

# Water Treatment Residues and Methanogenic Activity in Wastewater-Sludge

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The effect of adding alum water treatment residues (WTR) on the methanogenic activity in the digestion of primary domestic wastewater sludge was evaluated through laboratory experiments in sedimentation columns, using total suspended solids (TSS) concentrations from 0.37 to 1.23 g/L. The addition of WTR to primary clarifiers can benefit its effluent water quality in terms of colour, turbidity, chemical oxygen demand (COD), and TSS. However, the presence of WTR can negatively influence the production of methane gas during organic sludge digestion in primary clarifiers, for concentrations of TSS between 14.43 and 25.23 g/L and of VSS between 10.2 and 11.85 g/L. The activity of the *Methanothrix* sp., curved bacilli, methanococci, and *Methanosarcina* sp. decreases considerably after 16 days of anaerobic digestion, and methane production seems to only be associated with fluorescent methanogenic bacilli.

Keywords: alum sludge ; anaerobic bacteria ; methane production ; specific methanogenic activity ; water treatment residues ; methanogenic bacteria

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## 1. Introduction

The production of drinking water in water treatment plants (WTP) involves the removal of several suspended and dissolved compounds, such as inorganic compounds (e.g., clay, silt, and sand) and organic compounds, consisting of humic substances which change the colour and turbidity of water, such as planktonic organisms, bacteria, protozoa, and viruses. In order to remove inorganic and organic compounds from water, several reagents can be used, such as coagulants, which destabilise the organic particles leading to the formation of precipitates called water treatment sludge or water treatment residues (WTR) <sup>[1]</sup>. These residues are essentially accumulated in the decanters and filters of the WTP, varying in their volume between 0.1% and 1.5% of the total volume of the treated water <sup>[2][3]</sup>. Flocculants, and tanks for chemical solutions preparation, also produce small amounts of residues during washing cycles <sup>[4]</sup>.

Currently, there are several alternative options for WTR valorisation being studied, for example, for the production of ceramic materials, bricks, cement, and concrete materials <sup>[5][6][7]</sup>, physical-chemical correction of soils <sup>[8][9][10]</sup>, used as adsorption material for the removal of phosphorous <sup>[11][12]</sup> and heavy metals <sup>[13]</sup>, and its recirculation at WTP for reducing the use of coagulants <sup>[14][15][16][17][18]</sup>. Several authors have studied the advantages of introducing WTR in wastewater treatment plants (WTP) and the removal of pollutants from stormwater runoff, having concluded that this procedure would help the sedimentation process in primary settlers <sup>[4][17]</sup>, as well as the removal of organic matter, phosphorous, and heavy metals <sup>[18][19][20]</sup>. Granulation techniques for particle enlargement have been tested to avoid the clogging problem when WTR is applied in biofilm reactors <sup>[21]</sup>.

The anaerobic conversion of sludge organic materials in primary settlers involves several pathways, including methanogenesis, which leads to the production of biogas (a mixture of methane and carbon dioxide, which is a useful, renewable energy source). During the last few years, the production and conversion of methane have received more attention, as they can reduce carbon emissions via the production of renewable energy. The methane-producing capability activity can be followed through laboratory tests, which are useful for evaluating systems' performance and its stability, as well as for defining the suitable operating conditions for good behaviour of anaerobic systems <sup>[22]</sup>.

The specific methanogenic activity (SMA), or specific sludge activity, is determined by the methane production rate, or substrate depletion rate, and amount of sludge <sup>[4][22]</sup>, normally expressed in terms of mass of methane as COD per volatile suspended solids (VSS). The innovation of this work is the evaluation of the impact of WTR on methane production in primary settlers. Therefore, the main objective of the work was to evaluate the potential effects of adding WTR on the performance of primary clarifiers (i.e., on the sedimentation characteristics of primary sludge and on the effluent water quality), as well as on the production of methane gas.

## 2. Evaluation of the Sedimentation Performance of Primary Clarifiers

The experiments for evaluating the impact of WTR on the performance of primary clarifiers were set up in four sedimentation test columns (Figure 1). A mixture of WTR from a WTP clarifier (WTR-C, 19.9%) and from filters washing (WTR-F, 80.1%) from the WTP of S. Carlos (SP, Brazil) was used. Aluminium sulphate is the main coagulant for water treatment. Domestic wastewater (DWW) samples were collected at the sewer network of the city of S. Carlos (SP, Brazil). Table 1 shows the volumes used in the experiments.

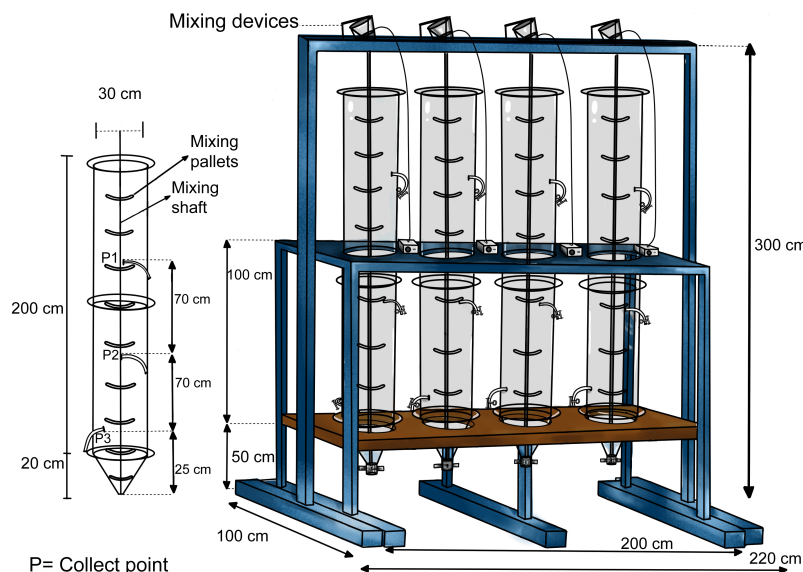


Figure 1. Test columns: (a) picture and (b) schematic representation.

Table 1. Volumes of WTR and DWW used in the sedimentation experiments.

Samples	CC	Columns with WTR					
		TC1		TC2		TC3	
	(% of Volume)	Volume (%)	TSS (g/L)	Volume (%)	TSS (g/L)	Volume (%)	TSS (g/L)
DWW	100	92.76	0.137	92.76	0.137	92.76	0.137
WTR-C	0	1.44	0.88	1.44	2.71	1.44	5.24
WTR-F	0	5.80	0.23	5.80	0.23	5.80	0.23

WTR = water treatment residues; CC: control column; TC: testing columns; DWW: domestic wastewater; WTR-C: water treatment residues from clarifiers; and WTR-F: water treatment residues from filters.

The TSS concentration for WTR-F was 0.23 g/L, and for WTR-C, it was 2.71 g/L. From the WTR-C sample, two others were prepared with a lower TSS (0.88 g/L) and higher TSS (5.24 g/L), to predict possible daily variations in the ratio of wastewater/WTR-C, as suggested by [4]. Thus, WTR-C samples with TSS of 0.88, 2.71, and 5.24 g/L were used in the columns TC1, TC2, and TC3. The TSS concentration of the raw WTR-F, in the three TC, was kept constant with the initial value (0.23 g/L). The volume percentages of wastewater, WTR-C, and WTR-F were kept constant, and only the TSS of the WTR-C was changed. The fourth column was fed only with wastewater, and this was the control column (CC). The total volume of each column was 130 L (120.6 L were occupied with DWW, and 9.4 L with WTR).

At times 0, 60, and 120 min of operation, composite samples of supernatant were collected along the columns (i.e., composed of the same volumes of aliquots collected from the three collection points), these were considered representative of the sedimentation columns. These samples were characterized in terms of pH, COD, colour, turbidity, total solids (TS), TSS, FSS, VSS, total coliforms (TC), E. coli, and worms and eggs of parasitic helminths (PH), for evaluating the effect of the WTR on the effluent water quality of primary clarifiers. The analyses were performed according to the Standard Methods [26], with the modified Bailenger method used for pH quantification [23].

## 3. Batch Experiments for Evaluating the Methanogenic Activity in Primary Sludge

After the sedimentation experiments were finished, a sludge similar to that produced in primary clarifiers was obtained, which was designated by water treatment residues from primary clarifiers (WTR-PC), at the bottom of each column. The WTR-PC of each TC was then mixed with a digested anaerobic sludge (DAS), an inoculum with a SSV concentration of 9 g/L, collected from the anaerobic WWTP digester from the city of Piracicaba (SP, Brazil). The four mixtures were placed in four digestion flasks (DF) of 620 mL each, defined as DF1, DF2, DF3, and DFC for the WTR-PC, coming from the

columns TC1, TC2, TC3 and CC, respectively. These experiments were used for evaluating the impact of the WTR-PC on the production of methane gas (methanogenic activity assay, with SMA calculation). The methodology proposed by Aquino [24] was followed, which included the determination of methane gas production, with duplicate tests at a thermostat-controlled temperature of 30 °C. The WTR-PC and DAS were characterized in terms of pH, COD, TS, TSS, FSS, and VSS, using the Standard Methods [25].

The concentration of methane gas produced was measured daily through chromatography, using an equipment Alpha MOS PR 2100 USA) equipped with FID detector and capillary column. During these experiments, samples were collected on the 8th and 16th days after incubation, using a needle and syringe, for optical microscopy analysis, at 10× and 40× through an Olympus BX 60 microscope (Japan).

The SMA calculation was performed for each methane gas concentration. This was through the angular coefficient of the linear equation, obtained from the maximum slope curve, after plotting the volumes of methane, in terms of COD consumed, divided by the concentration of VSS of the inoculum (i.e., in gCOD-CH<sub>4</sub> per gVSS.d), as mentioned in [22][24][26].

## 4. Effect of WTR on the Sedimentation Characteristics of Primary Sludge and on the Effluent Water Quality of Primary Clarifiers

The characteristics of the WTR and the DWW samples used in the TC are shown in Table 2. For the WTR, between 78.3% and 80.2% of TSS is composed of fixed solids, i.e., most of the solid material is formed by inorganic substances (e.g., silt, sand, clay, and metal hydroxides). The fraction of VSS varied between 19.8% and 21.7%, which is contrary to that observed in the DWW samples, where 85.4% of the TSS is of the volatile type, due to the presence of organic matter.

Table 2. Characteristics of the WTR and the DWW before the sedimentation experiments.

Parameter	WTR Samples before the Experiments				WTR Samples after Mixing Used in Each Column				DWW
	WTR-F	WTR-C			CC	WTR-C + WTR-F			
		TC1	TC2	TC3		TC1	TC2	TC3	
Turbidity (NTU)	172	576	1836	3565	Only DWW; No WTR	260	477	745	78
TSS (g/L)	0.23	0.88	2.71	5.24		0.37	0.72	1.23	137
FSS (g/L)	0.18	0.70	2.16	4.20		0.29	0.57	0.98	20
VSS (g/L)	0.05	0.18	0.55	1.04		0.08	0.15	0.25	117
pH	7.3	6.7	6.7	6.7		6.9	6.9	6.9	6.9
COD (mg/L)	62	268	1048	962		78	263	197	381

WTR = water treatment residues; CC: control column; TC: testing columns; DWW: domestic wastewater; WTR-C: water treatment residues from clarifiers; WTR-F: water treatment residues from filters; TSS: total suspended solids; FSS: fixed suspended solids; VSS: volatile suspended solids; and COD: chemical oxygen demand.

After mixing the WTR with the DWW, the TSS concentrations at the beginning of the experiments were 0.37, 0.72, and 1.23 g/L for TC1, TC2, and TC3, respectively. The results of Table 2 also show that the biomass (expressed in VSS), solids contents (as TSS), and the organic matter (expressed in COD) were diluted after joining the WTR-C, WTR-F, and DWW for the columns' testing. The reduction of TSS was 58%, 73.4%, and 76.5% when both residues were mixed in TC1, TC2, and TC3, respectively, or COD was 70.9%, 74.9%, and 79.5%, and turbidity was 54.8%, 74% and 79.1%.

After the sedimentation period of 2 h, for the columns with WTR, an increase of the removal efficiency (RE) of solids in the liquid effluent, for any point P1, P2, and P3, as the TSS concentration was increased in the WTR-C, was seen. The final liquid effluent TSS at P3 was 104, 106, 90, and 120 mg/L for TC1, TC2, TC3, and CC, respectively. Therefore, the increase in WTR at primary clarifiers increases the sedimentation of the organic sludge and improves the effluent water quality in terms of TSS.

The RE of colour, TSS, and COD for the CC was below 20%, and the turbidity actually increased. The colour removal in the TC with WTR was 16.9%, 19.5%, and 33.3%, for TC1, TC2, and TC3, respectively, whilst for turbidity the RE was 15.9%, 17.2%, and 19%. For TSS, the RE was 22.6%, 28.5%, and 31.4%, and for COD, the RE was 17.8%, 21%, and 23.4%. The solid removal in the three columns was mainly in the form of VSS. There was a reduction of the total coliforms (TC) and E. coli content after contact with WTR, but the analysis was inconclusive for worms and eggs of parasitic helminths (PH).

## 5. Effect of WTR on the Methane Production from Primary Sludge

The characterization of the WTR-PC and the DAS for the SMA experiments is shown in Table 3. The fraction between biomass present in both materials (fraction VSSWTR-PC/VSSDAS) changed between 0.21 and 0.25 g/g.

Table 3. Characteristics of the WTR-PC and DAS before the experiment for determining the SMA.

Digestion Flasks	Composition of the Digested Flasks									Volume of Deionized Water (mL)
	WTR-PC					DAS				
	Vol. (mL)	pH	TSS (g/L)	FSS (g/L)	VSS (g/L)	Vol. (mL)	TSS (g/L)	FSS (g/L)	VSS (g/L)	
DFC	120.5	7.0	14.43	3.23	11.20	57.20	87.73	40.50	47.23	122.3
DF1	132.4	7.2	17.42	7.22	10.20	57.20	87.73	40.50	47.23	110.4
DF2	113.9	7.1	23.28	11.43	11.85	57.20	87.73	40.50	47.23	128.9
DF3	118.4	7.2	25.23	13.83	11.40	57.20	87.73	40.50	47.23	124.4

WTR-PC = water treatment residues from primary clarifiers; TSS: total suspended solids; FSS: fixed suspended solids; VSS: volatile suspended solids; DAS: digested anaerobic sludge; DFC: digestion flask control; and DF: digestion flask.

The cumulative SMA, throughout the 60 days of experiments, is presented in Figure 2a. It is noted that the highest amount of methane was produced in the DFC, which did not receive WTR (cumulative value of 0.077 gCOD-CH /gVSS.d). The DF with WTR showed a decrease in methane production as the TSS concentration increased, reaching a difference of 16.6%, 19.6%, and 26.4% between DF1, DF2, and DF3 and the control flask DFC, respectively. The cumulative values for the DF with WTR were 0.06 gCOD-CH /gVSS.d (DF1), 0.057 gCOD-CH /gVSS.d (DF2), and 0.052 gCOD-CH /gVSS.d (DF3). Therefore, negative interference can occur in methane production in sedimentation tanks, when WTR with TSS between 14.43 and 25.23 g/L, and VSS between 10.2 and 11.85 g/L are used.

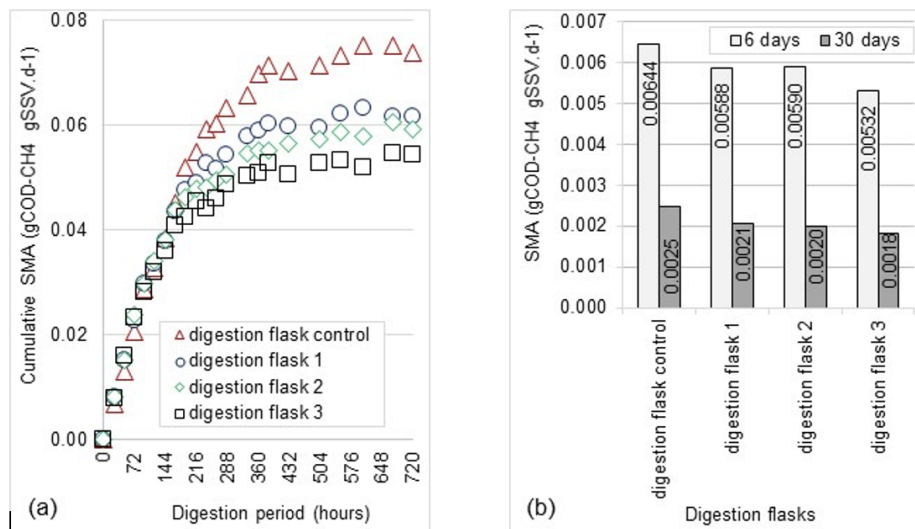


Figure 2. SMA production: (a) cumulative SMA for each DF and (b) SMA for 6 and 30 days

Figure 2b presents, for comparison, the production of methane gas for 6 and 30 days of digestion, considering the greater slope of the production curves for each DF shown in Figure 3. Higher methane production without WTR (from 0.0025 to 0.006 gCOD-CH<sub>4</sub>/gVSS.d) can be seen, and this decreases as TSS increases in DF1, DF2, and DF3. The difference between methane production in the DFC and the other flasks was 8.7%, 8.4%, and 17.3% for DF1, DF2 and DF3, respectively.



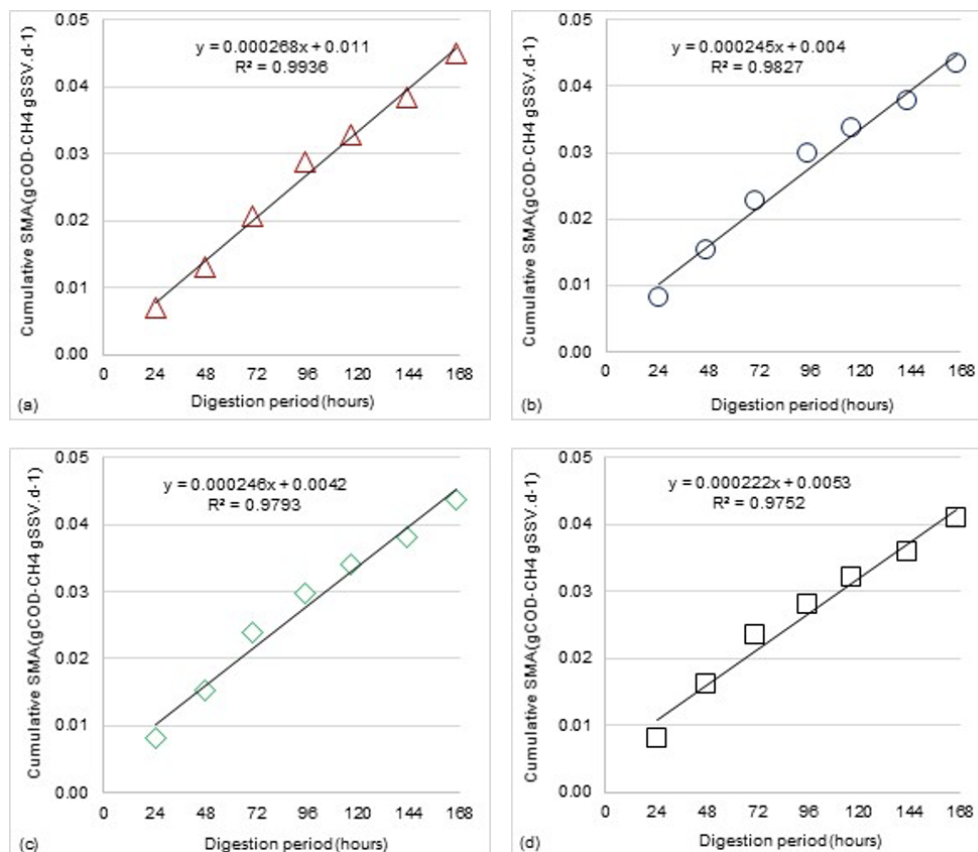


Figure 3. Determination of the angular coefficient of the curve section with the highest slope of the methane production: (a) DFC; (b) DF1; (c) DF2; and (d) DF3.

The values of SMA of Figure 2b show that there was negative interference of WTR in the production of methane gas along the two scenarios (6 and 30 days of digestion). However, production of gas still occurred, suggesting that some methanogenic microorganisms were physiologically active even in the presence of aluminium oxides.

For volumetric relationships between total sludge and anaerobic sludge up to 86%, there was no significant decrease in methane production. However, for higher volumetric ratios, a significant inhibitory effect was registered. The temperature and the ratio between organic sludge and alum sludge seems to interfere in the production of methane and thus should be controlled in order to reduce the damage in anaerobic bacteria.

The microscopic analysis on the DF showed that, after 8 days of incubation, a predominance of methanogenic rod-shaped bacteria, which are fluorescent and produce methane via the hydrogenotrophic pathway (Figure 4).

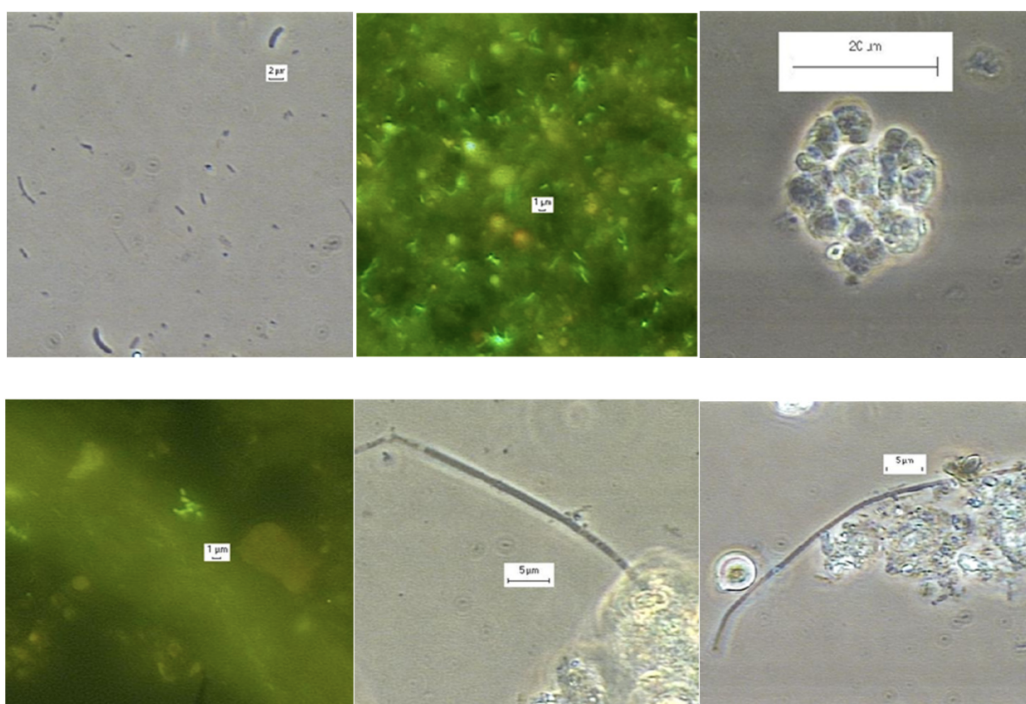


Figure 4. (a) Bacteria in the form of curved bacilli similar to sulphate reducing agents in DFC; (b) fluorescent bacteria in the form of rods; (c) methanosarcina sp. present in DF1; (d) few fluorescent methanococci; and (e,f) methanothrix sp present in DF2 and DF3.

Bacteria of the genus *Methanothrix* sp. were lower in relation to rod-shaped bacteria. Methanogenic bacteria of the genus *Methanosarcina* sp., methanococci, which are fluorescent, and bacteria in the form of curved bacilli, similar to the sulphate reducers, were also found in small quantities in all flasks. In the DF1, DF2, and DF3, a decrease in the bacteria of the genus *Methanothrix* sp. was observed, whilst the bacteria in the form of rods had a slight increase, with higher values for the DF1. In these three reactor flasks were found, in small quantity, methanogenic bacteria in the form of cocoons and *Methanosarcina* sp.

*Methanosarcina* species, such as *Methanosarcina lacustris* and *Methanosarcina mazei*, are metabolically versatile with respect to substrate utilization for methane production, using different pathways and a variety of substrates for methanogenesis, such as H<sub>2</sub> together with CO<sub>2</sub>, acetate, methanol, and methylamines [27].

After 16 days of incubation in the DFC, there was a predominance of fluorescent methanogenic bacilli and non-methanogenic bacilli; only a few *Methanothrix* sp. bacteria were found, and no methanococci and *Methanosarcina* sp. was detected. In DF1, DF2, and DF3, more fluorescent bacilli and only a few *Methanothrix* sp. was observed. The fluorescent bacilli were higher at the DF1. In the three flasks, some curved bacilli, similar to sulphate reducing agents, were found along with fungal hyphae and few nonfluorescent bacilli.

Microbiological analyses only confirm the existence of species and genus of bacteria, with participation in the reactions of methanogenesis. Although there was a decrease in the production of methane gas after adding aluminium-based WTR, probably due to the destruction and inhibition of part of the anaerobic consortium, anaerobic bacteria with methane gas capacity still remained in the DF after 16 days of anaerobic digestion. The genus *Methanothrix* sp. and the curved bacilli decreased considerably with the introduction of WTR, whereas the methanococci and *Methanosarcina* sp. no longer appear after 16 days of incubation.

Fluorescent methanogenic bacilli and non-methanogenic bacilli were detected in both samples and did not appear to have been affected by the introduction of WTR after 16 days of reaction. Overall, it seems the time of biodigestion influenced the diversity of bacteria and Archaea communities. The biodiversity of methanogenic microorganisms was significantly decreased over time, which indicates that *Methanothrix* sp. and curved bacilli seem susceptible to the addition of WTR in the long term. Therefore, fluorescent methanogenic bacilli seem to be the main bacteria responsible by keeping methane production, after adding WTR for 16 days of anaerobic digestion.

The results of this research seem to indicate that the use of WTR in wastewater treatment plants can be useful, either for improving the conditions for settling in primary settlers, or for improving the quality of the effluent for secondary biological treatment, or also for improving the production of methane.

## 6. Conclusions

Adding alum WTR at primary clarifiers, for TSS concentrations between 0.37 and 1.23 g L<sup>-1</sup>, increases the sedimentation of the organic sludge and improves the effluent water quality in terms of colour, turbidity, COD, and TSS. The removal of pathogenic microorganisms was not significant. It can cause negative interference in methane production when inorganic sludge with TSS between 14.43 and 25.23 g L<sup>-1</sup> and VSS between 10.2 and 11.85 g L<sup>-1</sup> are used for organic sludge digestion. The ratio between organic sludge and alum sludge seems to interfere in the production of methane and should be controlled, in order to inhibit anaerobic bacteria. The activity of *Methanothrix* sp. and the curved bacilli is considerably affected by the introduction of WTR, whilst methanococci and *Methanosarcina* sp. disappear after 16 days of incubation. Fluorescent methanogenic bacilli seem to be the main bacteria responsible for methanogenesis, after 16 days of anaerobic digestion.

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