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Impact of Alum Water Treatment Residues on the Methanogenic Activity in the Digestion of Primary Domestic Wastewater Sludge

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Abstract: 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) [1]. 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 [2,3]. Flocculants, and tanks for chemical solutions preparation, also produce small amounts of residues during washing cycles [4].

According to Ren et al. [3], the main destination of this waste is sanitary landfills, although they are still available in drying ponds, soils, or even in surface water, which increases the risk of negative environmental impact on the environment, such as changes in soil characteristics, and water quality in water resources. Brazilian Law No. 12.305/2010 [5] on “National Solid Waste Policy” has raised the need to concern ourselves with the management of this type of waste. The European Union has classified WTR as a “residue” with code 19-09 [6] in the list of waste (LoW). Therefore, reusing WTR is a practice for adding value in products for as long as possible and eliminates the disposal of the waste, which is

in accordance with the principles of the SDG 12 (Sustainable Consumption and Production) of the United Nations Sustainable Development Goals (SDGs).

Currently, there are several alternative options for WTR valorisation being studied, for example, for the production of ceramic materials, bricks, cement, and concrete materials [7–9], physical-chemical correction of soils [10–12], used as adsorption material for the removal of phosphorous [13,14] and heavy metals [15], and its recirculation at WTP for reducing the use of coagulants [16–18]. 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 [4,19], as well as the removal of organic matter, phosphorous, and heavy metals [20–22]. Granulation techniques for particle enlargement have been tested to avoid the clogging problem when WTR is applied in biofilm reactors [3,23].

Most of the previous studies looked at the impact of using WTR for removing chemical oxygen demand (COD), phosphorous fractions, nitrogen compounds, total suspended solids (TSS), and total volatile solids (TVS). Sharma et al. [24] evaluated the potential for reuse of iron-rich WTR, as a replacement for commercial iron salts in anaerobic digestion in a primary settler and an activated sludge process, having observed an increase in dissolved iron (II) and iron (III)hydroxides, which produced the rise of pH due to the release of alkalinity. A lower internal recirculation of phosphate concentration in the reject water and the reduction of sulphide in the digested liquid were also observed.

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 [25]. The specific methanogenic activity (SMA), or specific sludge activity, is determined by the methane production rate, or substrate depletion rate, and amount of sludge [4,25], 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. Materials and Methods

2.1. Columns Experiments for Evaluating 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).

Testing columns (TC) were built in acrylic, each one with: a diameter of 0.3 m, a height of 2.2 m, and a volume of 130 L. The lower part is a conical structure of 0.2 m in height, with a collection device for sediment removal. Three collection points (P1, P2, and P3), with a distance of 0.7 m, were included for sampling. Inside the columns, there was a mechanical shaft for keeping the materials mixed during the experiments.

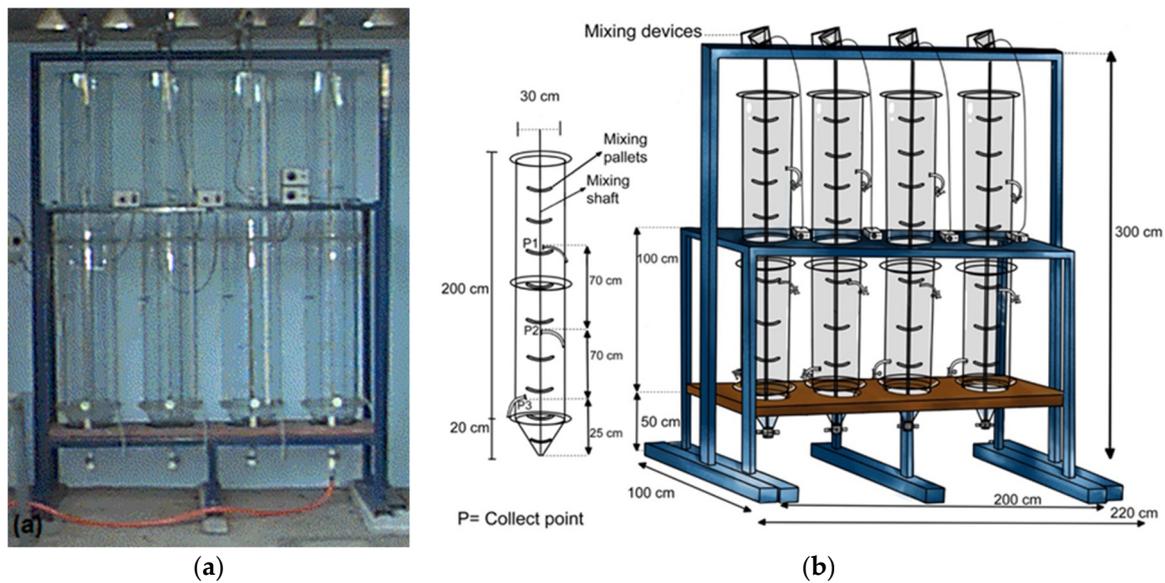


Figure 1. Test columns: (a) picture and (b) schematic representation.

The volume of WTR used in the experiments was estimated from the average volume of drinking water produced in the WTP (1,121,522 m³/month), and the average volume of WTR that was generated at the WTP for producing that volume of water (69,939 m³/month). The volume of wastewater used in the experiments, was forecast from the volume that would theoretically be produced after the use of drinking water (around 897,218 m³/month). Thus, the volumes used in experiments were: 92.76% of wastewater and 7.24% of WTR (1.44% of WTR-C and 5.80% of WTR-F), as presented in Table 1. The samples of DWW, WTR-C, and WTR-F were characterised individually, and after mixing in the TC, for determining the pH, turbidity, TSS, fixed suspended solids (FSS), VSS, and COD, according to the Standard Methods [26].

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

Samples	CC (% of Volume)	Columns with WTR					
		TC1		TC2		TC3	
		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). The columns' materials were mixed for 30 min at a 300 s⁻¹ velocity gradient, simulating the mixing conditions in primary settlers of WWTP, as suggested by [4]. After this time, the mixers were switched off, and then water samples were collected

at P1, P2, and P3 for intervals of between 20 min and 2 h. These samples were analysed for the determination of TSS concentration in order to evaluate the variation of the production of solids along a primary settler (i.e., for assessing the sedimentation characteristics of primary sludge).

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 [27].

2.2. Batch Experiments for Evaluating the Methanogenic Activity in Primary Sludge

The sludge potential for biogas production was seen through the methanogenic activity, which provides information on methane gas production, as well as the presence of inhibitory compounds and slowly degradable, or even non-biodegradable, organic matter.

After the sedimentation experiments were finished (point 2.1.), 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 DF4 for the WTR-PC, coming from the columns TC1, TC2, TC2 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 [28] 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 [26].

The DF reaction volume was 300 mL, including WTR-PC, DAS, and deionized water. The VSS ratio of WTR-PC and of DAS was kept at 0.5. Before the flasks were closed, their contents were bubbled with pure nitrogen (100%). The DF were then shaken continuously at 150 rpm in a Nova Etica shaker, model 430 (Brazil) for 30 days. 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, and the procedures suggested in [4,29]. Hydrogen was used as the carrier gas. Gas samples (0.5 mL) were collected from the DF through a sampler-injector and introduced into the column chamber.

As 1 mole of any gas occupies a volume of 22.7 L in the NTP (20 °C and 1 atm) and, according to the ratio $\text{CH}_4 + 2\text{O}_2 \Rightarrow \text{CO}_2 + \text{H}_2\text{O}$, 1 mole of methane consumes 2 moles of O_2 or 64 g of COD, it can be said that 1 g of destroyed COD is equivalent, at NTP, to 0.355 L of methane (22.7/64). For the test performed at local pressure of 690.7 mmHg, the volume occupied by 1 mole of methane is 27.72 L. Thus, it is possible to say that 0.433 L of methane produced is equivalent to 1 g of COD destroyed.

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_4 per gVSS.d), as mentioned in [25,28,30].

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), at the Laboratory of Biological Processes

of the EESC-USP (S. Carlos, Brazil). This was to identify the microfauna present in the methanogenic activity experiments.

3. Results and Discussion

3.1. 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), being compatible with the values found by [22,31], which describes most of the solid's content in the WTR as inert. 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		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	Only DWW; No WTR	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%.

The TSS concentration variation at the liquid effluent, along each column over 2 h of sedimentation is presented in Table 3. The TSS concentration for the liquid effluent at any point (P1, P2, and P3), and for time 0, was 167, 203, 227, and 137 mg/L for TC1, TC2, TC3, and CC, respectively. This reflected an increase in the TSS concentration at the water effluent for the TC, where more WRs were added. However, 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.

Table 3. Variation of TSS concentrations in the liquid effluent collected along each column for 2 h of sedimentation.

Time (Minutes)	TSS (mg/L)											
	CC			TC1			TC2			TC3		
	P1	P2	P3	P1	P2	P3	P1	P2	P3	P1	P2	P3
0	137	137	137	167	167	167	203	203	203	227	227	227
20	128	136	118	156	154	148	170	176	166	178	176	160
40	134	136	130	156	148	144	146	140	134	130	140	110
60	132	128	124	138	136	130	126	118	110	112	112	110
80	130	128	126	146	138	132	124	116	110	128	118	114
100	134	124	122	124	124	114	114	114	112	114	112	106
120	132	132	120	130	122	104	108	108	106	102	98	90
RE (%)	0.75	0.75	12.41	22.15	26.94	37.72	46.79	46.79	47.78	55.06	56.82	60.35

TSS: total suspended solids; CC: control column; and TC: testing columns.

The variation of the effluent quality in the four TC, after one and two hours of sedimentation, is presented in Table 4. It can be noted that the RE of colour, TSS, and COD for the CC is 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. Fragoso and Duarte [32] have observed higher reductions (40–50% for COD, 70% for TSS, and 45% TVS) when 200–300 g/L of WTR is mixed in a biological reactor for treating wastewater from an olive oil mill. Nair and Ahammed [33] obtained 74% removal of COD, and a higher removal for turbidity (89%), but in an upflow anaerobic sludge blanket reactor (UASB), for the treatment of urban wastewater.

Table 4. Effluent water quality after 60 and 120 min of sedimentation.

Parameter	Time = 0 min				Time = 60 min				Time = 120 min			
	CC	TC1	TC2	TC3	CC	TC1	TC2	TC3	CC	TC1	TC2	TC3
pH	6.9	7	7	7.1	6.9	7	7.1	7.1	6.9	7	7.1	7.2
Colour (Pt-Co)	1535	1675	2005	2050	1620	1604	1460	1290	1419	1275	1236	1024
Turbidity (NTU)	78.0	79.4	92.8	96.4	79.9	70.3	69.5	66.3	79.1	65.6	64.6	63.2
TSS (mg/L)	137	167	203	227	114	120	114	110	112	106	98	94
FSS (mg/L)	20	43	60	80	16	14	26	26	16	18	20	18
VSS (mg/L)	117	124	143	147	98	106	88	84	96	88	78	76
COD (mg/L)	381	365	342	367	382	332	308	330	357	313	301	292
TC × 10 ⁵ (NMP/100 mL)	185	185	185	185	IC	IC	IC	IC	2.76	2.76	2.55	2.31
<i>Escherichia coli</i> × 10 ⁵ (NMP/100 mL)	75	75	75	75	IC	IC	IC	IC	1.19	1.05	0.73	0.62

CC: control column; TC: testing columns; TSS: total suspended solids; FSS: fixed suspended solids; VSS: volatile suspended solids; COD: chemical oxygen demand; TC: total coliforms; IC: inconclusive.

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). Di Bernardo et al. [34], carrying out similar experiments with an alum WTR, observed higher ER for TC and *E. coli*, similar ER for colour, turbidity, COD, and TSS, and satisfactory removal of nitrogen, phosphorus, and heavy metals. This situation can be explained by different mixing conditions in CT, as well as different WTR and DWW characteristics.

Therefore, adding WTR to primary clarifiers improves the effluent water quality in terms of colour, turbidity, COD, and TSS.

3.2. 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 5. The fraction between biomass present in both materials (fraction VSS_{WTR-PC}/VSS_{DAS}) changed between 0.21 and 0.25 g/g.

Table 5. 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-CH₄/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-CH₄/gVSS.d (DF1), 0.057 gCOD-CH₄/gVSS.d (DF2), and 0.052 gCOD-CH₄/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.

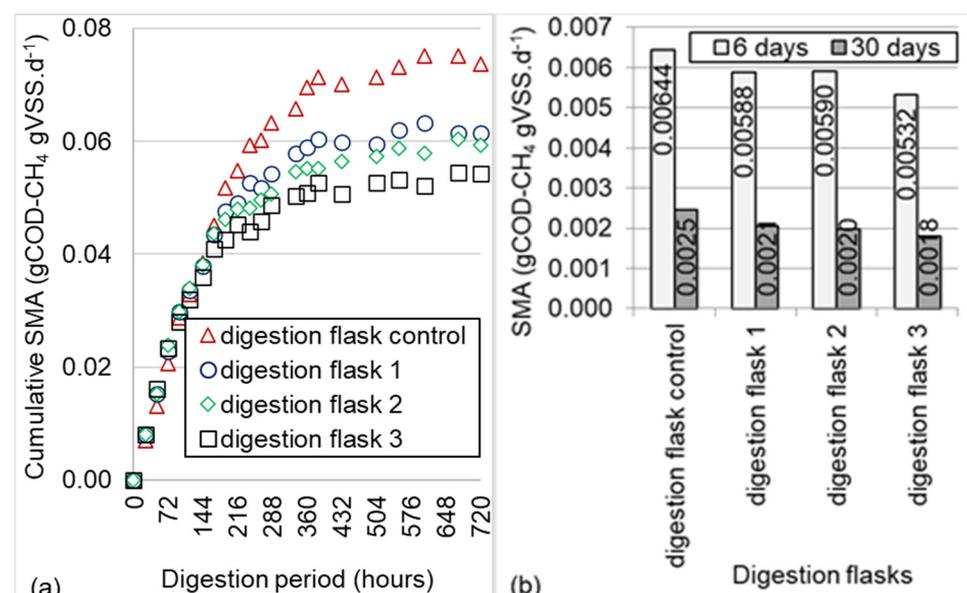


Figure 2. SMA production: (a) cumulative SMA for each DF and (b) SMA for 6 and 30 days.

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-CH₄/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. This circumstance may be associated with some

interference of the increase of metal aluminium, which increased from TC1 to TC, as the TSS increased and must have also increased from DF1 to DF3. Aluminium ion has been reported to have some interference on biogas generation (less 21% in volume) during anaerobic digestion of sludge but with a lower impact compared with iron metal (less 36% in volume) [35], for additions of 0.1 g/L of each metal salt.

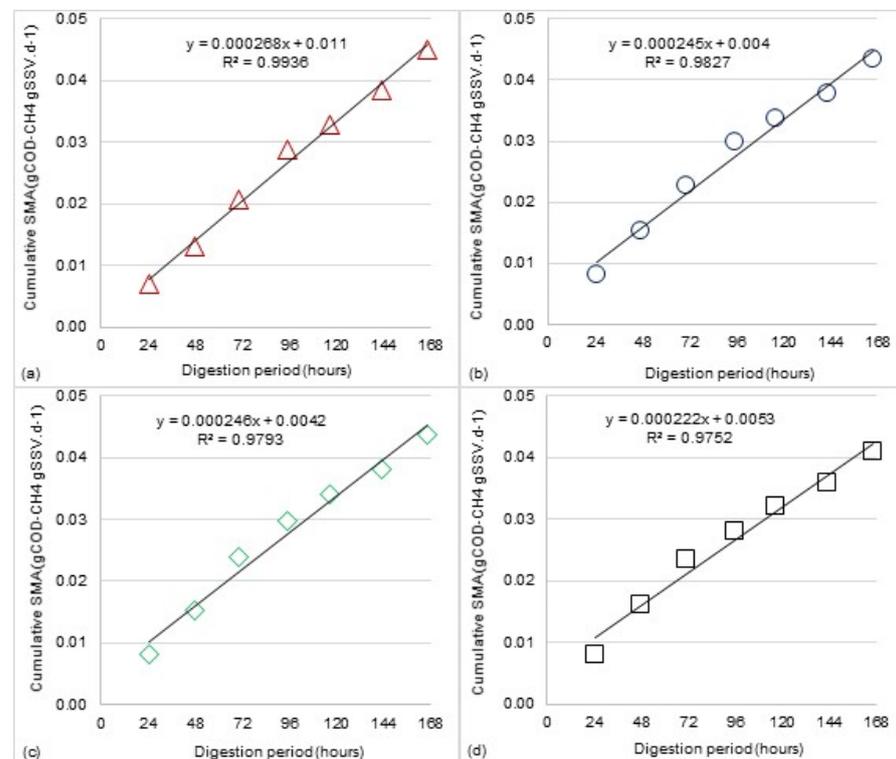


Figure 3. Determination of the angular coefficient of the curve section with the highest slope of the methane production: (a) DFC; (b) DF1; (c) DF2; and (d) DF3.

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. Alvarez and Cervantes [36], in studies on the effect of alumina particles in methanogenesis, concluded that the reduction of methane production in the presence of aluminium oxides, might be explained by the cell membrane damage of some anaerobic bacteria species. As mentioned by Simon-Deckers et al. [37], aluminium ions releasing aluminium oxides can induce both production of intracellular reactive oxygen species, causing damage in the cell membrane, and/or the production of free radicals under dark conditions, which could also affect cellular viability. WTR can reduce dehydrogenase activity in anaerobic and aerobic excess activated sludge [38].

This situation is different from that observed by Carvalho [19], who did not verify negative interference in methane production in similar trials, but using a ferric chloride WTR, probably because the iron ions did not damage the cellular membrane of methanogenic bacteria. Pradhan et al. [39] explains that biogas production is higher and more stable with iron coagulant, in comparison with aluminium coagulant, presumably due to the reduced formation of hydrogen sulphide. Escobar [40] evaluated methane production in an anaerobic biodigester, with the addition of alum WTR-F. 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 regimented.

Da Silva et al. [30] compared the methanogenic activity in sludge digestion coming from the treatment of textile and food effluents, having observed higher SMA ($0.17 \text{ gCOD-CH}_4/\text{gVSS.d}$, for food sludge; $0.10 \text{ gCOD-CH}_4/\text{gVSS.d}$, for textile sludge), and higher methane production (337 mL for food sludge; 3 mL for textile sludge). Kayranli and Ugurlu [41] also found higher values for different temperatures ($0.74 \text{ gCOD-CH}_4/\text{gVSS.d}$ (25°C), $0.70 \text{ gCOD-CH}_4/\text{gVSS.d}$ (15°C), and $0.68 \text{ gCOD-CH}_4/\text{gVSS.d}$ (10°C)) in experiments with acetic acid as substrate. This showed that both the SMA and the substrate removal decrease as the temperature decreases. Punal et al. [42] carried out batch experiments at 35°C with $0.15\text{--}1.5 \text{ g/L}$ of total organic carbon, and biomass from hybrid UASB, obtaining an SMA of $0.25 \text{ gCOD-CH}_4/\text{gVSS.d}$ for suspended biomass.

Therefore, 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. Alvarez and Cervantes [36] showed that coating aluminium oxide nanoparticles decreased their toxicology effect in methane production bacteria.

The microscopic analysis on the DF showed that, after 8 days of incubation, the predominance of methanogenic rod-shaped bacteria, which are fluorescent and produce methane via the hydrogenotrophic pathway (Figure 4), which is also carried out by [43,44].

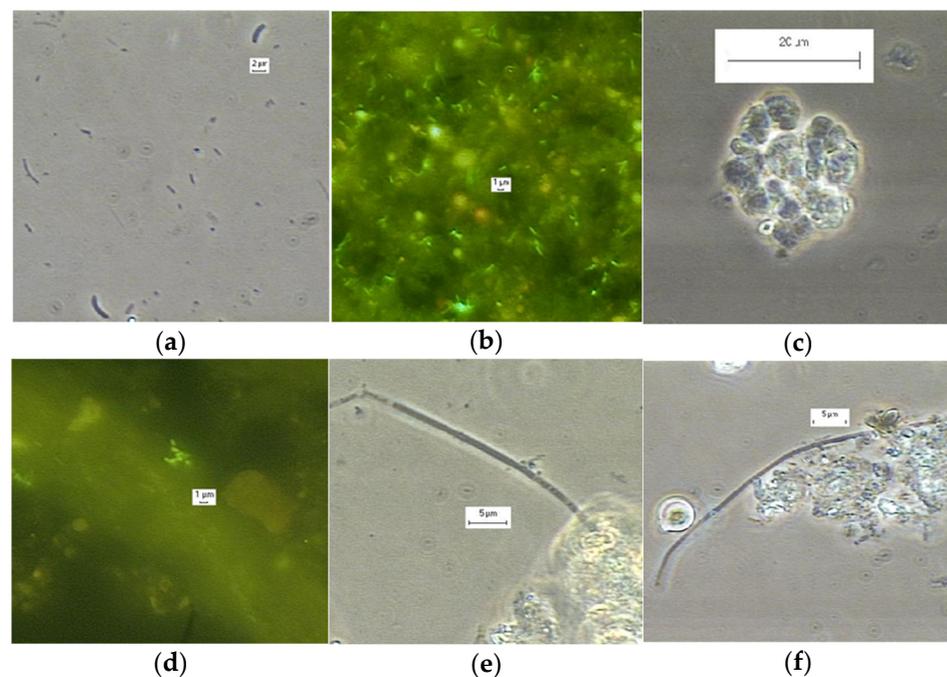


Figure 4. (a) Bacteria in the form of curved bacilli similar to sulphate reducing agents in DFC; (b) fluorescent bacteria in the form of rods; (c) *methanosarcina* sp. present in DF1; (d) few fluorescent methanococci; and (e,f) *methanothrix* sp. present in DF2 and DF3.

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 quantity 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 coconuts and *Methanosarcina* sp. In DF3, unlike DF1 and DF2, no curved bacilli were found, similar to sulphate reducing agents. *Methanosarcinales* and *Methanomicrobiales* were described as dominant in the archaeal microbiome of long term, semicontinuous anaerobic digesters bioreactors, fed with food waste, in phases where acetate was the main volatile fatty acid accumulated [45]. *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 H₂ together with CO₂, acetate, methanol, and methylamines [46].

After 16 days of incubation in the DFC, there was a predominance of fluorescent methanogenic bacilli and non-methanogenic bacilli; only a few *Methanotherix* sp. bacteria were found, and no methanococci and *Methanosarcina* sp. was detected. In DF1, DF2, and DF3, more fluorescent bacilli and only a few *Methanotherix* 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 *Methanotherix* 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 indicate that *Methanotherix* sp. and curved bacilli seem susceptible to the addition of WTR in the long term. The decrease in methanogenic bacteria activity over time was also reported by 45 during the anaerobic digestion of lignocellulosic biomass [46]. 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.

4. Conclusions

Adding alum WTR at primary clarifiers, for TSS concentrations between 0.37 and 1.23 g L⁻¹, 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⁻¹ and VSS between 10.2 and 11.85 g L⁻¹ 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 *Methanotherix* 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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