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EXPERIMENTAL GUIDELINES FOR THE DETERMINATION OF OPTIMAL INOCULUM TO SUBSTRATE MIXING RATIOS IN ANAEROBIC DIGESTION

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Out of the processes involved in wastewater treatment, anaerobic digestion has been receiving increased attention due to the prospect of recovering methane rich biogas. However, in most situations, the requirements for an optimal production of quality biogas are not met. This raises the challenge to find solutions to enhance the process to exhibit better outputs in said conditions. A research direction targets the ratio of microorganism populations (inoculum) to amount of dissolved chemicals that constitute their source of nutrition (represented by the substrate). The current paper is aimed at identifying such a mixing ratio in the case of an artificial wastewater closely resembling municipal sewage and to expand on the knowledge of nutrient bioavailability.

Keywords: wastewater treatment, biogas, anaerobic digestion.

Abbreviations

AMPTS: Automatic Methane Potential Test System
AnMBR: anaerobic membrane bioreactor
BMP: biochemical methane potential
BOD: biochemical oxygen demand
COD: chemical oxygen demand
I/S: inoculum to substrate ratio
MBR: membrane bioreactor
Nml: millilitres in normal conditions (0°C, 1.013 bar)
TN: total nitrogen (Kjeldahl)
TP: total phosphorus
WWTP: wastewater treatment plant

1. Introduction

In recent years, an increasing focus has been placed on "future-proofing" the technologies and services that mankind relies on, effectively rendering them more sustainable, more reliable. A vector for positive change is represented by the

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research sector, with actors from both industrial (R&D segments) and academic (universities) environments reaching out to surmount the challenges posed.

In wastewater treatment, modelling and simulation of the different processes involved go hand in hand with experimental determinations, as in most cases the models require fine tuning and particularization to the studied situation [1],[2]. There are numerous guidelines for laboratory determinations and rules of good practice, but some areas, such as the determination of sludge/wastewater mixing ratio, can prove problematic as they tend to be tailored to each specific case [3],[4],[5]. Moreover, it is relatively commonly known in the scientific community that experiments heavily reliant on biological processes can quickly run awry due to the number of factors that need to be controlled, an aspect felt more closely in small scale experiments. As such, an attempt was made at streamlining the process of determining an optimal mixing ratio for municipal sludge and the wastewater that serves as a food source for the microorganism communities, with methane production as a guiding mark.

In this paper the focus was placed on the identification of the best ratio of inoculum to substrate, further expressed as I/S, as a general methodology, which is the preliminary step in carrying out experiments pertaining to most BMP determinations. This was achieved by testing different COD or mass based ratios for a given set of sludge and wastewater, respecting typical municipal conditions. The base of comparison for assessing a successful ratio was experiment behaviour (aiming for stability) and methane production. The information presented herein is also meant to provide an information bridge between wastewater and chemistry specialists, as in most advanced wastewater treatment applications the shared knowledge base is vital. The current paper is part of a larger project aimed at assessing biogas generation using MBRs (membrane bioreactors), constituting a preliminary step in the on-going experimental work. This step of the research was carried out at Saxion University of Applied Sciences, in Enschede, The Netherlands, during an Erasmus scholarship.

2. Equipment

This section presents a succinct description of the equipment used in the determinations. Due to the nature of the experimental work that had to be carried out, the laboratory equipment had to offer the possibility of running multiple experiment instances (multiple combinations of sludge and wastewater). Considering this aspect, the AMPTS was chosen for the determination of the optimal mixing ratio. The AMPTS (Automatic Methane Potential Test System, Fig.1), developed by Bioprocess Control (BPC), is an established tool, generally used when testing the interaction between inoculum and substrate. It consists of three units: the sample incubation unit, the CO_2 fixing unit and the gas volume measuring device.

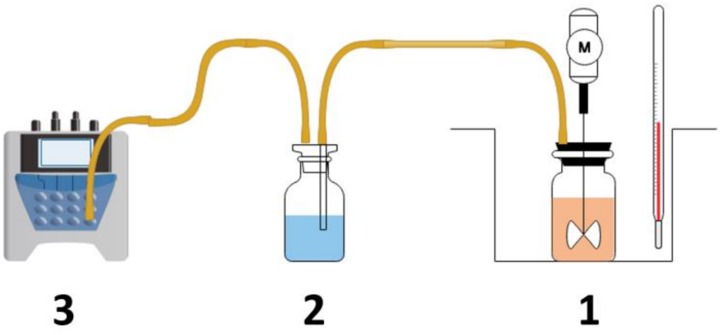

Fig.1. AMPTS general view. 1: sample incubation unit, 2: CO_2 fixing unit, 3: gas measuring unit

The sample incubation unit comprises a thermostatic water bath which houses 15 glass bottles with a volume of 500 mL each acting as reactors. The bottles are sealed with a rubber stopper in such a way that the gas produced is directed to the CO_2 fixing unit through connectors. Through a stirrer driven by a motor connected to the gas measuring unit, the contents of each individual reactor are mixed, the stirring speed being adjustable from a computer. The CO_2 fixing unit contains 15 glass bottles with a volume of 100 mL each, filled with a base. Following the recommendations of the equipment producer, a 3M NaOH solution was used, with a few drops of thymolphtalein added to indicate when the chemical binding capability of the solution was exhausted. The gas produced in the reactors reaches the NaOH solution, the acidic fraction is retained and (theoretically) only the methane reaches the third unit, the gas measuring unit. The last element of the equipment is also connected to a computer which allows a certain degree of experimental control and data visualisation in the form of graphs and tables. The gas measurement in itself is accomplished via the implementation of the principles of liquid displacement and floatability. Within the water tank that comprises the body of the gas volume measuring unit there are 15 calibrated injection flow cells, corresponding to the 15 bottles of the CO_2 fixing unit. These moves, allowing a set volume of gas to flow, the movement being read and recorded in the integrated embedded data acquisition system, further control and visualisation being offered through a PC connection. The AMPTS also calculates corrections for the measured volume of gas, the recorded value being in Nml (millilitres in normal conditions). During the experiments presented in the current work, two such AMPTS were used at the same time.

3. Experimental determinations

In this paper, the preliminary determinations that led to the main body of experimental work are detailed. The scope of this stage was to identify a combination of wastewater and sludge, expressed through a ratio, that would exhibit the best methane output. The general diagram that provides the outline for the experiments leading up to the test carried out with the main installation (the AnMBR, anaerobic membrane bioreactor) are presented in Fig.2. The current work covers the first two blocks. The preliminary analysis focused on identifying the compounds present in the inoculum and substrate that interact during the anaerobic digestion process leading to methane. As such, determining the COD value of the two streams involved was needed, in order to obtain a rough base of estimation for the expected methane output. COD tests quantify the amount of organic matter that can be used by the microorganism communities from the inoculum as a food source, the value of this parameter being used in calculating a theoretical value for the methane yield (0.35 L/gCOD). After obtaining an expected value for the methane, and considering that the target was obtaining an I/S ratio leading to maximum output (highest volume), the ratio tests were carried out. Once the ratio was identified, it was used in other series of experiments that had other objectives, not related to the currently presented work.

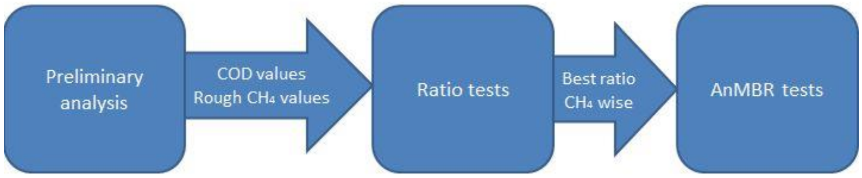

Fig.2. Experiment diagram

In order to ensure as much as possible constant composition for the wastewater used in the experiments, the following recipe for synthetic wastewater was used [6]:

- glucose 150 mg/L
- sodium acetate 300 mg/L
- peptone 30 mg/L
- NH_4Cl 140 mg/L
- KH_2PO_4 35 mg/L
- $MgSO_4 \cdot 7H_2O$ 30 mg/L
- $FeSO_4 \cdot 7H_2O$ 5 mg/L

This recipe was chosen due to its relative closeness to the composition of actual municipal wastewater and stability during the experiments [6]. The wastewater prepared according to the recipe would have the following approximate water quality parameters:

- BOD 200 mg/L

- COD 400 mg/L
- Ammonia 20-30 mg/L
- TN 40-60 mg/L
- TP 10 mg/L

The specified parameters were determined using Hach Lange test cuvettes using their respective standards, LCK 1014, LCK 338, LCK 554 and LCK 348, available on the Hach Lange website. This theoretical set of values is important when considering that the dissolved organic carbon is the main available food source for the microorganisms that constitute the sludge and is involved in the actual obtaining of methane, with the other compounds acting as secondary food sources sustaining the growth of the microorganism communities. Considering that a large number of experiments was to be carried out and working with anaerobic conditions implies a certain speed in filling and handling reactors, a 100 times concentrated version of this wastewater recipe was made and stored in glass bottles at 4 °C, to be diluted as required.

As the experimental installation included an acidic fraction trap (acted as an aqueous filter for the produced biogas and retained CO_2 and other such gases allowing just CH_4 to pass through) a binder had to also be prepared. The binding agent was a solution of NaOH 3M with thymolphthalein added as an indicator to assess the binding capability. The four-step process that culminates with the production of methane is presented in a simplified form in Fig.3. This was used as a rough guideline for the experiment preparation, as, essentially, finding a suitable I/S ratio means identifying a balance between the chemical species that are present initially in the substrate and the ability of the microorganisms constituting the inoculum to degrade them to methane, as this compound was used as reference to quantify the rate of success for the experiments.

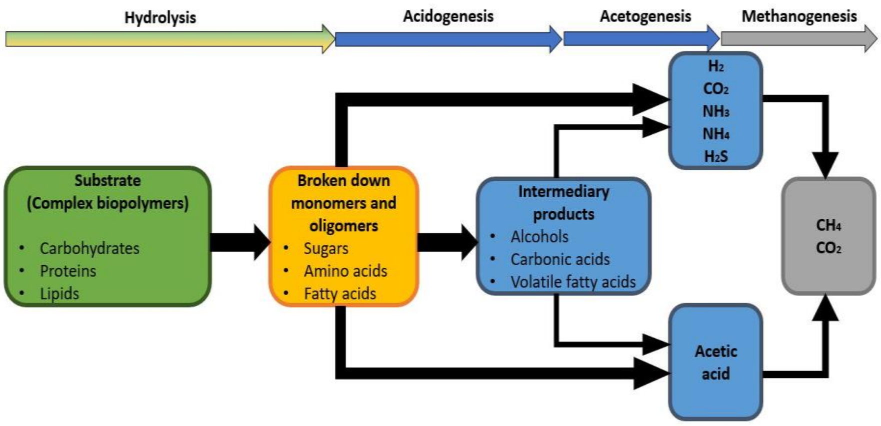

Fig.3. Anaerobic degradation four step process [7]

3.1 Preliminary analyses

Before starting the ratio tests, a set of preliminary analysis had to be carried out in order to assess the actual composition of the wastewater to be used, further referred to as substrate, and of the sludge, referred to as inoculum. As the research is based on municipal sewage conditions, sludge from a municipal wastewater treatment plant in Enschede, The Netherlands, was obtained. In order to determine the water quality parameters, Hach Lange cuvettes were used. First and foremost, a testing procedure was determined. This consisted in determining the best sampling, filtering and testing method, in respect to obtaining data as accurate as possible. The samples were obtained using sterile pipette tips and centrifuged for 5 min at 1500 rpm, after which they were left to settle. After separation, the clarified topmost layer was used for the actual determinations. Hach Lange cuvettes were used according to the instructions provided by the kits. In order to determine the best sequence of testing, series of cuvettes were analysed and the series that exhibited the smallest deviation in value from test to test was used. This is important to note, as with all spectrophotometric determinations, suspended particles can cause interference and lead to incorrect assumptions.

The preliminary COD values for the wastewater and sludge, averaged out across triplicates were 446 mg/L for the wastewater and 800 mg/L for the sludge. Considering the nature of the anaerobic reactions, the starting pH was also determined as 6.5 for the wastewater and 7.3 for the sludge. As these values are in the "safe zone" considered in literature, no starting pH buffer was used in the beginning of the experiments [8]. COD and BOD tests were used to quantify the amount of organic matter present in both wastewater and sludge, thus providing a base for estimating the potential methane production, with a theoretical maximum of 0.35 L/gCOD derived from the conversion of acetate to methane and carbon dioxide. During the experiments, a decision was made to only use COD determinations as a method of controlling the tested scenarios, in order to introduce a degree of robustness to the procedure. Considering that most wastewater treatment guides offer a COD breakpoint of 2000 mg/L from where the methane production is adequate, an insubstantial amount was expected to be recorded as generated. As the estimated production of methane is relatively low, raising the COD was considered in order to improve the visibility of the modifications brought to the ratio setup. As cost effectiveness was considered when obtaining the methane, the experiments were carried out in the mesophilic temperature range at 35 ± 0.5°C.

3.2. Ratio tests

As stated in the introduction of the paper, the aim was to identify a successful I/S ratio, with process stability and methane yield used as checks. When carrying out experiments revolving around anaerobic digestion, one of the methods of assessing stability is the comparison between the methane yield graphs obtained from the experiment and those that are typical for similar scenarios. A reference graph is shown in Fig.4.

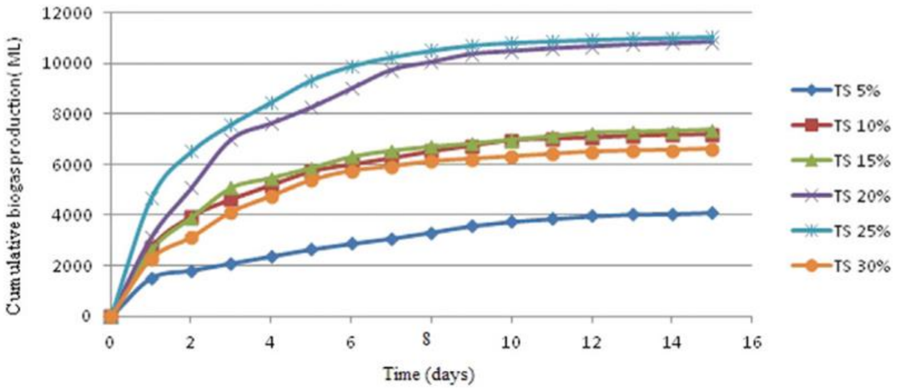

Fig.4. Graph showing typical methane production patterns [9]

In Fig.4 the typical pattern of biomethane production is outlined. In the first 3 to 4 days a type of burst production is recorded, after which the methane generation follows a steadier pattern, called a plateau. The patterns are typical for a multitude of scenarios, including different substrates and temperature regimes.

In addition to the production pattern check, the methane yield obtained from the experiments was compared to the theoretical maximum of 0.35 L/gCOD. This value is derived from the conversion of acetate to methane and carbon dioxide. Based on the preliminary determinations, the ratios for the first experiment were calculated. In order to ensure statistical significance, all the experiments were carried out as triplicates, with 3 reactor bottles constituting a tested ratio. Moreover, as per the recommendations found in the AMPTS manual, the headspace of each reactor bottle was targeted to be 100 mL.

The ratios for the first experiment were calculated based on the COD content, as follows:

- Ratio 1 (R1): inoculum to substrate (I/S) ratio of 0.428;
- Ratio 2 (R2): I/S ratio of 1;
- Ratio 3 (R3): I/S ratio of 1.5.

For this experiment, after a period of 10 days, the values recorded by the AMPTS reached an average value of 20 Nml of produced methane, which is under the threshold for generating graphs. This was attributed to the reduced COD value of the reactor contents. Two short term experiments were carried out with an increased I/S ratio, as can be seen in Table 1.

As in the previous cases, the AMPTS did not record significant data. This was expected, as the sludge was withdrawn from a segment of the wastewater treatment plant prior to dewatering and was quite diluted with the average COD of the inoculum and substrate mix being well under the recommended value of 2000 mg/l for viable methane production.

Table 1

Short term ratios

	I/S experiment 1	I/S experiment 2
R 1	1.5	3
R 2	2	4
R 3	3	6

At this point, a decision was taken to enhance the sludge used in the experiments, prior to applying the different mixing ratios of inoculum and substrate, in the form of COD enhancers. This would, in theory, have emulated a more concentrated, pre-fed sludge, that exhibits more activity, or a dewatered, higher strength substrate. The first addition of this type consisted in reaching a concentration of 1 g/l sugar and 2 g/l starch by adding the said components in the sludge prior to filling the reactors. The higher I/S ratios (3; 4; 6) were tested again using the enhanced sludge. This time, the AMPTS recorded an average of 70 Nml of produced methane, but the generation pattern was erratic, and as such, the experimental results were not taken into consideration.

For the following experiments, a new batch of sludge was used, taken from the recirculation system of the wastewater treatment plant. The new sludge was thicker and considering that microorganisms use suspended sediments as a support, a higher concentration in terms of microorganisms was hypothesised. As such, it was expected that the methane generation patterns obtained from the experiments using this new batch of sludge would follow more recognizable patterns, comparable to literature. At this point, a decision was made to switch from COD based ratios to mass based ratios. The reactors from the AMPTS were filled according to Table 2.

Table 2

Reactor contents

	L1	L2	L3	L4	L5
Contents	Blank (400g sludge)	Blank ++ (sludge, 2g/L sugar, starch 4g/L, gelatine 2g/L)	75/25 (300g sludge + 100g wastewater)	50/50 (200g sludge + 200g wastewater)	25/75 (100g sludge + 300g wastewater)

A series of COD and pH determinations were done in order to correlate the methane production patterns to the basic chemical properties. These determinations are presented in Table 3.

Table 3

pH and COD determinations

Line / Parameter	pH	COD [mg/l]
L1	7.01	695
L2	6.5	5482
L3	7.08	784
L4	7.03	483
L5	6.95	314

Considering these values, the second line, L2, was expected to produce the highest values of methane. However, as can be seen from Fig.5 the methane generation did not fit the normal, expected patterns typical of anaerobic digestion.

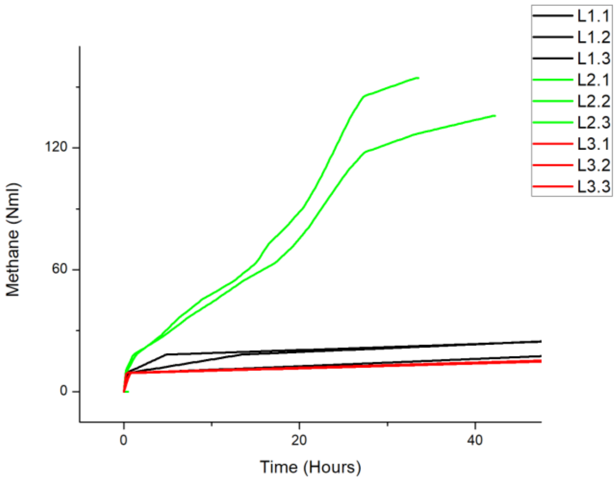

Fig.5. Fourth experiment progress check

This graph was generated from the .csv datasets obtained from the AMPTS. The operating regime was mesophilic at 35 °C. From the 15 reactor bottles that were set up at the start of the experiment, only 9 recorded activity, as can be noted from the presence in the graph of only L1, L2 and L3. This was expected, as L4 and L5 had a very low COD concentration, and, coupled with the relatively low volume of the reactors, would generate trace amounts of methane in time, that would pass undetected by the AMPTS. One of the reactors from the L2 group stopped exhibiting activity shortly after passing the 30-hour mark, which triggered the assumption that an inhibitory effect was in action. The behaviour of the blank (L1) and the 75/25 I/S ratio (L3) is relatively normal, when considering the very low organic matter content, as expressed by a low COD concentration. Another AMPTS had been operating with the same reactor contents, but at a lower temperature of 25 °C. A graph obtained from that experiment is presented in Fig.6. In this case, the generation pattern is more recognizable, following a curve typically found in literature. This was thought to be at least in part due to the sludge having been sampled from a municipal wastewater treatment plant that operated in the lower end of the mesophilic regime.

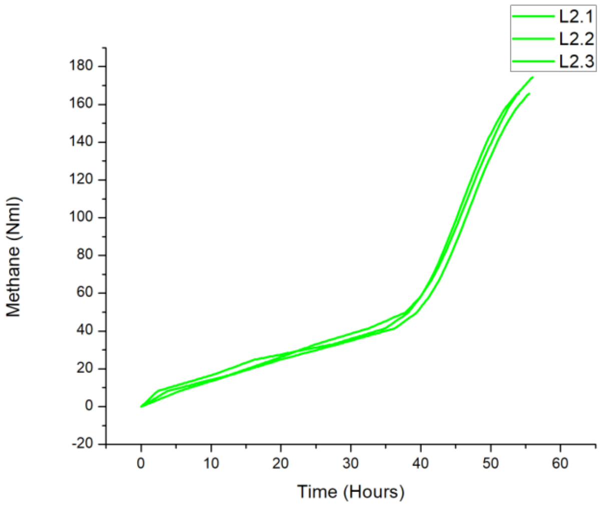

Fig.6. Fourth experiment progress check, 25 °C

However, out of the 15 reactor bottles that were set up at the start of the experiment only 3 reactors, corresponding to the L2 group, with the highest COD, exhibited activity. In order to determine other plausible causes for the underperformance of the reactors, samples were taken from the L2 reactors, as being the ones with the highest COD. A lowered pH value was observed, 4.72 averaged across the tested triplicates. At this point, a decision was made to feed all the reactors a pH-buffer, booster solution, consisting of 5 mL NaOH 3M and 10 mL wastewater concentrate topped to 100 mL with distilled water. From this solution each reactor was fed 1 mL. The results for the AMPTS operated at 35 °C can be seen in Fig.7. After the addition of the feed, all the reactors, with the exception of the ones from group L2, started exhibiting activity. The reactors from the groups L4 and L5, previously dormant, showed a slow but steady increase in production, whereas in the case of groups L1 and L3 the increase in production was more pronounced, attributed to the fact that they were still active from a microbiological perspective at the time the addition was performed. However, in the case of the reactors from group L2, taking into account the low pH that was measured, the microorganisms' activity was considered to had been affected strongly enough that the feeding solution could not restart a proper digestion process. One of the reactor bottles from L2 that appeared at the time to resume activity, as can be seen in Fig.7, exhibited an increase in production of under 10

Nml in a span of roughly 70 hours, which is insufficient to properly consider as active.

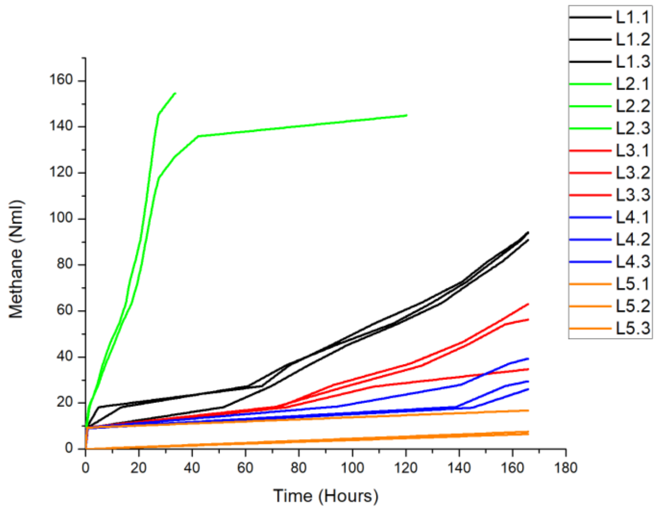

Fig.7. Fourth experiment end of run, 35 °C

The effect of the addition on the reactor operating in the lower mesophilic conditions at 25 °C was insignificant, with no change recorded neither through the continuously updated graphs the AMPTS display nor by consulting the datasheets with the actual values. Therefore, for further experiments, a decision was made to discard setting up experiments in the lower mesophilic regime and focus on the 35 °C reactors. At this point a decision was made to switch to using a different approach regarding the experiments, an increase in COD being targeted. As such, the next experiment had the following setup:

Table 4

Reactor contents

	L1	L2	L3	L4	L5
Contents	Blank (400g sludge)	Blank ++ (sludge, starch 3g/L, gelatine 5g/L)	75/25 (300g sludge + 100g wastewater; sludge with starch 3g/L, gelatine 5g/L)	50/50 (200g sludge + 200g wastewater; sludge with starch 3g/L, gelatine 5g/L)	25/75 (100g sludge + 300g wastewater; sludge with starch 3g/L, gelatine 5g/L)

Sugar was removed as an additive due to its propensity to trigger a drop in pH during degradation, thus destabilizing the anaerobic processes. The additions of 3 g/L starch and 5 g/L gelatine were applied to the sludge prior to mixing. The decision to increase the gelatine concentration was taken in order to account for some specifics of the inoculum that was used in the experiments. Since the sludge was taken from a local wastewater treatment plant that treated domestic effluents, it was acclimated to working in certain conditions. A report was requested from the plant operator, in order to make positive adjustments to the substrate. From the report, a recommendation to increase the protein content of the feeding stream was received, which translated into an increase in the gelatine concentration. The starch content was lowered due to its propensity of coagulating into mechanically stable formations within the reactors, which is detrimental to the anaerobic digestion processes (microorganisms need to be able to freely access the compounds produced by the different communities, any impermeable barrier can cause chemical by-product build-ups and could trigger inhibition).

The determinations at the start of the experiment are seen in Table 5.

Table 5

pH and COD determinations

Line / Parameter	pH	COD [mg/L]
L1	7.1	272.8
L2	6.95	5348
L3	7.03	3740
L4	7.07	3003.5
L5	7.08	1797

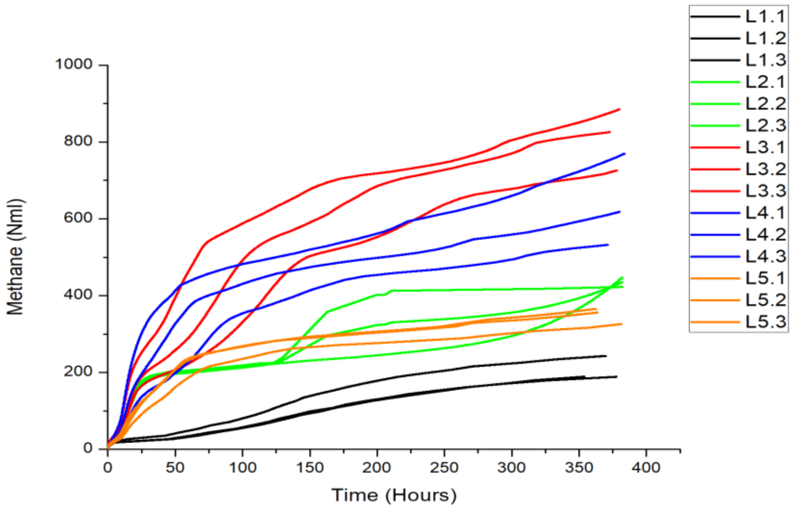

Fig.8. Fifth experiment end of run

This experiment was carried out at 35 °C. The L3 group (red lines), representing a 75/25 or 3:1 I/S ratio, appeared to be the most successful, with an average recorded value of 760 Nml. Considering the starting COD value of the group, 3740 mg/L, and the theoretical methane production of 0.35 L/gCOD, the maximum yield under ideal conditions would be 1309 Nml. However, considering that this value is ideal, and that the experiments were not carried out with optimisation as a target, this result is considered a success. The L4 group had an average corrected value of produced methane of 600 Nml. The theoretical methane output for this case, starting from the COD value of 3003.5 mg/L is 1051 Nml. Thus, it was considered that this ratio was also successful. Lastly, for the L5 setup, an average of 320 Nml of methane was produced, compared to the theoretical 628 Nml calculated from a COD of 1797 mg/L. The blanks exhibited a behaviour that is considered to be within normal parameters. The production patterns for all the setups also follow forms that are comparable to those found in literature.

4. Results and discussion

In order to classify the I/S ratios the gap between recorded and theoretical methane productions was considered. According to this criterion, L5 (the 25/75 or 1:3 I/S ratio) is the best, followed by L4 and L3. This is largely due to the sludge being adapted to function with lower strength feeds, with lower COD values. Further research should be carried out with sludge that is adapted slowly to elevated organic matter loads. However, in the case of the currently presented experiments, an elevated rough methane production was desired, and as such the order of classification is reversed, with L3 (the 75/25, 3:1 I/S ratio) representing the best ratio, followed by L4 and L3.

Among the main elements identified to cause low methane output, the shift in pH towards the acidic domain and low COD values are considered to be the most important. pH is extremely important to the methanogenic microorganisms, as their optimal value is around 7.2, with growth stopping altogether in the case where values reach less than 5 or more than 8.5 [10]. Interestingly enough, the activity was restarted in a significant number of reactors by the addition of the pH correcting buffer, which serves to underline the importance of this parameter. From general speciality literature, in order to have a reliable production of methane, the COD of an anaerobically treated aqueous stream has to have a value of at least 2000 mg/l [11]. This does not mean that under this value there would be no generated methane, but that the production could be difficult to measure. When considering methods of enriching the carbon content, care should be taken in selecting the components to be introduced, as the conversion of sugar can trigger acidification in a manner that is inhibiting for the other processes to develop. Also, it should be mentioned at this point, that most recent research targeted at optimising methane rich biogas production favours the use of TOC, total organic carbon, as being more relevant to expressing the actual available carbon source for the microorganisms, without chemical by-product interference during analysis [12],[13]. TOC could not be determined at the time the experiments were carried out, although COD stands as the currently most used parameter in industry standards.

Another aspect to be discussed is the microorganisms' density in the inoculum. Without properly determining the communities living inside a given sample through microscopy, assumptions have to be made regarding the number of microorganisms and their state (inhibited or not). However, for the scope of this project, bacterial counting was not deemed necessary (adhering to low end working conditions). As a general rule when conducting anaerobic digestion experiments, the sludge samples are kept at 4 °C and then slowly brought to the temperature regime that the experiment is conducted at, in order to reacclimatise them. Then, the sludge should undergo a period of adaptation, where trickle feeding is usually used. This lengthens the experiments considerably, and for the purpose of the current work this process was heavily accelerated, which leads to the supposition that most of the experiments were conducted in a partly inhibited state. However, when taking into account that only the trickle feeding process was waived and that the sludge was subjected to working environments similar to those in the wastewater treatment plant it was sampled from (albeit at higher COD concentrations), the results of the current experiments are considered to be in the range of validity.

When considering the applicability of the ratios deemed to be satisfactory, some aspects pertaining to the objective of the work have to be discussed. As the experiments presented here are part of a larger project aimed at using anaerobic membrane bioreactors to obtain methane, the focus was placed less on achieving high treatability and more on effectively feeding the sludge, maintaining process stability and using the microorganism communities as "biological methane factories" to obtain the highest yield, the procedure that leads to the ratio accomplishing the aforementioned goals being the scope of this paper.

5. Conclusions

The current presented work was targeted at identifying an optimal inoculum to substrate mixing ratio in anaerobic digestion in order to provide an effective guideline that can be followed in other similar determinations. By following a strict testing regime and upholding rigorous laboratory practices, most major sources of error that usually manifest in conducting experimental work in this kind of applications are eliminated. A diagram summarising the steps needed to identify a potential I/S ratio, as exemplified in the current paper, is presented in Fig.9. First and foremost, the focus must be clearly identified, either being methane yield (highest recorded volume), process stability or optimal feed-to-output ratio (the measured methane output as close as possible to the theoretical value). Process stability can also imply a focus on treatability (removal of different compounds in the substrate without having the anaerobic digestion process reach an inhibited state). A canvassing procedure should follow, in order to observe the compounds present in the inoculum and substrate. If the chemical compositions of both streams align with the target of the research, the first ratio tests can be performed. If not, and adjustments by way of pH or nutrient corrections are not possible, a re-evaluation is needed for the targets (if the inoculum-substrate pair is imposed in the project requirements) or a change of either inoculum or substrate can be performed, if there are no restrictions regarding this.

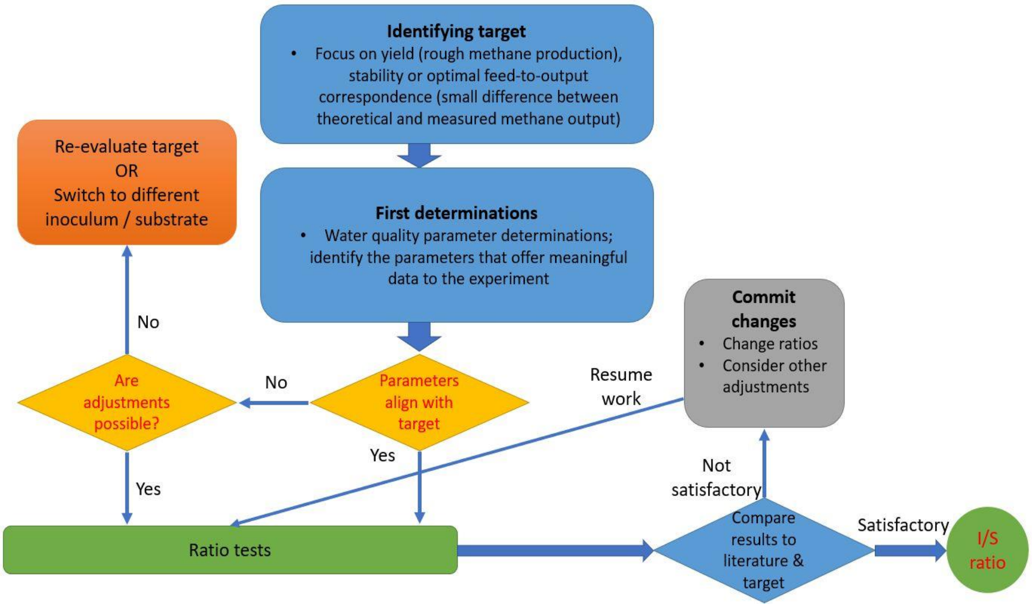

Fig.9. I/S identification process steps

The results obtained from the first ratio tests should be compared to the literature to check their validity and afterwards to the target of the I/S identification. If the results are unsatisfactory, changes should be made either to the ratios themselves or by carrying out small adjustments (additions or feeds), and the experiments resumed, thus obtaining an iterative step. Otherwise, if the results are satisfactory, it can be stated that the I/S ratio was identified. This testing protocol was outlined with relatively low-end conditions in mind, meaning either laboratories that do not have high end equipment and consumables or highly trained personnel. As such, the sequence of experimental work presented here can be perceived as a guideline, with the possibility to be used as teaching material for students or training for laboratory technicians.

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