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Research article

The influence of different pretreatment methods on biogas production from *Jatropha curcas* oil cake

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ABSTRACT

Drought and pest resistance, together with high oil content in its seeds, make *Jatropha curcas* a good oil source for biodiesel. Oil cake from *J. curcas* is not suitable for animal feeding and thus may be profitably used for additional energy production by conversion into biogas; however, the anaerobic digestion process must be optimized to obtain good efficiency. We subjected oil cake to thermal and acidic pretreatment to deactivate protease inhibitors and partially hydrolyze phytate. We then digested the samples in batch conditions to determine the effects of pretreatment on biogas production. Thermal pretreatment changed the kinetics of anaerobic digestion and reduced protease inhibitor activity and the concentration of phytate; however, biogas production efficiency was not affected (0.281 m^3 kg^{-1}). To evaluate the possibility of recirculating water for SSF hydrolysis, ammonium nitrogen recovery from effluent was evaluated by its precipitation in the form of struvite (magnesium ammonium phosphate).Concentration of ammonium ions was reduced by 53% (to 980 mg L^{-1}). We propose a water-saving concept based on percolation of *J. curcas* cake using anaerobic digestion effluent and feeding that percolate into a methanogenic bioreactor.

1. Introduction

Growing world energy demand and the incoming depletion of fossil fuels has increased interest in development of renewable energy sources. Renewable fuels may be obtained from biomass by anaerobic digestion and transesterification of vegetable oils. Due to its advantages (such as drought and pest resistance and high oil content in its seeds), *Jatropha curcas* is considered a suitable candidate for biodiesel production in dry climate (Achten et al., 2007). The oil cake remaining after the removal of oil, however, cannot be utilized as a fodder due its toxicity (Martínez-Herrera et al., 2006); production of additional energy from this substrate seems a reasonable solution. Several technologies may be used to achieve this: anaerobic digestion, alcohol fermentation, pyrolysis, and combustion (Liang et al., 2010; Şen and Kar, 2011; Staubmann et al., 1997). The two latter methods offer high energy recovery, but anaerobic digestion enables the recovery of nitrogen from this material in the form of ammonium ions, which may be used as fertilizer (Batstone and Virdis, 2014). Nitrogen recovery is valuable, especially in developing countries with limited access to synthetic fertilizers. However application of this technology in dry climate arises two problems: high water demand and low efficiency in case of *J. curcas* (Chandra et al., 2012; Jabłoński et al., 2015; Staubmann et al., 1997).Plants from the *Jatropha* genus contain many substances regarded as antinutritional and toxic (Martínez-Herrera et al., 2006), including phytate and protease inhibitors that reduce animals' digestion rate of *Jatropha* seeds' biomass (Makkar et al., 1998). The concentration of these two compounds in *J. curcas* seeds is much higher than in oil cakes derived from other oil plants. Since the digestion process in animal intestinal tracts, in many aspects, resembles the anaerobic digestion of organic matter, it is probable that protease inhibitors and phytate may cause reduced biogas production efficiency from *Jatropha* oil cake.

The first goal was the determination of the presence of antinutritional compounds in Jatropha oil cake. The second goal was the selection of pretreatment method efficiently removing or inactivating the detrimental compounds. The next goal was the determination of impact of pretreatment on the anaerobic digestion process.

The efficiency of anaerobic digestion of Jatropha oil cake may be improved by application of percolation technology, based on closed

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water circulation (Kothari et al., 2014). However this solution has potential drawbacks. Degradation of nitrogen-rich substrate result in release of ammonium ions, thus recirculation of process water may result in accumulation of ammonium, what may result in process collapse (Yenigün and Demirel, 2013). Moreover the organic fertilizer obtained with this method would contain low concentration of nitrogen since ammonium ions are not bound with solid fraction of anaerobic sludge. This problems may be resolved by chemical precipitation of ammonium ions. One of possible solutions is the formation of struvite ($(NH_4)Mg[PO_4] \times 6 H_2O$) (Uysal et al., 2010).

Another problem concerning the anaerobic digestion of *J. curcas* in dry regions of the world is the demand for processed water; traditional wet digestion would consume a significant amount of water. The solution to this problem may be application of a combined digestion system, in which water is used in a closed circuit (Kothari et al., 2014); however, in the case of protein-rich oilcake digestion, the resulting accumulation of ammonia will inhibit digestion (Yenigün and Demirel, 2013). Thus, ammonium nitrogen removal is required. One possible solution is the precipitation of ammonium ions in the form of struvit mineral, which may then be recovered and used as a fertilizer (Uysal et al., 2010). Struvit is a mineral salt ($(NH_4)Mg[PO_4] \times 6 H_2O$) formed at about pH 9.

Optimal utilization of *Jatropha* cake should exploit all potential biomass components (chemical energy and minerals) with minimal energy input and water usage. This could be achieved by: optimization of pretreatment methods, increasing the efficiency of biogas production; recovery of minerals used as fertilizer from the effluent and water recirculation within the system; and the use of the remaining biomass and the recovered minerals as fertilizer. The aim of this work was to evaluate different pretreatment methods on the protease inhibitors and phytate and determine the kinetics and efficiency of biogas production from *J.curcas* oil cake. The recovery of ammonium nitrogen from the effluent was evaluated by struvite precipitation. We then proposed the concept of water-saving digestion technology.

2. Materials and methods

2.1. Jatropha curcas oil cake

J. curcas oil cake was obtained from the Chemical Department of the Wrocław University of Technology (WUoT). *J. curcas* seeds were purchased from EKOMOTOR Sp. z o.o. in 2011. The oil from *J. curcas* whole seeds (with shells) was removed by cold pressing in the laboratory press in the Chemical department of WUoT. After oil pressing, the oil cake was ground in a MKM 6000 grinder (BOSH). This material was used for further experiments.

2.2. Oil cake pretreatment

16.7 g samples of oil cake were suspended in 33 mL of 0.1 $mol \cdot L^{-1}$HCl or in 33 mL of 0.1 $mol \cdot L^{-1}$NaCl. Pretreatment incubation conditions are presented in Table 1. After incubation, the samples were cooled to room temperature. NaOH was added to the samples containing HCl to neutralize the solution. The samples were stored at −20 °C until further use.

Table 1
Incubation time at different pretreatment conditions [min.].

Additive	Temperature [°C]			
	20	70	100	115
HCl	60	60	60	15
NaCl	60	60	60	15

2.3. Anaerobic digestion of pretreated samples

Anaerobic biodegradation tests were prepared in 120 mL serum bottles. 50mLof the inoculum and 0.5 g of the substrate sample were placed in each serum bottle (in control bottles, the substrate was omitted). The inoculum material was obtained from a laboratory anaerobic reactor fed with cow manure. In the next step, the air was removed from the bottles by flushing them with nitrogen gas. The digestion test took place at 37 °C. The samples were stirred manually, every 24 h, just before the gas measurements. Produced gas was measured every 24 h across 21 days by water displacement (Kida et al., 2001). The picture of measuring device is attached as supplementary material. All the samples were prepared in quadruplicate. The amount of biogas produced from biomass was calculated as the difference between the production in the sample bottles and the production in the blank bottles (without addition of substrate). Biogas volumes were calculated for standard state (100 kPa, 273.15 K).

2.4. Precipitation of struvite

For the precipitation of struvite, solid particles were removed from the sludge by centrifugation (10,000 RCF for 10 min). Supernatant was transferred to fresh test tubes and stored at 4 °C before further use. For precipitation experiments, 1 $mol \cdot L^{-1}$solutions of $MgCl_2$ and Na_2HPO_4 were prepared. To precipitate struvite, 1 mL of MgCl solution and 1 mL of Na_2HPO_4were added to 10 mL of supernatant and NaOH solution (40% w/w) was added to obtain a pH of9. After the precipitation occurred, the total nitrogen concentration in the solution was determined.

2.5. Oil cake composition analysis

The dry weight, ash, and volatile solids were prepared by standard methods for the examination of water and wastewater (Clesceri et al., 1998). The organic nitrogen was prepared according to the Kieldahl method (Persson, 2008). The total lipid concentration was determined by extraction of dry samples with petroleum ether, 60/80 fraction (from POCH), in a Soxleth apparatus. 0.5 g of sample was dried at 105 °C for 4 h, placed in a Soxleth apparatus, and extracted with 100 mL of petroleum ether for 3 h. The solvent was removed from the extract with a vacuum evaporator. The obtained extract was weighted on analytical scales. Dietary fiber analysis was performed with detergent extraction method (van Soest, 1967).

2.6. Determination of trypsin inhibitor activity

Protease inhibitor activity was evaluated by measuring trypsin activity in a casein assay in the presence of oil cake extracts. To obtain extracts, 5 mL of 0.9% (w/v) NaCl were added to 5 mL of oil cake suspension after pretreatment. The samples were incubated at 20 °C for 30 min with shaking and then centrifuged at 20,000 RCF for 10 min. The obtained supernatant was filtered through a 0.22 μm filter and transferred to a new test tube. Samples were stored at 4 °C for 3 days before further analysis.

The trypsin activity was determined as follows. 50 μL of trypsin solution (80 $\mu g \cdot mL^{-1}$) in 1 $mmol \cdot L^{-1}$HCL and 80 $mmol \cdot L^{-1}$ $CaCl_2$were mixed with 25 μL of 0.1 $mol \cdot L^{-1}$ $CaCl_2$, 50 μL of diluted oil cake extract, and 175 μL of 0.2 $mol \cdot L^{-1}$Tris/HCL buffer (pH = 7.5). The samples were incubated at 37 °C for 5 min, after which 500 μL of 0.5% (w/v) casein solution were added to the samples. The reaction was stopped after 15 min by addition of 500 μL of 10% (w/v)

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