ANAEROBIC DIGESTION IN SANITIZATION OF PIG SLURRY AND BIOMASS IN AGRICULTURAL BIOGAS PLANT

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INTRODUCTION

Nowadays, there is a constant need to increase food production due to still growing human population. It causes new techniques and methods of intensive agriculture and animal husbandry to be developed. This necessary progress causes an important problem, which is a huge amount of waste products, including pig slurry which is produced by pig farms.

Pig slurry is a liquid mixture of pig feces and urine, which is produced by dilution of manure in livestock production process (Zimny, 2003). According to Polish Law of 10 July 2007 about fertilizers and fertilization pig slurry is valuable natural organic fertilizer, but it should be managed in suitable way because it is a potential threat for environment due to high content of nitrogen (1.5 – 9.2 g/dm³) and phosphorus (0.2 – 2.5 g/dm³) compounds. The excessive emission of these substances to the environment can cause a pollution of water and soil (Marszałek et al., 2013). Slurry also contains a large number of microorganisms, mainly from animal feces, but also from external sources. The main slurry bacteria is Enterobacteriaceae family, especially Enterobacter genus, and fecal coliforms. Most of them are opportunistic pathogens for people and animals and can cause infections. In slurry also obligatory pathogens, such as Salmonella typhi, can be found (Iannotti et al., 1982; Podkówka, 2012). For those reasons proper management of pig slurry is very important. Annual production of pig slurry in Poland is estimated at 35-38 millions of m³ (Stankiewicz, 2010). Most of it is poured out on fields as a fertilizer, but 30% of wastes is a supplement for anaerobic digestion in biogas production process. The aim of this study was to determine the role of anaerobic digestion in sanitization, especially in the aspect of fecal bacteria, of biomass from biogas plant, which contains pig slurry produced by industrial pig farm located in the West Pomerania in Poland.

MATERIAL AND METHODS

Biological samples – characteristics, source and preparation

The source of material for the study was an agricultural biogas plant, which uses a pig slurry from nearby pig farm and maize silage to produce biogas. As a fuel for generator and stove, biogas is immediately converted to heat and electricity, which is defined as a high-efficiency cogeneration. Technological process runs in a continuous system with hourly addition of substrates. During one cycle defined as one fermentation, enterococci in the sample were undetectable. Results of this study proved anaerobic fermentation to be an effective way to neutralize microbial threat, that is pig slurry.

Keywords: Anaerobic digestion, pig slurry, sanitization

ABSTRACT

Pig slurry is one of the production manure, which should be managed properly because of environmental threats it can cause. Pig slurry contains a wide range of microorganisms, most of which are opportunistic or obligatory pathogens for people and animals. Spreading it on fields without control can cause microbial contaminations of water and soil. Use of pig slurry as substrate in anaerobic digestion can be an effective way of sanitization. In this work role of methanogenic fermentation in pig slurry sanitization was laboratory examined. Study materials were biological samples: 1 sample of raw slurry and 3 samples of fermented biomass from different stages of fermentation. Total number of coliforms was determined by MPN (most probable number) method, and presence of enterococci was verified in each sample. Study have shown that anaerobic digestion reduced total number of coliforms from initial amount of \(7.0 \times 10^3\) [MPN/ml] in raw slurry to \(3.7 \times 10^3\) [MPN/ml]. Total reduction was 99.47%. Moreover, after first fermentation, enterococci in the sample were undetectable. This study proved anaerobic fermentation to be an effective way to neutralize microbial threat, that is pig slurry.

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positive result – presence of coliforms in examined culture. Lack of at least one trait was considered as negative – absence of coliforms in examined culture. For each sample (I,II,III,IV) three last dilutions in which growth was observed were taken into account. Numbers of positive results was compared with McCrady’s tables for 2 tubes variant, and the MPN was obtained from them.

In order to confirm the results and obtain single colonies of coliforms for further microscopic analysis, cultures were prepared by streaking method, from two tubes of each sample (I, II, III, IV) that were considered as positive for coliforms presence. A slightly selective and differential medium for isolation of coliforms was used – ENDO Agar (BTL Sp. z o.o., Łódź). Cultures were incubated for 24 h in 37°C. After incubation in each plate presence or lack of characteristic for enterococci growth colonies was examined. Then, for confirmation of results, 10 selected characteristic colonies from plates marked as positive were screened on selective and differential for Enterococcus genus medium – Bile Esculin Agar (BTL Sp. z o.o., Łódź). Cultures were incubated for 24 h in 37°C. Afterwards, from selected single colonies identified as Enterococcus genus 2 microscope slides were prepared and stained by Gram method (Wawrzikiewicz et al., 1983). Slides were examined with optical microscope (Olympus, Japan) and photographed.

RESULTS AND DISCUSSION

Most probable number of faecal coliforms

The results of the analysis indicate significant reduction of faecal coliforms number in each sample from subsequent stages of biogas production process. In the first fermentation in FT the most probable number of bacteria was reduced from 7.0 x 10^5 [MPN/ml] in raw pig slurry (I) to 2.4 x 10^6 in fermenting biomass (II). Another fermenting process in PT reduced number of coliforms to 2.4 x 10^5 in fermented biomass (III). In sample collected from lagoon (IV) the most probable number of bacteria was 3.7 x 10^5. The results of each sample are presented in Table 1. The total reduction of coliforms was 99.47% of the initial value from raw slurry. The percent reduction from each stage is shown in Figure 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Serial dilution</th>
<th>MPN/ml</th>
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<tbody>
<tr>
<td>I</td>
<td>10^4</td>
<td>10^5</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>III</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>IV</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>


On plates with ENDO agar medium growth of small, opalescent colonies, which had a colour from pink to dark red was observed. Growth like this is characteristic for lactose positive bacteria from Enterobacteriaceae family. Two selected single colonies were prepared and examined by microscopic analysis (Figure 2).

Figure 3 Microscopic view of faecal enterococci isolated from examined biomass. Arrows (↑) shows characteristic for streptococci, chain-like arrangement of bacteria.

DISCUSSION

Côté et al. (2006) examined indicator microorganisms and opportunistic pathogens reduction efficiency in pig slurry during the anaerobic digestion, and similar results were obtained. Samples were collected from industrial pig farms of Canada, and the fermentation was performed in laboratory scale in a continuous process at 20°C for 20 days without feeding. It was observed that the anaerobic digestion resulted in the reduction of faecal coliforms by 97.94-99.99%. Such a high level of reduction at such a low temperature of the process was achieved probably due to small-scale experiments, and the lack of a constant supply of fresh manure, which always delivers new inoculum of microorganisms. For that reason level of reduction of bacteria in industrial scale installation examined in current work was a bit lower.
Puchajda & Oleszkiewicz (2003, 2004) examined reduction of pathogens in two different installations: one- and two-stage anaerobic digestion. Both processes were carried out in a continuous system, and the cyclic addition of the slurry. It was found that the two-stage process carried out in a system of two fermenters – first in thermophilic conditions and second, mesophilic - was significantly more effective in reducing coliforms than one-stage process. For this reason two-stage installation (both fermenters, FT and PT, in mesophilic conditions) examined in current work obtained so effective reduction of coliforms.

Peu et al. (2006) also obtained results showing reductive effects of anaerobic digestion through the liquid fraction of the pig slurry after separating off the solid effluent was studied. Biomass was stored under anaerobic conditions, and the fermentation occurred spontaneously, without the industrial use of the produced biogas. Molecular methods used in the experiment allowed for a very detailed analysis of the microorganisms population in fermenting slurry. Analysis have shown that the percentage of bacteria number (enterococci and coliforms) significantly decreased during the first 20 days of anaerobic digestion. Moreover, soil that was fertilized with fermented slurry was examined. Number of enterococci and coliforms in there was below the detection limit.

Cao et al. (2013) in laboratory conditions examined influence of anaerobic digestion on number of microorganisms in pig slurry. 180 days of continuous fermentation of pig slurry samples collected from Jingang Academy of Agricultural Sciences pig farm showed significant reduction of the total number of microorganisms in pig slurry used in experiment. The initial viable count of 1.73 x 107 CFU/ml was reduced to 5.75 x 102 CFU/ml after fermentation. In the study the temperature of the process was highlighted as one of the main reducing factors, therefore reduction of Bacillus is such a small level because this genus is able to produce spores, and survive high temperature conditions. Similar results were obtained by Rui et al. (2014). Collected from 13 industrial biogas plants located in different regions of China, biological samples of fermenting biomass were tested. Material was analyzed for different bacteria content and the content of ammonium and phosphate ions. It has been found that the ratio of the content of these ions is the main factor conditioning composition of the microbial community in tested biomass. Authors pointed out that high proportion of ammonium ions to phosphate ions in biomass is largely beneficial for genera such as Clostridium and Bacillus. Probably this is one of the reasons of small reduction of these microorganisms showed in a current work.

Anaerobic digestion shows reducing effect of coliforms not only in pig slurry. Manyli-Loh et al. (2014) investigated the survival rates of bacterial pathogens before, during and after mesophilic anaerobic digestion of a dairy manure from one of a dairy farms in South Africa. Investigation showed that pathogens such as Campylobacter, E. coli and Salmonella inoculated to the digester were reduced from the initial level of 10.1 x 103, 3.6 x 103, 7.4 x 103 CFU/g to concentration below the detection limits, as same as in the current work. Authors obtained similar value of 90-99% reduction of a health importance pathogens. Pandey & Soupir (2011) also examined reduction of Escherichia coli in cattle manure used in production of biogas. Comparing conditions of anaerobic digestion: psychrophilic, mesophilic, and thermophilic fermentation showed that E. coli inactivation during the thermophilic process was 15-17 times more effective than in other thermal conditions, which confirms that the temperature has the biggest impact on sanitization efficiency in examined biogas plant.

CONCLUSION

The use of pig slurry as a feedstock in the production of biogas is an effective method of sanitizing it, due to significant coliforms reduction effect of anaerobic digestion. Moreover, research has indicated that fermentation can reduce number of enterococci in pig slurry to value below the detection limit. Determining the exact value of this reductions would require further quantitative analyzes conducted in this regard. What factors, and to what extent, cause reduction of the total number of microorganisms in pig slurry should also be examined. Production of biogas from pig slurry in agricultural biogas plants has important environmental meaning, because it reduces emission of faecal opportunistic pathogens by intensive livestock production.

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REFERENCES


