ELSEVIER

Bioresource Technology 82 (2002) 205–208

BIORESOURCE TECHNOLOGY

Short communication

The effects of certain antibiotics on biogas production in the anaerobic digestion of pig waste slurry

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Received 10 July 2001; received in revised form 6 September 2001; accepted 10 September 2001

Abstract

Antibiotics commonly used in the treatment of pigs – amoxicillin trihydrate, oxytetracycline hydrochloride and thiamphenicol – were added at different concentrations to aliquots of pig waste slurry plus anaerobic sludge in serum bottles. The biogas production and methane concentration in the headspace were monitored to determine the effect of the antibiotics on the anaerobic process. With thiamphenicol significant differences in methane production were found for concentrations of 80 and 160 mg l^{-1} slurry. Compared to the control, only minor differences in methane production were noted in the bottles to which amoxicillin (60 and 120 mg l^{-1}) had been added. Methane production was about the same for the bottles with different oxytetracycline concentrations (125 and 250 mg l^{-1}) and for the control.

Keywords: Anaerobic digestion; Antibiotics; Biogas; Inhibition; Methane; Pig slurry

1. Introduction

Antibiotics are widely used in pig farms as feed additives, to promote growth and to prevent infection, and at therapeutic level to treat animals that are ill. Certain antibiotics contain carriers that allow them to target specific parts of the animal organism (the intestine, the respiratory system, etc.) while others act at a general level. Some antibiotics become active when metabolised, whereas others operate in an initial active form. In either case, a fraction of the ingested antibiotics may be excreted in an active form (Gamel-El-Din, 1986) and is then found in the wastewater from the farm.

The overuse of antibiotics is the primary cause of high antibiotic concentrations in the slurry sent to treatment plants. A second factor is the quantity of water and the methods used to clean out pig houses. Since the most frequently used biological process in wastewater treatment plants is the anaerobic digester, it is possible that these antibiotic concentrations could have negative effects on the mixed populations of anaerobic bacteria: they could determine the selection of cultures or reduce their rate of growth, and therefore might have a significant influence on both the degree of degradation of the organic load of the waste and on the production of biogas. Problems of this nature have often been noted by those working in the field and have sometimes been reported in the literature (Hobson and Shaw, 1976; Poels et al., 1984). Unfortunately, there are not many experimental studies on the effects of antibiotics on the performance of anaerobic digestion. Furthermore, several of the antibiotics investigated in scientific works have now been banned and replaced by alternatives. Hilpert et al. (1981) studied the sensitivity of 10 strains of methanogens to 28 antibiotics. Runs carried out by the agar diffusion test revealed that only some of the antibiotics investigated inhibited methanogens. The biological activity of the anaerobic digestion of pig slurry was found to be reduced by the presence of lincomycin (Fisher et al., 1981) and some other antibiotics (Poels et al., 1984; Varel and Hashimoto, 1982). In studying the anaerobic digestion of cattle manure, Blotevogel and Jannsen (1988) found that carbadox, lasalocid and monesin inhibited biogas production, while avoparcin did not. The potential inhibitory effect of some antibiotics (chloramphenicol, chlortetracyclin, tylosin, erythromicin) was studied by Camprubi et al. (1988) in batch and semicontinuous experiments. In the antibiotic concentration range studied, chlortetracyclin, tylosin and erythromycin did not inhibit methanogenic activity, but considerable inhibition by chloramphenicol was found.

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0960-8524/02/$ - see front matter
PII: S0960-8524(01)00162-6

Since many of the other antibiotics studied, chloramphenicol included, are now banned, we decided that it was important to address these issues with regard to antibiotics currently in use on pig farms. This paper examines three antibiotics – amoxicillin trihydrate, oxytetracycline hydrochloride (henceforth referred to as simply amoxicillin and oxytetracycline, respectively) and thiamphenicol – to study their influence on the anaerobic digestion of pig waste slurry in laboratory scale reactors (serum bottles). Gas production was used as a direct indicator of the vitality of the anaerobic digester process. It should be pointed out that no experimental work in this area has been found in the literature for the above mentioned antibiotics other than a study of the biogas production from cow manure contaminated with oxytetracycline hydrochloride (Gamel-El-Din, 1986).

2. Methods

All experiments were carried out batch-wise with sludge obtained fresh from the second anaerobic digester of a private plant for treating pig slurry. The slurry used came from pigs that had not been dosed with antibiotics for a considerable time. A 50-ml aliquot of sludge was placed into each of the 160-ml serum bottles. The bottles were capped with butyl rubber stoppers crimped with an aluminium seal. After sealing, the headspaces were flushed with nitrogen gas to remove traces of oxygen. Then the serum bottles were incubated in a rotary shaker (70 rpm) at 37 °C. Biogas production was monitored for a few days to check the activity in each of the three cultures. Next 10 ml of fresh pig slurry, in which different concentrations of antibiotics had been dissolved, was added to two of the serum bottles. Control cultures were prepared in the same way without dissolving antibiotics in the content of the bottle: 50 ml of digested sludge plus 10 ml of fresh slurry. Each experiment was performed in duplicate.

The main characteristics of the fresh pig slurry were: COD 18 g l^{-1}, total solids 9.7 g l^{-1}, volatile solids 6.1 g l^{-1} and pH 7.5. The applied concentrations of the antibiotics in the experiments were (in mg l^{-1}): thiamphenicol 80, 160; amoxicillin trihydrate 60, 120; oxytetracycline hydrochloride 125, 250. The concentrations of antibiotics chosen for this study reflect theoretical concentrations calculated on the basis of the practical dosage of the antibiotic, the number of pigs potentially treatable and the quantity of water used to clean out pig houses on the farm from which we took the slurry.

Biogas production was monitored as time elapsed by puncturing the rubber stopper of the serum bottles to measure internal gas pressure (Varel et al., 1980). Methane concentration in the headspace gas was determined by gas chromatography using a Supelco Carboxen 1000 stainless steel column (450 cm × 0.3 cm) with packing material of 60–80 mesh size. A thermal conductivity detector was used. The detector and the wire temperatures were 250 and 300 °C, respectively. Helium was used as a carrier gas at 30 ml min^{-1}.

3. Results and discussion

The results obtained in all the experiments are shown in Figs. 1–3. All the results presented are the means of two independent assays. Fig. 1 reports methane production in the experiments performed with the addition of thiamphenicol compared with the control curve. In all the serum bottles methane production was observed: there was a gradual increase in methane production for the control bottle, i.e., bottle without thiamphenicol. After nearly 100-h incubation period, methane concentration in biogas for the control reactor was already very close to the maximum value observed of 75%: there was a slight increase in methane production for the other two bottles. But in the bottles with higher thiamphenicol

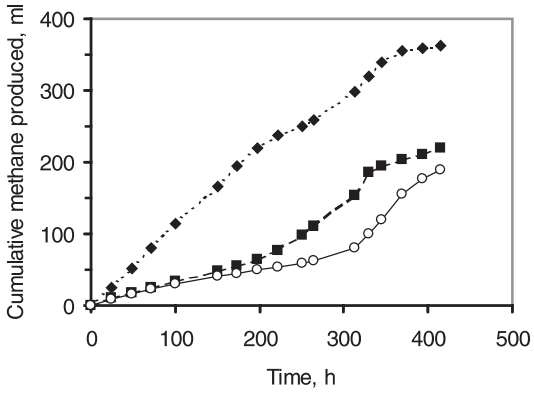

Fig. 1. Effect of the initial thiamphenicol concentration on accumulated methane production during batch fermentation in 160-ml serum bottles. Symbols for antibiotic concentration (in mg l^{-1}): (◆) 0, (■) 80, (◯) 160.

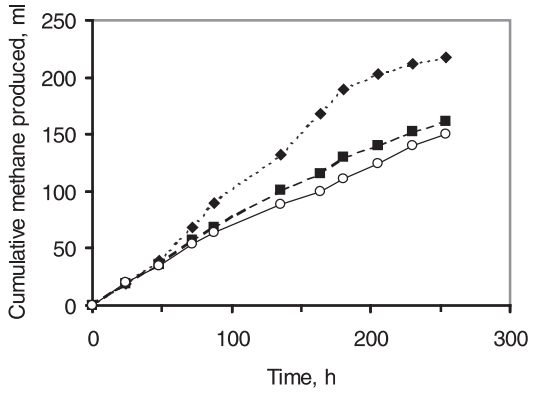

Fig. 2. Effect of the initial amoxicillin concentration on accumulated methane production during batch fermentation in 160-ml serum bottles. Symbols for antibiotic concentration (in mg l^{-1}): (◆) 0, (■) 60, (◯) 120.

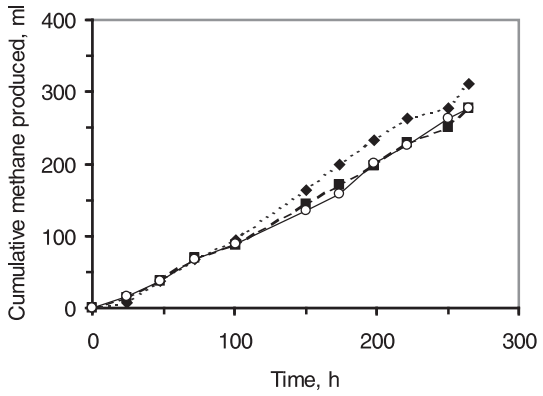

Fig. 3. Effect of the initial oxytetracycline concentration on accumulated methane production during batch fermentation in 160-ml serum bottles. Symbols for antibiotic concentration (in mg l^{-1}): (◆) 0, (■) 125, (◯) 250.

concentration there was lower methane production. The biogas methane maximum percentage, was also in these cases equal to 75%, and was reached after about 320 and 360 h in the bottles with 80 and 160 mg l^{-1} of thiamphenicol, respectively. The analysis of the patterns of the three curves in Fig. 1 suggests that methane production was preceded by an adaptation period necessary for the microbial cultures to develop resistance to the antibiotic. It seems that the adaptation period lengthened with increasing thiamphenicol concentrations added to the test bottle.

When amoxicillin was added, the rates of methane production showed similar trends for each of the bottles (Fig. 2). The differences in methane production may be attributed to the differences in antibiotic concentration added; in particular, as the amoxicillin concentration increased, methane production decreased. In fact at 60 and 120 mg l^{-1} of amoxicillin the methane production represented 75% and 68%, respectively, of the control values at the end of the 250-h incubation period. Confirmation of this seemed to be provided by data (not shown) on the methane percentage in the biogas: the higher the amoxicillin concentration in the bottles, the lower the methane concentration.

We speculate that, for both thiamphenicol and amoxicillin, the increase in the concentration of added antibiotic causes a greater inhibitory effect on one or more of the major metabolic bacterial groups active in methane fermentors. For example, Poels et al. (1984) have reported an increase in the accumulation of volatile fatty acids and so a decrease in gas production upon the application of increasing amounts of antibiotic to methane digestions.

The effect of adding oxytetracycline to the cultures is shown in Fig. 3. Methane production curves overlapped for the cultures developed in the presence of antibiotic, while production was only slightly higher for the control bottle. If we consider that the methane concentration in the biogas for the three bottles was identical during the runs, we can conclude that in the range of antibiotic concentrations studied, both the acid-forming and methane-forming bacteria seem not to be affected by the presence of oxytetracycline. Gamel-El-Din (1986) obtained similar results in testing its effects on the anaerobic digestion of cow manure.

In this preliminary study we present some new results. Then we can conclude that: thiamphenicol added to anaerobic cultures had a considerable effect on methane production when compared to the control culture; amoxicillin had a lower but significant inhibitory effect on the methane production related to the concentration of the antibiotic; oxytetracycline seemed not to exert any effect on the methane production. The above results constitute further confirmation of what others have found (Hobson and Shaw, 1976; Poels et al., 1984), i.e., that the presence of antibiotics in waste slurry from pig farms can create problems in the treatment of wastewater by anaerobic digestion, especially where the production of biogas is concerned. Our results also lead us to conclude that for a given antibiotic it is not possible to predict a priori the degree of inhibition for methane production. Moreover, the results obtained with antibiotics that cause inhibition do not allow us to state which part of the anaerobic community (methane- or acid-producing bacteria) is affected by antibiotics. For this reason, further experimentation with measurement of volatile fatty acids is needed.

Acknowledgements

The authors thank Antonello Viola for his assistance with HPLC measurements. This research was carried out in part with the financial contribution of the Regione Autonoma della Sardegna and of the Italian Ministero dell'Università e della Ricerca Scientifica.

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