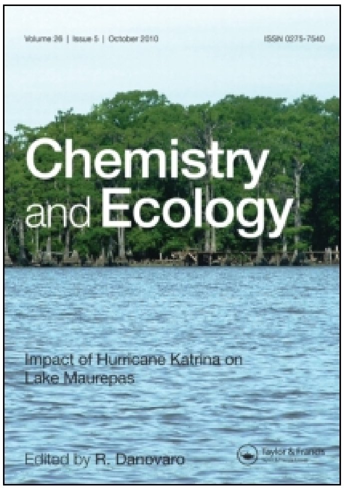

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INFLUENCE OF IRON, NICKEL AND COBALT ON BIOGAS PRODUCTION DURING THE ANAEROBIC FERMENTATION OF FRESH RESIDUAL BIOMASS

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We have investigated the dependence of the rate of the production of biogas upon the concentration of nickel, cobalt and iron at sub-toxic concentration and monitored its composition as amount of hydrogen, methane and carbon dioxide. The distribution of the added metals between the liquid and solid phase has also been monitored.

The results of our investigations show that the addition of any of the listed metals to the sludge may cause the production of a higher amount of biogas and influence the methane or carbon dioxide percentage. Conversely, the effect on the hydrogen production depends upon the metal added, the age of the active sludge used, and its adaptation to the susbtrate. As a general feature, during the acidogenesis phase, nickel reduces, while iron increases, the percentage of dihydrogen in the biogas, while cobalt has no influence.

Keywords: Biogas; Residual fresh biomass; Anaerobic fermentation; Influence of metal ions; Nickel; iron; Cobalt

1 INTRODUCTION

Treatment of municipal residual fresh biomass is an issue of debate because landfilling and agricultural applications are strictly regulated in EU countries, while incineration is questioned in some countries for its environmental impact. Commonly, such materials, as well as industrial analogues and sludges, are treated by aerobic or anaerobic digestion. In the aerobic digestion process, which is relatively costly essentially due to the continued aeration (Bernard and Gray, 2000), micro-organisms consume the biological degradable fraction with no recovery of energy. The anaerobic digestion of organic compounds to methane is an exoergonic process, with low efficiency because of low biodegradability of the solids, long retention times (20–30 days) and moderate efficiency (30–50%) (Weemaes *et al.*, 2000). It is considered to be an alternative to organic waste treatment in Northern Europe, and different technologies (for temperature, solids content, type of reactor) are exploited that are advantageously integrated with landfill and incineration (IEA, 1994). Most energy is recovered as methane, which can be separated from the biogas produced by bacteria, and

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TABLE I Energetics of Some Reactions Occurring in the Anaerobic Fermentation of Fresh Biomass.

H_2-producing reactions	*AG^0 (kJ per mol)*	*H_2-consuming reactions*	*AG^0 (kJ per mol)*
$CH_3CH_2OH + H_2O \rightarrow CH_3COO^- + H^+ + 2H_2$	+9.6	$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$	−131.0
$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + 3H_2 + HCO_3^-$	+76.1	$4H_2 + 2CO_2 \rightarrow CH_3COO^- + H^+ + 2H_2O$	−94.9
$CH_3CH_2CH_2COO^- + 2H_2O \rightarrow 2CH_3COO^- + H^+ + 2H_2$	+48.3	$H_2 + S^0 \rightarrow H_2S$	−33.9
$C_7H_5O_2^- + 7H_2O \rightarrow 3CH_3COO^- + 3\ H^+ + 3H_2 + HCO_3^-$	+53.0	$H_2C(NH_3^+)COO^- + H_2 \rightarrow CH_3COO^- + NH_4^+$	−86.0
$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + CO_2 + 3H_2$	+76.0	$4H_2 + SO_4^{2-} + H^+ \rightarrow HS^- + 4H_2O$	−151.0
$CH_3COO^- + H^+ + 2H_2O \rightarrow 2CO_2 + 4H_2$	+94.9		
$CH_3CH(CH_3)CH_2COO^- + CO_2 + 2H_2O \rightarrow 3CH_3COO^- + 2H^+ + H_2$	+25.2		
$CH_2OHCOO^- + H^+ + H_2O \rightarrow 2CO_2 + 3H_2$	+19.3		
$C_6H_5OH + 5H_2O \rightarrow 3CH_3COO^- + 3H^+ + 2H_2$	+10.2		
$CH_3CH(NH_3^+)COO^- + 2H_2O \rightarrow CH_3COO^- + NH_4^+ + CO_2 + 2H_2$	+2.7		

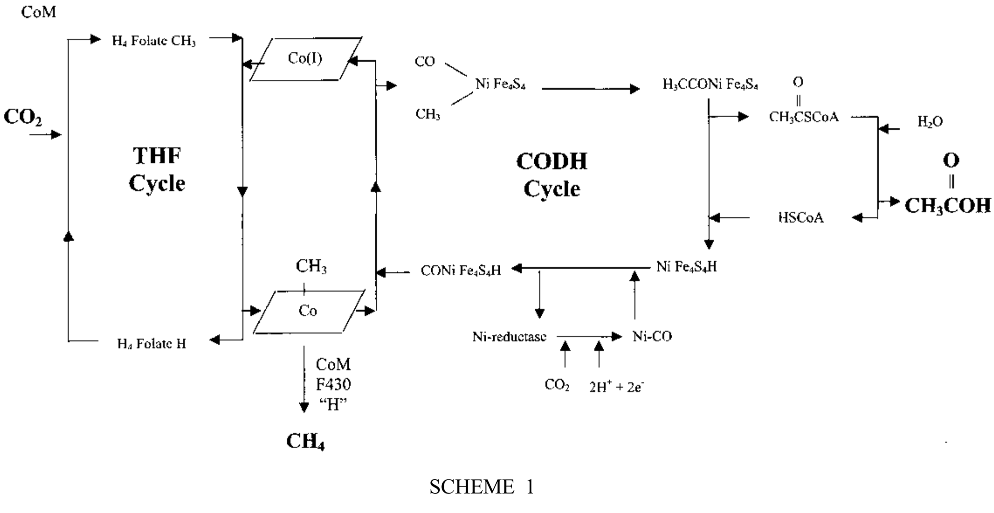
used as an energy source. The conversion of organic compounds into methane requires different bacterial communities and a complex metabolic food chain (Zehnder, 1978; Zeikus, 1983; Schink, 1983). As a matter of fact, the biomass undergoes several steps (depolymerization, acidogenesis, acetate formation, methanogenesis, methanation of CO_2) and in the whole process, H_2 and acetate are the most important intermediates utilized by the methanogenic bacteria. Hydrogen is a very important precursor in methanogenesis and H_2-consuming bacteria maintain a low H_2 partial pressure, allowing the realization of key biological reactions which would not occur for thermodynamic reasons at higher H_2 partial pressure (Zehnder, 1978). The degradation of fatty acids and alcohols, in the anaerobic digestion of organic compounds, is accomplished by a syntrophy between H_2-producing bacteria and H_2-consuming methanogenic archaea (Schink, 1983). This syntrophic partnership creates conditions for a better use of the energy content of primary substrates (Thauer *et al.*, 1997).

The aim of this work was to investigate the role of iron, nickel and cobalt in the production of biogas during the anaerobic digestion of a sludge. These metals were chosen considering their role in the anaerobic metabolism during the methanogenic fermentation. In fact, they constitute the active centre in several enzymes which play a key role in the complex methanation process (Tab. I).

In particular, nickel is the active centre of the methyl-coenzyme M reductase (known as F430) and several H_2-consuming hydrogenases (Walsh and Orme-Johnoson, 1987; Aresta *et al.*, 1988) as well as acetate formation enzymes (Aresta *et al.*, 1998; Albracht *et al.*, 1994; Ellefson and Wolfe, 1981; Rouviere *et al.*, 1985). Iron is part of different hydrogenases (H_2 uptake or evolution), and of the carbon monoxide dehydrogenase (CODH) enzyme, a central enzyme in the formation of acetic acid by anaerobic bacteria, that contains Fe_4S_4 (Aresta *et al.*, 1998; Rouviere *et al.*, 1985; Adams, 1990; Qiu *et al.*, 1994). Cobalt is part of cobalamin which catalyses the transfer of methyl-groups (Hippler and Thauer, 1999). All these enzymes work together for the production of methane and carbon dioxide during the anaerobic digestion of sludges (Schonheit *et al.*, 1979; Aresta *et al.*, 2002) (Scheme 1).

2 MATERIALS AND METHODS

Two batch reactors, each of 2 L capacity, were used to create the anaerobic conditions for the methanogenic fermentation. Each reactor was made of Pyrex glass, with a spherical base and



SCHEME 1

a double-wall in which water was circulated at the constant temperature of 37 °C. Each reactor was magnetically stirred at a speed of 250–500 rpm. The biogas produced in each reactor during the fermentation was collected into a 2.5 L gasometer. The analysis of biogas was made using a Carlo Erba gas chromatograph equipped with Cromosorb SII column 3 m × 2.1 mm (ID) and TCD detector.

An anaerobic sludge taken from a municipal water-treatment plant was used in this work to start the digestion of fermentable biomass. It had a 5% total solid. The feed was a mixture of various vegetable residues (fruits and legumes, essentially), with a composition similar to that of a domestic fresh-waste. It was homogenized in order to favour the biodegradation. Feed was added once a week at a rate of 3%. Metals were added separately in three different tests. Metal-compounds were dissolved in deareated water. Each addition was 1 mL of $NiCl_2 \cdot 6H_2O$ or $CoCl_2 \cdot 6H_2O$ or $FeCl_2 \cdot 6H_2O$ solution $1\,mol\,L^{-1}$. $NiCl_2 \cdot 6H_2O$ was purchased from Carlo Erba, $CoCl_2 \cdot 6H_2O$ from J. T. Backer Chemicals and $FeCl_2 \cdot 4H_2O$ from Aldrich Chemical Company.

The addition of nickel and iron was done one week after the anaerobic fermentation in the reactors was started, cobalt was added to the sludge after 18 days. During the first 10 days of biogas production the acidogenesis phase is prevalent in methanogenesis, so it is possible to monitor the H_2 production. The total amount of metals in the reactor did not reach the toxic concentration. Metal content of the liquid and solid phase was measured at the start and after each addition according to the IRSA analytical method (IRSA, 1985) by using the Atomic Absorption Spectrometer Perkin Elmer 3110. A sample of the sludge was withdrawn under controlled conditions (avoiding O_2 admittance) and the solid was separated by centrifugation. The liquid and solid were processed as indicated above. The metal ions in the sludge very slowly accumulated in the solid phase, either due to the formation of extracellular polysaccharides that may adsorb metals, or due to adsorption on solids.

The effect of each metal was monitored at two different time intervals as '8 hour' (the analysis of biogas was done every 30 min for 8 hours), and a '5 days' (the analysis was made every 24 hours for 5 days). The first one was useful for studying the effect of each addition (metal or feeding) during the first 8 hours after the addition, monitoring the acidogenesis phase of the fermentation (production of H_2). The second test was used to study the effect of each metal addition on CH_4 and CO_2 production. All data in the figures are the average of three measurements with standard deviation represented as error.

3 RESULTS AND DISCUSSION

Each of the metals tested in this work (Fe, Co, Ni) produces a different effect on the composition of biogas, as expected on the basis of the role of the enzymes in which they are present. Nickel, iron and cobalt have been added to the sludge either separately or in combination, always at sub-toxic concentration.

In a typical case, the starting concentration of metal ions in the sludge was 6, 13.6 and 4.46 ppm for Ni (II), Fe (II) and Co (II), respectively, in the liquid phase. Their level in the solid phase was 14.6, 54.12 and 13.43 ppm under the same conditions. The addition of 0.5 mL of 1 M $NiCl_2$ to the reactor filled with 1.7 L of sludge produced an instantaneous increase of the metal concentration in the liquid phase up to 22.4 ppm, from initial 6 ppm. The concentration in the solid phase rised to 16.08 ppm from 14.6 ppm. Figure 1 shows the trend of metal concentration in solution and in the solid phase during a 5 days check.

It is quite evident that the addition of the free metal chloride causes a rapid increase in the concentration in solution and the metal remains available in solution for days. The transfer

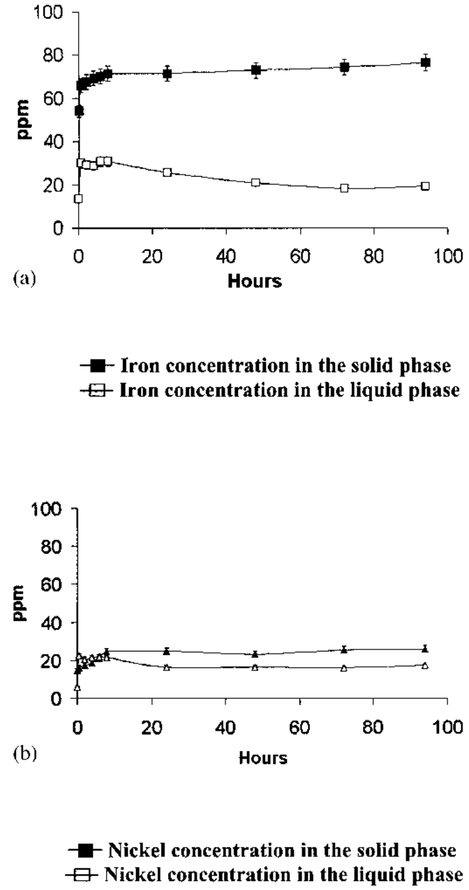


FIGURE 1 Distribution of Iron (a) and Nickel (b) in the solid and liquid phase.

from the solution to the solid phase starts slowly after 10 hours. After several days the metal concentration in solution is within 60% of the value measured soon after the addition. Therefore, during the test of the 8 hours (which starts soon after the addition of metal and feeding) the bacteria really feel the increase of the concentration of the metal available in solution. The response to such metal increase is thus a real cause–effect process. The effect of metals on H_2 production was quite evident only when fresh sludges were used, *i.e.* during the starting phase of fermentation, which means not more than 10 days after the start of the fermentation process and during the first 10 hours after the addition of the metal or feed.

The increase of concentration of Ni in solution, caused a decrease of dihydrogen in the gas phase (Fig. 2).

This observation agrees well with the role of nickel as part of the active site in H_2-consuming hydrogenases (Albracht, 1994). Therefore, the addition of Ni (II) promotes a greater activity of such H_2-consuming hydrogenases. Conversely, when iron was added, the increase of H_2 production in the batch reactor was observed within 2.5 hours after feeding (Fig. 3), in comparison to the control that had a lower H_2 production. These data also agree with the role of iron in H_2-evolving hydrogenases, which promote the formation of H_2 (Adams, 1990). Such metal-depending effects were evident only when fresh feed was added and the system had not reached the optimum performance. Conversely, once sludges were aged, the evolution

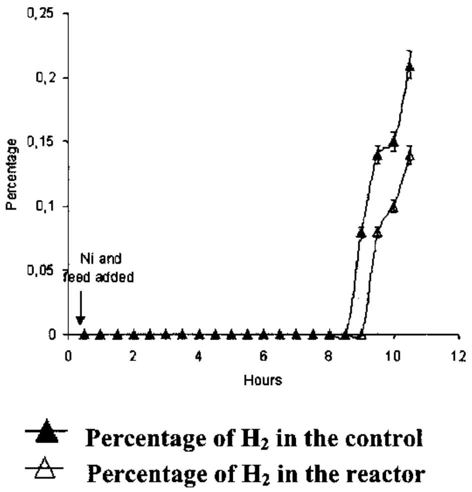


FIGURE 2 Effect of Nickel on H_2 production.

and accumulation of dihydrogen was not so evident when Ni or Fe were added. Nevertheless, the added metal ions under these conditions showed a positive influence on the methane formation, increasing the rate of formation and the amount of methane.

Notably, the addition of cobalt did not cause any variation of the H_2 production at any time after the start of the fermentation.

Figure 4 shows the composition of biogas produced after the addition of nickel (Fig. 4a), iron (Fig. 4b) or cobalt (Fig. 4c): in all cases an increase of the percentage of methane in the gas phase was observed.

All figures show that before the addition of the metals, both the reactor and control have the same or very close CH_4/CO_2 ratio that varies with the addition of feed. The increase of the ratio after the addition of feed can be explained considering the enhanced production of hydrogen that is made available also for direct CO_2 methanation. After the metal was added, a

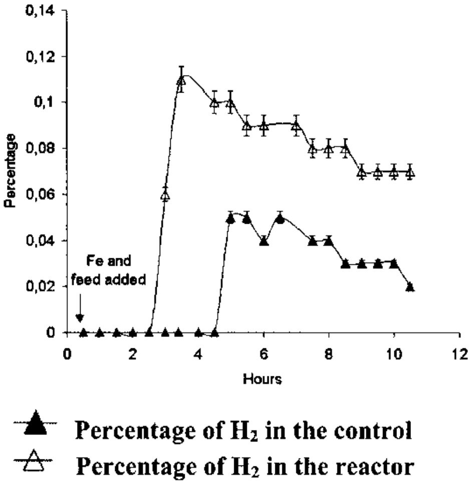


FIGURE 3 Effect of iron on H_2 production.

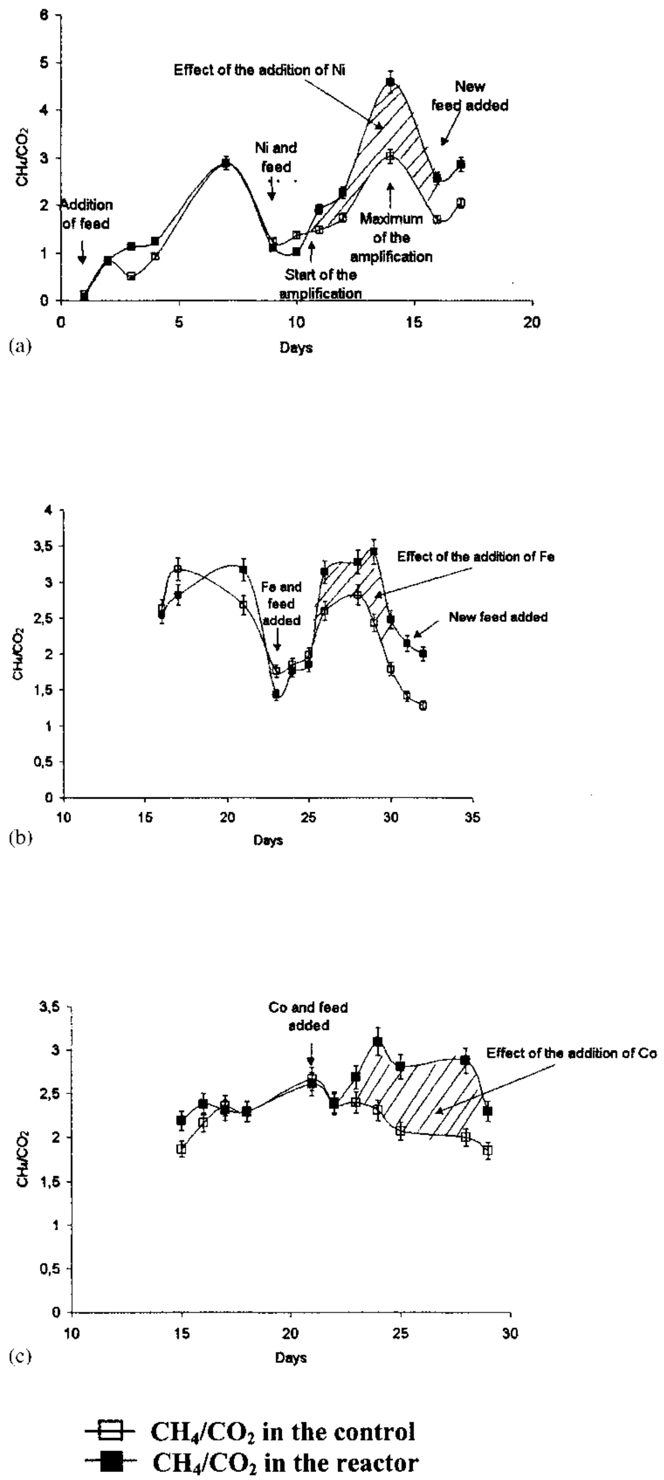


FIGURE 4 Effect of Nickel (a), Iron (b) and Cobalt (c) on CH_4 production.

net increase of the CH_4/CO_2 ratio was observed in the reactor with respect to the control. Typically, the amplification started within 2 days after the addition of the metal and reached the maximum around the fifth/sixth day. The difference maintained quite strongly for 7–10 days also during the phase of decrease of methanation. If more feed were added, the amplification was again evident. After 20–30 days the added metal still seemed to quite affect the CH_4/CO_2 ratio. It is important to note that during such a period the concentration of the metal in the liquid phase in the reactor was always higher than that in the control and slowly decreased with respect to $t = 0$, due to the transfer to the solid phase (see Fig. 2). The increase of the CH_4/CO_2 ratio is the effect of two concurrent facts: the decrease of the CO_2 content and the increase of the methane percent in the gas mixture. However, the increase of the CH_4/CO_2 ratio is generated by a more effective hydrogenation of CO_2 to methane. Nickel has a quite interesting effect as it increases the CH_4/CO_2 ratio up to a maximum of 4.5. Such ratio is well above the value of 3, typically found in a good quality biogas as shown by the control (see Fig. 4).

All the metal ions used in this work are active centres of enzymes involved in methanogenic metabolism, as can be seen in Table I. In particular, Ni is more effective than Fe and Co in increasing the CH_4/CO_2 ratio. Notably, the addition of Ni reduces the amount of H_2 in the reactor and, consequently, causes an increase of the CH_4/CO_2 ratio, as shown in Figure 4a. The effect of Co is clearly according to its role in corrins implied as methyl-transfer agents that improve the CH_4 production. Anyway, the CH_4/CO_2 ratio maintains around the range of 3. The addition of Fe clearly improves the CH_4/CO_2 ratio. Its role can be explained considering the Fe_4S_4-protein implication in the formation of hydrogen that makes more H_2 available for CO_2 methanation. Among the three metal ions, nickel has, thus, the most spectacular effect (compare Fig. 4a–c).

4 CONCLUSIONS

These results suggest that the addition of Fe, Ni and Co could be beneficial for improving the methanation process within given concentration limits. These findings suggest that the natural biogas composition may have been influenced by the nature of the walls of the reservoirs. The presence of leacheable Ni, Fe, Co ions in the rocks may affect biogas quality positively or negatively.

It may also be suggested that the use of industrial waters which contain metals like Fe, Ni, Co in the suitable concentration range may be worthy for the anaerobic fermentation of fresh biomass and for ameliorating the performance of the bioreactor.

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