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Effect of trace element supplementation on the mesophilic anaerobic digestion of foodwaste in batch trials: the influence of inoculum origin

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Abstract

Batch anaerobic trials using a source-separated food waste as a substrate with inoculums of different origins were carried out under mesophilic conditions. Reactions were operated both with and without trace element (Co, Mo, Ni, Se, W) supplementation. Supplementation with trace metals had either neutral or slightly negative effects with inoculums originating from reactors with a high background level of metals, such as those for the co-digestion of biowaste and waste activated sludge. For inoculums from reactors treating food waste only, which inherently contain low levels of trace metals, supplementation with these metals increased methane production. In particular, Mo concentrations in the range of 3-12 mg per kg dry matter and Se concentrations of 10 mg per kg dry matter increased methane production to as high as 30-40%. Supplementation with a metal mixture (Co, Mo, Ni, Se and W) increased the methane production to the range 45-65% for inoculums with low background concentrations of trace metals. These findings may have an important impact in the commercial production of methane from food waste.

Keywords anaerobic digestion, food waste, inoculum, mesophilic, trace elements

1. Introduction

The introduction of separated collection for different fractions of municipal solid waste (MSW), in addition to subsidies for renewable energy production, have been the main drivers for the implementation of the anaerobic digestion (AD) of biowaste in recent years [1]. Food/kitchen waste originating from door-to-door collections contains low levels of inert materials (plastic, glass, stones …) and is a suitable substrate for AD, enabling biogas productions in the range 160-180 m^3 per tonne of raw waste treated [2]. Based on this figure, complete anaerobic digestion of the biowaste collected in the EU-27 area could generate approximately 35,500 MWh per day of electric power, assuming an organic material collection rate of 0.2 kg per person per day [3]. This increased biogas generation would contribute significantly to the production of renewable energy and the mitigation of greenhouse gas (GHG) emissions.

Food and kitchen waste generally contains low concentrations of trace elements, especially metals:, this may lead to the failure of the anaerobic digestion process [3]. Several specific trace metals, including Co, Ni, W, Se and Mo, are essential for the enzyme cofactors involved in the biochemistry of methane formation and are needed in a balanced anaerobic digestion process [4]. Limiting the metals required by the enzymes may disturb the total process. As reported in literature, Co [5,6,7] Mo, [8,9], Ni [10,11,12,13,14], Se [15,16] and W [17,18] are all involved in the methane production biochemical process.

The methanogenic requirement for trace elements, both for acetoclastic and hydrogenotrophic microorganisms, is not fully understood, presenting a serious impediment for the commercial applications of anaerobic digestion processes, which fundamentally require process reliability.

Recently, both Demirer and Scherer [19] and Schattauer et al. [20] reviewed the issues involved with the lack of trace elements in the anaerobic digestion process. In the large body of literature reviewed by these authors, only a few papers reported on the effects of trace element supplementation on the anaerobic digestion of food waste or similar substrates. Several of these studies [3, 21-28] reported on the beneficial effects of the addition of multiple metal mixtures without delineating the supplementation effects for each individual metal, making it difficult to completely define the process characteristics. To further complicate this situation, the different trace metal additions were sometimes determined on a wet weight basis, while others were determined on a dry weight basis or on a volatile solids basis or a COD content or removal basis. Extensive studies in the literature also suggest that for digestive processes using source materials containing large amounts of metals, such as sludge or manure [20, 37], trace elements do not limit methane production.

In this study, we determined the effects of the addition of the trace metals Co, Ni, Mo, Se, and W, both as single elements and in mixed cocktails, for mesophilic anaerobic digestion batch trials using food waste as the only substrate. To assess the effects of different trace metal background levels on digestion performance, two sources of inocula were used in the experiments. The first source was from a reactor co-digesting waste-activated sludge and food waste. The second source was from a reactor treating only food waste.

2. Material and Methods

2.1 Experimental design

The effect of trace metals addition on methane production was studied using biochemical methane potential (BMP) trials. The substrate to inoculum ratio was kept at levels of approximately 0.3-0.4 on a VS basis, according to the guidelines given in Angelidaki et al. [29]. The tests were performed under mesophilic (37°C) conditions. At the end of the incubation period, pH, total and volatile solids, COD, total and partial alkalinity, ammonia and TKN measurements were performed according to the *Standard Methods for the Examination of Water and Wastewater* [47]. The total and soluble concentrations of the tested trace metals were determined according to EPA methods 6020A and 3051A. The volatile fatty acids (VFA) content was monitored using a gas chromatograph (Carlo Erba instruments), with hydrogen as the gas carrier, equipped with a Fused Silica Capillary Column (Supelco NUKOL^{TM}, 5 m x 0.53 mm x 0.5 mm film thickness) and a flame ionisation detector (200°C). The analysed samples were centrifuged and filtrated with a 0.45 µm membrane.

Based upon a survey of the literature, the effects of supplementation with the metals Co, Mo, Ni, Se, and W were examined. The amounts of the trace metals to be added were determined by the quantities of each metal typically found in food waste, taking into consideration the typical requirements reported in literature. Four concentration levels were used to characterise the process and determine whether there are possible threshold effects or an inhibition level. The supplementation concentrations were calculated on a dry mass basis.

The substrate used in this study was food waste (FW) collected in the Treviso municipality in north-eastern Italy. This material originated from a door-to-door collection scheme and was composed primarily of kitchen waste [30,31]. This food waste was minced and mixed to form a homogeneous mixture. The inoculums came from two different reactors: a mesophilic full-scale reactor [32] treating separately collected food waste and waste-activated sludge and a pilot scale reactor treating separately collected food waste only. Both the reactors operated under mesophilic conditions. The full-scale reactor operated with a typical hydraulic retention time of approximately 25 days with an organic loading rate of approximately 2 kgVS/m^3 per day. The organic load had approximately equal portions of food waste and waste-activated sludge. The pilot scale reactor was inoculated and operated with food waste only. The applied OLR was approximately 1.5 kgVS/m^3 per day with an HRT of approximately 40 days.

For the two inoculums, the specific methane activities (SMA) on acetate were similar, despite their differing origins and operational conditions. The biomass using the full-scale reactor inoculum (A) operated with an SMA in the range of 0.1-0.15 g COD per g VS per day, while the biomass using the pilot scale reactor inoculum had an SMA in the range of 0.12-0.18 g COD per g VS per day at 37°C. These values are consistent with the literature data [29].

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2.2 Determination of the amount of trace metal addition

The representative concentrations for Co, Mo, Ni, Se, and W in both the anaerobic biomass and the food waste are shown in Table 1. Comparing the levels required by anaerobic bacteria with the concentrations observed in food waste, the requirements for addition were calculated. The gap between these two values is shown in the last column of table 1.

The amounts of trace metals to be used in the batch tests were selected based upon data available in the literature, as reported in Table 1. The difference between the concentrations reported for anaerobic biomass and the concentration levels found in food waste was used to calculate the addition requirements. These calculated requirements were then adjusted to facilitate the weighing of the different salts to be supplemented in the batch trials. These values were set as the benchmark levels. Both lower and higher concentrations than the benchmark values were used to determine the possible presence of threshold effects for the tested elements.

Additional concentrations to be examined included values equal to 50, 100 and 200% of the calculated requirements. To stress the system, an additional concentration at 1000% of the baseline values, shown in Table 1, was also examined. As shown in Table 1, representative concentrations in anaerobic biomass reported for nickel, cobalt, and molybdenum were 11, 9 and 7 mg per kg of dry matter [33], respectively. The reported values for the selenium and tungsten concentrations were 1.5 [34] and < 0.1 mg per kg dry matter [21,25], respectively. The representative concentrations of these metals found in food waste were 2 mg/kgTS for Ni, 1 mg/kgTS for Mo, Se and W and 0.2 mg/kgTS for Co [3,21,25,35]. The trace metals were added in the form of the following salts: $NiCl_2 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $Na_2MoO_4 \cdot 2H_2O$, Na_2SeO_3, and $Na_2WO_4 \cdot 2H_2O$.

Metals were added both individually, to study the single addition effects, and in combination, to study the synergic effects.

3. Results and Discussion

3.1 Inoculums and substrate characterisation

The food waste and the two inoculums used in this experimentation were characterised for both macro parameters and trace metal concentrations. Table 2 shows the representative characteristics of the inoculums. Inoculum A originated from an anaerobic reactor treating food waste and waste-activated sludge. Inoculum B originated from an anaerobic reactor treating food waste only.

The measurements for the macro-parameters, including total and volatile solids, COD and nutrients, are representative of this type of waste [2,3]. Additionally, the trace metal contents were consistent with those reported by the DEFRA [3]. Co, Mo and Se were below the limit of detection of 2, 2 and 1 mg/kgTS, respectively, while Ni showed concentrations greater than those found in previous studies on food waste. In our study, the Ni concentrations averaged 9.6 mg/kgTS, which is appreciably higher than values reported in previous studies that ranged from levels below the limit of detection [25,36] to 2 mg/kgTS [35]. This increased value of Ni may be due to a Ni contamination occurring during the collection and transportation in containers or trucks.

The characteristics of inoculum A and B differed appreciably. Inoculum A, originating from a reactor treating both food waste and waste activated sludge, showed greater levels of total and volatile solids, a very low level of volatile fatty acids, and a relatively high concentration of trace metals. Compared with the representative values found in inoculum B, these concentrations were typically two to four times higher for most trace metals and up to ten times for Se.

This difference can be ascribed to the presence of waste activated sludge in the reactor feed. Clearly, inoculum B, which originated from a reactor treating food waste as the sole substrate, showed lower levels of contamination. Se, in particular, was lower than the limit of detection of 1 mg/kgTS. The values found for inoculum B were similar to those values characterising food waste, the only substrate fed to this reactor. Only Ni and Mo showed greater concentrations in inoculum B than in the food waste.

3.2 Methane production in the batch trials

The specific methane production values (as m^3 per kgVS fed) at 37°C measured using the BMP tests and carried out in the different conditions using inoculum A and B are reported in Table 3. The values shown in Table 3 are the averages from the batch bottles experiments measured in triplicate and the relative standard deviations.

Using inoculum A, the digestion of food waste as the sole substrate generated approximately 0.76 cubic meter of biogas per kilogram of VS added (57% CH_4). With the exception of a Se addition, the supplementation with Co, Mo, Ni, and W gave no or negative responses. The observed values for the specific methane production were consistently lower than ones observed in the control (see data in Table 3 and Figure 1). Compared with the control, adding a mix of metals resulted in reduced methane production, regardless of the concentrations tested. Only the addition of Se, up to 2 mg per kg of dry matter, improved methane production. This observed increase was approximately 10%, a value that is insufficient to justify the addition of this metal at large scale.

The effects of the trace elements supplementation are shown in Figure 1. For the most part, the trace metals supplementation had a negative effect. The additions of Co, Mo, and W showed consistently negative responses, including decreases in methane production as high as 20% for Mo and W at 60 and 10 mg/kgTS, respectively. The addition of Ni resulted in an almost imperceptible improvement for a dose of 5 mg/kgTS and a negative response for higher doses. The only trace element to improve methane production was Se, whose addition resulted in an increase in production for doses of 0.5, 1 and 2 mg/kgTS. At the high dose of 10 mg/kgTS, Se decreased methane production. The trace elements cocktails also decreased methane production.

This work suggests that the supplementation of trace metals to a biomass already containing relatively high background levels of trace elements, such as the inoculum originating from the co-digestion plant, does not improve methane production. These data showed toxic effects with a clear decrease in methane production. Increasing metal concentrations decreased methane production. These results are consistent with those reported by Ishaq et al. [37] indicating that the supplementation of unneeded metals may have a negative effect on acetoclastic methanogenesis or on the other metabolic pathways of an anaerobic digestion process.

For the batch trials using inoculum B, the addition of any metal or mix of metals was beneficial, with high variability. Several dose levels (20 mg/kgTS for Ni, 100 mg/kgTS for Co, 2 mg/kgTS for Se, and 10 mg/kgTS for W) improved methane production by approximately 10%. Other dosages, including Mo in the range 3-12 mg/kgTS, Se at 10 mg/kgTS and the metals mix supplementation, consistently improved methane production, with the metals mix reaching improvement levels in the range of 60-70% (see data in Table 3 and Figure 2).

In these experiments, a threshold effect could be observed. Supplementing the digestion at 10 times greater than the baseline levels gave the best results for the Co, Ni, Se, and W additions. There was also a synergistic effect from the addition of all the trace metals (see Figure 2).

Both for W and Se, the addition of 10 mg/kgTS determined an incease in methane production of approximately 10% compared to range of 0.5 - 2 mg/kgTS (see Figure 2).

The results for Se were consistent with those found for the tests with inoculum A. Considering the different background levels of Se in the two inoculums (9.1 and < 1 mg/kgTS for inoculum A and B, respectively), Se addition was beneficial with a total concentration of approximately 10 mg/Kg TS in the batch trials. Only for inoculum A the "threshold effect" was observed, indicating that there were limits to the concentration needed to ensure an increase in methane production.

Experiments testing the addition of the trace metals mix at levels of 50%, 100% and 200% of the basic requirements, shown in Table 1, were also performed. Supplementation with the cocktail resulted in increased methane production from 45% to 67% of the SMP found for the control production values (see Table 3). These results have the potential to significantly improve the commercial application of the anaerobic digestion process for food waste, as these improvement levels can positively affect the process efficiency and improve the economic balance of a given treatment plant.

To clarify the contribution of molybdenum to methane production for inoculum B, batch trials both with and without Mo addition in the trace metals mix tests were carried out (Figure 3). The concentrations of the metal mix, both with and without Mo, were 100% of the typical requirement for anaerobic biomass (Table 1). The methane production values observed in the two cases, together with the control, are shown in Figure 3. Adding Mo to the metals mix increased the methane production from 1005 ml ± 16 to 1288 ml ± 41, confirming the fundamental role exerted by this element.

These results partially confirmed the previous findings of other researchers. Hinken et al. [33] showed that trace element concentrations in biomass depend on several characteristics, including the operation time of the AD reactors, the amount of substrate and the type of substrate feed. Trace element concentrations depend on the history of the biomass plant. As a consequence of the different trace metals supplementations, inoculum A and inoculum B demonstrated different trace metal requirements and different responses. The concentration of the trace elements of the different biomass sources should be analysed, and anaerobic batch tests should be carried out with adequate trace elements dosages. Supplementation with unneeded metals has a negative effect on methane production [37]. Supplemented at levels of 1000% of the trace metal requirements, inoculum A decreased methane production (Figure 1), demonstrating an unambiguous inhibitory effect.

Our results are also consistent with those of Lindorfer et al. [38]. An anaerobic biomass taken from a municipal sludge digester (co-digestion plant with foodwaste) contained sufficient metals content so that a further addition of metals did not significantly improve methane production. Selenium only marginally improved methane production. Biomass from a mono-digestion pilot plant treating only foodwaste had an insufficient trace metal content. In this case, any addition of the trace metals, alone or in combination (Ni, Co, Mo, Se, W together), improved methane production. For inoculum B, the experiments performed using 100% of the trace metal requirements, both with and without Mo addition, showed that Mo is a key trace element in the anaerobic methanation of food waste (Figure 2 and Figure 3).

The findings of this study are only in partial agreement with several previous studies on the effect of the supplementation of trace elements for the AD of food waste.

Banks et al. [3,28] concluded that selenium and cobalt are the key elements essential for long-term process stability and are present in insufficient quantities in foodwaste. Selenium in particular was found to be essential in promoting VFA removal under high ammonia concentrations. In particular, they reported that concentrations of Se and Co of 0.16 and 0.22 mg per kg food waste (wet weight), respectively, represented the minimal requirements at moderate digester loadings (< 3 kgVS/m^{3}day).

In the present study, Se was also found to be an essential trace metal, and its addition showed a positive effect on methane production for both inoculums (inoculum A and inoculum B), starting from dose levels of 0.5 mg/kgTS or 0.12 mg/kg food waste, a value equivalent to the one found in Banks et al [28]. The effect of Se, however, was found to be only relatively important. Co, on the other hand, made small contributions to methane production with a modest increase of approximately 10% at a very high dose (100 mg/kgTS).

Limited studies have been conducted on the effects of tungsten supplementation. Feng et al. [24] showed that the highest methane production for the anaerobic digestion of foodwaste was associated with the addition of Se and W in combination with Co. Our results indicate that tungsten is also important in methanogenesis. Inoculum A showed a sufficient W content (5.21 mg/kgTS), and tungsten addition did not improve methane production. Conversely, inoculum B contained an insufficient amount of W (2.68 mg/kgTS), and the supplementation of 10 mg/kgTS tungsten resulted in an 10.3 % improvement in the methane production on average.

Both Banks et al. [3,28] and Feng et al. [24] reported that cobalt is as an important metal for methanogenesis. Our results indicate that inoculum A, with a 7.4 mgCo/kgTS content, contained a sufficient level of cobalt. Inoculum B with 2.9 mgCo/kgTS has cobalt deficiencies that were remedied using a 100 mgCo/kgTS addition, which increased the methane production by 11%, compared to the control test.

In another important study, Uemura [26] showed that nickel is the most important trace element for the anaerobic digestion of the organic fraction of municipal solid waste. In our study, a 100 mgNi/kgTS addition to inoculum B increased methane production by 15%, while the addition of the same concentration to inoculum A decreased methane production by 7%. The Ni content in inoculum A was twice the Ni content of inoculum B (Table 2). Several studies suggested that trace metals content at too high of a concentration are toxic for anaerobic biomass [34, 37].

However, this study demonstrated that Mo has a dramatic impact on the process. In reviewing the literature, only Lo et al. [25] showed that molybdenum addition over the broad concentration range of 0.044-52.94 mg/l had the potential to enhance the methane production from the organic fraction of municipal solid waste. Compared with the control and the other single metal supplementations (see Figure 2), Mo addition resulted in the largest improvement in methane production for inoculum B.

In addition to role of Mo in the biochemical reactions (Mo is present in the common enzyme formate dehydrogenase [9]), this trace metal is also used in the management of the anaerobic reactors. By inhibiting sulphur bacteria, Mo reduces the competition with the methanogenic bacteria [39]. The addition of molybdenum to the digesters has been reported to decrease the rate of sulphate reduction with a correspondingly lower sulphide level and increase the production of methane [40,41,42]. Mo inhibition of the sulphur reducing bacteria could result in a lower presence of HS^-, allowing a greater availability of the essential trace metals to the methanogens instead of being precipitated as insoluble sulphides.

To identify the possible fate of the trace metals added to the system, the presence of these elements in the liquid phase was measured at the very end of the batch trials. It was found that the levels of concentrations were particularly low, regardless of the initial addition level. In particular, selenium and tungsten were below the limit of detection of 0.01 mg/l. Molybdenum was approximately 0.01 mg/l. Cobalt was approximately 0.02 mg/l, and nickel was approximately 0.05 mg/l. Compared with the metal additions, these levels are clearly lower, suggesting that the supplemented metals disappeared. In addition to the obvious biological use, the metals may have precipitated or adsorbed onto the anaerobic biomass.

4. Conclusions

For the mesophilic anaerobic digestion of food waste, the addition of trace metals (Co, Mo, Ni, Se and W) demonstrated that in the batch trials used in our experiments, these metals could improve the methane production. Two inoculums, isolated from two sources containing different levels of metal concentrations, were studied to characterise the effects of the supplementation on reactor performance. For the inoculum from an anaerobic reactor treating food waste as the sole substrate, any level of supplementation was beneficial. When added at levels ranging from 3-12 mg/kgTS, Mo in particular showed a 43% improvement over the control system. The addition of cocktails containing all the trace metals improved reactor performance up to 67%.

The addition of trace metals to an inoculum containing high background levels of those trace metals failed to improve reactor performance.

The only trace metal to improve the methane production for both inoculums of differing origins was selenium.

These results suggest that either trace metal supplementation or the implementation of a co-digestion option to increase the availability of these elements could improve methane production at the industrial scale.

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5. References

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Figures & Tables

Table 1. Determination of trace metal addition in anaerobic digestion trials

Trace metals	Trace metals in anaerobic biomass, *mg/kg TS*	Trace metals in foodwaste, *mg/kg TS*	Trace metals addition used in this study, *mg/kg* TS_{in}*
Ni	11 [33,34,44,45, 46]	2 [21,35,36]	10
Co	9 [25,33,37,44,45,46]	0.2 [3,21,25]	10
Mo	7 [25,33,45]	1 [3,21,25,36]	6
Se	1.5 [4,34,45,]	1 [3,21]	1
W	<0.1 [3, 21,25]	1 [3,21,25]	1

* The amount of trace metals added were calculated based on VS content of substrate (in this case Organic Fraction of Municipal Solid Waste)

Table 2 : Characteristics of foodwaste, inoculum A, and inoculum B used in the batch trial

		FOODWASTE		**INOCULUM A**		**INOCULUM B**	
Parameter	**Units**	**Average**	**Standard deviation**	**Average**	**Standard deviation**	**Average**	**Standard deviation**
Total Solid	gTS/kg	278.6	± 1.9	41.2	± 1.2	12.5	± 0.5
Volatile Solid	gVS/kg	221.1	± 2.9	23.4	± 1.5	7.0	± 0.3
VS %TS	%	79.3	± 1.5	56.7	± 2.3	55.0	± 1.0
COD	mgCOD/gTS	980.45	± 27.91	629.76	± 8.43	571.50	± 8.06
Total Phosphorus	mg P /gTS	1.63	± 1.21	14.40	± 2.35	9.20	± 0.28
Total Nitrogen (as TKN)	mg TKN/gTS	30.00	± 12.76	41.90	± 2.99	42.80	± 1.84
N-NH$_3$	mg NH$_3$/l	386	± 4	640	± 49	884	± 44
pH		5.3	± 0.1	7.6	± 0.1	8.3	± 0.1
Partial Alkalinity	mgCaCO$_3$/l	nd	nd	5339	± 24	5040	± 24
Total Alkalinity	mgCaCO$_3$/l	5094	± 29	7343	± 35	7767	± 24
Volatile Fatty Acids	mgCOD/l	8784	± 400	144	±100	2500	± 100
Nickel (soluble)	mg/l	0.54	± 0.01	0.05	± 0.01	0.05	± 0.01
Nickel (total)	mg/kgTS	9.6	± 2	47.0	± 2	24.2	± 2
Cobalt (soluble)	mg/l	0.053	± 0.005	0.018	± 0.005	< 0.005	nd
Cobalt (total)	mg/kgTS	< 2	nd	7.4	± 2	2.9	± 2
Molybdenum (soluble)	mg/l	0.10	± 0.01	< 0.01	nd	< 0.01	nd
Molybdenum(total)	mg/kgTS	< 2	nd	15.9	± 2	4.0	± 2
Selenium (soluble)	mg/l	0.32	± 0.01	0.02	± 0.01	< 0.01	nd
Selenium (total)	mg/kgTS	< 1	nd	9.1	± 1	< 1	nd
Tungsten (soluble)	mg/l	< 0.1	nd	< 0.1	nd	< 0.1	nd
Tungsten (total)	mg/kgTS	1	± 1	5.2	± 1	2.7	± 1

Table 3. Specific Methane Production. SMP: cubic metres of methane produced per kilogram of volatile solids present in the substrate added

			INOCULUM A		**INOCULUM B**	
Metal added	**Concentration**	**Units**	**Average**	**Standard deviation**	**Average**	**Standard deviation**
Control		m^3/KgVS$_{in}$	0.434	± 0.040	0.338	± 0.030
Ni	5mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.451	± 0.011	0.365	± 0.004
Ni	10mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.424	± 0.006	0.367	± 0.011
Ni	20mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.425	± 0.007	0.380	± 0.003
Ni	100mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.407	± 0.008	0.390	± 0.001
Co	5mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.426	± 0.004	0.344	± 0.004
Co	10mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.419	± 0.010	0.343	± 0.021
Co	20mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.415	± 0.014	0.342	± 0.007
Co	100mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.403	± 0.009	0.376	± 0.006
Mo	3mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.434	± 0.002	0.481	± 0.041
Mo	6mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.433	± 0.003	0.483	± 0.043
Mo	12mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.427	± 0.006	0.470	± 0.042
Mo	60mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.346	± 0.003	0.372	± 0.003
Se	0.5mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.459	± 0.011	0.384	± 0.003
Se	1mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.445	± 0.011	0.380	± 0.001
Se	2mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.493	± 0.030	0.378	± 0.004
Se	10mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.393	± 0.010	0.430	± 0.018
W	0.5mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.405	± 0.010	0.347	± 0.002
W	1mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.407	± 0.009	0.345	± 0.006
W	2mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.403	± 0.009	0.347	± 0.001
W	10mg/kgTS$_{in}$	m^3/KgVS$_{in}$	0.346	± 0.008	0.374	± 0.002
Metal mix	50% of typical requirement	m^3/KgVS$_{in}$	0.405	± 0.005	0.487	± 0.033
Metal mix	100% of typical requirement	m^3/KgVS$_{in}$	0.418	± 0.005	0.566	± 0.018
Metal mix	200% of typical requirement	m^3/KgVS$_{in}$	0.397	± 0.009	0.543	± 0.054

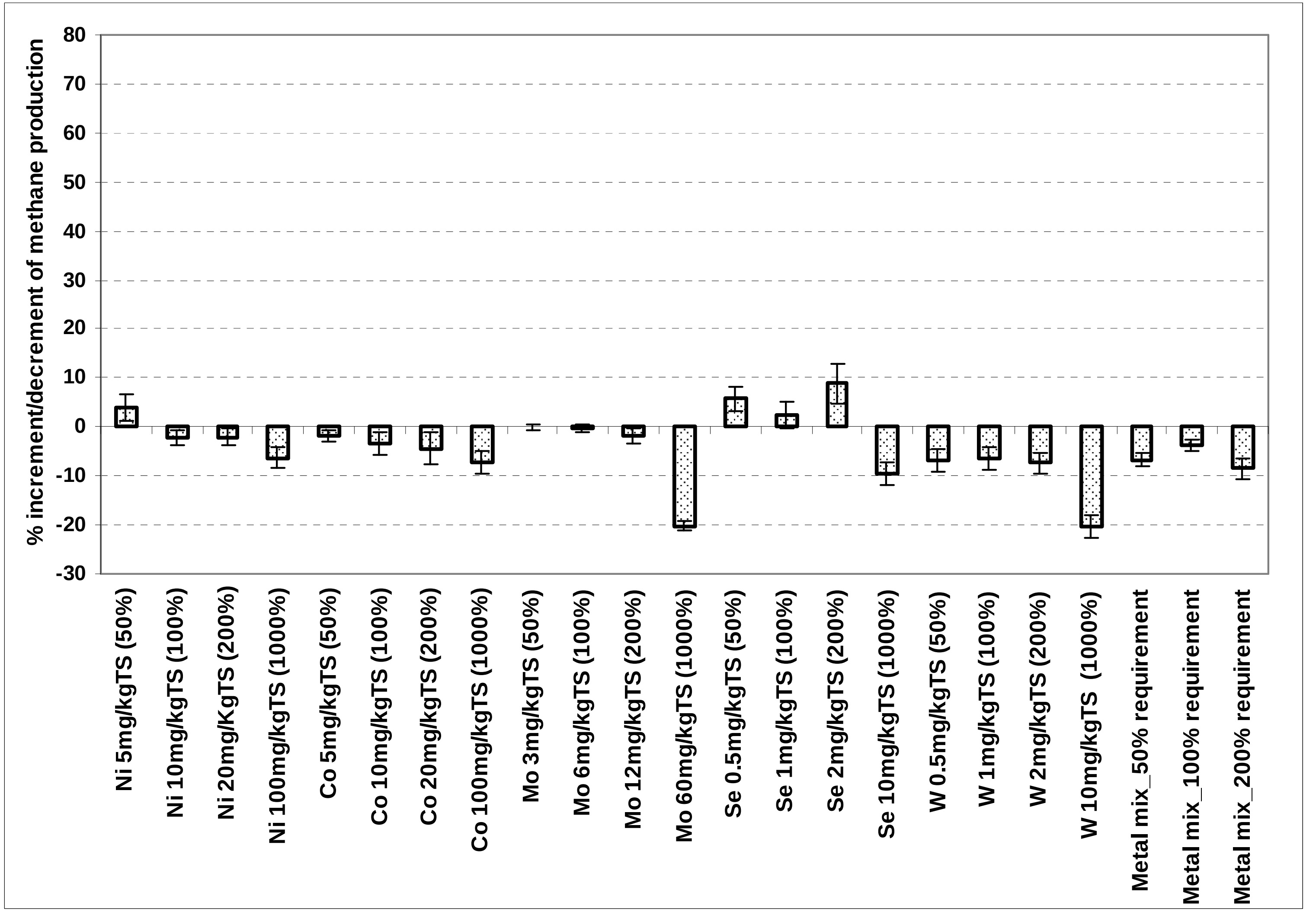

Figure 1. Histogram of increment or decrement on methane production of different condition tested compared to control test, expressed as a percentage. Results referred to Inoculum A

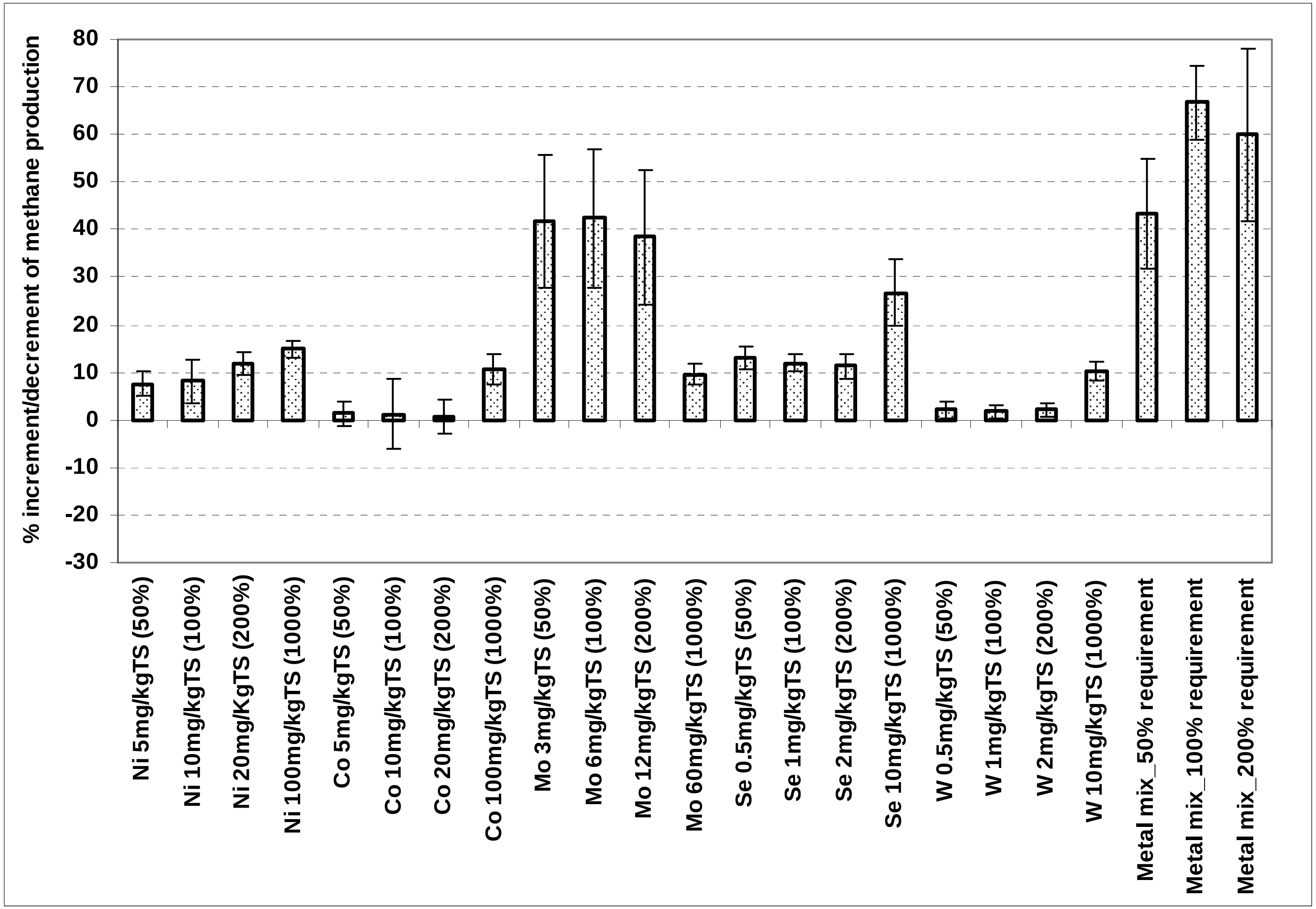

Figure 2. Histogram of increment or decrement on methane production of different condition tested compared to control test, expressed as a percentage. Results referred to Inoculum B

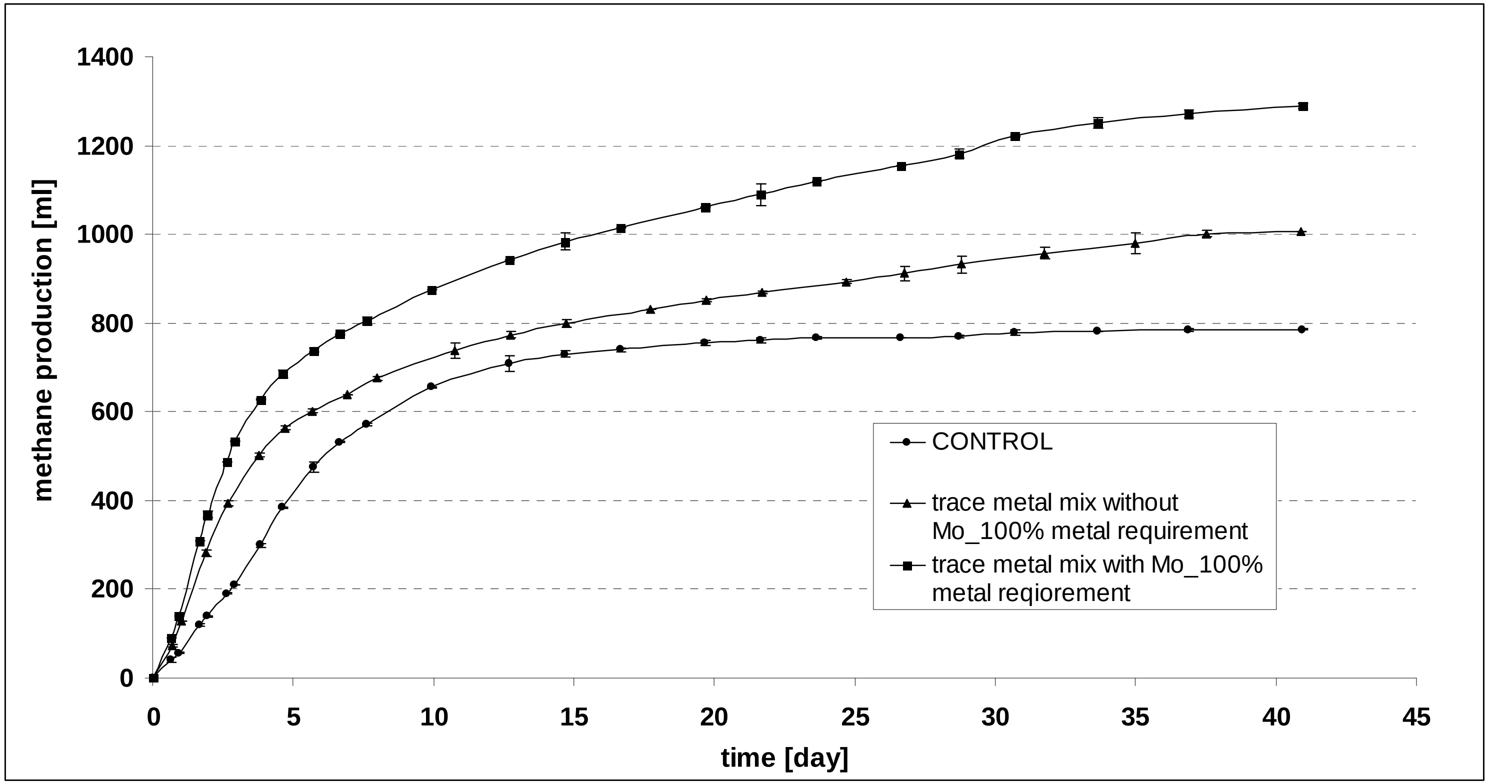

Figure 3. Cumulative curves of net methane production of control test, trace metal mix without Mo at 100% of trace metal requirement, trace metal mix with Mo at 100% of trace metal requirement. Error bars indicate 95% confidence intervals