

# 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
- Anaerobic digestion has been an optimal way to use those organic wastes
- Cattle manure usually 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 represents an important source of bacterial resistance and the spread of antibiotic resistance genes

# OBJETIVES

- To evaluate the potential effect of the presence of antibiotics at different concentrations on:
  - ✓ the biomethanization process in mesophilic conditions
  - ✓ the bacterial communities involved
  - ✓ the fate of those antibiotics throughout the anaerobic digestion process

# MATERIALS AND METHODS

- Cattle manure mixed with the animal bedding was used as anaerobic digestion substrate
- The sludge from a wastewater treatment plant located inside the same farm was used as inoculum
- The presence of antibiotics of Sulfonamides and Tetracyclines families was analysed in cattle manure and sludge by solid phase extraction followed by UPLC-MS/MS analysis

# MATERIALS AND METHODS

Initial estimated concentration of antibiotics (n=3)									
ng/kg	SDZ	STZ	SP	SMZ	STOL	SMX	SDX	OTC	TC
<b>Control (Manure)</b>	0,07±0,03	ND	ND	0,04±0,02	0,11±0,03	0,75±0,43	0,07±0,08	1,17±0,02	74,57±8,19
<b>Inoculum (Sludge)</b>	33±1	1,86±0,07	0,36±0,03	13±2	ND	30±3	1,3±0,03	3,8±0,9	7±1

- Manure showed lower estimated concentrations of antibiotics than the sludge except for Tetracycline (TC)

Antibiotics added					
ng/kg	SDZ	SMZ	SMX	OTC	TC
<b>Control</b>	0	0	0	0	0
<b>Manure x2</b>	0,14	0,08	1,5	2,5	150
<b>Manure x3</b>	0,2	0,12	2,25	3,5	223,71
<b>Manure x4</b>	0,28	0,15	3	4,66	298,28

- The estimated concentration of antibiotics in manure were duplicated, triplicate and quadruplicate to study the potential effect on anaerobic digestion process

# MATERIALS AND METHODS



Homogenisation



Substrate homogenised



Inoculum

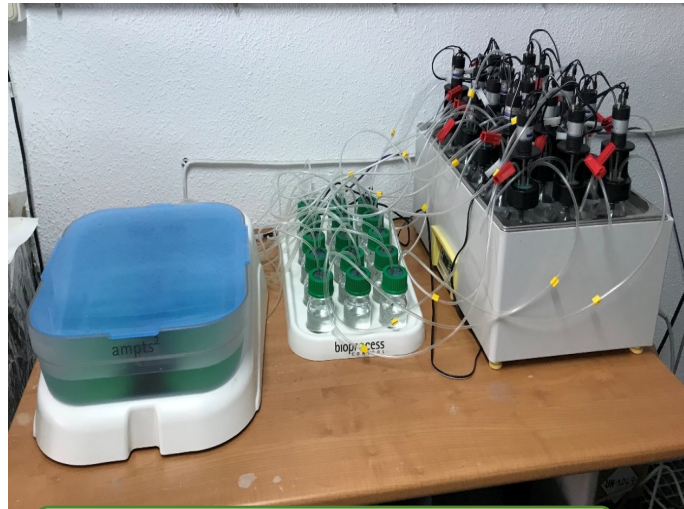
## Control parameters

VS (APHA, 2005)

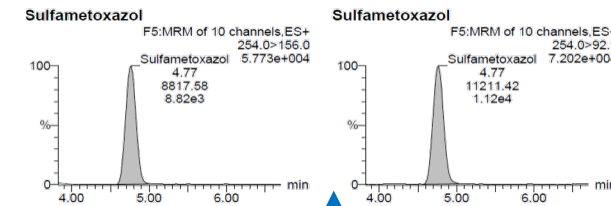
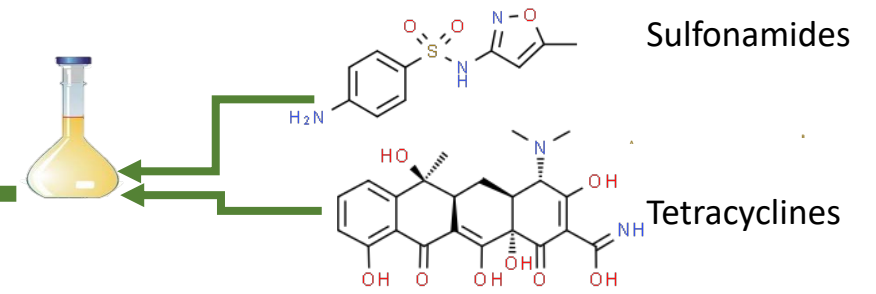
**Chemical Oxygen Demand (COD)** by spectrophotometer of Hanna instruments (Smithfield, RI 02917 USA) (James W. O'Dell, 1993)

**Total Kjeldhal nitrogen, ammonia nitrogen and volatile acids** by titration using KjeldFlex K-360 coupled with TitrinoPlus (Büchi Labortechnik, Flawil, Suiza), Total, partial and intermediate **alkalinity** (Ripley L. E., 1986).

The anaerobic digestion took place in micro digestors (500 mL) in batch on continuous basis in triplicate at 37 °C. The ratio Inoculum/substrate was 2:1. The volume of methane was daily measured using an equipment AMPTS II (Bioprocess Control, Lund, Sweden). The digestions were finished when the daily methane production was lower than 1% of the production of the day before.



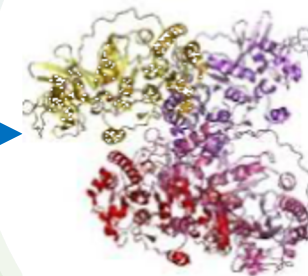
Anaerobic digestion



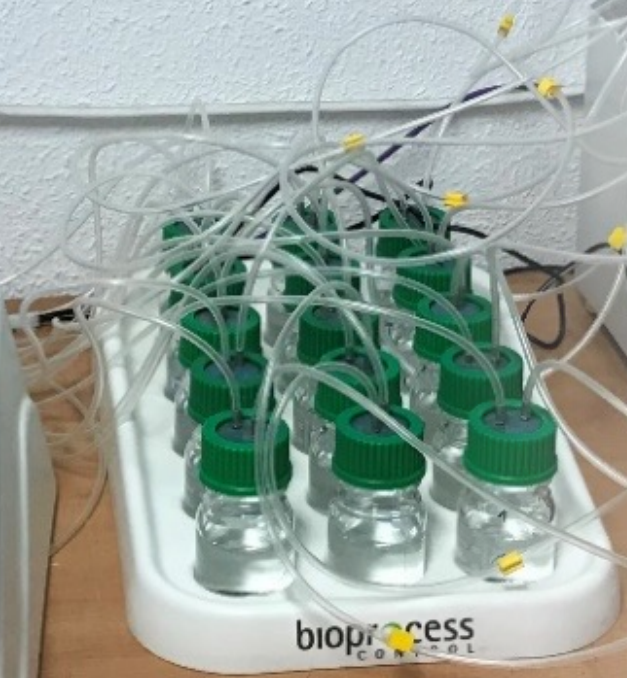
Antibiotics analysis

DIGESTATES

RNA extraction and sequencing (16S)

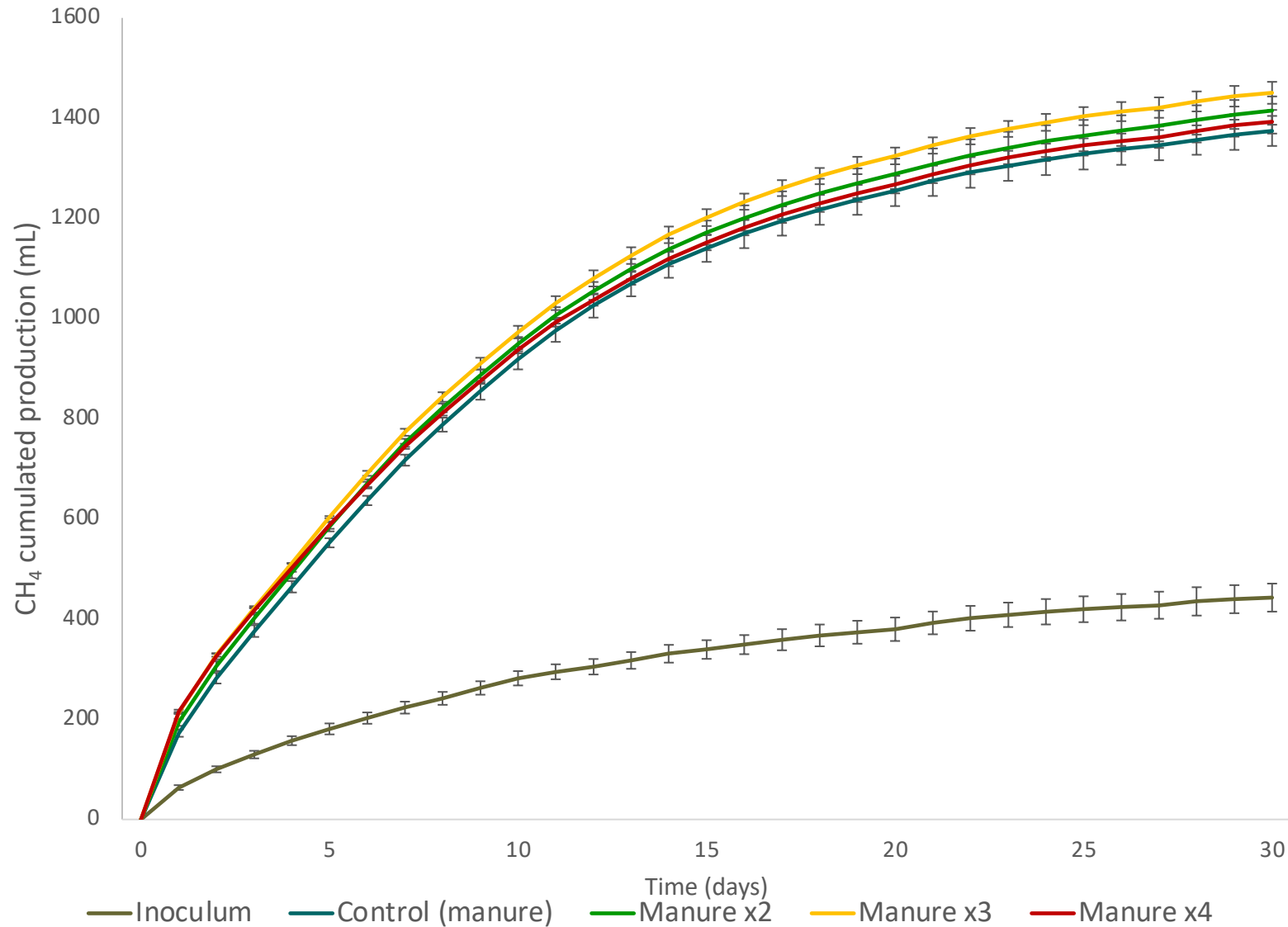






Results

# RESULTS: Methane cumulated production

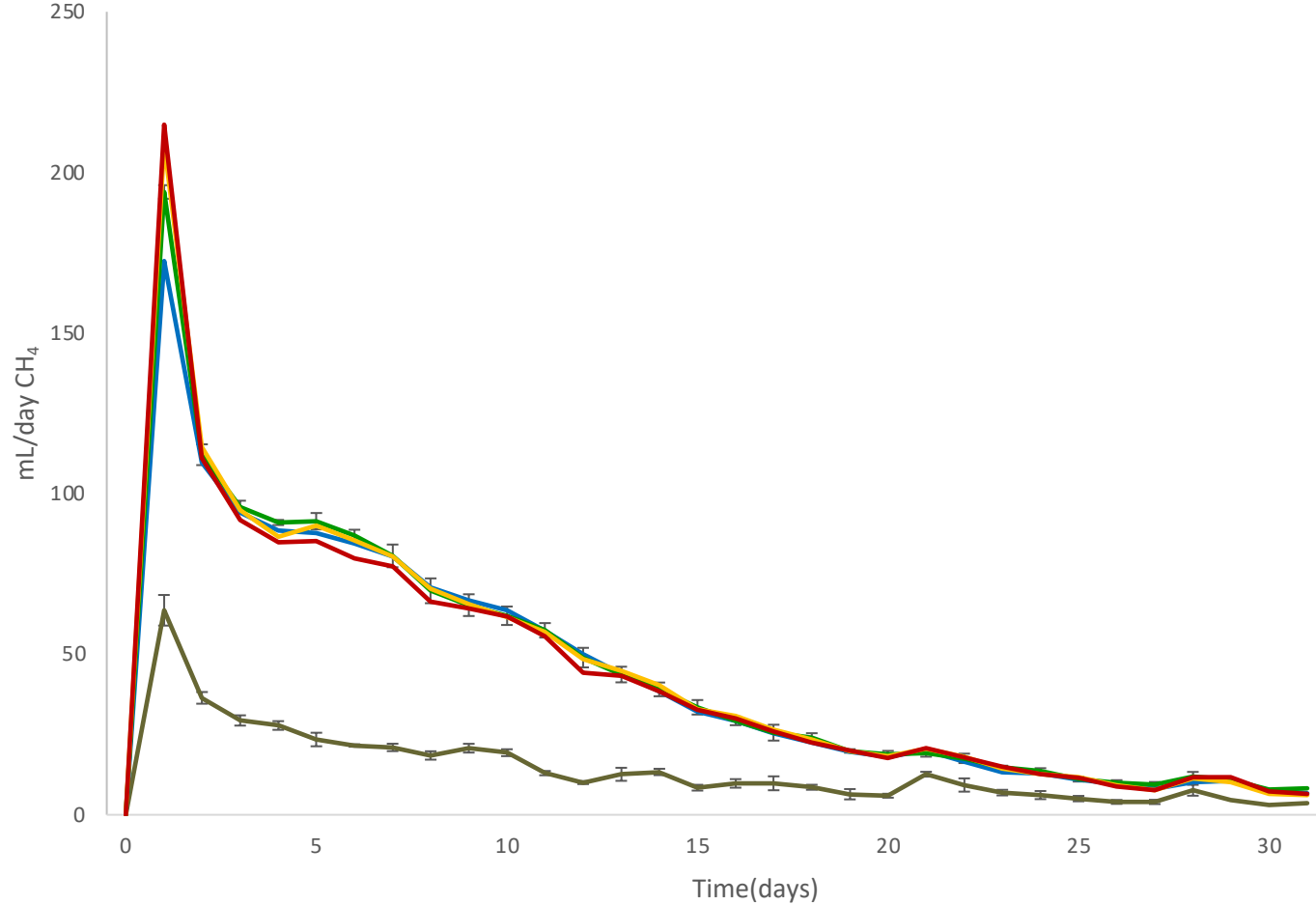


The presence of antibiotics of the sulfonamide and tetracycline families did not affect the biomethane production.

Manure x3 was the treatment with higher production but it was not significant



# RESULTS: Methane daily production



— Inoculum — Control (manure) — Manure x2 — Manure x3 — Manure x4

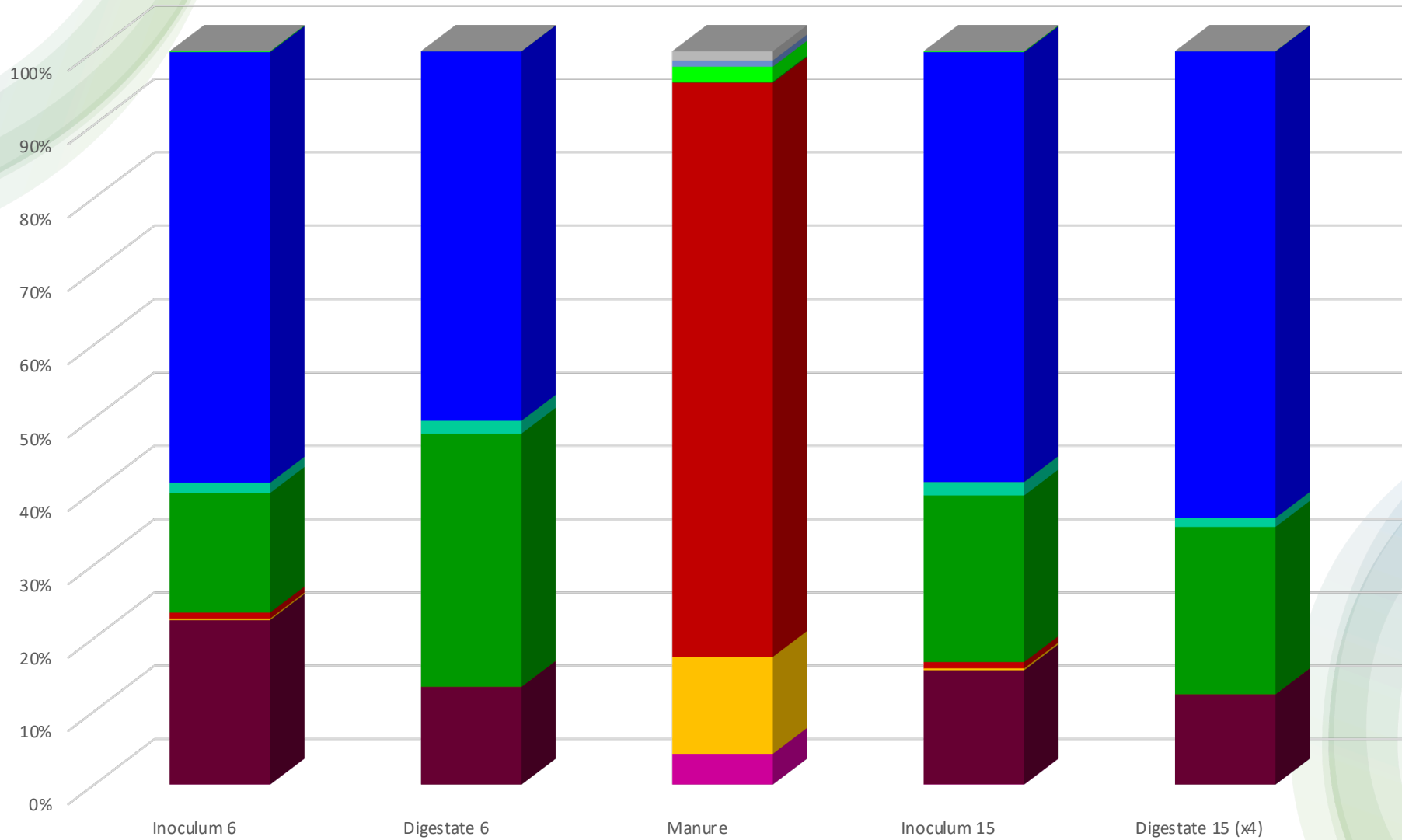
- Biomethane production reached a peak at day 2.
- At the beginning manure x4 was the treatment that produced more CH<sub>4</sub>, but later all of the treatments including the control followed the same pattern

## RESULTS: Antibiotic analysis of the digestates

Digestates								
ng/kg	SDZ	STZ	SP	SMZ	SMX	SDX	OTC	TC
<b>Inoculum</b>	0,9±0,4	0,6±0,1	0,4±0,1	8,0±0,4	0,7±0,1	2,9±1,5	1,12±0,01	0,1
<b>Control (Manure)</b>	0,9±0,3	0,5±0,1	0,5±0,1	6,9±0,1	0,8±0,1	2,59±0,02	0,8±0,1	0,09±0,04
<b>Manure x2</b>	1,0±0,3	0,7±0,3	0,5±0,1	26±8	0,8±0,1	4,88±0,04	0,57±0,01	ND
<b>Manure x3</b>	1,0±0,4	0,7±0,2	0,6±0,2	8±1	1,0±0,4	1,3±0,4	1,1±0,2	0,105
<b>Manure x4</b>	1,0±0,2	0,6±0,1	0,52±0,02	8,6±1,9	0,7±0,1	3,0±0,4	0,9±0,3	0,2

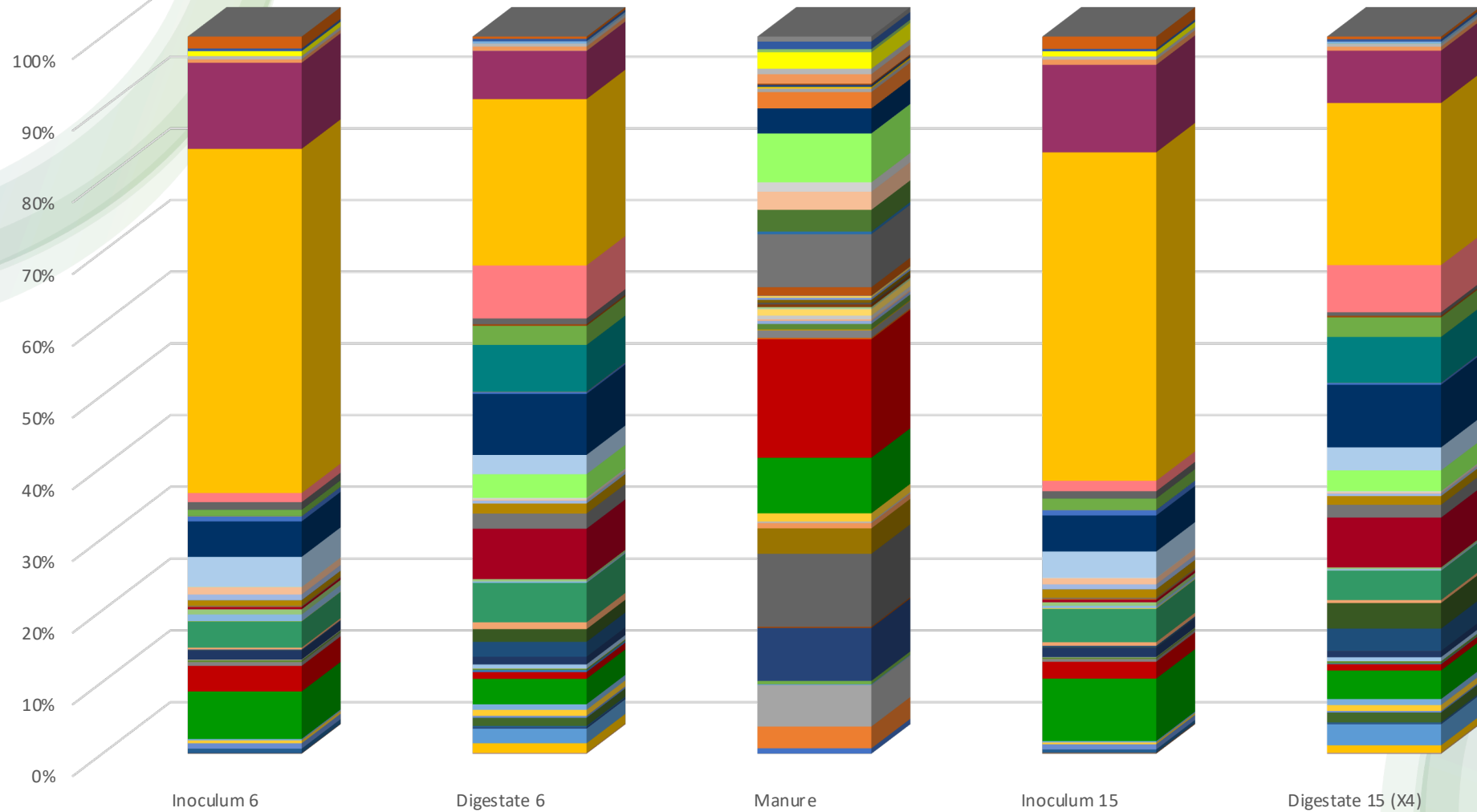
- Sulfonamides were kept throughout the anaerobic digestion process
- Tetracyclines had their concentration decreased at the end of the process

# RESULTS: Relative abundance Archaea (Genus)

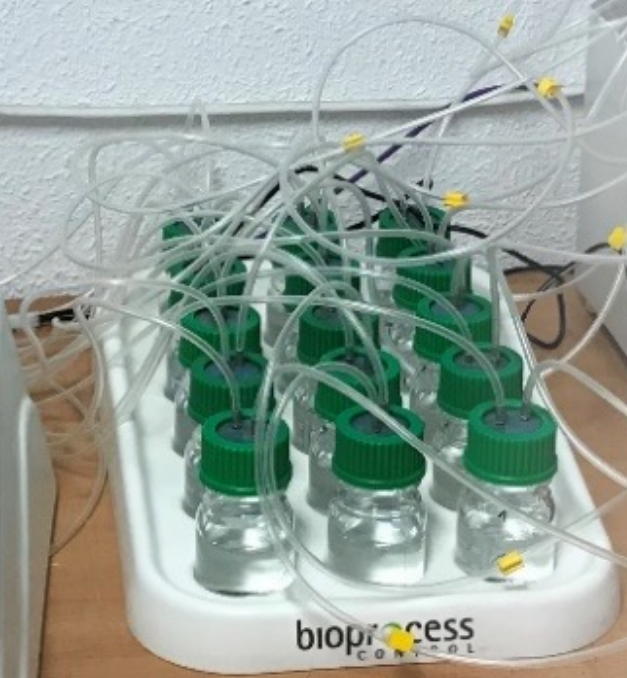


- Candidatus\_Methanoregula
- Methanimicrococcus
- Methanobacterium
- Methanobrevibacter
- Methanococcus
- Methanogenium
- Methanomasiliicoccus
- Methanomethylovorans
- Methanoplanus
- Methanosaeta
- Methanosarcina
- Methanosphaera
- Methanospirillum
- vadinCA11

# RESULTS: Relative abundance Bacteria (Genus)



- |                      |                  |                    |                     |                    |                 |                    |                   |                     |
|----------------------|------------------|--------------------|---------------------|--------------------|-----------------|--------------------|-------------------|---------------------|
| ■ 5-7N15             | ■ Acholeplasma   | ■ Acinetobacter    | ■ Aequorivita       | ■ Alcanivorax      | ■ Anaerofilum   | ■ Arcobacter       | ■ Bacillus        | ■ Bacteroides       |
| ■ BF311              | ■ BHB21          | ■ Blvii28          | ■ Caldico probacter | ■ Carnobacterium   | ■ Cellvibrio    | ■ Chryseobacterium | ■ Cloacibacterium | ■ Clostridium       |
| ■ Corynebacterium    | ■ Cryomorphia    | ■ Desulfomicrobium | ■ Desulfovibrio     | ■ Desulfuromonas   | ■ Devosia       | ■ Dietzia          | ■ Epulopiscium    | ■ Facklamia         |
| ■ Fibrobacter        | ■ Gelidibacter   | ■ Jonesia          | ■ Kos motoga        | ■ Lactobacillus    | ■ Lactococcus   | ■ Lu teimonas      | ■ Lutispora       | ■ Lysobacter        |
| ■ Methanocorpusculum | ■ Methanoculleus | ■ Methanosaeta     | ■ Mogibacterium     | ■ Mycobacterium    | ■ N09           | ■ Niabella         | ■ Oligella        | ■ Paludibacter      |
| ■ Pelotomaculum      | ■ Planomicrobium | ■ Prevotella       | ■ Propionicimonas   | ■ Proteinclasticum | ■ Pseudomonas   | ■ RFN20            | ■ Rhodobacter     | ■ Sedimentibacter   |
| ■ SHD-231            | ■ Sphaerochaeta  | ■ Sphingobacterium | ■ Sphingobium       | ■ Sporosarcina     | ■ Streptococcus | ■ Streptomyces     | ■ Sulfurimonas    | ■ Syn troph obacter |
| ■ Syntrophomonas     | ■ T78            | ■ Thermomonas      | ■ Thiobacillus      | ■ Treponema        | ■ Trichococcus  | ■ Turicibacter     | ■ vad in CA02     | ■ vad in CA11       |
| ■ vad in HB04        | ■ W5             | ■ Wautersiella     |                     |                    |                 |                    |                   |                     |



Conclusions



# 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
- Except for tetracycline, antibiotics used in this work went throughout the process without being degraded
- Archaea and Bacteria genus from inoculum prevail in digestates meanwhile those from manure were not present in them

The background features two large, overlapping, curved decorative elements. One is a light blue arc in the upper right, and the other is a light green arc in the lower left. Both have a soft, multi-layered gradient effect.

Thank you very much for your attention