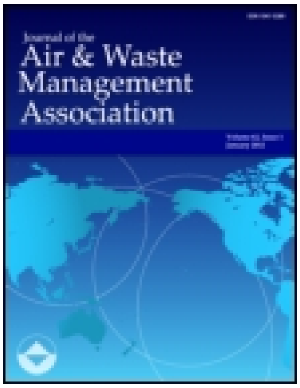
This article was downloaded by: [UOV University of Oviedo]
On: 23 September 2013, At: 05:26
Publisher: Taylor & Francis
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK




Journal of the Air & Waste Management Association
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/uawm20

# Anaerobic Mesophilic Treatment of Cattle Manure in an Upflow Anaerobic Sludge Blanket Reactor with Prior Pasteurization

Elena Marañón [a] , Leonor Castrillón [a] , Juan José Fernández [a] , Yolanda Fernández [a] , Ana Isabel Peláez [b] & Jesús Sánchez [b]

[a] Department of Chemical and Environmental Technology and Institute of Industrial Technology of Asturias, Higher Polytechnic School of Engineering, University of Oviedo, Gijón, Spain

[b] Department of Functional Biology and Biotechnology Institute of Asturias, Faculty of Medicine, Oviedo, Spain
Published online: 28 Feb 2012.

To cite this article: Elena Marañón , Leonor Castrillón , Juan José Fernández , Yolanda Fernández , Ana Isabel Peláez & Jesús Sánchez (2006) Anaerobic Mesophilic Treatment of Cattle Manure in an Upflow Anaerobic Sludge Blanket Reactor with Prior Pasteurization, Journal of the Air & Waste Management Association, 56:2, 137-143, DOI: 10.1080/10473289.2006.10464448

To link to this article: http://dx.doi.org/10.1080/10473289.2006.10464448

PLEASE SCROLL DOWN FOR ARTICLE

Taylor & Francis makes every effort to ensure the accuracy of all the information (the "Content") contained in the publications on our platform. However, Taylor & Francis, our agents, and our licensors make no representations or warranties whatsoever as to the accuracy, completeness, or suitability for any purpose of the Content. Any opinions and views expressed in this publication are the opinions and views of the authors, and are not the views of or endorsed by Taylor & Francis. The accuracy of the Content should not be relied upon and should be independently verified with primary sources of information. Taylor and Francis shall not be liable for any losses, actions, claims, proceedings, demands, costs, expenses, damages, and other liabilities whatsoever or howsoever caused arising directly or indirectly in connection with, in relation to or arising out of the use of the Content.

This article may be used for research, teaching, and private study purposes. Any substantial or systematic reproduction, redistribution, reselling, loan, sub-licensing, systematic supply, or distribution in any form to anyone is expressly forbidden. Terms & Conditions of access and use can be found at http://www.tandfonline.com/page/terms-and-conditions

**TECHNICAL PAPER**

ISSN 1047-3289 *J. Air & Waste Manage. Assoc.* **56**:137–143
Copyright 2006 Air & Waste Management Association

# Anaerobic Mesophilic Treatment of Cattle Manure in an Upflow Anaerobic Sludge Blanket Reactor with Prior Pasteurization

**Elena Marañón, Leonor Castrillón, Juan José Fernández, and Yolanda Fernández**
*Department of Chemical and Environmental Technology and Institute of Industrial Technology of Asturias, Higher Polytechnic School of Engineering, University of Oviedo, Gijón, Spain*

**Ana Isabel Peláez and Jesús Sánchez**
*Department of Functional Biology and Biotechnology Institute of Asturias, Faculty of Medicine, Oviedo, Spain*

Downloaded by [UOV University of Oviedo] at 05:26 23 September 2013

## ABSTRACT
Different autonomous communities located in northern Spain have large populations of dairy cattle. In the case of Asturias, the greatest concentration of dairy farms is found in the areas near the coast, where the elimination of cattle manure by means of its use as a fertilizer may lead to environmental problems. The aim of the present research work was to study the anaerobic treatment of the liquid fraction of cattle manure at mesophilic temperature using an upflow anaerobic sludge blanket (UASB) reactor combined with a settler after a pasteurization process at 70 °C for 2 hr. The manure used in this study came from two different farms, with 40 and 200 cows, respectively. The manure from the smaller farm was pretreated in the laboratory by filtration through a 1-mm mesh, and the manure from the other farm was pretreated on the farm by filtration through a separator screw press (0.5-mm mesh). The pasteurization process removed the pathogenic microorganisms lacking spores, such as *Enterococcus*, *Yersinia*, *Pseudomonas*, and coliforms, but bacterial spores are only reduced by this treatment, not removed. The combination of a UASB reactor and a settler proved to be effective for the treatment of cattle manure. In spite of the variation in the organic loading rate and total solids in the influent during the experiment, the chemical oxygen demand (COD) of the effluent from the settler remained relatively constant, obtaining reductions in the COD of ~85%.

### IMPLICATIONS
The industrialization of cattle farming had led to surplus manure that cannot be used as fertilizer in certain areas with an insufficient farming surface for it to be applied to the land. Furthermore, new bedding methods have given rise to manure with a higher water content, thus increasing management problems. In this paper, a possible treatment for this waste (solid-liquid separation, thermal treatment, and anaerobic digestion) is studied. The results will enable the design of a treatment plant for the cattle manure surpluses generated in different regions in northern Spain, where there are large populations of dairy cattle.

## INTRODUCTION
Cattle manure is a waste that is rich in organic matter, particulate matter, nitrogen (fundamentally ammonium), potassium, and calcium. It also contains variable amounts of other components, such as heavy metals,[1] among which the principal ones are generally iron (Fe), manganese (Mn), zinc (Zn), and copper (Cu).[2]

Cattle manure is likewise known to contain pathogens. Because of the fact that the digested residue can be recycled, its use as a fertilizer must be proven to be hygienically safe for both people and animals. Manure may contain pathogenic bacteria of different species such as *Salmonella*, *Listeria*, *Escherichia coli*, *Campylobacter*, *Mycobacteria*, *Clostridia,* and *Yersinia*. Several of these bacteria are persistent, and many of them may cause infections in both animals and people.

*Salmonella* is one of the most likely pathogens to be spread in the environment by animal slurry. *Salmonella* may survive in slurry for 77 days and grow at temperatures ranging from 6 to 47 °C. *Listeria monocytogenes* is primarily considered a foodborne pathogen and may survive and even grow from 1 to 45 °C. Regarding *E. coli*, cattle are the main reservoir of these bacteria, which are able to multiply in bovine feces at 22 and 37 °C. *Mycobacterium paratuberculosis* is found worldwide; the bacteria are excreted in the feces of infected animals and mainly spread to other animals by contaminated water. *M. paratuberculosis* is highly resistant to various environmental conditions.[3] *Campylobacter*, one of the major bacterial causes of gastroenteritis in people, is fairly sensitive to anaerobic digestion. Because of its spore-forming capacity, *Clostridium spp*., like other spore-forming bacteria, is very resistant. Spores can survive for many years in the environment. *Yersinia enterocolitica* causes acute enteritis, mainly in children. It may grow at temperatures approaching 0 °C.

Animal waste, and particularly manure, has been used for many centuries to maintain soil fertility. However, intensive animal production in recent years has resulted in high concentrations of animals in small areas, producing large amounts of waste with insufficient nearby land for its application.

Different autonomous communities located in the north of Spain have large populations of dairy cattle. In the case of Asturias, the greatest concentration of dairy farms (in this case, the cows are usually kept in stables) is found in the areas near the coast, where the elimination of cattle manure by means of its use as a fertilizer may lead to environmental problems.

As mentioned above, cattle manure contains high levels of particulate matter. A possible solution for the treatment of manure is a preliminary solid-liquid mechanical separation.[4] Various farms use mechanical separator screens for this purpose. This type of separation removes solids to a large extent, as well as some of the biodegradable matter.

Anaerobic treatment is an alternative way of treating animal waste. The biogas produced represents an energy source that can contribute to the self-sufficiency of the farm, and the digested waste, "digestat," can be applied as a fertilizer. In Europe, an increasing number of biogas plants use manure as an energy source.[5,6] Most conventional digesters used for animal waste treatment are continuously stirred-tank reactors.[7,8] In Denmark, some of the industrial biogas plants have a separate pasteurization step where the substrate is heated to 70 °C for 30 or 60 min.[6] In Swedish biogas plants, undigested substrate is heated to 70 °C for 60 min in a separate batchwise step before anaerobic digestion. In Germany and Austria, presanitation treatment at 70 °C for 20 min before mesophilic or for 30 min before thermophilic anaerobic digestion is recommended.[3]

Among the high-rate reactors, the upflow anaerobic sludge blanket (UASB) reactor is the one most widely used.[9,10] The anaerobic codigestion of a mixture of oil mill effluent with swine manure[11] and the anaerobic treatment of the liquid fraction of hen manure[12] using UASB reactors were studied, obtaining good results. In the case of cattle manure, the authors of the present paper had previously studied its anaerobic treatment in the mesophilic and thermophilic range using UASB-type reactors at laboratory scale with good results, obtaining high organic matter removal rates.[2,13]

The aim of the present research work was to study anaerobic treatment of the liquid fraction of cattle manure (which was previously pasteurized at 70 °C for 2 hr) at a mesophilic range using a UASB reactor combined with a settler tank. The manure used in this study was produced on two farms, with 40 and 200 cows, respectively. A continuous operation was planned to obtain steady-state operation data.

## EXPERIMENTAL METHODS

### Equipment

The reactor used was made of transparent polyvinyl chloride and consisted of two cylindrical sections, the lower one being jacketed and separated from the upper one by a deflecting ring to facilitate phase separation (Figure 1). The upper body had a larger diameter and contained the gas collector, as well as outlets for the effluent, recycling, and other uses. Two side-outlets for samples were arranged along the lower body at two different heights. The volume of the reactor up to the triphasic separator was 9 L. After the reactor, a settler was used with the aim of removing the solid that passed the anaerobic treatment. The settler had a volume of ~5 L and an i.d. of 0.20 m.

### Analytical Methods

The physicochemical parameters analyzed in the liquid cattle manure were pH, total solids (TS), volatile solids (VS), chemical oxygen demand (COD), ammonium nitrogen ($N\text{-}NH_4^+$), phosphate ($PO_4^{3-}$), volatile acidity (VA), total alkalinity (TA), and metals, and in the solid fraction were pH, humidity, organic matter, total nitrogen (N), total phosphorus (P), COD, carbon (C)/N ratio, and metals. Standard methods were used.[14]

The metals were determined by atomic absorption on a PerkinElmer model 3110 spectrophotometer. Pathogens were determined before and after the pasteurization treatment. All of the samples were inoculated in different culture media with the aim of ascertaining the presence/absence of pathogens. Inoculation was carried out as recommended by the manufacturer, Oxoid, who supplied the culture media used. The culture media used are described below.

*Glucose, Asparagine, Yeast Extract Medium.* This is a relatively poor medium that is adequate for the growth of many microorganisms with scant nutritional requirements, such as *Actinomycetes* (*Nocardia* and *Streptomyces*), among others.

*KF Medium.* This is a selective, differential medium containing sodium azide, which permits the detection of *Enterococci*.

*Hektoen Medium.* This is a selective, differential medium for isolating enterobacteria species such as *Shigella* and *Salmonella* and lactose-fermenting coliforms.

*Tergitol Medium.* This is a selective, differential medium for the detection and enumeration of *E. coli*, *Enterobacter*, *Salmonella*, *Shigella*, *Proteus*, and *Pseudomonas*. It is incubated at 37 and 44.5 °C to distinguish between total and fecal coliforms, respectively.

*SPS Medium.* This is a selective medium used to isolate and enumerate *Clostridium perfringens*.

*L-PALCAM Medium.* This is a selective, differential medium for detecting and identifying *L. monocytogenes*.

Yersinia *Base Medium.* This is a selective medium for *Y. enterocolítica*. At the same time, the presence/absence of *Yersinia* was corroborated by biochemical and antibiotic resistance tests performed in the Microbiology Service I, Central University Hospital, Oviedo, Spain.

### Operating Mode

The cattle manure used in this study came from two farms, with 40 (farm A) and 120 (farm B) cows, respectively. The cows are kept in free stall barn stables on these farms. Samples were taken on farm A from the liquid manure cesspit, with attempts always made to collect

Downloaded by [UOV University of Oviedo] at 05:26 23 September 2013

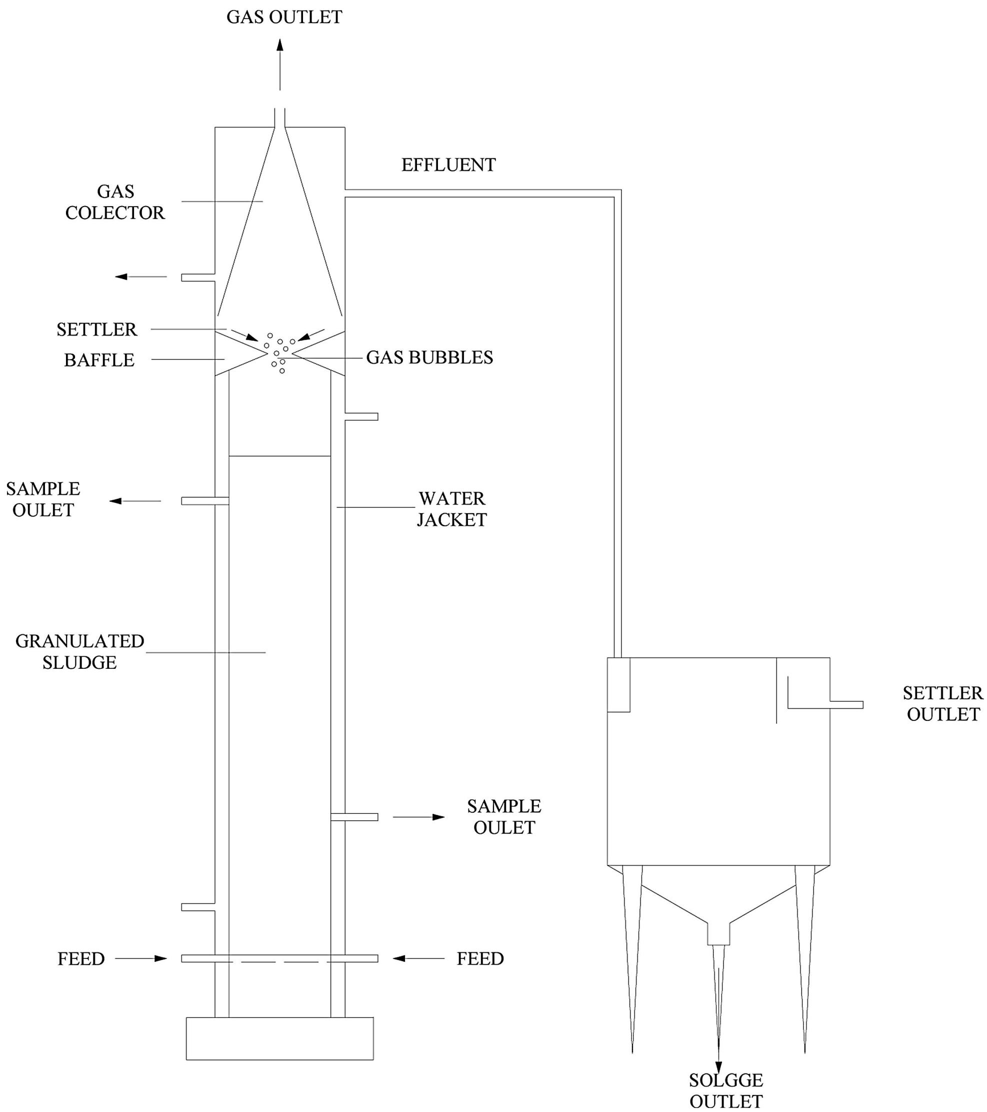



**Figure 1.** UASB reactor and settling tank used in the experiments.

Downloaded by [UOV University of Oviedo] at 05:26 23 September 2013

these after prior agitation of the cesspit. This manure was pretreated in the laboratory by filtration through a 1-mm sieve, with ~60% of the total filtered volume passing through this sieve (liquid fraction A) and the rest being retained by the filter (solid fraction A). Regarding solids, ~80% of the TS present in the raw manure is retained by the filter with the rest passing to the filtered liquid.

Farm B had a fan press screw separator (Fan Separator Company) with a 0.5-mm mesh to separate two fractions, with ~70% of the total filtered volume passing through the screen (liquid fraction B) and the rest being retained (solid fraction B). Liquid fraction A was stored under refrigeration, whereas liquid fraction B was kept at room temperature in a 50-L cylindrical tank, with the aim of studying the evolution of the physicochemical characteristics of the manure over time to simulate the behavior of the manure in the cesspit.

After pretreatment (either in the laboratory or on the farm), the cattle manure was heat-treated at 70 °C for 2 hr with the aim of removing pathogenic microorganisms. Pasteurization can be run either before or after anaerobic digestion and can be run batchwise or continuously.[3] The batch method was chosen because it is more easily controlled in terms of temperature and time. Pasteurization was always the first step during our experiments. The effect of manure pasteurization on the reduction of different types of pathogens and microorganisms was studied. Another reason for carrying out prior pasteurization of manure is the effect on the soluble organic matter; part of the organic matter can be hydrolyzed during heating. Total and soluble COD were analyzed before and after pasteurization in six samples of liquid cattle manure with a high content of organic matter.

The effluent from the pasteurization process was introduced into the UASB reactor at a mesophilic temperature (37 °C). The reactor used for these experiments had been used previously in other experiments with cattle manure at a mesophilic range, where the influence of the hydraulic residence time (HRT) on the treatment was studied,[2] finding that an HRT of 14 days was adequate for the treatment. Thus, the same HRT was used in this work.

During the first part of the experiments, the cattle manure used was from farm A. From day 245 of operation, the manure from farm B was used. Because this manure

**Table 1.** Composition of the liquid fraction of cattle manure.

| Parameter | Farm A | Farm B |
|---|---|---|
| pH | 7.6 | 7.4 |
| Total solids (g/L) | 34.2 | 57.8 |
| Volatile solids (g/L) | 21.7 | 36.7 |
| Total COD (g $O_2$/L) | 37.8 | 60.7 |
| N-$NH_4^+$ (g/L) | 1.3 | 2.2 |
| P total (mg $PO_4^{3-}$/L) | 405 | 810 |
| Metals (mg/L) | | |
| Fe | 82.80 | 123.50 |
| Mn | | 35.00 |
| Zn | 25.20 | 34.75 |
| Cu | 5.31 | 2.92 |
| Ni | 0.21 | 0.26 |
| Pb | 0.49 | 0.37 |
| Cd | 0.10 | $<$0.05 |
| Cr | 0.26 | 0.15 |

**Table 2.** Composition of the solid fraction of cattle manure.

| Parameter | Farm A | Farm B |
|---|---|---|
| pH | 7.93 | 8.8 |
| Humidity (%) | 83.1 | 77.8 |
| Organic matter (% d.m.) | 86.1 | 82.4 |
| Total N (% d.m.) | 2.7 | 1.5 |
| % P total (g $PO_4^{3-}$/100 g d.m.) | 0.7 | 0.5 |
| COT (% dry matter) | 51.6 | 49.4 |
| Relation C/N | 18.9 | 33.6 |
| Zn (mg/kg) | 249.9 | 101.5 |
| Fe (mg/kg) | 80.7 | 685.8 |
| Cu (mg/kg) | 60.7 | 23.1 |
| Ni (mg/kg) | 1.1 | 3.4 |
| Pb (mg/kg) | 1.3 | 4.5 |
| Cd (mg/kg) | 0.9 | 0.5 |
| Cr (mg/kg) | 0.4 | 1.2 |
| Mn (mg/kg) | | 133.5 |

Downloaded by [UOV University of Oviedo] at 05:26 23 September 2013

had a higher COD (60 g $O_2$/L), the effluent from the settler was initially recirculated at a ratio of 1:3, being progressively decreased with the aim of progressively increasing the organic loading rate (OLR) and the solid content.

As reported previously by other authors, operating problems, such as flotation, may arise when operating with high-organic loads.[9] This was the reason for introducing a settler (HRT of ~7 days), namely to retain the solids that were not eliminated in the UASB reactor.

In both cases, the pH of the influent to the UASB was kept at ~7 by means of the addition of hydrochloric acid at a dose of ~1.6 mL of concentrated hydrochloric acid per liter of influent. The previously described parameters were determined in the pasteurized manure used as feed for the reactors, as well as in the effluents from the UASB and settler. TS and VS were measured at two different points inside the reactor, with the aim of characterizing the quantity of biomass. Likewise, metals were determined in the reactor lower sampling outlet. Samples were taken approximately once per week, with the exception of COD, VA, and TA of the effluent of the reactor, which were taken twice per week; pH of the influent, which was determined every day; and metals, which were determined eventually.

## RESULTS AND DISCUSSION

### Characterization of Cattle Manure

The characteristics of the cattle manure depend on different factors related to the management and practices used on the farm. Among the influencing factors, the type of cesspit (open or closed) is one of the most important. Another important factor is the possible mixing of the waste with household sewage or with the rinsewater used to clean the installations or milking equipment, which leads to dilution of the waste. The type of cattle, the animal's diet, the time of year, and the duration and conditions of storage are also influencing factors. Tables 1 and 2 show the composition of the liquid and solid fractions of the cattle manure used in this study. The liquid fraction of cattle manure from farm A presents a TS content of ~34 g/L; farm B presents higher values, ~58 g/L. The percentage of VS versus TS was ~63% in both cases. The COD on farm A varied at ~38 g/L and on farm B, at ~61 g/L. Another parameter that must be taken into account in anaerobic treatment is the ammonium content, because high quantities can be toxic to the microorganisms present.[15] Values of 1.3 and 2.2 g/L were found for farms A and B, respectively. These values, although high, do not suppose inhibition or toxic effects with respect to anaerobic bacteria. Of the metals analyzed (Fe, Mn, Cu, Zn, nickel [Ni], cadmium [Cd], lead [Pb], and chromium [Cr]), the major ones present in both manures were Fe, Mn, Zn, and Cu.

Table 3 shows the evolution, at room temperature, of soluble and total COD of the cattle manure from farm B with time. As can be seen, soluble and total COD decreased with time, the decrease being greater in soluble COD (~24% for total COD and 39% for soluble COD), because soluble organic matter is easily biodegraded.

The solid fractions of cattle manure present moisture contents of between 77 and 83%. The majority of the dry matter is organic matter, with percentages of between 82 and 86%, indicating the high level of biomass, as can be seen in Table 2. The C/N ratio varied between values of ~19 on farm A and 33 on farm B. Cattle manure from farm B contains higher quantities of straw (used in bedding), which means that higher quantities of C are present. This solid waste can be stabilized in a composting plant. However, depending on the C/N ratio, other C-rich

**Table 3.** Variation of total and soluble COD during storage.

| Days of Storage | Total COD (g $O_2$/L) | % Total COD Removed | Soluble COD (g $O_2$/L) | % Soluble COD Removed |
|---|---|---|---|---|
| 1 | 61.2 | | | |
| 8 | 60.3 | 1.5 | | |
| 15 | 59.6 | 2.6 | | |
| 49 | 59.4 | 2.9 | 17.6 | |
| 57 | 53.9 | 3.0 | 16.4 | 7.0 |
| 70 | 51.1 | 12.0 | 13.8 | 21.8 |
| 86 | 49.0 | 20.0 | 12.1 | 31.4 |
| 94 | 46.6 | 23.9 | 10.8 | 38.8 |

**Table 4.** Bacterial colony-forming units in the different media used, before and after the pasteurization process.

| Culture Medium | Type of Microorganism | M1 (CFU/mL) | M2 (CFU/mL) |
|---|---|---|---|
| GAE | *Actinomycetes* and other spore-forming bacteria | $5.7 \times 10^6$ | $30 \times 10^5$ |
| KF | *Enterococci* | $1.7 \times 10^5$ | 0 |
| Hektoen | *E. coli*, *Enterobacter*, *Salmonella*, *Shigella*, *Proteus*, and *Pseudomonas* | $1.2 \times 10^5$ | 0 |
| SPS | *Clostridium perfringens* | Confluent | $3 \times 10^5$ |
| L-PALCAM | *L. monocytogenes* and other bacteria | $10^4$ | $10^3$ |
| *Yersinia* agar | *Yersinia* and other bacteria | $10^2$ | 0 |
| Tergitol 37°C | Total coliforms | $1.5 \times 10^5$ | 0 |
| | *E. coli* | $1.2 \times 10^5$ | |
| Tergitol 44.5°C | Fecal coliforms | $1 \times 10^4$ | 0 |
| | *E. coli* | $8 \times 10^3$ | |

*Notes:* CFU = colony-forming units; M1 = sample before heating; M2 = sample after heating.

materials, such as pruning waste, may have to be added. In this case, the waste from farm B has the adequate C/N ratio[25–35] without the need to add C-rich wastes.

## Pasteurization Process

The effect of moderate heating of manure (70 °C for 2 hr) on the reduction of the following microorganisms was studied: *Actinomycetes* and low-nutritional requirement bacteria, *Enterococcus*, *Enterobacter*, *Salmonella*, *Shigella*, *Proteus*, *Pseudomonas*, *C. perfringens*, *Listeria*, *Yersinia*, total and fecal coliforms, and *E. coli*. Table 4 shows the results of bacterial reduction after the process. From these results, it is possible to conclude that the heating process produces complete elimination of most of the groups analyzed and partial reduction of others. Bacteria growing in glucose, asparagine, yeast extract (GAE), SPS, and L-PALCAM media are less affected by heating, whereas no growth was appreciated in the rest of the selective media used. The values obtained show a wide variation in reduction values (0–40% in GAE, 65–80% in the SPS medium, and 30–80% in the L-PALCAM medium). This result could be attributed to the different storage times of the manure or to the particular niche occupied by the bacteria during the heating process.

In the case of the Hektoen, L-PALCAM, and *Yersinia* media, which allow us to differentiate *Shigella-Salmonella*, *Listeria*, and *Yersinia*, respectively, growth of colonies was observed before heating. However, none of these colonies showed the growth characteristics of any of the former genus, as observed when additional immunological tests (Latex Test FT0203A, Rapid Test FT401M, and Oxoid) were carried out on several colonies from each of the specific *Salmonella* and *Listeria* media. The absence of *Yersinia* was corroborated by biochemical and antibiotic resistance. Therefore, in the absence of these bacteria, we cannot conclude any heating effect. Heating is expected to be less effective on spore-producing bacteria, such as *Clostridium*, and, to a certain degree, *Actinomycetes*. It may be concluded that the heating process is generally efficient on microorganisms lacking spores, such as *Enterococcus*, *Yersinia*, and coliforms. Other bacteria, such as *Pseudomonas*, were also detected in the Tergitol plates before heating (not shown in Table 4) and were also eliminated by this treatment.

**Table 5.** Variation of total and soluble COD (g $O_2$/L) in the pasteurization process of the liquid fraction of cattle manure.

| Sample | Total COD before Pasteurization | Total COD after Pasteurization | Soluble COD before Pasteurization | Soluble COD after Pasteurization |
|---|---|---|---|---|
| 1 | 59.6 | 61.6 | | |
| 2 | 59.4 | 59.8 | 17.6 | 20.2 |
| 3 | 53.9 | 53.2 | 16.4 | 20.9 |
| 4 | 51.1 | 57.9 | 13.8 | 21.4 |
| 5 | 49.0 | 49.0 | 12.1 | 14.2 |
| 6 | 46.6 | 50.2 | 10.8 | 14.6 |

Pasteurization of manure is also expected to have an effect on soluble organic matter. Table 5 shows the influence of the pasteurization process on the total and soluble COD of the six samples of liquid cattle manure. In general, the total COD of the cattle is slightly higher after pasteurization. This may be attributed to the fact that, during heating, partial evaporation can concentrate the organic compounds. On the other hand, the soluble COD increased slightly during the pasteurization step. Thus, the ratio of soluble COD to total COD increases during the pasteurization step, thus tending to improve the anaerobic digestion process.

## Performance of the UASB Digester

One of the problems in the anaerobic digestion of cattle manure in UASB reactors is the high level of solids of this biowaste. TS and VS were determined throughout the experiment in the influent to the reactor, the effluent from the settler, as well as inside the reactor, where samples were taken from two outlets located at different heights. Because of the problems encountered in filtering the manure through a 0.45-μm filter, determination of suspended VS, considered to be indicative of the microbial concentration,[16] was not carried out.

To determine the solids inside the reactor, ~100-mL samples were taken at the lower exit and ~75-mL samples at the upper exit. This operation served at the same time to carry out purging of the sludge, allowing the growth of the biomass throughout the reactor to be controlled. When the concentration of solids inside the reactor was very high, the volume of sludge to be purged at the lower sampling outlet was increased, thus impeding excessive accumulation of solids inside the reactor.

As can be seen in Figure 2, the content of TS in the influent varied throughout the study over a wide range, between values of 26.9 g/L at the beginning of the experiment and 51.2 g/L at the end of the experiment. The content of TS in the effluent of the settler was <22.8 g/L during the entire experiment. The average value for the influent was 35.4 g/L and for the effluent 14.9 g/L, which means a reduction of 57.9%. With respect to the solids content at the two different heights inside the reactor, it can be observed that, as expected, the concentration decreases with height. With regard to total VS as an approximation for the biomass content, the ratio VS/TS was

Downloaded by [UOV University of Oviedo] at 05:26 23 September 2013

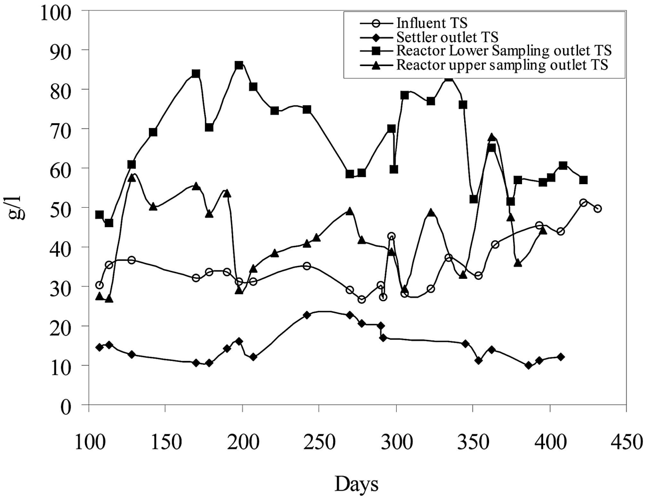



**Figure 2.** TS in the influent, effluent, and inside the UASB reactor.

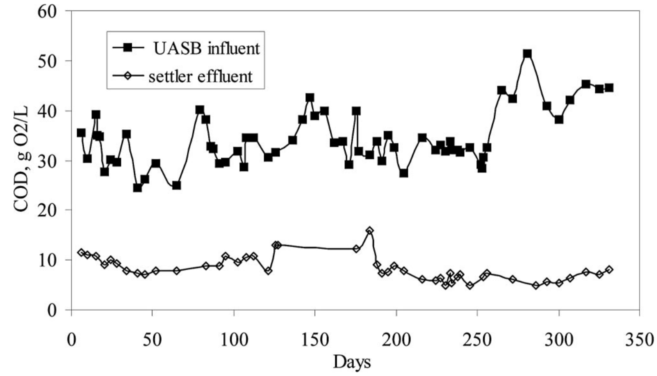



**Figure 3.** Variation in COD during the anaerobic biodegradation and settling process of cattle manure.

Downloaded by [UOV University of Oviedo] at 05:26 23 September 2013

constant in all of the determinations carried out, at ~0.6 at both sampled heights.

In this study, because of the variation of the COD of the influent, the OLR varied widely between values of 1.5 and 3.7 kg COD/m$^3$/day, for a HRT of 14 days. Figure 3 shows the variation of the COD during the anaerobic biodegradation of cattle manure plus settler.

It should be noted that, from day 245 onwards, when the reactor began to be fed with cattle waste from farm B, the performance of the reactor was observed to worsen because of the increase in both solids and COD. However, this did not affect the characteristics of the final effluent from the settler, with COD values of ~6.4 g $O_2$/L (Figure 3). During this period, the average reduction in COD in the reactor and in the settler was 58 and 85%, respectively. If these results are compared with those from previous research,[2] where filtered cattle manure from a different farm was likewise treated in a UASB reactor at mesophilic range, although without using a settler, for similar values of the OLR (3.68 kg COD/m$^3$/day) but smaller values of TS to those found in this work (30 g/L vs. 40 g/L), the reactor was found to perform better (higher removal of COD, average 69%). Therefore, the use of a settler after the reactor overcomes the possible fluctuations in the effluent because of the aforementioned problems, such as high values in the OLR, solids, and COD of the feed, which can lead to sludge flotation in the reactor and high COD values at the exit of the reactor.

Heavy metal ions are accumulated in anaerobic sludge outside the bacterial cells by precipitation and adsorption mechanisms and inside the cells by microbial absorption.[17] Table 6 presents analytical values (in two different samples, sample 1 and sample 2, in which the cattle manure belong to the farm B) of the main metals in the influent and the effluent of the UASB reactor, as well as in the sludge of the reactor (lower sampling outlet) and settler effluent. A decrease in the concentration of metals can be observed in the process because of the deposition of metals inside the reactor. Similar results were obtained in a previous study.[2] Table 7 presents the concentration of minor metals presented in the influent and effluents (sample 1). In general, the metal content in the settler effluent is slightly lower than in the influent to the UASB.

With respect to ammonium, the content in $NH_4^+$-N in the reactor influent varied between 600 and 1700 mg/L and, in the settler effluent, between 550 and 1220 mg/L. Although the ammonium content was high, these amounts did not perturb the smooth running of the anaerobic process. Ammonium removal could be because of the evaporation of $NH_3$ produced in the process, bearing in mind the pH value of ~8 in the effluent from the UASB reactor and from the settler. In previous research,[18] it was found that ammonia volatilization from uncovered slurry ranged from zero at subzero temperatures to 30 g N/m$^2$/day$^{-1}$ during the summer.

## CONCLUSIONS

The composition of cattle manure depends on the kind of management used on the farm. COD of the liquid fraction of cattle manure ranged from values of ~38 to 60 g $O_2$/L. Solids content ranged from values of ~34 to values of 58 g/L.

**Table 6.** Main metals content (mg/L) in the influent and effluent of the anaerobic process, in the anaerobic sludge, and in the settler effluent (farm B).

| | Zn | | Fe | | Mn | | Cu | |
|---|---|---|---|---|---|---|---|---|
| Sample | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 | Sample 1 | Sample 2 |
| Reactor influent | 19.2 | 14.7 | 95.2 | 80.1 | 26.5 | 20.7 | 2.4 | 4.6 |
| Reactor effluent | | 6.0 | | 20.5 | | 7.2 | | 3.1 |
| Settler effluent | 3.7 | 1.7 | 11.3 | 8.1 | 2.2 | 1.5 | 0.4 | 0.7 |
| Reactor sludge | | 115.1 | | 246.6 | | 62.5 | | 15.0 |

**Table 7.** Other metals content (mg/L) in the influent and effluent of the anaerobic process and in the settler effluent (farm B).

| Sample 1 | Ni | Pb | Cd | Cr |
|---|---|---|---|---|
| Reactor influent | 0.24 | 0.36 | 0.05 | 0.13 |
| Reactor effluent | 0.22 | 0.35 | 0.05 | 0.16 |
| Settler effluent | 0.07 | 0.15 | 0.01 | 0.02 |

Soluble and total COD decreased over time during storage, the decrease being greater for soluble COD (~24% for total COD and 39% for soluble COD). This finding implies that part of the organic matter is hydrolyzed during the storage.

Heating to 70 °C for 2 hr removes the microorganisms that do not possess spores, such as *Enterococcus*, *Yersinia*, *E. coli*, total and fecal coliforms, and *Pseudomonas*. Nevertheless, the bacteria that do have spores are only reduced by this treatment. Because *Salmonella* or *Listeria* were not detected in the sample before pasteurization, no conclusion can be drawn as to the effects of heating on these microorganisms. However, given the similar characteristics of said bacteria to those sensitive to heating, it can be assumed that these potential pathogens would also be eliminated, if present.

The pasteurization process slightly increases the total COD of the cattle manure because of partial evaporation of water during the heating. Soluble COD also increases after the thermal treatment because of hydrolyzation, thus increasing the ratio of soluble COD to total COD.

The value of the COD in the reactor effluent was influenced by the high level of solids in the influent. Influents to the reactor with a similar OLR (3.7 kg COD/$m^3$/day) but a higher solids content (40 g/L vs. 30 g/L) produced a decrease in COD removal (58% vs. 69%).

The use of a settler after the UASB reactor overcomes possible fluctuations in the effluent because of high values in the OLR, solids, and COD of the manure, which can lead to sludge flotation in the reactor and high COD values at the exit of the reactor. The average COD removal obtained for a HRT 14 was 85% (average OLR was 3.1 kg COD/$m^3$/day).

Downloaded by [UOV University of Oviedo] at 05:26 23 September 2013

## ACKNOWLEDGMENTS

This work was funded by the Spanish Ministry of Science and Technology, contract no. REN2002–01969 TECNO. The authors thank Dr. Javier Méndez, from the Microbiology Service I, Central University Hospital, Oviedo, Spain, for help in the identification of bacteria from *Listeria* and *Yersinia* media.

## REFERENCES

1. Nicholson, F.A.; Chambers, B.J.; Williams, J.R.; Unwin, R.J. Heavy Metal Contents of Livestock Feeds and Animal Manures in England and Wales; *Biores. Technol.* **1999**, *70*, 23-31.
2. Marañón, E.; Castrillón, L.; Vazquez, I.; Sastre, H. The Influence of Hydraulic Residence Time on the Treatment of Cattle Manure in UASB Reactors. *Waste Manage. Res.* **2001**, *19*, 436-441.
3. Sahlström, L. A Review of Survival of Pathogenic Bacteria in Organic Waste Used in Biogas Plants; *Biores. Technol.* **2003**, *87*, 161-166.
4. Moller, H.B.; Sommer, S.G.; Ahring, B.K. Separation Efficiency and Particle Size Distribution in Relation to Manure Type and Storage Conditions; *Biores. Technol.* **2002**, *85*, 189-196.
5. Ahring, B.K.; Angelidaki, I.; Johansen, K. Anaerobic Treatment of Manure Together with Industrial Waste; *Water Sci. Technol.* **1992**, *25*, 211-218.
6. *Progress Report on the Economy of Centralized Biogas Plants*. Danish Energy Agency: Copenhagen, Denmark, 1995.
7. Keshtkar, A.; Ghaforian, H.; Abolhamd G.; Meyssami, B. Dynamic Simulation of Cyclic Batch Anaerobic Digestion of Cattle Manure; *Biores. Technol.* **2001**, *80*, 9-17.
8. Keshtkar, A.; Meyssami, B.; Abolhamd, G.; Ghaforian, H.; Khalagi Asadi, M. Mathematical Modeling of Non-Ideal Mixing Continuous Flow Reactors for Anaerobic Digestion of Cattle Manure; *Biores. Technol.* **2003,** *87*, 113-124.
9. Lettinga, G.; Hulshoff Pol, W. UASB-Process Design for Various Types of Wastewaters; *Water Sci. Technol. Water Sci. Technol.* **1991**, *24*, 87-107.
10. Fang, H.; Wai-Chung, D. Anaerobic Treatment of Proteinaceus Wastewater under Mesophilic and Thermophilic Conditions; *Water Sci. Technol.* **1999**, *40*, 77-84.
11. Angelidaki, I.; Ahring, B.K.; Deng, H.; Schmidt, J.E. Anaerobic Digestion of Olive Oil Mill Effluents Together with Swine Manure in UASB Reactors; *Water Sci. Technol.* **2002**, *45*, 213-218.
12. Kalyuzhny, S.; Fedorovich, V.; Nozhevnikova, A. Anaerobic Treatment of Liquid Fraction of Hen Manure in UASB Reactors; *Biores. Technol.* **1998**, *65*, 221-225.
13. Castrillón, L.; Vázquez, I.; Marañón, E.; Sastre, H. Anaerobic Thermophilic Treatment of Cattle Manure in UASB Reactors; *Waste Manage. Res.* **2002**, *20*, 350-356.
14. American Public Health Association, American Water Works Association, and Water Pollution Control Facilities. *Standard Methods for the Examination of Water and Wastewater, 20th ed*. American Public Health Association: Washington, DC, 2001.
15. Hansen K.H., Angelidaki, I.; Ahring, B.K. Anaerobic Digestion of Swine Manure: Inhibition by Ammonia; *Water Res.* **1998,** *32*, 5.12.
16. Solera, R.; Romero, L.; Sales, D. Measurement of Microbial Numbers and Biomass Contained in Thermophilic Anaerobic Reactors; *Water Environ. Res.* **2001**, *73*, 684-690.
17. Ginter, M.O.; Grobicki, A. Analysis of Anaerobic Sludge Containing Heavy Metals: A Novel Technique; *Water Res.* **1995**, *29*, 2780-2784.
18. Sommer, S.G. Ammonia Volatilisation from Farm Tanks Containing Anaerobically Digested Animal Slurry; *Atmos. Environ.* **1997**, *31*, 863-868.

### About the Authors

Elena Marañón and Leonor Castrillón are associate professors in Environmental Engineering, Yolanda Fernández is a postdoctoral researcher, and Juan José Fernández is a Ph.D. student in the Department of Chemical and Environmental Technology and Institute of Industrial Technology of Asturias at the University of Oviedo. Jesús Sánchez is an associate professor in microbiology and Ana Isabel Peláez is a postdoctoral researcher in microbiology, both in the Department of Functional Biology and Biotechnology Institute of Asturias at the University of Oviedo. Address correspondence to: Elena Marañón, Dept. of Chemical and Environmental Technology and Institute of Industrial Technology of Asturias, Higher Polytechnic School of Engineering, University of Oviedo, 33203 Gijón, Asturias, Spain; phone: 34-98-5182027; fax: 34-98-5182337; e-mail: emara@correo.uniovi.es.