Effects of Nickel, Cobalt, and Molybdenum on Performance of Methanogenic Fixed-Film Reactors†

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Received 6 March 1981/Accepted 3 June 1981

The conversion of acetic acid to methane and carbon dioxide by a mixed methanogenic population from an anaerobic fixed-film digester was stimulated by the addition of nickel (100 nM) and cobalt (50 nM) and especially by the addition of these elements in combination. Molybdenum addition (50 nM) was only slightly stimulatory when added in combination with both nickel and cobalt. The addition of these trace metals to anaerobic fixed-film digestors, which treat food processing waste, greatly enhanced reactor performance. Total gas and methane productions were increased 42%, greater volumes of waste could be effectively treated, and reactor residence time was shortened. However, the lag period for reactor start-up was not reduced. Tests showed that reactor performance was increased because trace nutrient addition allowed accumulation of a thicker methanogenic fixed film.

Methanogens, particularly those that convert acetate to methane and carbon dioxide, are dependent on iron for growth and activity (6, 16, 18). Similar requirements for other transition metals have also been demonstrated in methanogens. The growth of a strain of acetate-fermenting Methanosarcina sp. was stimulated by the presence of cobalt in basal medium (9). The purified F-430 factor from both Methanobacterium thermoautotrophicum and Methanobacterium bryantii has been shown to contain nickel (21). Also, the growth of M. thermoautotrophicum on H2 and CO2 as the sole sources of energy and carbon, respectively, was found to be dependent on trace amounts of nickel, cobalt, and molybdenum (14).

It has been established that acetate is the precursor of approximately 70% of the methane in anaerobic digestors (7, 15) (hydrogen and carbon dioxide account for the remainder), yet little is known about the stimulation of acetate conversion to methane by trace quantities of these metals. This paper reports on the effect of nickel, cobalt, and molybdenum on the methanogenic population of fixed-film reactors treating food processing waste. Factors such as methane production, reactor loading rates, reactor start-up, and fixed-film distribution were studied.

MATERIALS AND METHODS

The stimulatory effect of nickel, cobalt, and molybdenum on acetate conversion was determined by using the overflow from a methanogenic fixed-film reactor which treats bean blanching waste, as described elsewhere (17). Acetic acid, an equimolar mixture of sodium acetate, potassium acetate, ammonium acetate, and glacial acetic acid, was added to the vials with a hypodermic syringe to obtain final concentrations of 100, 50, and 50 nM, respectively. These levels are well below the concentrations generally considered to be environmentally acceptable for waste water effluents. These concentrations were chosen because they were found to be sufficient for the growth of M. thermoautotrophicum (14). Tests were made in triplicate, and the results were compared with those of control vials. Acetic acid, as an equimolar mixture of sodium acetate, potassium acetate, ammonium acetate, and glacial acetic acid, was added to the vials to a concentration of 67 mM to maintain the pH in the range of 6.5 to 7.2. The acetic acid concentration was maintained between 17 and 67 mM by subsequent additions of glacial acetic acid. The rate of conversion of acetate to methane and carbon dioxide was determined frequently by the method of Khan and Trottier (8) for analysis of volatile acids by gas chromatography.

The effect of these trace metals on methanogens in down-flow, anaerobic fixed-film fermentors after the reactors reached a maximum level of waste treatment and methane production was also determined. The construction, operation, and performance of these reactors has been described elsewhere (17). Nickel, cobalt, and molybdenum were added to concentrations of 100, 50, and 50 nM, respectively, to the bean blanch-
ing waste treated in two established fixed-film reactors. One of these fermentors was an 8-ft (243.84-cm), single-channel reactor which had a total volume of 1.4 liters, whereas the other fermentor was a 35-liter, 4-ft (121.92-cm), multichannel reactor. The volatile acid concentration in these fermentors was analyzed daily, and the feed rate was adjusted to keep these acid levels in the range of 200 to 400 mg/liter. Reactor performance was judged on the basis of gas production and hydraulic retention time.

To obtain a more unequivocal assessment of the effects of trace metal addition on the performance of anaerobic fermentors, four new single-channel, downflow, fixed-film reactors were set up in accordance with the basic design of van den Berg and Kennedy (17). Inert, needle-punched polyester (Texel Inc., Beauce-Nord, Quebec, Canada) was used as the film support material, rather than the potter’s clay used in the two established fermentors mentioned above. (Clay contains iron and other minerals [W. D. Murray and L. van den Berg, J. Appl. Bacteriol., in press] which may be slowly released owing to the cation-exchange capability of clay (5), and such a release might mask the effect of added trace metals.) The reactors were made by sewing the needle-punched polyester around 2-ft (60.96-cm), stainless steel, wire frames (triangular in cross section) and inserting these into glass tubes (diameter, 5.1 cm) which were then sealed with rubber stoppers to ensure the exclusion of oxygen. This design allowed the development of fixed methanogenic film on both sides of the material. The reactors were flushed with nitrogen gas and inoculated with effluent from an established laboratory fermentor acclimated to bean blanching waste as feed. Two fermentors were fed bean blanching waste, and the other two were fed the same waste supplemented with the usual concentrations of nickel, cobalt, and molybdenum. The initial low feed rate (hydraulic retention time, 15 days) was gradually increased to keep the volatile acid level near 300 mg/liter. Daily gas production was observed, and volatile acids and methane concentrations were determined by gas-liquid chromatography. After 80 days of operation, the reactors were carefully drained, and the film supports were removed and air dried overnight. The distribution of fixed film was then determined by oven drying, ashing, and weighing measured sections of the fixed film cut from along the length of the support.

RESULTS AND DISCUSSION

The conversion of acetic acid to carbon dioxide and methane by methanogens which came from a fixed-film fermentor was stimulated by the single additions of nickel and cobalt, but not molybdenum (Fig. 1a). When these trace metals were tested in combinations, nickel and cobalt together stimulated methanogenesis to a much greater extent than would be expected when they were added individually (Fig. 1b). Nickel and cobalt are therefore both required in quantities above those present in bean blanching waste for the promotion of growth and activity of some or all of the methanogenic population capable of converting acetic acid to methane. The individual addition of one of these metals alleviated the requirement for that compound. However, activity was then limited by a requirement for the second transition metal. Molybdenum slightly increased methanogenic activity, but only when added in combination with both nickel and cobalt. This slight stimulation of methanogenesis indicates a requirement for molybdenum which may have been largely met by existing trace amounts of this compound in the reactor effluent.

In-reactor methanogenesis was also stimulated by the addition of these metals (Table 1). Total gas and methane production increased approximately 42% in both the 1.4- and 35-liter reactors. To maintain volatile acid levels near 300 mg/liter, the feed loading rate for these two fermentors had to be raised with a concomitant reduction in hydraulic retention time. Methane production increased in proportion to loading rate.

The start-up of the new reactors was not affected by the addition of nickel, cobalt, and...
molybdenum, but ultimate performance was affected (Fig. 2). The initial development of the methanogenic population, as indicated by the changes in loading rate and methane production, was identical in all four fixed-film fermentors. After 40 days of reactor operation, however, differences in fermentor performance became evident. The reactors receiving bean blanching waste without additions attained a maximum level of waste treatment of 1,550 ml/day (ca. 16.3 g of chemical oxygen demand per day) and a maximum production of methane of 5.2 liters/day. The activity of the methanogens in the reactors receiving nickel, cobalt, and molybdenum continued to increase and eventually leveled off after 78 days of operation at a loading rate of 3,000 ml/day (31.5 g of chemical oxygen demand per day) and a methane production rate of 10 liters/day. The natural trace mineral concentration in bean blanching waste was therefore sufficient to meet the requirements of the methanogenic population during reactor start-up, but this concentration became limiting as the methanogenic fixed film continued to develop.

The distribution of fixed film along the polyester support material was not uniform along the length of the column (Fig. 3). Fixed-film development reached maximum between 10 and 18 cm from the top of the reactor, was uniform from approximately 25 to 45 cm, and became rapidly thinner towards the bottom of the column. This distribution was the same for all fermentors whether or not they received trace metal additions. The effect of nickel, cobalt, and molybdenum addition was to increase the overall thickness of the film. Mixing studies indicated that the flow rate of feed down the column and the release of gas upward were sufficient to assure thorough mixing of carbon source and required nutrients. The large accumulation of fixed film near the top of the reactor may have been due to luxury uptake of the trace metals or to the rapid uptake and depletion of some limiting trace nutrient other than nickel, cobalt, or molybdenum.

Although Schonheit et al. (14) were able to show that the growth of *M. thermoautotrophicum* was dependent on the presence of trace amounts of nickel, cobalt, and molybdenum in synthetic growth medium, no growth dependence for nickel could be demonstrated where the growth medium had been in contact with stainless steel fermentor parts. Similarly, the nickel

<table>
<thead>
<tr>
<th>Reactor</th>
<th>Gas production (liter/day)</th>
<th>Hydraulic retention time (days)</th>
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<tbody>
<tr>
<td></td>
<td>Total Methane</td>
<td></td>
</tr>
<tr>
<td>1.4 liters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before addition</td>
<td>13.7</td>
<td>8.0</td>
</tr>
<tr>
<td>After addition</td>
<td>19.3</td>
<td>11.4</td>
</tr>
<tr>
<td>35 liters</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before addition</td>
<td>196.0</td>
<td>115.6</td>
</tr>
<tr>
<td>After addition</td>
<td>280.0</td>
<td>165.2</td>
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\*50 days after start of trace metal addition.  
\*17 days after start of trace metal addition.
dissolved from stainless steel syringe needles by H₂S was found to be sufficient for the maintenance of the growth of this bacterium in medium not supplemented with nickel. The bean blanching waste used in this study was obtained from a commercial cooking process during which the beans were blanched in stainless steel vats (Canadian Canners Ltd., Burlington, Ontario). The trace minerals leached from the beans during blanching and those acquired from contact with stainless steel equipment, however, were not sufficient to meet the requirements of the methanogenic microorganisms in the established fixed-film reactors. The early leveling off of waste treatment efficiency and methane production appears to have been due to trace mineral limitation.

The physiological role of nickel in plants and animals has been well documented (3, 4, 10, 12), but little is known of the nickel requirements of bacteria. The fact that this element is generally not considered essential for bacterial growth (14) may be due to trace nickel contamination of medium chemicals. Nickel has been shown to be essential for the growth of *M. thermoautotrophicum* (14) and *Alcaligenes eutrophus* (13), as well as being an essential component of the carbon monoxide dehydrogenase of *Clostridium pasteurianum* (2) and the F-430 factors of *M. bryantii* and *M. thermoautotrophicum* (9). Unlike nickel, cobalt has been proven to be generally required by bacteria. It is needed for the synthesis of corrinoid compounds (14) and has been found as a component of transcarboxylase in *Propionibacterium shermanii* (11). Similarly, molybdenum is important as the prosthetic group of some bacterial enzymes (1).

The trace concentrations of nickel, cobalt, and molybdenum in most media may be sufficient for the unlimited proliferation of bacteria. However, this may not be the case in industrial fermentors, in which the microorganisms are retained in the reactor, and the development of a large biomass may quickly deplete trace quantities of required elements. Accordingly, even rich feeds may have to be fortified with additional trace nutrients to obtain optimum digestor performance.

**ACKNOWLEDGMENTS**

The expert technical assistance of Milan Muzar and Dave Cameron is gratefully acknowledged.

**LITERATURE CITED**


