Effect of sulfonamides and tetracyclines in biomethanization processes and on methanogenic bacterial communities

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Introduction

Agricultural wastes are a mixture of organic materials full of nutrients and energy that should not be wasted in landfills. Manure represents an important agricultural waste due to its huge production everywhere. Anaerobic digestion has been an optimal way to use those organic wastes for a long time (Deublein, and., Steinhauser, 2011), it provides an efficient alternative to waste disposal as well as a renewable energy source (Noor et al., 2021). However, manure also contains low concentrations of pharmaceuticals such as antibiotics used for treating animal infections. The spread of antibiotics in the environment due to direct or indirect application of manure on soil is responsible for bacterial resistance and the spread of antibiotic resistance genes (Zhang et al., 2021).

The aim of this work was to assess the potential effect of the presence of antibiotics at different concentrations on the biomethanization process in mesophilic conditions and on the bacterial communities involved as well as the fate of those antibiotics throughout the process. The substrate used in this work was cow manure and the inoculum, sludge from a wastewater treatment plant. The presence of sulfonamide and tetracycline antibiotics in the cattle manure and inoculum was previously analysed, and then it was doubled, triplicated, and quadruplicated by spiking the manure to evaluate the eventual effects on the anaerobic digestion process.

Bacteria involved in the process were determined and studied by DNA 16 S amplicons sequencing. Digestates still contained antibiotics, mainly sulphonamides that were not affected by the process. By contrast, the concentration of tetracyclines decreased after the anaerobic digestion.

Materials and Methods

The cow manure used was obtained from a livestock farm located in Burgos (Spain), and the anaerobic sludge from a wastewater treatment plant located inside the same farm was used as inoculum. The ratio inoculum: substrate was 2:1 (w:w) based on volatile solids of each one (VS). The control parameters were VS (APHA, 2005), Chemical Oxygen Demand COD quantified by colorimetry using a spectrophotometer of Hanna instruments (Smithfield, RI 02917 USA) (O'Dell J.W., 1993), Total Kjeldhal nitrogen, ammonia nitrogen and volatile acids by titration using KjelFlex K-360 coupled with TitrinoPlus (Büchi Labortechnik, Flawil, Suiza), and total, partial and intermediate alkalinity (Ripley L. E., 1986).

In order to evaluate the eventual effects of antibiotic concentration on the digestion process, three aliquots of the cow manure were taken and spiked with different amounts of antibiotics to double, triplicate, and quadruplicate the concentration of antibiotics found in manure which ranged between 0.1 and 75 ng kg^{-1}.

The anaerobic digestion of the spiked and non-spiked manure took place in micro digestors (500 mL) in batch on continuous basis in triplicate at 37 ºC. The volume of methane was measured using an equipment AMPTS II (Bioprocess Control, Lund, Sweden). The experiments were finished when the daily methane production was lower than 1% of the production of the day before.

The process of 16S and archaea amplicons generation and sequencing on Illumina Miseq equipment was carried out at the Genomics Unit of the Madrid Science Park. Antibiotics were analysed by ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS/MS) after a solid phase extraction (SPE).

Results and Discussion

The presence of antibiotics of the sulfonamide and tetracycline families did not affect the biomethane production in this assay. The taxonomic profile of bacteria and archaea in the digestates was more similar to the profile of the sludge, used as inoculum, than that of the manure, the substrate for anaerobic digestion. Preliminary data showed a resistance of these taxa to the presence of these antibiotics, possibly due to the higher presence of antibiotics in the inoculum. So that, neither these bacteria, nor their performance in the anaerobic digestion of the manure were

therefore affected. Other researchers found similar results concluding that the presence of some antimicrobials did not affect the biomethane production at low concentrations (mg kg^{-1}). However, high concentrations of these compounds decreased the methane yield (Koniuszewska et al., 2021).

The cumulate CH_4 production can be seen in Figure 1, the methane yield of the different treatments was very similar.

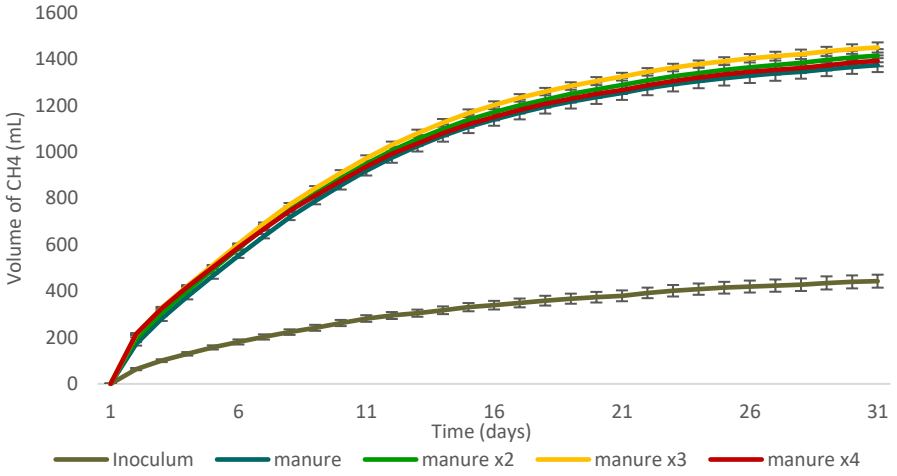

Figure 1 Cumulated methane production in the assay: manure x2 (concentration of antibiotics duplicated), manure x3 (concentration of antibiotics triplicated) and manure x4 (concentration of antibiotics quadruplicated) (n=3). Error bars represent standard deviation)

Sulfonamides were not affected by the digestion but a high decrease in tetracycline concentration was observed, even in the digestors with the quadruplicated concentration.

In the digestates obtained at the end of the process, bacteria and archaea from manure were replaced by those of them present in the inoculum.

Conclusions

Biomethane production by anaerobic digestion from cattle manure did not result affected by the presence of low concentration of antibiotics of sulfonamide and tetracycline families. It was demonstrated in this work since manure and inoculum presented low concentration of them, enough for bacteria and archaea to perform normally in the anaerobic digestion and biomethane production. In addition, except for tetracycline, antibiotics used in this work went throughout the process without being degraded, so more research should be done in this respect as those low concentrations of antibiotics are everywhere and this is considered a major concern due to the potential risk of antimicrobial resistant bacteria and the spread of antimicrobial resistance genes.

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