CONTINUOUS ANAEROBIC BIODIGESTION OF THE LIQUID SUBSTRATE EXTRACTED FROM PINEAPPLE STUBBLE IN BIODIGESTERS OF 0.1 AND 4.6 L

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Abstract

The pineapple crop represents the most important agricultural activity in Costa Rica, from the economic point of view, represents 32% of agricultural exports. The sowing area is estimated at 58,000 hectares. However, this activity generates a considerable amount of biomass in the order of 340 metric tons per hectare, of which 26% corresponds to fruit and 74% to organic agricultural waste (OAW), so called pineapple stubble. This is a generator of environmental problems because it is not treated adequately in the field, where it is opted for the use of herbicides to cause its degradation and then incorporate it into the soil, a practice that has generated problems of contamination of groundwater and the pest of the stable fly Stomoxys Calcitrans. The stubble of the pineapple has the highest energy potential, within the OAW that are generated in the agricultural activity in the country as mentioned by Coto in 2013. The use of pineapple stubble juice was evaluated as a substrate for biodigestion in mesophilic conditions, using the Hohenheim methodology with volumes of 100 ml and in 6 liters CSTD biodigesters. Three types of substrate were evaluated: 100% stubble juice, a mixture of 90% juice and 10% cow manure and a mixture of 80% juice and 20% cow manure. The substrate of 100% pure pineapple stubble juice showed the best performance in biogas production. In the Hohenheim test the accumulated production was 320 ml, while the 9010 mixture produced 280 ml and the mixture 80 -20 produced 250 ml. In the CSTD reactor test, the substrate 100% stubble juice had an accumulated production of 170 liters; the 90-10 mixture produced 60 liters. The 80-20 mixture collapsed from day 20, ceasing gas production. The behavior of the pH and the FOS/TAC test showed stable behavior during the process in all cases. It was demonstrated that pure stubble juice is a good substrate for the biodigestion process; with which the technology of anaerobic biodigestion is presented as an important alternative for the use of this kind of OAW.

Key words: *anaerobic biodigestion, pineapple stubble, biogas production, renewable energies, etc.*

INTRODUCTION

The export of fresh pineapple from Costa Rica reached a value of 942 million dollars in the year 2017. This means an increase in national income of 7.9% more than the year previous and produced a total of 32 thousand direct jobs (CANAPEP 2018) [2]. In global terms, the FAO (2018) registered Costa Rica as the main pineapple exporter in the world in 2016: placed on the market 2,930,661 tonnes of pineapple that represented 11.35% of the market world. [6]
With respect to the planted area, there is a discrepancy between CANAPEP (2018) that declares 44,500 hectares and the survey with remote sensors made by PRIAS in 2016 that identified 58,607.5 hectares (MINAE 2017) [11].
Each hectare generates a quantity of stubble estimated at 250 tonnes, representing an organic agricultural waste with a high value energy that is not being used.
According to Coto (2013) the Costa Rican pineapple sector does not consider the viability technological and economic use of the waste produced by their farms, which puts highlight the need to investigate this bioenergetic potential, especially from the anaerobic digestion of pineapple stubble. [4]
The anaerobic biodigestion process is a complex process in which the organic matter is degraded by a series of metabolic interactions performed by a microbial community, acting in concert in a proper

environmental conditions, in which take place the develop of microorganisms of vital importance, as well as the substrate that serves as a source of feeding for bacteria and carbon for the generation of methane gas as mentioned by Khanal in 2008 [9].

Pineapple stubble as substrate

Extracts in water of pineapple stubble represent 11% m/m of the findings of Irías in 2014[8]. Pineapple residues have high contents of holocelluloses [3, 8] and high acidity [8].

Both characteristics hinder the anaerobic biodigestion of pineapple waste because the celluloses they are not digestible and the microorganisms responsible for the process do not tolerate acidity. As was indicated by McMorrow *et al* in 1969, who worked with pineapple waste from canned fruit in Hawaii, the liquid phase of the pineapple substrate has a relatively high content of sugars, but is low in organic acids. In the initial phases of the biodigestion of the pineapple liquid substrate, the microorganisms are converted into nucleic acids [10].

Subsequently, the pH increases and the fatty acids decay slightly with a concomitant improvement in the quality of the gas (35% methane). According to Aworanti *et al (*2018) the pineapple substrate showed an increase in the production of biogas as the total solids content and the temperature of the biodigester increased in the ranges of 4 to 8% and 40 to 60°C, respectively [1].

The purpose of the experiments described here was to establish the productive potential of biogas produced from the juice extracted from the pineapple stubble on a laboratory scale by means of continuous feeding tests following the VDI 4630 standard. (Ingenieure Verein Deutschland 2006) [7].

MATERIALS AND METHODS

The pineapple stubble used in these trials came from the Piñales de Santa Clara farm, located in San Gerardo de Río Cuarto (10°25'50.42 "N, 84°08'48.38" W, 161 masl). The soils of the farm belong to the order of the inceptisols. The pineapple stubble was subjected to a pre-treatment consisting of two stages: first, the cutting of the fibers with a chopping machine or "chipper"; second, the extraction of stubble juice with a sugar cane mill ("trapiche"). The pressing operation was repeated two consecutive times. The determination of total solids (ST) was made from three subsamples to have three repetitions.

The crucibles were previously brought to constant weight, placing them in a muffle at 550°C for 45 minutes. Once cooled (in a desiccator), the crucibles were weighed in vacuum. 20 ml of the sample was added to each crucible and weighed again, then placed in a furnace at a temperature of 105°C for six hours to reach constant weight. After being cooled the samples were weighed again.

Subsequently, for the determination of the volatile solids (SV), the samples were placed for 45 min in a muffle at a temperature of 550°C. Both ST and SV were determined by mass differences using an analytical balance.

Continuous biodigestion tests were carried out applying two different types of tests. In one case, the Hohenheim fermentation test was used in 100 ml syringes and, in another, the test was applied using 6 liter bioreactors (gross volume) with a functional volume of 4.6 L. The measurement of the volume of gas produced by the biodigesters of 6 liters was made with a gasometer Ritter and, the determination of the quality of the gas with the help of a Multitec 560, Sewerin.

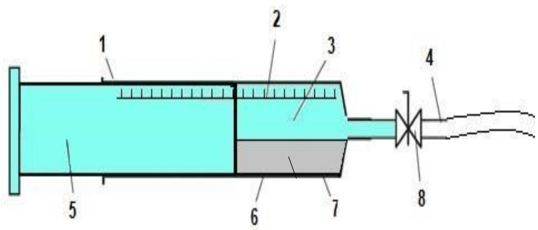
Fermentation test with Hohenheim Method.

Figure 1 shows the schematic representation of the syringes used in the Hohenheim fermentation test.

This method does not need an additional gas sampling tube. Between measurement periods, the biogas remains in the syringe, which also serves as a fermentation chamber. The biogas produced from the disintegration of the substrate displaces the plunger of the syringe, which allows measuring the amount of gas generated in a given time.

Gas losses are avoided by placing hoses on the tip of the syringes, which are sealed by presses.

Due to the limited volume of gas produced, in this test it was not possible to determine the quality of the biogas.



1 Lubricant and sealer 5 Plunger
2 1cc scale for measuring gas
6 Glass syringe
3 Gas chamber 7 Substrate
4 Hose 8 Press

Fig. 1. Schematic diagram of the Hohenheim fermentation test.
Source: Adapted from VDI 4630 [6]

Figure 2 shows the assembly of the test done in the laboratory installed in the EEFBM. The syringes were placed inside a chamber with temperature control and mechanical agitation. The temperature used was 37°C and the relative humidity was set at 80%. This methodology allows testing of different substrates simultaneously with several repetitions. Although the Hohenheim methodology is designed to perform batch tests, this time continuous tests were run, for which it was necessary to extract an amount of effluent equal to that of substrate fed (6 ml daily), to maintain the volume of constant matter in the syringes. In the first feeding, the syringes were inoculated with 5 ml of effluent from an active biodigester and the substrate corresponding to each treatment, in accordance with the VDI 4630 standard [7].

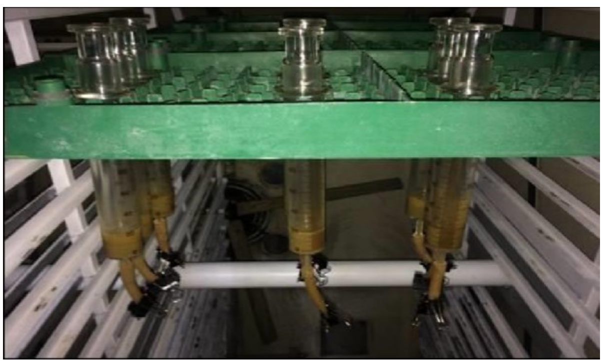
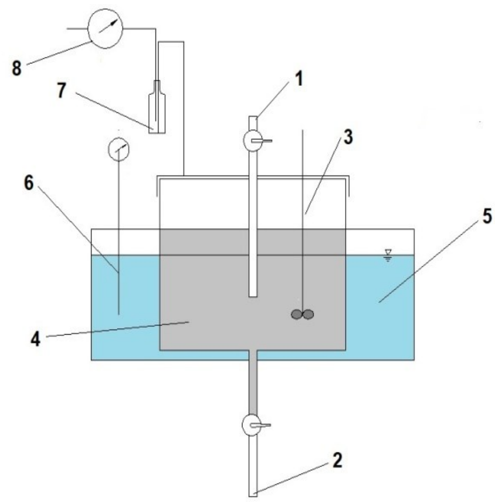


Fig. 2. Assembly of the Hohenheim fermentation test in an incubator with relative humidity and fixed temperature
Source: Own elaboration.

The treatments consisted of three types of substrate: pure pineapple juice and its mixture with dung in the proportions of 90:10 and 70:30. The trial had three repetitions of each treatment. It started on January 29, 2018 and included observations of the volume of gas produced during 96 hours. In these tests, due to its characteristics of using very small amounts of substrate, it was not possible to perform pH or FOS/information over a relatively long period to analyze the behavior of the process with various types of substrate under the same operating conditions. The test was carried out in three 6 liters biodigesters placed in a water bath and provided with internal stirring, temperature control, connection with the Ritter gas meters and the Sewerin brand biocontrol unit, as shown in Figure 3. Each Biodigester was fed with the same substrates (treatments) already indicated in the Hohenheim fermentation section. The temperature condition was mesophilic (37 ° C on average).



Legend:
1 Substrate entry
2 Substrate output
3 Agitator
4 Substrate
5 Bath Maria
6 Temperature sensor
7 Water trap
8 Gasometer

Fig. 3. Schematic diagram of the fermentation test in 6 liters biodigesters.
Source: Own elaboration.

An amount of 4 L inoculum was initially placed in each digester. Substrate feeding started with small amounts (0.5 kg ST / m³ and day) and was increased every two days by 0.5 units, according to VDI 4630 procedure. However, when observing that there was no biogas production and that the values of FOS/TAC were very low, it was decided to reduce by 30% the amount of substrate feeding trying to bring such values to the range of 0.3-0.4.

The parameters measured daily were pH, temperature, FOS/TAC balance and gas production. The pH was measured according to the norm using "peachimeter", which was calibrated with buffers of pH 4.0 and 7.0. The FOS/TAC determination was carried out with the HACH titration test, using the titrator AT 1000. The gas production was measured with a Ritter drum gasometer of 0.5 L.

Fig. 4. Assembly of fermentation test with 6 liters biodigesters.
Source: Own elaboration.

RESULTS AND DISCUSSIONS

The three analyzed substrates were characterized in terms of their ST, SV and pH content (Table 1). The stubble juice showed the highest levels of ST and SV, as well as the lowest pH value. In general, as the dung content of the substrate was increased the content of ST and SV decreased and the pH value increased. In all cases, the content of ST and SV were within the range of operation of a biodigester of the completely agitated type, which is less than 10%. Frequent agitation homogenized both the distribution of solids within the digester and its temperature. This condition occurred both in the test with syringes and in that of the 6-liter biodigesters. Despite the low pH values initially observed, there was no need to make any correction.

Table 1. Content of total solids (ST), volatile solids (SV) and pH of the three substrates analyzed

Treatment	Total solids (ST) (%)	Volatile solids (ST) (%)	pH
Pineapple stubble juice	4.102	3.102	4.22
90% juice and 10% dung	3.8975	2.943	4.31
70% juice and 30% dung	3.4885	2.625	4.51

Source: Own elaboration.

Figure 5 shows the variation of the pH of each treatment as a function of the time of the substrate at the entrance and its exit (effluent) from the 6 L biodigesters.

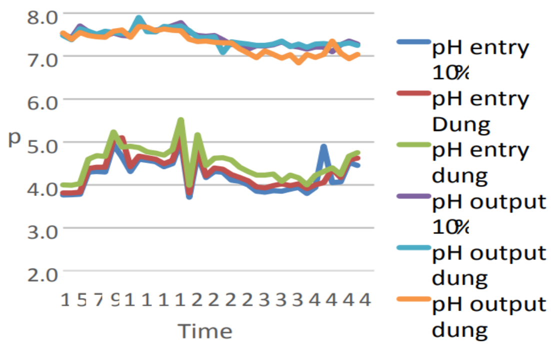


Fig. 5. Variation of the pH of the feeding substrates and the effluent of the digester during the tests.
Source: Own elaboration.

Figure 6 indicates the FOS/TAC balance of each treatment observed in the biodigesters of 6 L as a function of time.

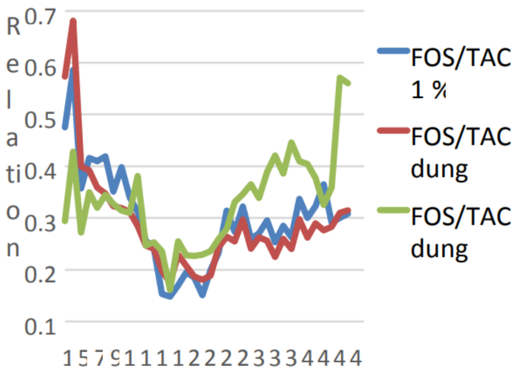


Fig. 6. Variation of the ratio FOS / TAC in the digesters during the tests.
Source: Own elaboration.

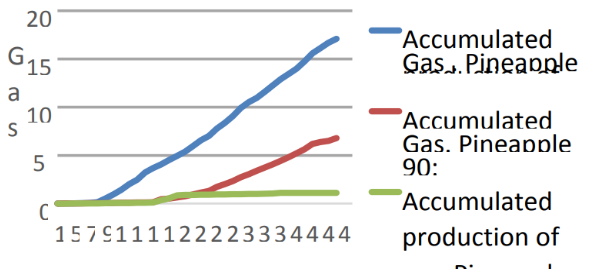


Fig. 7. Gas production of the treatments against time in digesters of 6 L.
Source: Own elaboration.

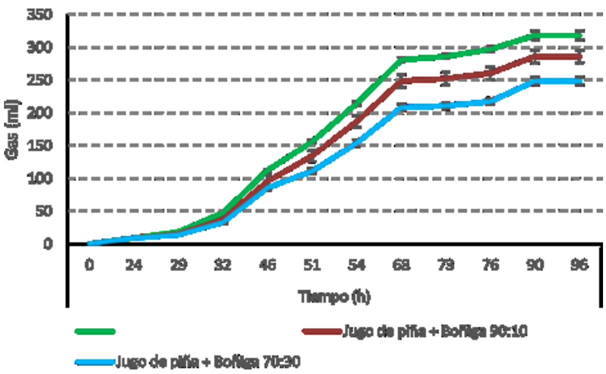


Fig. 8. Gas production of the three treatments as a function of time in Hohenheim fermenters of 100 ml. The daily diet was 6 ml.
Source: Own elaboration.

According to their characterization (Table 1), the treatments used are adequate to process them with wet anaerobic biodigestion. Despite the relatively low pH of all the treatments, it was not necessary to make acidity corrections using alkaline substances. This showed that the communities of microorganisms managed to carry out the disintegration of the substrate despite its initial acidity and that the system possesses a good buffering capacity of the pH. Figure 5 showed that the intervention of the microorganisms managed to neutralize the pH of the substrate. Therefore, it is possible to conclude that the behavior presented by the three treatments is stable, keeping the reactor within the ideal range of operation. This ensures the balance between the phases of the biodigestion process, that of methane production, since methanogenic bacteria are inhibited in conditions of pH lower than 6.7.

The FOS/TAC test is an indicator to evaluate the biodigestion processes in a reactor in operation. The TAC value is an estimate of the buffer capacity of the sample and the FOS value corresponds to the content of volatile fatty acids. The value of the FOS/TAC ratio is an indication of the stability of the anaerobic process of degradation of organic matter. As a general reference for a stable operation values are assumed in the range of 0.15 to 0.45. Values below this range could indicate a condition called "alkalosis" or inadequate feeding, which could lead to an increase in pH values and a reduction in the content of organic acids. All this would harm the hydrolysis and the formation of acids. On the contrary, a value higher than the indicated range is an indicator of a condition called "acidosis" in which there is an accumulation of fatty acids, which can cause acidification of the system as mentioned by Voß in 2009 [14]. According to the empirical values provided by Deula - Nienburg [5], the maximum production of biogas occurs when the value of the FOS/TAC balance ranges from 0.3 to 0.4. Above this range there would be an overload of organic matter that will be higher the higher the value obtained. Above 0.6 the load of organic matter would be excessive. Values below the range are an indication that food is precarious. Below 0.2 the biomass load is considered very low as found by Mézes *et al.*, in 2011 [11].

Initially the values of this parameter were high (Figure 6). Therefore, according to the recommendation, the amount of biomass fed must be reduced. However, the amount of biomass added was actually low, since the recommendations of the VDI 4630 standard were being followed. As the trial progressed, the amounts of biomass fed instead of being reduced increased and the FOS/TAC values They tended to go down until they reached the ideal range. This behavior can be attributed to the fact that the initial stage of the process is in a transition in which the process of biodigestion is not totally stable because the microbial population has not developed enough quantity or variety. Under these conditions, the concentration of volatile fatty acids (VFA) and the alkalinity of the system have not reached an equilibrium.

Therefore, the condition of the digester can not be assessed only based on the FOS/TAC balance.

According to Rosato (2017) this titration method (the FOS/TAC) allows to obtain some information about the order of magnitude of the concentration of the VFA and the alkalinity of the system [13]. However, the parameters must be analyzed separately within a context, which is the state of the observed system, characterized by a set of variables and not only the FOS/TAC balance. According to Rosato (2017), in the case of a single-step biodigester (the whole process is carried out in the same container or container, that is, without separation of the stages in several digesters), it is necessary to monitor at least twenty parameters to characterize its state adequately and thus know the margin of stability and efficiency [13]. Therefore, maintaining the FOS-TAC relationship in the range of accepted values does not necessarily allow the stable operation of the system. By contrasting the behavior of the pH (Figure 5) and the FOS-TAC ratio for treatments 1 and 2 during the first seven days the system (Figure 6) there was a transition process in which the values of FOS / TAC did not faithfully reflect the state of the system, since the pH values indicated a balanced system. Note that biogas production started on day seven for 100% juice treatment and on day 19 for 90:10 treatment (juice: dung). This last treatment showed a significant increase on day 25 (diauxiatic behavior).

From the above, it can be inferred that before starting the production of biogas, the system went through a transition stage in which the microbial population was in the process of growing and adapting to the feeding substrate and, once a number condition had been reached. and type of bacteria (that is, once the consortium of bacteria was defined and stabilized), they achieved a growing and sustainable gas production. Once the recommended FOS/TAC range of 0.3 to 0.4 was reached, the trend continued to decrease until day 25, when an upward trend began (Figure 6). This behavior responded to the fact that, based on the values obtained and the Deula - Nienburg recommendation, the quantities of substrate fed were systematically increased in search of a positive response.

By observing the behavior of biogas production (Figures 7), it can be seen that the systems operate normally, which indicates that they are "healthy". The treatment of pure juice showed a growing and sustained gas production from day seven, coinciding with the moment in which the system reached optimal values of FOS/TAC (Figure 6). At that time the pH was also in the optimal range (Figure 5). The treatment with more dung (70% juice and 30% dung) had a very different behavior than the other two treatments, presenting higher FOS/TAC values than the other two. He also showed abrupt elevations above 0.5 when the other two treatments were in the recommended range. The gas production of the continuous test with Hohenheim biodigesters showed the significant behavior of the three treatments after 51 hours of observation. In this case, the treatment with more content of dung did not show a behavior inconsistent with the pattern observed in the other two treatments. In all cases, there was a production of biogas proportional to the content of pineapple stubble juice from the substrate.

CONCLUSIONS

Under soil, but especially climate conditions of the year 2016 and the technology used, Premium wheat varieties tested had values of protein content between 13.7% and 15.9%. The average value of the six analyzed varieties was 14.6%. The lowest protein content was recorded in the 4- Atrium variant, 13.7% and the highest in 5 Arnold variant, 15.9%. Arnold variety was the only one to exceed the protein content of control (15.2%). Variants 2-Bitop, 5- Arnold and 6-Joseph obtained protein content values that exceeded the calculated average value of the varieties. Concerning gluten content of varieties, the average value was 29.9%, surpassed only by the variant 2-Bitop and the Arnold variety. Bitop variety was the variety that also recorded the highest content in gluten, 31.8%. As a witness, all varieties studied added a gluten content of between 1 and 3.1%.

In terms of hectoliter weight values, the highest value, 80.8%, was recorded in the 2-

Bitop variant. All the studied varieties exceeded the value of hectoliter weight recorded by control, (75.1%), the recorded increase being between 3.3 and 5.7%. All recorded values were over 78%.

The mass of one thousand grains had the lowest value, 35.44 g, for Fulvio 3-variant and the highest, 43.06 g, for 2- Bitop variant.

Regarding the profitability obtained by the studied varieties, it overcome in all variants the production of control, the differences being very significant and consisted in crop profitability ranging from 568 to 1,666 kg/ha. The highest production was recorded in Midas 1 variant, 8,158 kg/ha and the lowest at Arnold 5 variant, 7,060 kg/ha. As compared to average production, the 1 variant Midas obtained the highest crop profitability, 693 kg/ha, a very significant increase.

Compared to the production average, Fulvio 3 variant and Atrium 4 variant did not show any significant differences. Compared to the production average of the six varieties (7,465 kg/ha), 5 variant Arnold and 6 variant Josef made very significant harvest minuses, resulting in production differences of 973 kg/ha and 405 kg/ha, which means, in relative values, a minus production of 5.4 to 13%.

All the studied variants have confirmed the excellent value of the varieties regarding some qualitative indices but also regarding the recorded productions.

REFERENCES

[1]Aworanti, O. A., Agarry, S. E., Ogunleye, O. O., 2018, Biomethanization of Cattle Manure, Pig Manure and Poultry Manure Mixture in Co-digestion with Waste of Pineapple Fruit and Content of Chicken-Gizzard- Part I: Kinetic and Thermodynamic Modelling Studies. Open Biotechnol. J. 2017, 12,36–53,

[2]CANAPEP, 2018, Estadísticas de valor de las exportaciones de piña. https://canapep.com/estadisticas/, Accessed March 1st, 2018.

[3]Chaiprasert, P., Bhurmiratana, S., Tanticharoen, M., 2001, Mesophilic and Thermophilic Anaerobic Digestion of Pineapple Cannery Wastes. Thammasat Int. J. Sc. Tech. 2001, 6, 1–9.

[4]Coto Chinchilla, O., 2013. Valoración actualizada de Residuos Agrícolas Orgánicos como fuente de energía en Costa Rica. FITACORI, 2013.

[5]DEULA-Nienburg, Our Knowledge-Your success, http://www.deula-nienburg.de/en/home.html, Accessed March 1st, 2018.

[6]FAO, 2018, Statistics. www.fao.org/faostat, Accessed March 1st, 2018.

[7]Ingenieure Verein Deutschland, 2006, Fermentation of organic materials (VDI 4630); Beuth Verlag GmbH: Dusseldorf, 2006.

[8]Irías A, G. L., 2014, Composición química de la biomasa residual de la planta de piña variedad MD2 proveniente de guacimo, limón. Cienc. y Tecnol. 2014, 30(2), 27–34.

[9]Khanal, S.K., 2008, Anaerobic Biotechnology for Bioenergy Production; Principles and Applications. USA, Wiley-Blackwell. 301 p.

[10]McMorrow, M. J. K., Young, R. H. F., Burbank, N. C., Lau, L. S., Klemmer, H. W., 1969, Anaerobic Digestion of Pineapple Mill Wastes. Water Resources Research Center, University of Hawaii at Manoa. WRRC Technical Report 27. https://scholarspace.manoa.hawaii.edu/handle/10125/6814, Accessed March 1st, 2018.

[11]Mézes, L., Györgyi, B., Erika, S., Mihály, P., János, B, János, T., 2011, Novel approach on the basis of FOS/TAC method. Anbals of Oradea University. Fascicula Environment Protection, Vol. XVII,. 713-718.

[12]MINAE, 2017, Manejo de cambio de uso en paisajes productivos. , http://mocupp.org/sites/default/files/do cumento-mocupp-es.pdf, Accessed March 1st, 2018.

[13]Rosato, M., 2017, A critical review of VFT/TA (FOS/TAC) method. Sustenable Thecnologies S.L. 2017.http://www.worldbiogasassociation.org/wpcontent/ uploads/2017/08/FOS-TAC-EN.pdf, Accessed March 1st, 2018.

[14]Voß, E., Weichgrebe, D., Rosenwinkel, K., 2009, FOS/TAC – Deduction, Methods, Application and Significance. Internationale Wissenscharttskonferenz. Biogas Science – science meet practice, LfL Bayern, 2009. 2-4. 12.09, Erdin.

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