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PIG MANURE DIGESTION ASSAYS UNDER ANAEROBIC CONDITIONS IN FLUIDIZED BED REACTORS

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Abstract: A study of the pig manure degradation and biofilm development under moderate psicrophilic conditions (20°C) was carried out on two pilot lab scale anaerobic fluidized bed reactors (AFBR), where biolite and activated coal were used as supports, respectively. The soluble total organic carbon (TOC) reduction in the treated waste was 40% for both reactors, working with a high organic loading rate (OLR) of 10 kg $TOC \cdot m^{-3} \cdot d^{-1}$. The percentage of organic carbon removal rose until 90% when the organic loading rate decreased up to 3 kg $TOC \cdot m^{-3} \cdot d^{-1}$ (treating in this case only manure proceeding from the piglet room). The study shows imperceptible contents in acetic, propionic and butyric acids in the treated manure, whereas fatty acids of longer chain are detected. Microbial population developed inside reactors seems unable to degrade these long chain acids as fast as short chain ones because the biofilm is thinly populated by acidogenic bacteria. On the contrary, it seems to be large acetogenic and methanogenic population that carries out short chain fatty acid degradation. Kinetic studies reveal the existence of a remaining organic fraction in the anaerobic pig waste degradation caused by inhibition of hydrolysis step. Scanning Electron Microscopy shows a wide variety of microcolonies of different trophic populations which are located in crevices and other regions sheltered from hydraulic shear forces.

INTRODUCTION

It has been estimated that a high percentage of the continental water pollution is caused by cattle wastes. The elimination of these waste products by indiscriminate application to the land or direct spill to water channels has caused the appearance of pollution in numerous points [11]. In the cattle sector the most serious case of environmental impact is the one caused by pig farms. In the last few years a great increase of the number of heads has occurred, which has derived in an increase of the amount of pig manure. Traditionally, this waste was used to fertilize cultivated fields, but the intensification of the sector has caused a noticeable tendency to the animal concentration in specialized farms distributed in heterogeneous forms with high index of heads per farm. These farms do not have a ground surface extensive enough to spill all the manure produced without causing damages to the environment, which makes difficult the management of this waste [8].

The increase of the number of intensive cattle farms with great dimensions, but with a reduced number of workers per farm, has also caused the adoption of different systems for manipulation of wastes in liquid state to reduce the time inverted in transport, storage and other operations. These factors cause the impossibility of managing the manure in the traditional way. For this reason, nowadays it is necessary the treatment of the effluents generated in these intensive pig farms.

The elimination can be made in diverse ways, each one with a characteristic incidence on the environment. Anaerobic digestion occurs naturally in liquid manure systems. The lack of oxygen and abundance of organic matter in liquid manure provide the proper conditions for anaerobic bacteria to survive [2]. The process of anaerobic digestion consists of three steps. The first one is the hydrolysis. This step breaks down the organic material to usable-size molecules. The second step is the conversion of decomposed matter into organic acids. And finally, the acids are converted to methane gas.

In the last years a better knowledge of the thermodynamic, microbiological and kinetic bases of the anaerobic process has allowed the design of equipment that can compete with other alternative techniques, specially in the case of processing of high loading wastes, as it is the case of cattle wastes [3, 6, 9, 13]. The anaerobic treatment processes have many advantages with regard to conventional aerobic process, nevertheless the anaerobic organisms grow very slowly, reason why, to operate with a reasonable volume of reactor and short hydraulic residence times, the biomass must be immobilized into the reactor or recirculated in the system. The development of new generation anaerobic reactors, that retain or immobilize great amounts of biomass, has allowed the extended application of this type of digesters [7]. The industrial digesters of pig manure operating until today have been fundamentally stirred tank reactors and plug flow reactors, but, once the validity of the anaerobic process has been demonstrated we should study alternative reactors that offer better benefits. For this reason, at the present time it tends towards the development of processes that operate with immobilized or retained biomass. The fluidized bed reactor is one of them.

In these reactors, mixed culture bacteria are made to grow as a film on the surface of equal inert carrier particle. These particles are then maintained inside the reactor in a fluidized state using the energy of the incoming influent stream. The linear velocity of the influent is kept above the minimum fluidization velocity, so that the film-covered particles are always in motion. The substrate present in the liquid phase diffuses into the biofilm and gets converted to volatile fatty acids and, ultimately, to methane. These products then diffuse out through the biofilm into the bulk liquid. The mixing and mass transfer achieved in these reactors are excellent and this is the reason why these systems allow for working with higher organic loading rate than the other reactors used in the anaerobic wastewater treatment.

The main aspect to consider when operating with fluidized bed reactors is the size of the support particle, required for the operation of these technologies. These particles must have a uniform geometry and size to obtain a homogenous bed fluidization throughout the digester. It must be chosen a support that allows a fast start up, in which the biofilm is developed with a great stability, economical and that presents a high resistance to physical, chemical and biological attack.

The main aim of the present work was to study the pig manure liquid fraction (filtered manure) degradation and biofilm development under moderate psycrophilic conditions on anaerobic fluidized bed reactors (AFBR) using different carriers as supports. Enzymatic treatment methods for increasing the degradation rate of manure were also investigated in this study.

EXPERIMENTAL

The experimental set up is shown in Figures 1 and 2. The installation consists of two AFBR equipped with a gas collector. Each reactor is a Plexiglas column 64 mm in inner diameter and 96 cm of total height with a conical upper settling zone. Pig manure to be treated (previous filtration) and the recycle flow are pumped into the reactor via an inlet tube, with downward distribution holes, located across the reactor and 5 cm from the base. Liquid goes upward through a bed formed by stones with diameters between 1 and 5 mm (aleatory sample) in order to carry out a uniform flow distribution across the bed. Due to the difficulties found in the systems to measure the recirculation flow, the parameter followed was the fluidized bed height, set always at 70 cm. Gases generated in the organic matter degradation process are collected via an outlet at the top of the reactor in a gas-liquid-solid separator.

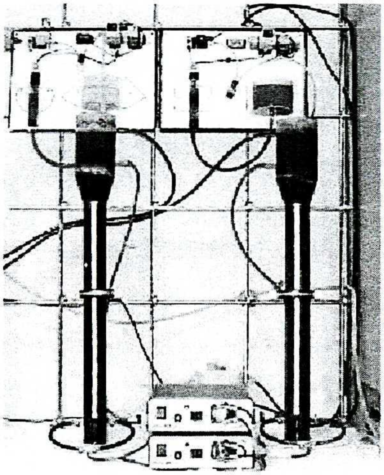

Fig. 1. Experimental set-up

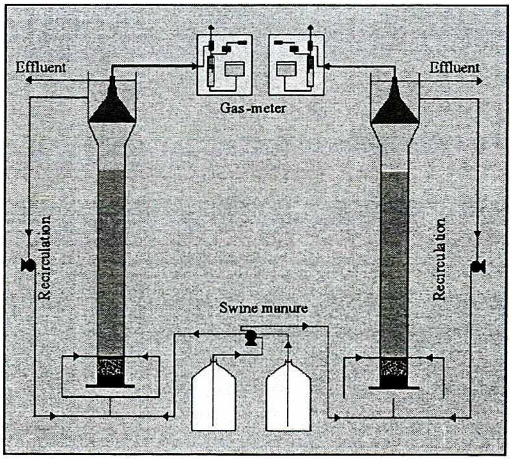

Fig. 2. Schematic diagram of the anaerobic fluidized bed reactors

The reactors were filled with two types of different supports up to a height of 40 cm, in order to study the influence of this parameter in the evolution of the system. The supports used were biolite (Rb) and activated coal (Rc), both with an average diameter of 300 μm and an apparent density of 1150 and 1250 $g \cdot dm^{-3}$, respectively. The high specific area of both materials, 0.6 $m^2 \cdot g^{-1}$ for biolite and 0.8 $m^2 \cdot g^{-1}$ for coal, their low density and a finely porous structure, convert them in excellent solid carriers to be used in biological processes with fluidized beds [5, 9, 13].

In order to study the influence of enzymes on manure degradation a series of experiments were carried out in a temperature-controlled room that was maintained at 25°C. The assays were carried out on three 500 cm^3 digesters, placed on an agitator simulating discontinuous reactors (Fig. 3). The small digesters were filled with different manure samples and enzymatic compounds. During the experiments the concentration of different volatile fatty acids (acetic and propionic) and organic matter content (TOC) were followed as a measure of the evolution of manure degradation in the presence of hydrolitic enzymes.

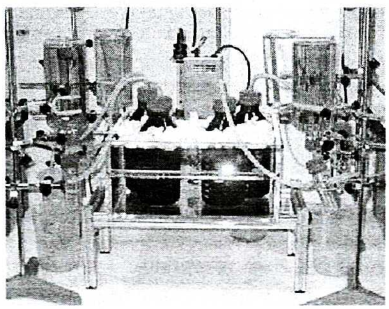

Fig. 3. Enzymatic assays set-up

Analytical determinations were performed according to Standard Methods [1]. The volatile fatty acids were analyzed by chromatography with a Shimadzu GC-17A gas chromatograph after centrifugation and acidification with concentrated phosphoric acid. Total organic carbon (TOC) was determined using an analyzer with non-scattered infra-red detection Shimadzu TOC 5000A (SHIMADZU Co., Kyoto, Japan). Samples for scanning by electron microscopy (SEM) were fixed with 3% (v/v) glutaraldehyde in 0.1 M cacodylate buffer (pH 7.2) and dehydrated through a graded series of ethanol solutions. The samples were then dried to their critical point and coated with gold. SEM micrographs were taken with a Jeol 30 JSM-T3000 scanning microscope (JEOL, Ltd., Tokyo, Japan).

RESULTS AND DISCUSSION

Lab-plant evolution

The feed used in the start-up and first operational period (until day 175) was the general effluent from the farm, sampled from a storage pool (average storing time 1 week). This manure was previously filtered with a 1 mm mesh sieve to be introduced in the reactors. The start up was fast in both reactors. In 20 days an organic matter removal of 70% was reached in Rc (Fig. 4), although working with low organic loading rates, around 1 kg $TOC \cdot m^{-3} \cdot d^{-1}$ (Fig. 5), to avoid the destabilization of the systems, which are very sensible to organic overloads in the first operation stages. On day 44, an accidental gasoline spill on the manure pool caused a slight destabilization of the systems that were fed with the contaminated effluent. After their recovery, the organic loading rate fed to the systems was increased progressively, reducing the hydraulic retention time. After 100 days, the system was operated with organic loading rates next to 10 kg TOC·m^{-3}·d^{-1} but the percentage of organic matter removal dropped, in this case, to 45%.

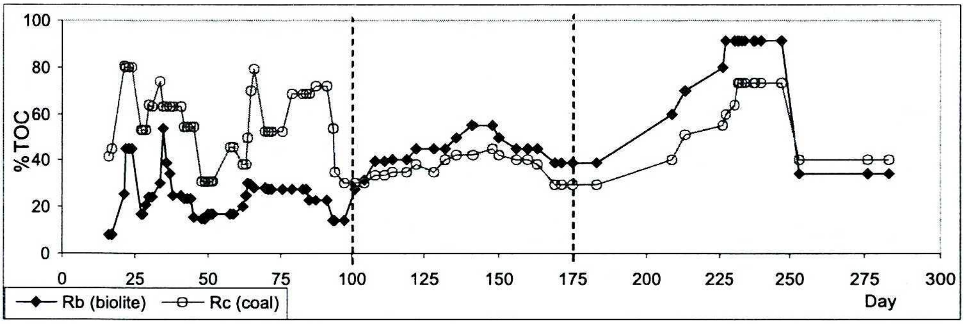

Fig. 4. Evolution of Organic Matter Removal

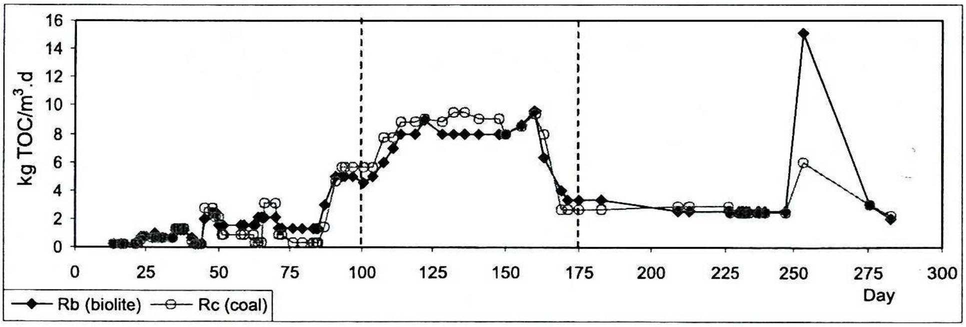

Fig. 5. Evolution of Organic Loading Rate

The higher initial remotion of organic matter observed in Rc (reactor with granular coal as support), as contrasted with Rb (reactor with biolite as support), was probably due to adsorption phenomena on the coal, because with further experimentation, the percentage of TOC removal in both systems tended to be the same and, even, the initial situation was reversed, as on day 100, where Rb produced higher removal yields.

In order to study the influence of the type of manure on the behavior of the systems, it was used, from day 175 and until the end of the experiment, manure coming from the piglet room. It can be observed in Figure 4 that the percentage of organic matter removal increased considerably, reaching a removal percentage of 73% in Rc and 91% in Rb, and showing the higher bio-degradability of this type of manure. The explanation can be associated with the fact that the pig, in the piglet growth-phase, has a more active metabolism than in other productive growth-phases; thus, the manure, in this case, is more easily digested and has a smaller percentage in recalcitrant organic matter. That facilitates its degradation by anaerobic digestion.

On day 253, an episode of organic overload was detected along with the consequent destabilization of the systems. In Rb, the OLR rose from 2.5 to 15 $kg \cdot m^{-3} \cdot d$ in less than 24 hours, which produced a drop in the percentage of organic matter removal from 91% to 34%. In Rc, an organic loading rate increment from 2.5 to 6 $kg \cdot m^{-3} \cdot d$ destabilized the system, resulting in organic matter removal dropping from 73% to 40%. None of the systems recovered completely, despite the stable feeding of manure for the next 30 days.

After destabilization, the attached volatile solid concentration (AttVS) on the biolite was 35 $g \cdot dm^{-3}$ whereas in the coal, it was 15 $g \cdot dm^{-3}$. This last value must be taken into account with certain reservation given the difficulty to calculate volatile solids concentration adhered to the coal and due to the loss of weight that suffers the material at high temperatures. Nevertheless, the difference between both values is enough to indicate the lower tendency to the microorganisms adhesion on the coal compare to that on the biolite. The differences on biomass concentration between both reactors were maintained throughout the process, as can be observed in Table 1.

Table 1. Evolution of Attached Volatile Solids

Operation day	AttVS biolite [$g \cdot dm^{-3}$]	AttVS coal [$g \cdot dm^{-3}$]
62	26	8
122	31	12
195	35	15

Another fact needs to be considered as it can help to explain the smaller percentage of organic matter removal reached in Rb when the experimentation advances and the smaller amount of biomass adhered on the coal: the coal granules tended to break and erode. Breakage causes the appearance of small particles, which were washed out with the effluent, while erosion produces the superficial smoothing of granules, which reduced microbial adhesion.

The evolution of pH in the reactors along the experiment is presented in Figure 6. It can be observed that this parameter stays practically constant throughout the experimentation in the range 7.5–8, being the values of pH in the effluents slightly higher than in the feeds. The lower values of pH in the reactors were detected in the period in which they were fed with manure from the piglet room, because of the lower pH of this type of manure, sometimes close to 6 (even after 1 week storage).

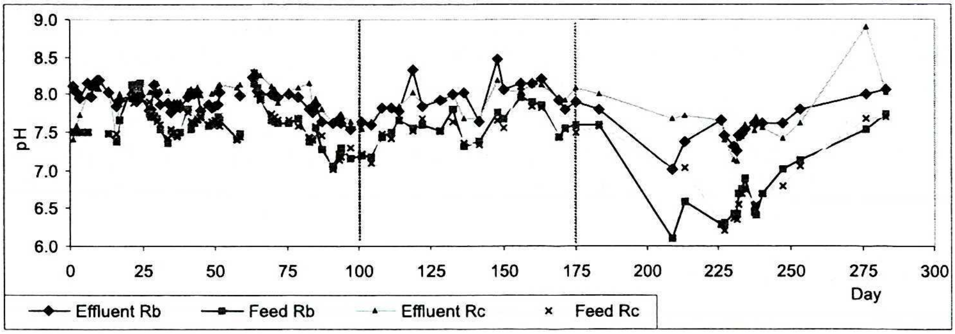

Fig. 6. Evolution of pH

Hidalgo [4], working with fluidized bed reactors, observed that with pH values in the reactor under 6.0 the biomass becomes inactive, blocking the anaerobic process. In this case, microorganisms remain adhered to the support but take a long time in recovering their activity. On the contrary, with pH values over 8.5 (example, day 275 in Fig. 6) a great part of the biomass is taken off from the carrier and is washed out with the reactor effluent, but the microorganisms that remain adhered to the support surface become active as soon as the pH returns to a neutral value. Thus, the system recovers better after an abrupt increment of the pH than after a decrease.

The results show that the biomass development in the systems is only able to degrade, when the reactors work with low hydraulic retention times, a part of the components of the pig manure used as feed, while the slower degradation kinetic compounds remain practically inalterable. A volatile fatty acids analysis of the effluent of these reactors revealed an imperceptible content in propionic, butyric, valeric, caproic and heptanoic acids, in spite of their concentration in the feed, in some cases, being higher than 2000 $mg \cdot dm^{-3}$. This fact seems to demonstrate that acidogenesis and acetogenesis are not the limiting steps of the global rate of the anaerobic digestion process. A high acetic acid reduction was also observed, in some cases near 100%, so, although the methanogenesis step is slower than the two previously mentioned, it is not the limiting step. Most of the organic matter in the effluent are carbohydrates of high molecular weight (cellulose compounds and lignin) and fats that need a high residence time for carrying out its decomposition successfully [14]. Thus, the hydrolysis is the step that limits the global rate of the pig manure anaerobic digestion process.

Throughout the experimentation, the phosphorus retention was higher in the reactor using activated coal as support than in the one using biolite. In the first case, phosphorus reduction percentage in the effluent was higher than 75% while in the second case, it did not exceed 50%. Also, a considerable reduction of the iron and copper in the treated pig manure was detected, although in this case the percentage of removal, 90% for iron and 75% for copper, is similar for both reactors.

Kinetic assays

With the purpose of determining the reactors kinetic behaviour, a series of experiments were conducted with organic matter concentration and pH controlled at regular intervals. In agreement with Padmasiri *et al.* [12], these degradation assays are an appropriate way to determine the degradation rate and the higher removal yield for any compound. The Rc and Rb kinetic behavior studies were made by the injection, in both reactors, of a manure pulse, that supposed a total organic carbon (TOC) concentration of 2500 $mg \cdot dm^{-3}$, and carrying out a detailed analytical monitoring of its degradation process. As result of the analysis it is observed that the TOC concentration decreased in both digesters, in a fast and linear way during the first operation period, until arriving at a point in which a final organic carbon concentration of 500 $mg \cdot dm^{-3}$ that stays constant along the time, is reached. These results are showed in Figure 7a.

Admitting that the systems respond to a zero-order kinetic equation, $c = c_0 - k_0 t$, characterized by situations with high substrate concentration [10], an adjustment of the points in the linear zone provides the degradation kinetic constants for both systems. These constants were the same for Rb and Rc, 20 mg $TOC \cdot dm^{-3} \cdot h^{-1}$. The type of carrier used does not seem to affect, in this case, the degradation kinetic. A gradual increase of the pH

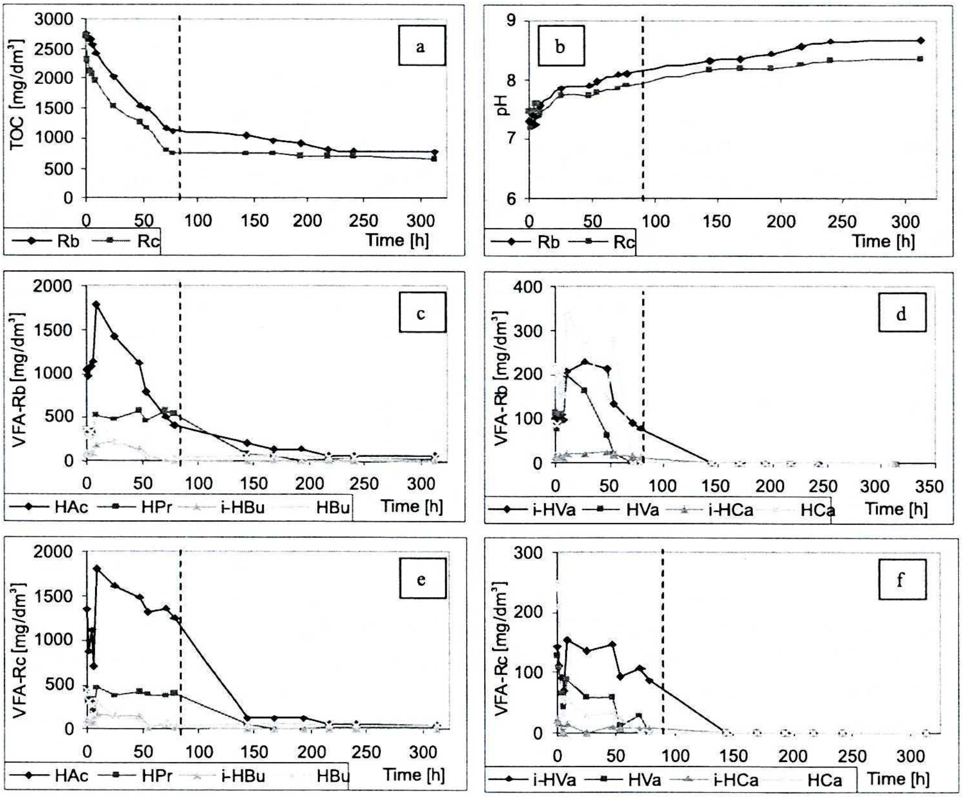

Fig. 7. Evolution of the degradation of a manure pulse:
7a – evolution of Total Organic Carbon (TOC) in Rc and Rb; 7b – evolution of pH in Rc and Rb; 7c and 7d – evolution of Volatile Fatty Acid (VFA) concentration in Rb; 7e and 7f – evolution of Volatile Fatty Acid concentration in Rc (HAc – acetic; HPr – propionic; i-HBu – iso-butyric; HBu – butyric; i-HVa – iso-valeric; HVa – valeric; i-HCa – iso-caproic; HCa – caproic)

in the process is also observed, because of the progressive consumption of the fatty acids present in the manure. This can be observed in Figure 7b.

Figures 7c, 7d, 7e and 7f present the volatile fatty acid consumption that takes place in each reactor. All the figures show an initial period in which the acids concentration increases, corresponding to the time when the system homogenization is carried out. The acetic acid (HAc) appears in greater concentrations inside the digesters (1750 mg·dm^{-3} at the beginning of the assay), followed by propionic (HPr), butyric (HBu) and iso-butyric (i-HBu) acids. The acids of longer chain such as valeric (HVa), iso-valeric (i-HVa), caproic (HCa) and iso-caproic (i-HCa) acids were present in lower concentrations (less than 200 mg·dm^{-3}). However, all of them were digested completely. Coinciding with the volatile fatty acids exhaustion in the reactors, the total organic carbon consumption suffered a slope change, beginning with a horizontal section due to the total paralyzation, at this point, of the digestion process.

Enzymatic assays

The hydrolysis step is considered the rate limiting step in the case of anaerobic manure digestion. This stage involves the depolimerization by hydrolytic enzyme action (cellulases, amylases, proteases) secreted by the fermentative bacteria, of high molecular weight compounds. The proteins are degraded to amino acids via polypeptides, the carbohydrates are transformed into soluble sugars (mono and disacharides) and lipids are turned into long chain acids and glycerin.

A series of assays were carried out with the aim of studying the influence that certain types of enzymes have on manure degradation and the effect that they exert on the activity of the bacterial population present in the fluidized bed reactors.

The assays were carried out on three 500 cm^3 digesters, placed on an agitator simulating discontinuous reactors. The small digesters were filled with 250 cm^3 of different manure samples (that supposes an initial TOC concentration in the digesters of 12000 $mg \cdot dm^{-3}$) collected from animals at a different growth-phase: fatten pigs, piglets and farrowing sows. The first digester (M-E) received a commercialized enzymatic compound (*Pankreoflat®*) with amylases (6000 Unit FIP/g), lypases (6000 Unit FIP/g) and proteases (400 Unit FIP/g); the second digester (M-Dig) received an enzymatic compound, specific for the degradation of manure, called *Epizymp®*. The third digester (M) received no enzymatic compound.

Each digester received also 50 cm^3 of bioparticles proceeding from the Rb reactor, which supposed an addition of 0.8 g of anaerobic biomass. During the experiments the concentration of acetic and propionic acids and organic matter content (TOC) were followed as a measure of the evolution of manure degradation in the presence of hydrolitic enzymes. Figure 8 shows the results obtained from piglet manure. Before the addition of the anaerobic microorganisms (t_i), the amount of organic carbon in the digesters remains constant and the addition of the microorganisms (t_i), which results in a drop in, very quickly at the beginning (and with a similar rate in the test with and without enzyme presence) and more slowly at the end. In the case of the test without enzymatic contribution (M), the reduction of organic carbon becomes practically paralyzed from day seventeen, with an organic remain of 5000 mg $TOC \cdot dm^{-3}$, showing that the microorganisms present in the digester are not able to degrade the manure. This fact does not happen in the test carried out with extra hydrolytic enzyme presence (M-E and M-Dig). In these cases, the degradation of the organic matter lasts throughout the experimental period (60 days).

On the other hand, the presence of an excess of hydrolytic enzymes reduces, slightly, the activity of acidogenic, acetogenic and methanogenic bacteria. The bacteria responsible for the propionic acid degradation were the most affected. This can be deduced from the final accumulation of this acid in the treated effluent, mainly in the M-Dig assay (1000 mg $HPr \cdot dm^{-3}$ on day 60). Nevertheless, the total percentage of organic matter reduction reached seems to compensate this slight diminution in the degradation rate of these products. The results can be generalized to the different types of manure studied, corresponding to animals at different growth-phases.

The enzymatic tests demonstrated a generalized increase in the fatty acid concentration inside the digesters, in those cases where the biomass has not been added, after the addition of enzymatic compounds. This increase was caused by the acceleration of the hydrolysis reactions that are catalyzed by the enzymes added. The breakage of molecules of long chain (cellulose compounds, lignin, fats, etc) took place with the consequent generation of shorter chain compounds, in this case, the fatty acids.

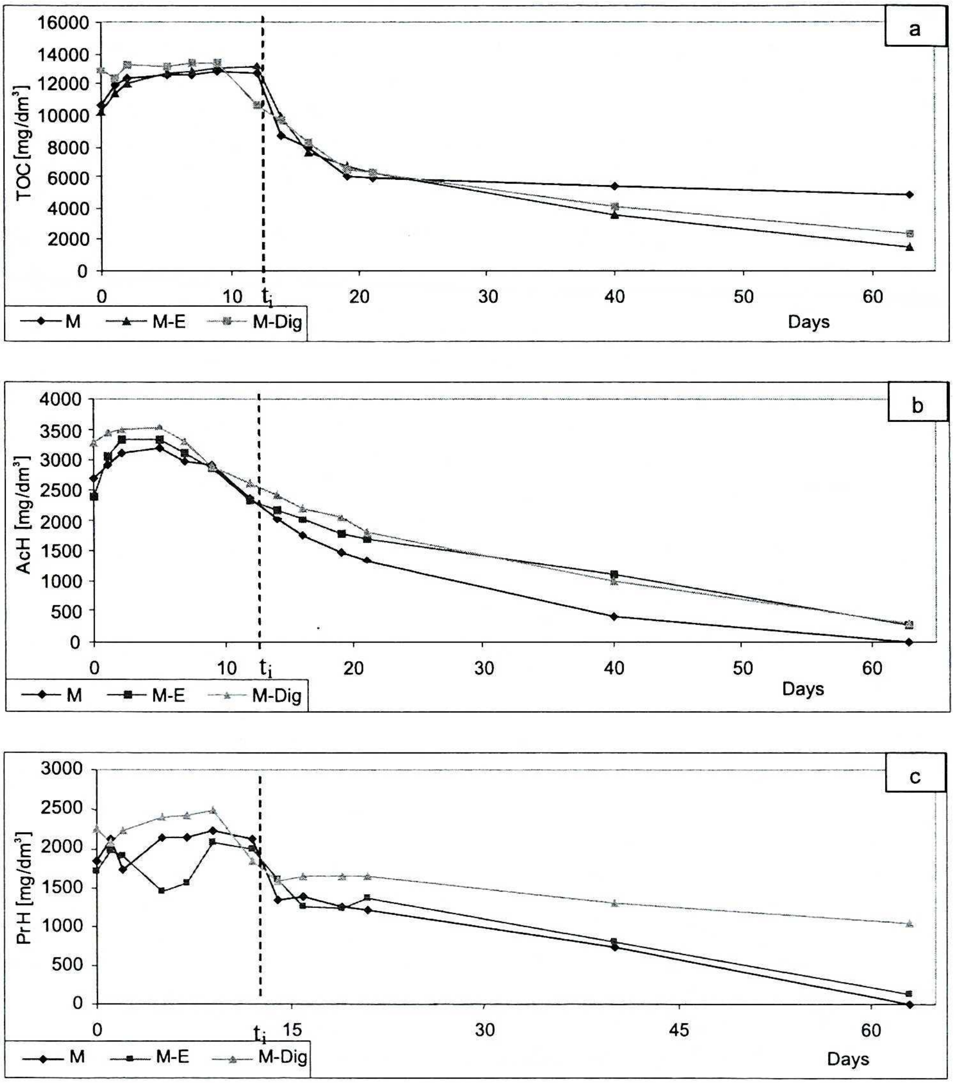

Fig. 8. Evolution of manure degradation in the presence of hydrolytic enzymes: a – TOC reduction; b – Acetic acid degradation; c – Propionic acid degradation

Different fatty acid contents were also observed in the manure based on its origin. The acetic acid appeared in greater concentration in all the samples of manure studied (manure from fatten pigs, piglets and farrowing sows), followed by butyric and propionic acids. The concentrations observed for these three major fatty acids in the three types of manure analyzed (fatten pigs, piglets and farrowing sows) are shown in Table 2.

Table 2. Volatile Fatty Acids in manure

	Fatten room [mg·dm^{-3}]	Piglet room [mg·dm^{-3}]	Farrowing sows [mg·dm^{-3}]
Acetic acid	8000–14000	2500–3000	4500
Propionic acid	2600–4400	1400–1800	1200
Butyric acid	2100–3000	1500–2600	800

Microscopic characterization

Scanning electron microscopy (SEM) was used to obtain detailed images of the support surface and the biofilm. In Figure 9, microphotos A and D show, respectively, the aspect of the biolite and the granular coal without biomass. In both photographs the great superficial porosity of these supports is visible, characteristic that confers them a high specific surface. Microphotos B and E show a general view of the colonized carrier surfaces from Rb and Rc, on day 180. At that time, the biomass concentration in Rb was 14 g AttVS·dm^{-3} (Attached Volatile Solids) and for Rc, it was slightly higher. Both supports produced a high colonization degree. Microphoto C(Rb) shows an image of the diverse morphotypes found in the colonized support surface. This photo reveals the presence of filamentous methanogenic forms, closely resembling *Methanosaeta* (*Methanotrix*) and *Methanosarcina*, microorganisms with rod-like morphologies and small coccoid bacteria mainly. Microphoto F(Rc) with higher resolution than B and E, shows the colonized surface where an accumulation of microorganisms can be observed inside the crevices and other regions sheltered from hydraulic shear forces.

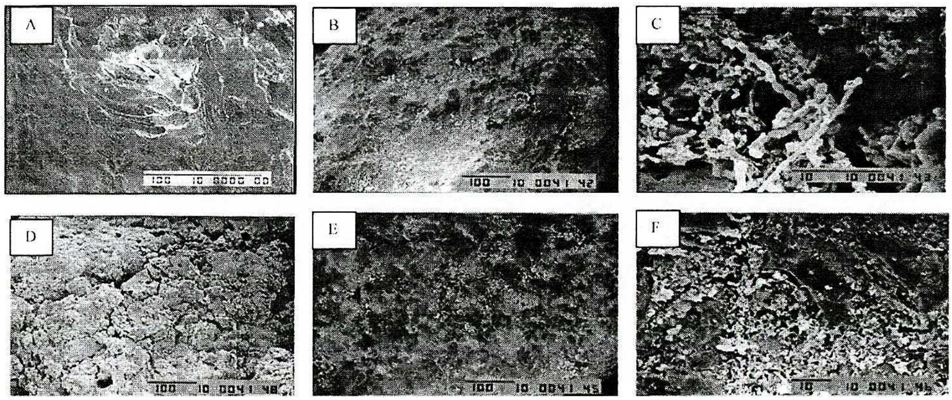

Fig. 9. SEM microphotographs showing:
1. Biolite samples: A – (X500) bar 20 μm, porous surface of clean biolite; B – (X200) bar 50 μm, general view of the completely colonized support; C – (X3500) bar 3 μm, bacterial population with presence of *Methanosaeta sp.*;
2. Granular coal samples: D – (X200) bar 50 μm, clean coal; E – (X200) bar 50 μm, general view of the colonized support; F – (X1500) bar 7 μm, accumulation of microorganisms inside crevices and other regions sheltered from hydraulic shear forces

CONCLUSIONS

From the research presented in this paper the following conclusions can be drown:

The FBR process is suitable for treatment of the liquid fraction of pig manure under moderate psycrophilic conditions which has been demonstrated at lab-scale. The maximum organic loading rate applied was, approximately, 10 kg TOC·m^{-3}·d^{-1} operating at the temperature of 20°C. The total organic carbon reduction under these conditions was approximately 45%. The performance of the anaerobic process without heating systems provides the opportunity to decrease or eliminate expenses in the treatment of organic wastes.

Admitting that the pig manure degradation responds to a zero-order kinetic equation, an adjustment of the points in the linear zone provide the degradation kinetic constant, that results to be the same irrespective of the carrier used. The assay reveals a zero-order kinetic constant of 20 mg TOC·m^{-3}·d^{-1}, approximately.

The hydrolysis stage was, clearly, the limiting step of the global rate of the manure anaerobic digestion process. The hydrolytic enzyme addition to the manure allows to improve the operational yields, favoring the degradation of high molecular weight compounds. Future work will be focused on this direction.

The study carried out with biolite and granular coal as supports, has revealed that these materials can be used successfully in the biological degradation of the organic matter proceeding from the pig manure liquid fraction. SEM microscopy studies show a high degree of biolite and granular coal colonization by the biomass. The great surface roughness, a characteristic that confers them a high specific surface, the geometric uniformity and the appropriated density for the fluidization of both materials turn them into suitable supports to be used in anaerobic fluidized bed reactors. Nevertheless, the higher mechanical resistance of the biolite seems to be more appropriate for this kind of process.

The growth of the biofilm in support particles takes place, mainly, inside the crevices and other regions sheltered from hydraulic shear forces.

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