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The biogas production potential from silkworm waste

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ABSTRACT

In view of the increasing demand of organic agriculture, utilization of waste and environmental protection, sericulture focuses not only on the cocoon production, but also on other ways that can benefit the farm's economy. It is necessary to find new sources of income for small-scale farmers not only through cocoon selling, but also by the multiple uses of by-products. Insect farming technology provides a cheap source of biomass, which may be a good material in biogas production.

Studies showed that the examined substrates, both silkworm breeding waste and caterpillar excreta, generate a biogas yield comparable to other substrates of agricultural origin, such as cattle, pig and chicken manures. Fermentation of silkworm excreta under mesophilic conditions produces 167.32 m^3/Mg TS of methane and 331.97 m^3/Mg TS of biogas, while fermentation of silkworm breeding waste yields 256.59 m^3/Mg TS of methane and 489.24 m^3/Mg TS of biogas. Moreover, the chemical composition of these raw materials was analyzed.

1. Introduction

For ages humans have utilized silk to produce textiles of great value and beauty. The major domesticated insect, which has been commercially exploited is the mulberry silkworm (*Bombyx mori* L.), accounting for the greatest share in total silk production (89%) (Patil et al., 2009).

The insect breeding is closely linked to the problem of waste, such as excreta and leaf waste. Small-scale farmers may produce 250–300 kg of silkworm waste, which is equivalent to 2500 kg farm manure and may be used to fertilize 0.067 ha farmland (Wenhua, 2001). Silkworm excreta have been successfully used as a good source of farm manure, due to their content of essential nutrients for plants.

Literature source reports that silkworm excrement has pharmaceutical and food commercial uses. In traditional Asian medicine excreta have been used as a therapeutic agent to treat infectious diseases, headache and abdominal pain, as well as lower LDL cholesterol and blood pressure (Tulp and Bohlin, 2004; Vimolmangkang et al., 2014). However, limited data are available on the bioactive compound profile of silkworm feces, although some interesting substances have been reported so far. The groups of lipids were identified, while chlorophylls, fatty acids, sterols, carotenoids, fatty alcohols and sterol glycosides predominated (Uzakova et al., 1987; Vimolmangkang et al., 2014). Due to the fact that silkworms excreta are rich in flavonoids, chlorophyll, alkaloids, carotenoids and lutein compounds, they show high antioxidant activity (Xu et al., 2014). Moreover, the high content of these compounds presents feces as a good source of a natural colorant for the food industry (Vimolmangkang et al., 2014). In industrial production 1 kg of chlorophyll is obtained from 200 kg of silkworm excrement (Wenhua, 2001).

It is obvious that all agricultural waste may be transformed into biogas. The advantages from transforming organic waste matter into biogas are numerous. The biogas production is not only a financial gain, but also the manure is much more homogenous. Defatted pupae of the mulberry silkworm were already tested as a feedstock of the biogas production (Viswanath and Nand, 1994), hitherto both of the presented substrates in this article have not been studied. Insect technology prepares waste mainly as food residue mixed with excreta. It is a high quality substrate to produce biogas through an anaerobic fermentation process, since it ensures a favorable environment for the development and optimal metabolic activity of bacteria involved in the process. This substrate contains only biodegradable organic matter, its C/N ratio is around the optimal 15–35 (Mao et al., 2015); additionally, it does not contain inhibitory compounds, e.g. detergents, antibiotics, antiseptics, which are toxic to bacteria (Dobre et al., 2014). However, obtaining an effective hydraulic retention time (HRT) and biogas production depend not only on the substrate

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composition, but also on the organic loading rate (OLR) and appropriate process temperature. OLR represents the amount of volatile total solids fed into a digester per day under continuous feeding. The process efficiency increases with an increase in OLR; however, an extremely high OLR inhibits bacterial activity (Mao et al., 2015).

The aim of this study is to test the chemical composition of the materials and analyze the biogas yield from substrates generated by mulberry silkworm farming. Moreover, the efficiency of biogas production under mesophilic conditions is compared with commonly used agricultural substrates. The presented study allows investors and farmers to easily estimate the amount of electricity and heat available from the sericulture substrate.

2. Materials and methods

2.1. Mulberry silkworm breeding

The mulberry silkworm (*Bombyx mori* L.) breeding was carried out at the Silkworm Breeding and Mulberry Cultivation Research Laboratory, the Institute of Natural Fibers and Medicinal Plants, Poznan, Poland. The breeding season started in mid-May 2016. To speed up the development of embryos the temperature in the breeding chamber was kept at 27 °C and the humidity at 60–70%. After the caterpillars hatch, the temperature was reduced according to larvae development to 22–23 °C during the 5th stage (Łochyńska, 2016). At the end of May caterpillars began to hatch. The population was kept in 30 plastic boxes, with 50 specimens per box. For 6–7 weeks larvae were fed 5–6 times per day with fresh leaves of white mulberry (*Morus alba* L.). The breeding conditions were matched to the larvae development stage, according to the Polish breeding method.

Two substrates were collected for the presented study: the breeding waste and the excrement of larvae. The waste of silkworm breeding was collected once a day during the active eating stages of the population, from the third to the fifth developmental stage of caterpillars. The breeding waste consisted mainly of mulberry leaf residues, petioles and in the 5th stage of larval development – fine mulberry twigs. The waste also contained a low share of caterpillar excrement. The excrements were collected once a day by screening the breeding waste through a sieve, from the third to the fifth developmental stage of larvae.

2.2. Chemical composition analyses

Analyses of the chemical composition of the raw materials were performed at the Faculty of Wood Technology, the Poznan University of Life Sciences, Poland, according to the PN-92/P-50092 standard for plant material (Waliszewska et al., 2015). The following parameters were determined:

- moisture content using the oven-dry (gravimetric) method,
- pH value of the breeding waste and excreta according to PB.40 ed. 7: 2010.
- content of cellulose according to Seifert using a mixture of acetylacetone and dioxane,
- content of lignin according to Tappi using concentrated sulfuric acid,
- content of holocellulose using sodium chlorite,
- pentosanes using the trihydroxybenzene method,
- contents of minerals were determined according to the DIN 51731 standards,
- N and C contents were determined according to PN-EN 15104:2011 and PN-EN 15289:2011.

Experimental materials were ground in a Pulverisette 15 laboratory mill, with the analytical fraction of 0.4–0.1 mm being separated on sieves.

2.3. Methods of biogas and methane production

The operating cost of the mesophilic process is much lower and the process is less sensitive to environmental changes than thermophilic fermentation (Chasnyk et al., 2015). Therefore, the analyzed substrates were fermented only under mesophilic conditions, which positively affects profitability of the biogas plant.

The analyses of biogas and methane efficiency were carried out at the Laboratory of Ecotechnologies, the Institute of Biosystems Engineering, the Poznan University of Life Sciences, Poland. The physical and chemical analyses were made in 3 replications, according to the Polish standard system: total solids (TS, dried mass) content within the Polish standard PN-75C-04616/01 (drying samples in triplicate for 24 h at 105 °C), and the content of volatile total solids (VTS, organic dried mass) in accordance with the PN-Z-15011-3 standard (combustion of samples at 550 °C for 3 h). The inoculum fermentation was also analyzed as the control. It was prepared by separating the liquid fraction of the digestate pulp from an operating agricultural biogas plant.

The Hydraulic Retention Time (HRT) was tested experimentally, based on the modified German standard DIN 38414/S8 as well as the standardized biogas guidelines issued by the Association of German Engineers in Dresden VDI 4630 (Cieślik et al., 2016; Dach, 2016). Biogas yield was recorded under standard conditions. HRT determines the residence time of the substrate in a biofermenter and it is the time required to complete the degradation of organic matter. It is associated with the microbial growth rate and depends on the process temperature, organic loading rate and substrate composition (Mao et al., 2015). The optimum temperature varies depending on microorganism groups. Mesophilic bacteria are stimulated at 35–37 °C, while thermophilic bacteria require 55–70 °C (Dobre et al., 2014; Mao et al., 2015). In the first stages of fermentation *Bacillus*, *Bifidobacterium*, *Pseudomonas* and *Clostridium* bacteria are the major cooperators (Shin et al., 2004). In the next phase *Syntrophobacter* spp. and *Syntrophomonas* spp. convert ethanol and volatile fatty acids into acetates, as well as hydrogen and carbon dioxide. The last stage (methanogenesis) involves autotrophic and heterotrophic methane bacteria, e.g. *Methanobacterium* spp., *Methanobrevibacter arboriphilus*, *Methanospirillum hungatei*, *Methanosphaera stadtmaniae*, *Methanococcus vannielli* and *Methanosarcina* spp. (Jha et al., 2015).

The experimental biogas production through anaerobic digestion was run in a multichamber biofermenter set. Each individual glass biofermenter was 2 dm^3 capacity. The biofermenters were placed in water at a controlled temperature (approx. 39 °C); hence, the tests were conducted under mesophilic conditions throughout the entire experiment (Dach, 2016; Dach et al., 2014). The fermentation inoculum, which is always added to the analyzed substrates, as well as the absence of oxygen created the perfect conditions inside the fermentation chamber to allow the methane production. Biogas produced in each separate biofermenter was transferred to cylindrical store equalizing gas reservoirs made from Plexiglas. Then inverted cylinders were immersed in water. However, the space between the gas and water was filled with the neutral liquid barrier, which prevented the dissolution of produced CO_2 in the water (Dach, 2016). The daily biogas production was recorded every day accurate to 0.01 dm^3. According to the respective standard the criterion for the completion of the experiment was daily biogas production below 1% of the total production obtained (Cieślik et al., 2016).

2.4. Energy calculations

The usefulness of selected waste from silkworm farming for energy purposes was assessed on the basis of the amount of excrement and breeding waste. In addition, knowing the biogas and