2.8	3.2	3.3	3.7
Total Organic Carbon (g/L)	2.4	1.0	1.5	1.2	1.1
Ammonia Nitrogen (g/L as NH ₃ ⁻)	0.87	0.86	0.85	0.78	0.82
Iron (mg/L)	140	170	160	293	303
Magnesium (mg/L)	190	180	177	857	773
Manganese (mg/L)	5.9	6.0	5.8	7.8	8.4
Silicon (mg/L)	82	115	120	433	397

527 (For initial samples N=3. For final samples N=2 for PC and N=3 for test sample). Values
528 highlighted in yellow are statistically different than the positive control final samples by paired t-
529 test (p-value not shown).

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