Bioresource Technology 153 (2014) 284–291

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Prevalence and persistence of potentially pathogenic and antibiotic resistant bacteria during anaerobic digestion treatment of cattle manure

Juliana Alves Resende [a], Vânia Lúcia Silva [a], Tamara Lopes Rocha de Oliveira [a], Samuel de Oliveira Fortunato [a], Jailton da Costa Carneiro [b], Marcelo Henrique Otenio [b], Cláudio Galuppo Diniz [a,*]

[a] Department of Parasitology, Microbiology and Immunology, Institute of Biological Sciences, Federal University of Juiz de Fora, 36036-900 Juiz de Fora, MG, Brazil
[b] Embrapa Dairy Cattle, Research Center, 36038-330 Juiz de Fora, MG, Brazil

HIGHLIGHTS

- Sustainable handling of anaerobic digestion and their effluents are needed.
- Effluents from biodigesters are widely spread into land used for the agriculture.
- Putative pathogenic bacteria persist after anaerobic digestion of cattle manure.
- Antimicrobial-resistant bacteria are prevalent in the effluents from biodigesters.
- Medically important bacteria imposes sanitary risks to the anaerobic digestion.

ARTICLE INFO

Article history:
Received 10 October 2013
Received in revised form 29 November 2013
Accepted 3 December 2013
Available online 11 December 2013

Keywords:
Anaerobic digestion
Biogas
Biofertilizer dairy cattle manure
Antimicrobial resistance
Pathogenic bacteria

ABSTRACT

Anaerobic digestion figures as a sustainable alternative to avoid discharge of cattle manure in the environment, which results in biogas and biofertilizer. Persistence of potentially pathogenic and drug-resistant bacteria during anaerobic digestion of cattle manure was evaluated. Selective cultures were performed for enterobacteria (ENT), non-fermenting Gram-negative rods (NFR) and Gram-positive cocci (GPC). Antimicrobial susceptibility patterns were determined and a decay of all bacterial groups was observed after 60 days. Multidrug-resistant bacteria were detected both the influent and effluent. GPC, the most prevalent group was highly resistant against penicillin and levofloxacin, whereas resistance to ampicillin, ampicillin-sulbactam and chloramphenicol was frequently observed in the ENT and NFR groups. The data point out the need of discussions to better address management of biodigesters and the implementation of sanitary and microbiological safe treatments of animal manures to avoid consequences to human, animal and environmental health.

© 2013 Elsevier Ltd. All rights reserved.

1. Introduction

As long as the need for increased food production leads to the dairy industry growth, environmental concerns related to cattle manure management, which includes residues discharge into soil and aquatic ecosystems favoring the spread of putative pathogenic microorganisms are pointed out (Sahlström, 2003; Venglovsky et al., 2009).

In this regard, prospective studies towards new strategies for safe disposal of large quantities of cattle manure should take into account the sanitary and microbiological risks (Karim et al., 2005). To avoid direct discharge of manure into soil and aquatic ecosystems anaerobic digestion is pointed out as a sustainable alternative resulting in production of biogas and biofertilizer, whilst reducing the microbial load of the surrounding environments (Bagge et al., 2005; Saunders et al., 2012).

However, cattle farming is frequently referred as a reservoir for potentially pathogenic and antimicrobial resistant bacteria or also, reservoir of antibiotic resistance genes (Munir and Xagoraraki, 2011; Thames et al., 2012). To increase production related to prophylaxis, infectious diseases treatment and/or growth promoters, antimicrobial drugs are widely applied in animal husbandry (Heuer et al., 2011). As an ecological consequence, the presence of zoonotic pathogens in the environments and unintentional selection of bacteria that are resistant to antibiotics could have important human and animal health consequences, mainly when

* Corresponding author. Address: Laboratory of Bacterial Physiology and Molecular Genetics, Department of Parasitology, Microbiology and Immunology, Institute of Biological Sciences, Federal University of Juiz de Fora, 36036-900 Juiz de Fora, MG, Brazil. Tel./fax: +55 32 2102 3213.

E-mail address: claudio.diniz@ufjf.edu.br (C.G. Diniz).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.12.007

end up in agricultural lands (Walczak and Xu, 2011; Costa et al., 2013).

As long as anaerobic digestion has been considered an attractive method to promote a clean fuel from renewable feed stocks, such as animal manure, to develop a well-established technology, the optimization of anaerobic digestion processes requires effective operative control and possible correlation with reduction of pathogens (Holm-Nielsen et al., 2009).

In Brazil, as the most part of the country is located in the tropical region, the climatic conditions are mostly in the mesophilic range, and the majority of the anaerobic digesters are operated at ambient temperature followed by land application of the effluent (Kunz et al., 2009). In this regard, recycling of cattle manure at ambient temperature, i.e. production of energy and fertilizer from anaerobic digestion, would be of commercial and environmental interests, and no literature is available considering sanitary and ecological safety. Indeed, it is already reported concerns on the persistency of potentially pathogenic and antimicrobial-resistant bacteria during biogas and biofertilizers production, considering other anaerobic digestion models, but at constant temperatures (Beneragama et al., 2012).

In this regard, this study was focused on the evaluation of persistence of clinically relevant bacteria and their susceptibility patterns to antimicrobial drugs during anaerobic digestion effluents in continuous pilot-scale biodigesters, to accesses the sanitary risks of the process concerning human, animal and environmental health.

2. Methods

2.1. Pilot-scale biogas reactor and sample collection

Four experimental continuous biodigesters operating at ambient temperatures, with a 60 day retention time, and 60 L working volume were used. Fresh dairy cattle manure was collected from the experimental Embrapa dairy cattle field located in Coronel Pacheco city, Minas Gerais state, Brazil. The biodigesters was fed daily with influent of dairy cattle manure mixed with cattle wastewater (final total solids concentration 3–4%).

Temperature was measured by using an ordinary mercury thermometer during the sampling. Total solids (TS), volatile solids (VS), and pH of influent and effluent samples were measured according to standard methods (APHA, 2005). Biogas produced was measured every week by gas chromatography (Agilent Technologies, 7820A). All measurements were done in triplicate and the averages were taken as representative values.

In total, 58 samples from the biodigesters were collected at different times, referred to as the influent ($n = 2$, before feeding the biodigesters), and effluent ($n = 56$, samples were taken every 3–5 days during the digestion). Influent and effluent samples (20 mL) were collected using sterile bottles between January–March (2012) and May–June (2012). All samples were brought to the laboratory and processed within 1 h after collection.

2.2. Microbiological quantitative methods

For bacterial counts, influent (1st day) and effluent samples (15th, 30th and 60th days) were collected and 10-fold serial diluted up to 10^{-8} in sterile saline solution (0.9% NaCl). Aliquots of 0.1 mL of each dilution were submitted to selective culture in different culture medium. The lowest dilution that produced microbial counts between 20 and 200 colonies was used to estimate the number of bacteria in the samples. The Gram positive cocci *Enterococcus* spp. were evaluated on Bile Esculin Agar (Himedia Laboratories, India) and *Staphylococcus* spp. on Hypertonic Manitol Agar (Himedia Laboratories, India) after incubation at 35.5 °C for 24 h. The Gram-negative bacteria were evaluated on Eosin-Methylene Blue Agar (Himedia Laboratories, India) after incubation at 37 °C for 24 h, and lactose fermenting (pink, purple or green metallic) and non-fermenting (colorless) colonies were counted. The experiments were performed in duplicates and results were expressed as mean bacterial counts.

2.3. Isolation and identification of bacterial samples

From the selective cultures for enterococci, staphylococci and Gram negative rods, three to five representative colonies were selected and sub-cultivated in Brain–Heart Infusion Agar (Himedia Laboratories) for stock by freezing and further experiments. For *Streptococcus* spp. isolation, the collected influent and effluent samples 10-fold serial diluted were streaked on sheep blood agar plates (Brain Heart Infusion supplemented with 5% of sheep blood) and incubated in a capnophilic atmosphere (5% CO_2). After incubation (18–48 h, 37 °C), pin point white colonies were selected.

The Gram positive cocci (staphylococci, enterococci and streptococci) were presumptively identified by morphotinctorial characteristics after Gram staining, as well as the ability to hydrolyze esculin, produce catalase and presence of zone of hemolysis. Species identification was performed using the commercial system BBL Crystal Rapid Gram-Positive ID Kit (Becton & Dickinson, USA), according to the manufacturer's instructions.

The Gram-negative bacteria were presumptively identified by morphotinctorial characteristics after Gram staining, as well as the ability of glucose, sucrose and lactose fermentation, oxidase and motility tests. Species identification was performed using API 20E (Bio Mérieux AB, Marcyl L'Etoile, France), according the manufacturer's instructions.

2.4. Antimicrobial susceptibility assays

The minimum inhibitory concentrations (MIC) for antimicrobial drugs were determined by the agar dilution method, according to the Clinical and Laboratory Standard Institute guideline (CLSI, 2012). Antibiotic stock solutions were added to melted Mueller–Hinton (Himedia) agar to obtain final concentrations ranging from 0.06 to 1024 $\mu g\, mL^{-1}$. The antimicrobial drugs were selected on the basis of microbial characteristics and clinical relevance as follows: (i) for Gram positive cocci catalase-positive (GPC/C+), penicillin (MedQuimica, Brazil), oxacillin (MedQuimica), vancomycin (MedQuimica), ampicillin-sulbactam (Cellofarm, Brazil), rifampin (Sigma Aldrich, USA), levofloxacin (Sigma Aldrich), trimethoprim-sulfamethoxazole (MedQuimica) and erythromycin (Sigma Aldrich); (ii) for Gram positive cocci catalase-negative (GPC/C−), penicillin, vancomycin, rifampin, levofloxacin and erythromycin; (iii) for Gram negative rods *Enterobacteriaceae* (ENT), ampicillin (Cellofarm), ampicillin-sulbactam, piperacillin-tazobactam (Novafarma, Brazil), cefepime (Biochimico, Brazil), meropenem (Biochimico), gentamicin (Novafarma, Brazil), amikacin (Teuto-Brasileiro Laboratorio, Brazil), levofloxacin, trimethoprim-sulfamethoxazole and chloramphenicol; and (iv) for non-fermenter Gram negative rods (NFR), piperacillin-tazobactam, cefepime, gentamicin, amikacin, trimethoprim-sulfamethoxazole, meropenem, levofloxacin and chloramphenicol.

The reference strains *Enterococcus faecalis* ATCC 51299, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were included as controls in the antimicrobial susceptibility assays for Gram-positive or Gram-negative bacteria and all tests were performed in duplicate. Using CLSI guidelines, the isolates were classified as sensitive, intermediate, or resistant to the tested antimicrobial agents (CLSI, 2012).

To determine the level of antibiotic resistance of the individual isolated bacteria, the multiple antibiotic resistance (MAR) index

Download English Version:

https://daneshyari.com/en/article/680740

Download Persian Version:

https://daneshyari.com/article/680740

Daneshyari.com