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# Methanogenic, Acidogenic and Hydrolytic activity tests of an anaerobic sludge of municipal wastewater plant

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**Abstract:** - The determination of methanogenic and non-methanogenic activity requires careful analysis because don't exist a standardized method to find this bioindicator. Therefore, we wanted to evaluate and optimize this analytical method for the characterization of anaerobic sludge. We did five experiments, where we changed variables such as agitation, nutrients supply, measurement time and substrate concentration. We have used 150 ml vials in which they were filled inoculum from anaerobic digester sludge. These vials were introduced in an incubator at 37 ° C, twice in a day we measure the pressure of biogas and the methane production. At the beginning and the end of the experiment, we measure OCD, volatile suspended solids (VSS), pH, and (VFA/AT) ratio. Finally, it was observed that experiment 5 without agitation, with nutrient supply and with seven days of measurement, was the best to obtain a better behavior in all specific activities. The calculation of these activities and the chemical analysis corroborate the previous result that we find in literature.

**Key-Words:** - Anaerobic digestion, sludge, Bioindicators, Methanogenic, Specific activity, methane.

## 1 Introduction

In recent years, the researchers have observed the behavior of anaerobic digesters in order to get ahead of critical situations. Early response to failures in the system can be very helpful to keeping the system in good condition and anticipating possible additions of external substrates. Anaerobic digesters not only are used to treat wastewater, also they treat different substrates that come from industries, doing co-digestion. For this, the wastewater plants must know the characteristics of their substrate, ideally continuously or discontinuously by measurement indicators. The methanogenic, acidogenic and hydrolytic activities showing the behavior of the inoculum, in order to anticipate possible decreases in reactor efficiency, or possible future inhibitions, which means, less generation of biogas and influent with high loads. As said Dong, effective monitoring and diagnosis of processes is a great challenge for anaerobic digestion reactors, which limits their stable operation. The effective process parameters are the basis for ensuring the process monitoring and control. Such indicators should be ideally accurate and sensitive to environmental fluctuations, reveal the change dynamics of reactor status [1]. Other researchers worked in this topic, for example, Soto and partners, obtain a good result using 125 ml vials and with glucose and cellulose as substrate [2].

On the other hand, Gomez and Villa did an experiment for various substrates like granular sludge used in septic tank and in anaerobic lagoon, obtained similar values of specific methanogenic activity (SMA) [3]. Ince and Anderson worked with SMA to the control of the organic load in the anaerobic system [4]. Can Liu, characterized the high solids anaerobic digestion using acetate, glucose, microcrystalline cellulose, hydrogen and carbon dioxide as substrates [5]. In addition, Jimenez optimized SMA in pig manure and rice straw co-digestion, he corroborates a positive effect of clay as an inorganic additive for stimulating pig manure anaerobic digestion, founding that in thermophilic range the behavior of SMA is better [6].

## 2 Problem Formulation

Do not exist a standard method of SMA, specific acidogenic activity (SAA) and specific hydrolytic activity (SHA) for anaerobic sludge, which gives better information of the reactor behavior than the traditional analysis. Boe exposes, that several parameters have been frequently adopted in the process monitoring, including pH, redox potential, ammonia, alkalinity, VFAs, biogas composition, microbial community and biogas production rate. In addition, he said that the Biogas production, for example, is an important parameter as it indicates

the overall performance of the process. However, it cannot be used to indicate process imbalance, since changes in the biogas production rate depend on the feed composition. Moreover, it has low sensitivity to overloading compared to other process indicators, with a decrease in biogas production often occurring after the process is severely inhibited or already broken down. Therefore, it is not an effective early warning indicator [7]. However, most of these parameters can only reveal the current reactor status, but it is often too late for effective process control once the threshold values are reached. Taking into account these diverse tests, we decided to probe the change of these variables in our laboratory to ensure the best way to use SMA for the characterization of anaerobic sludge.

## 2.1 Operational conditions

Various researchers in anaerobic digestion have worked to establish methods of specific activity (SMA). Normally, these researchers do experiments to find an effective method to use in anaerobic reactors. Generally, in these tests, the researchers proved various concentrations of substrates like sugars and they determined the SMA of various inoculums. They worked with variables like supply nutrients, temperature, and shaking of digesters. In our research, we used sugars like cellulose, glucose and sodium acetate as substrates. We used vials of 300 ml, in which we filled 150 ml of anaerobic digester sludge (Inoculum). We used three vials for each activity, for example in the first test we used 3 vials for inoculum, others 3 vials for SMA, for SAA other 3 vials and finally for SHA other 3 vials. In total for each test were 12 vials. Then, we supply nutrients as energy supply. For all vials, we used  $\text{KH}_2\text{PO}_4$  as a buffer and to neutralize pH. It is recommended doing a pretreatment which consists in heater and shaker the vials for thirty minutes before beginning de incubation. When de vials are ready, they were introducing into incubator-shaker in which the vials were heated at 37 °C and shake at 150 rpm. The same day we measured OCD, pH, ST, SV, and VFA/AT. One day later, we measure biogas pressure and methane production twice a day, for a period between 168 hours to 336 hours. When we retired the vials of the incubator, we did the same chemical analysis made at the beginning of the experiment. The differences in the measurement time are because is important to know the representative time for methane measurements if we want to use this method in the lab of a wastewater plant. In a critical situation, the operator should know the fast answer to take a decision in critical operational times.

### 2.1.1 Specific Methanogenic Activity

The SMA is used to know the sludge quality in anaerobic reactors, it is used like a tool that evaluated the behavior of the polluted biomass and determinates the maximum organic material. The principal challenge of this analysis is the capacity of the treatment, the toxicity of the determined substrate and the possibility of choosing an inoculum [8]. When it is knowing the SMA of the sludge, is possible establish the maximum capacity of the elimination of OCD (liquid phase), allowing to estimate, the maximum organic load, that is possible apply in anaerobic reactor, preventing its destabilization. The SMA is perhaps, more representative than the other activities because methane is the most important product in anaerobic digestion. We proved sodium acetate and acids (butyric, propionic, Valeric) as the substrate, and was evident that is better use acetate for SMA. Obviously, there are also hydrophilic methanogenic bacterium who obtain  $\text{H}_2$  from the acidification step, but if we take to account that only the 26 % of organic matter is converted in acids and lipids to produce hydrogen, is better pay attention to acetoclastic methane bacterium. The concentration of the acetate also was between 6 to 6,6 g/l, we found that the concentration of 6,6 g/l is the most adequate to take a representative curve of the SMA.

### 2.1.2 Specific Acidogenic Activity

In the Acidogenic phase, the big molecules like proteins, lipids or carbohydrates, are degraded for the action of the enzymes of acidogenic bacteria, obtaining the nutrients and energy necessary to survive. As “sub-products” they generate butyric, propionic and valeric acids and in few proportions, acetates. This phase not limit the anaerobic reaction but is important for the acetogenic bacterium. The evaluation of the acidogenic activity can give important information about biomass development and the dynamic behavior in anaerobic digesters [2]. The substrate used for acidogenic activity was glucose, which is considered as the main intermediate in the pathway of anaerobic digestion of carbohydrate complex organics. For determinate the concentration of Glucose, we proved at the beginning 6 g/l, but this concentration didn't representative to find best SAA method, so we increase to 7 g/l, observing better results.

### 2.1.3 Specific Hydrolytic Activity

In this phase of anaerobic digestion, the organic complex is biodegraded to monosaccharides and amino acids. The hydrolysis is carried out through extracellular enzymes produced with acidogenic

bacterium. This phase is very important for the whole process because is where the process begins the biodegradation of organic material. The hydrolysis of a few complex molecules is a limiting step in the anaerobic digesters. In this case, the determination of hydrolytic activity of anaerobic sludge and a specific substrate could be important in the selection of the best equipment in the control of process conditions [2]. For Hydrolytic activity, we used cellulose as a substrate because is the organic biomolecule more abundant in the biosphere. We evaluated for SHA a concentration of 5,62 g/l in the first test, follows the literature. However, this concentration was not representative, so we decided to prove 6 g/l, having great result.

## 2.2 Methodology

Five tests were made to find the best result for SMA, SAA, and SHA. The first test was carried out in fourteen days, with nutrients and shaker. We used for SMA, acetate (6 g/l) as the substrate, for SAA, glucose (6g/l) and for SHA cellulose (5,62 g/l) as can see in table 1.

Specific Activity	Substrate	Substrate g/l
Hydrolytic	Cellulose	5,6 - 6
Acidogenic	Glucose	6 -7
Methanogenic	Sodium Acetate	6- 6.6

Table 1. Substrates of specific activities

For the second test, we used the same substrates for fifteen days, but with different concentration for cellulose (6g/l). During the experiment, we did not add nutrients to avoid a higher microbial activity to the real growth, according to the methodology followed for Soto. Table 2 shows that in the second test the vials did not shake. In the third test, we changed the concentration of Acetate (6.6 g/l) and glucose (7 g/l) and we added nutrients. The 4 and 5 tests were carried out in seven days. We did these tests at the same time because we wanted to look the difference between shake and without shake. The concentration of substrates was the same of the third test.

Test	Nutrients	Shaker	Time (days)
1	x	x	14
2			14
3		x	7
4	x	x	7
5	x		7

Table 2. Parameters of the Tests

## 3 Problem Solution

For determinate an effective method of SMA, SAA and SHA, we first investigated other researches who proved changes parameters in the method. However, when we proved their concentration substrate and their experiment time, these data didn't give a representative result. For example, Lopez and their partners worked with glucose at 2 g/l. In the case of hydrolytic activity, it worked with the same substrate concentration [3].

### 3.1 Specific Activities

As can see in figure 1, the curve 5 is the representative curve because shows better behavior of SMA. Besides, we observed a good methane production, reaching 170 ml. The eliminated OCD of inoculum is smaller than OCD of SAA because the inoculum doesn't have substrate, its function is to be the white. We expected this behavior because the inoculum doesn't have so much energy and carbon source than the specific activities.

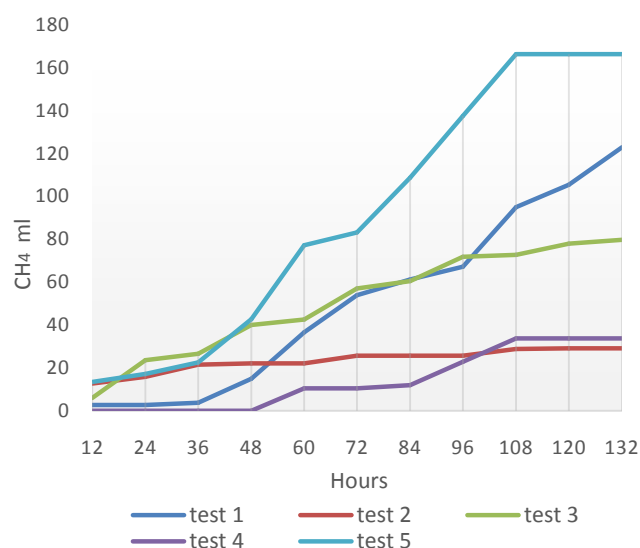


Figure 1. 5 tests of Specific methanogenic activity

In terms of eliminated OCD, the test that had better behavior was also the 5 and then the third as can see in figure 2. Although in test 3 the eliminated OCD get over inoculum OCD, is not more representative than test 5, because the difference of OCD between 3 and 5 tests is 5 g/l. Could be the bacteria in test 5 had the optimal conditions to biodegraded 5 grams more of organic matter. We can observe in figure 3, that test 5 was the best for SHA, because the eliminated OCD was higher than other tests.

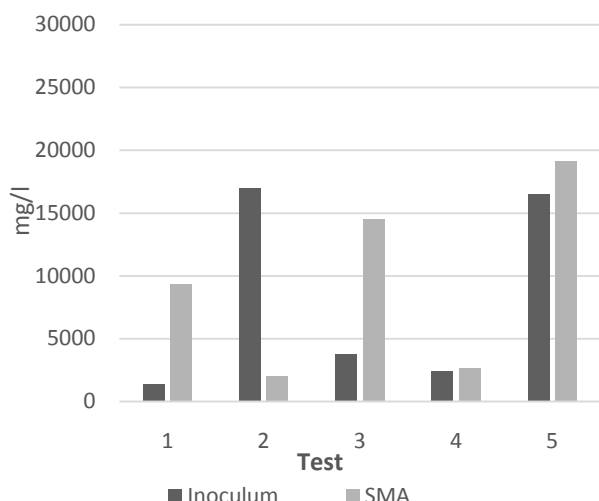


Figure 2. OCD eliminated of SMA

The inoculum also has a high eliminated OCD because these microorganisms found the optimal conditions. In the other tests, the relation between inoculum and activities were not optimal, apparently, the bacterium doesn't have the optimal conditions for their grown. Figure 4 shows that test 5 had better behavior for SAA than other tests. However, we can see that test 2 also had a great result but this OCD inoculum overcome the OCD of SAA.

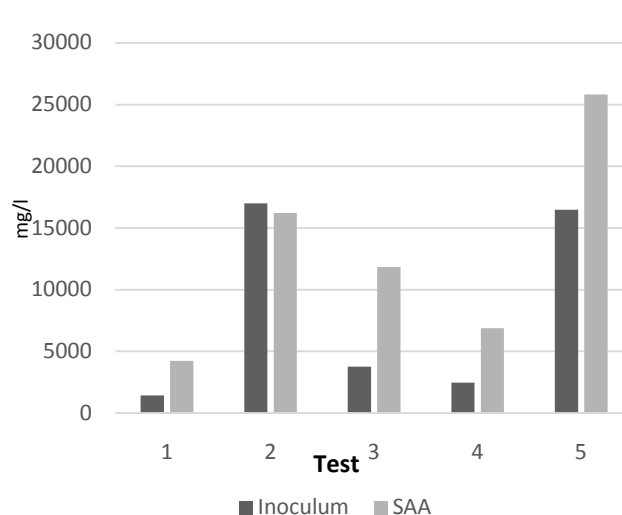


Figure 4. OCD eliminated of SAA

### 3.2. Chemistry parameters

Figure 5 support the result in SMA curve an in the OCD value, because test 5 had the best behavior that the other tests. The SHA had the higher value owing that in the hydrolytic phase, could be the bacteria destroyed the big molecules that can found in the sludge. If we pay attention to other activities, the SAA had a great eliminated SV, principally because the glucose gives the ideal conditions to bacteria for eliminated organic matter. The eliminated SV of SMA was less because in the methanogenic phase are not eliminate too much organic material, so the specialty of the methanogenic bacteria is producing methane from acetate and Hydrogen.

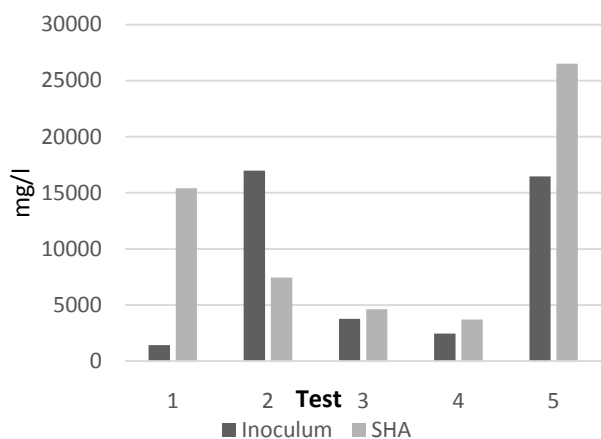


Figure 3. OCD eliminated of SHA

On the other hand, we also evaluated the eliminated volatile solids (VS), an important parameter in anaerobic digestion. In Figure 5 it can see the evolution of the eliminated VS in all test and in the inoculum. In whole tests, the VS of inoculum were less than all tests, because the inoculum did not have a substrate.

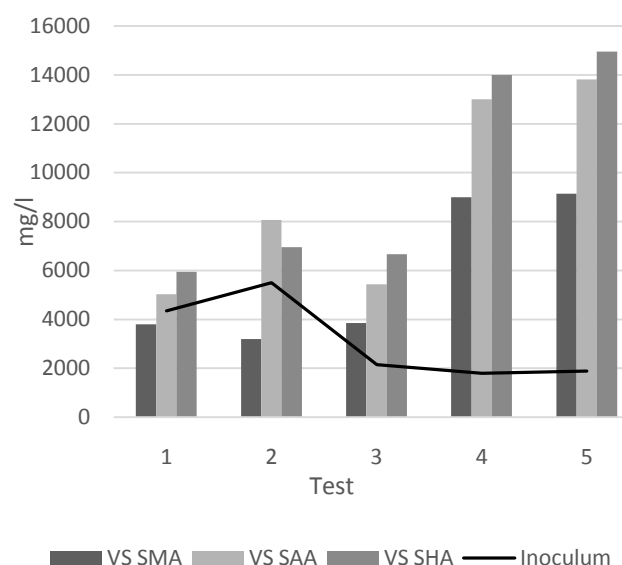


Figure 5. Eliminated volatile solids

Another important parameter of the anaerobic digestion is the rate between volatile fatty acids and alkalinity, generally this rate should be less than 0,4. As said Borja, The VFA/TA ratio can be used as an indicator of the stability of the process. When this ratio is lower than 0.4-0.5 (equivalents of acetic acid/CaCO<sub>3</sub> equivalents), it is considered that the process is working favorably without acidification risk [9]. This rate shows the equilibrium that should have the reactor between the offer of acids and the methanogenic capacity to use these acids and produce methane. Li carried out anaerobic digestion of food waste using a continuous stirred tank reactor (CSTR), and concluded that a combination of total VFA, the ratio of VFA to total alkalinity (VFA/TA) can reflect the metabolism of the digesting system and realize rapid and effective early warning [10].

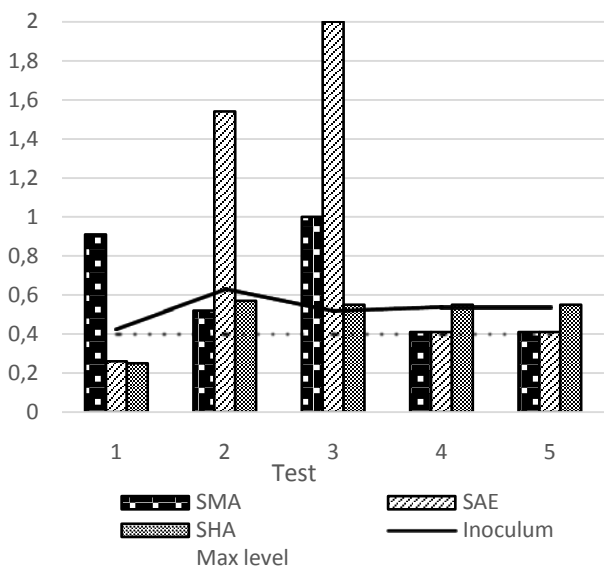


Figure 6. Initial VFA/AT rate

In this research, we did VFA at the begin and at the end of the tests. We can see in figure 6 that the 4 and 5 tests were in the optimal rate, except SHA, where the rate increase due to the high presence of acids in the inoculum that had a 0,5- 0,6 rate. Figure 7 shows that SMA was in the optimal rate, but SAA had a higher rate because there are many acids in the medium. We can observe at 1,2,3 tests, that even the inoculum was not in the optimal rate, whereby is normal that the rate in these tests was high. On the other hand, we can see in the fifth test, that was the best, the rate of SAA and SHA were higher, this is because in the hydrolytic phase is possible to obtain long chain fatty acid from hydrolysis. Is obviously that in SAA the offer of VFA was huge because in our experiment we wanted to specialize the bacteria, and this rate supports this specialty of bacteria in SAA.

### 3.3 Specific Activities Calculation

For the calculation of the specific activities we based in Mond Kinetic Model. The equation of the specific activity can be simplified into the zero-order model, that's means the kinetic behavior when the operational conditions exposed above maintained.

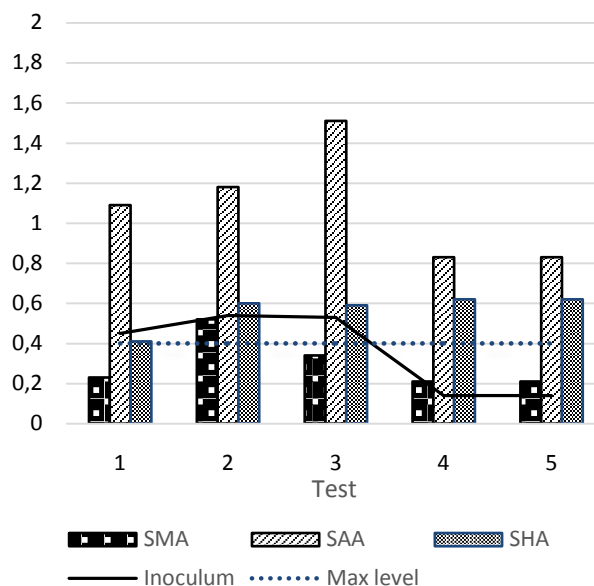


Figure 7. Final VFA/AT rate

The activity expressed as g COD\*g<sup>-1</sup>SSV\*d<sup>-1</sup> and calculated from the methane production velocity (dV<sub>CH<sub>4</sub></sub>/dt) form a substrate degradation rate (dS/dt) [8]. Is important to clarify that the SMA was calculated from CH<sub>4</sub> production and the other two activities were calculated from OCD. The calculation of the activity can do by the following methods:

- a. From substrate consumption rate:

$$(Ac_m)_s = -(dS/dt)/X_0 \quad \text{gCOD} \cdot \text{g}^{-1} \text{SSV} \cdot \text{d}^{-1} \quad (1)$$

In this case S is expressed as COD concentration

- b. From methane production:

$$(Ac_m)_{CH_4} = (dV_{CH_4}/dt) / (X_0 V_R f_1) \quad (2)$$

$$(Ac_m)_{CH_4} = X_0 \cdot t / f_1 \cdot V_R \cdot SSV \quad (3)$$

$$\text{gCOD} \cdot \text{g}^{-1} \text{SSV} \cdot \text{d}^{-1}$$

Where Ac is Activity, S is the limiting substrate, V<sub>CH<sub>4</sub></sub> is the cumulative methane production, V<sub>R</sub> the volume of the reactor, X<sub>0</sub> is the value of the slope in the period of time that the maximum speed is observed, and f<sub>1</sub> is a conversion factor which represents the OCD value of the unit of methane volume and it depends of temperature. For this calculation, we did an analysis of volatile suspended

solids (VSS) that is different than VS.  $f_1$  was obtained from a table of literature, where for 37°C is 418[11]. The results can see in table 3, where first and fifth tests were the closest to 1, where 1 is the value when all SSV are converted to CH<sub>4</sub>.

Test	SMA	I*	SAA	SHA
1	0.8	0.18	0.87	2.69
2	2.52	1.41	2.24	1.11
3	2.35	0.06	2.27	1.15
4	0.05	1.12	0.51	0.25
5	0,23	1.79	1.94	1.28

Table 3. Specific activity calculation. Activity in g OCD<sub>CH<sub>4</sub></sub>/g SSV\*d. SAA and SHA in g OCD/g SSV\*d.\*Inoculum

The values obtained in the research are like other experiments. For example, Soto calculation of the SMA was 0.97 OCD<sub>CH<sub>4</sub></sub>/g SSV\*d on average. The result for SAA were between 0,6-1,08 OCD CH<sub>4</sub>/g SSV\*d. On the other hand, another researcher as Lopez calculated the SMA of other substrates. They calculated 0,02-0,2 OCD CH<sub>4</sub>/g SSV\*d for anaerobic sludge, 0.8-1.5 OCD CH<sub>4</sub>/g SSV\*d for granular sludge and for anaerobic lagoon 0.03-0.1[3]. In addition, Jimenez obtains 1,31 value of SMA of co-digestion between pig manure and rice straw [6]. Table 3 shows that for SMA and SAA the first test has the best value and for SHA was the fifth test. If we focus on the results of the fifth test, it can see that the results are close to 1, that is the neutral state of the Specific Activity. However, for SMA the value was 0,23 that indicated a high diminution of SVV but a slow action to produce methane; as concluded Soto, it should be considered that the methanogenic step rate is usually lower than the hydrolytic or acidogenic one, especially when soluble substrates are considered [2].

#### 4 Conclusion

We evaluated the specific activities to improve the method of SMA, SAA, and SHA and find representative results. For the SMA method, we found that test 5, without shake, was the best. First test had the high second value. This result is supported by higher OCD eliminated, the SV eliminated and the correlation with VGA/AT. It is evident in these two tests, the importance of supply nutrients into the vials. The best concentration of Acetate was 6,6 g/l and we can observe the great behavior of sodium acetate as the substrate in SMA. On the other hand, for SAA and SHA the best result was the fifth test, principally by the higher OCD

eliminated and the SV eliminated. The best concentration of the glucose was 7g/l and for cellulose 6g/l.

With respect to the specific activity parameters, we found that is better make the method without shake, with supply nutrients and with a minimum 108 hours of experiment time (almost 5 days), because in figure 1 we can see in the fifth test, how the grown of methanogenic bacteria stop at 108 hours, and then its stabilized. The specific activity calculation shows that the values were like other researches and it is possible correlating with the chemical parameters.

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