University of Sopron

Theses of doctoral (PhD) dissertation

Optimization of anaerobic digestion processes regarding to the application of microalgae and microelement supplementation

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INTRODUCTION

Increasing energy demand of the world, the risk of depletion of fossil energy sources and their injurious impact on environment led to our coal-based society recognized the potential of renewable energy sources.

Versatile energy sources such as biomass, including biogas production, can play an important role next to solar, wind and hydropower utilization. However, recent studies predict, the presence of nowadays applied substrates will be reduced in the near future and new methods will come to the fore. Such an alternative solution could be the anaerobic digestion of microalgae, one of the main aim of the dissertation was to examine the possibilities of adaptability to current technologies.

The optimum operation of anaerobic digestion significantly influenced by the microelement status, thus further objective was to examine the effect on methane production of microelement supplementation in case of sugar beet pressed pulp.

Beside the flexible use of anaerobic digestion, complex utilization is a forward-looking issue in the sector. Due to the high inorganic content of fermented sludge, it can serve as fertiliser in agricultural and forestry fields. Apart from the positive impact on methane production, the accumulated heavy metals in the sludge could have adverse effects on the soil ecosystem. In order to quantify these effects I have investigated the phytotoxicity of anaerobic sludges via ecotoxicological test for white mustard and spring barley. To determine the accumulation of microelements, the spring barley test

was followed by microelement analysis of sludge, soil and barley biomass samples.

Aims of the dissertation

1. Examination the adaptability of *Chlorella vulgaris* and *Scenedesmus sp.* microalgae into recent anaerobic technologies. Determination of more favourable microalgae species for further codigestions.

2. Analysis of inhibitory effects during the semi-continuous lab scale anaerobic digestion by analytical methods. Following of metabolites by instrumental analytical technologies: HPLC-UV, GC-MS.

3. Investigation of codigestion of microalgae and different agricultural by-products, in order to determine the optimal operational parameters and the maximal organic loading rate.

4. Examination of the effect of different total solid content microalgae and used cooking oil substrate mixture on anaerobic codigestion. Following the disturbance of microbial consortia.

5. Examination the effect of microelement supplementation on methane production in case of sugar beet pressed pulp monodigestion.

6. Ecotoxicological evaluation of different microelement amended anaerobic sludges by two plant test organizations in order to determine the application

possibilities in agricultural and forestry fields furthermore monitoring the accumulation of microelements in sludges and soils.

MATERIALS AND METHODS

Experimental design

1. Monodigestion
- 3% and 10% N-containing media *Chlorella vulgaris* microalgae

- 3% and 10% N-containing media *Scenedesmus sp*. microalgae

2. Codigestion
- *Chlorella vulgaris* + used cooking oil/maize silage/ mill residue

- Effect of total solid content of substrate in codigestion of microalgae and used cooking oil

- Sugar beet pressed pulp/ maize silage (70%) + *Chlorella vulgaris* (20%), used cooking oil (10%)

3. Microelement supplementation
- Effect on methane production of sugar beet pressed pulp monodigestion, three groups due to the supplementation
a. non-supplemented: without supplementation
b. supplemented (2 $\mu L L^{-1} day^{-1}$ microelement supplementation),
c. supplemented+Fe (2 $\mu L L^{-1} day^{-1}$ microelement+ 82$\mu L L^{-1} day^{-1}$ Fe(III)-chloride solution of 40%)

- Ecotoxicology: SBPP, maize silage sludges: root elongation test on white mustard (*Sinapis alba*), biomass production of spring barley (*Hordeum vulgare* L.)

- Microelement analysis: sludges, soil, biomass

Anaerobic digestion

The anaerobic digesters were 1L working volume bottles (2.5 L capacity threaded brown bottles, Merck, Germany). The digesters were incubated in a water bath (Memmert WNB 14 Basic, Memmert GmbH. & Co.) at a constant temperature of 39°C. The anaerobic digester inoculum was active, obtained from a biogas plant (Sugar factory, Kaposvár, Hungary). The reactors were manually mixed three times per day. Produced biogas was collected by Tedlar® gas sampling bags; volume was measured with a Hamilton gas-tight syringe (Sigma Aldrich Co).

Table 1. Main parameters of applied substrates during anaerobic digestion

	TS%	VS%	C%	N%	S%	C/N
Sugar beet pressed pulp	20	91	47.04	3.38	0.30	14
Maize silage	30	29	46.92	2.25	0.23	21
Mill residue	88	81	44.28	2.66	0.25	17
Used cooking oil	100	99	82.57	0.08	0.001	1032

Microalgal biomass of Chlorella vulgaris (MACC-755) derived from the Mosonmagyarovar Algal Culture Collection (MACC) was produced in tubular

photobioreactors by Agro-Bioferment LLC in Hungary. The algal suspension was harvested using an Alfa Laval Clara 80 separator (Alfa Laval, Sweden).

Table 2. Main parameters of applied microalgae

	lipid (%/TS)	**protein (%/TS)**	**total carbohydrate (%/TS)**
Chlorella vulgaris (MACC-452) monodigestion	34.4	15.9	43.5
Scenedesmus sp. (MACC-401) monodigestion	23.7	24.3	24.7
Chlorella vulgaris codigestion	11.2	60.2	13.5

Analytical analysis

Titrated volatile fatty acid content was determined by own method (Rétfalvi et al., 2013).

The volatile fatty acid content of sludges was determine by HPLC-UV (Gynkotek M 480-as pump, TOSOH 6040 UV detector, 20 mL loop, Rheodyne 8125 injector, Aminex HPX-87H column). The microelement analysis was performed by ICP-OES (iCAP 6300 Duo ICP-OES, Thermo Fisher Scientific Inc., Waltham, MA USA), the CNP content was analysed by MAX CNS analyser (Elementar Analysensysteme GmbH, Germany) coupled WLD detector.

Determination of COD was carried out according to the Hungarian standard protocol (MSZ ISO 6060).

Determination of ammonium and total phosphorus were carried out according to the Hungarian standard protocols MSZ ISO 7150-1 and MSZ 488/18-77, respectively.

Ecotoxicological tests

Root elongation test of white mustard (*Sinapis alba*) (acute toxicity test) was applied according to the Slovakian standard of STN 83 8303:1999.

The effect of microelement accumulation on terrestrial flora was tested using the Slovakian standard STN EN 14735:2006-03 (83 8300). The static acute toxicity test was designed for investigation of spring barley (*Hordeum vulgare* L.) biomass in a growth chamber experiment.

Molecular characterization

Total environmental DNA was sequenced using the Ion Torrent PGM platform (Life Technologies, USA). Ion Torrent PGM fragment libraries of 200 nt were generated according to the appropriate protocols. Metagenomic DNA (1 μg) pooled from each sample was used for library preparation. DNA shearing and end-repair was achieved using the Ion Xpress Plus Fragment Library Kit, and DNA was purified using a PureLink PCR Purification Kit.

Statistical evaluation

Basic statistical analysis was applied by Microsoft Excel, analysis of variance was performed by STATISTICA 10 (Version 10, StatSoft, Tulsa, OK, USA), multivariate analysis (PCA) was execute by Microsoft Excel „Chemometrics-Add-In" program.

THESES

Thesis 1.

The nitrogen content of nutrient solution of microalgae cultivation has an impact on specific methane yield of anaerobic digestion of microalgae biomass. The volume and orientation of this effect are specific for microalgae species. In case of *Chlorella vulgaris* (MACC 452) increase of the nitrogen content of media from 3% to 10% resulted the volumetric methane yield surplus with 7.6%, and the specific methane yield increased by 59.8%, although in case of *Scenedesmus* (MACC 401) microalgae (same change in nitrogen content of media) we have found decreased volumetric methane yield with 54.2% and reduced specific methane yield with 9.6%.

Thesis 2.

Based on our semi continuous lab scale anaerobic digestion of sugar beet pressed pulp by addition of lyophilized *Chlorella vulgaris* microalgae and used cooking oil successful cofermentation can be achieved. On the basis of volatile solid (VS%) with *Chlorella vulgaris* ratio of 20% and used cooking oil ratio of 10% the specific methane yield increased by 18.4%, the volumetric methane yield reached 26.2% benefit compared to monofermentation of sugar beet pressed pulp. Regarding to the methane content of produced biogas 10.8% surplus was determined.

Thesis 3.

In cofermentation of lyophilized *Chlorella vulgaris* microalgae and maize silage with the ratio of 50-50% volatile solid 38.9% intensification can be achieved in specific methane yield by application of used cooking oil with the ratio of 70% maize silage, 20% of Chlorella vulgaris and 10% of used cooking oil.

Thesis 4.

By the effect of organic complex form of microelement supplementation the specific methane yield (regarding to volatile solid) was 11.0% higher in case of semi-continuous lab scale monofermentation of sugar beet pressed pulp. Further beneficial effect of supplementation was found; after an overloading of substrate the titrated volatile fatty acid content of anaerobic sludge decreased more rapidly, the sludge could be recovered in shorter time.

Thesis 5.

Microelement supplementation has an impact on ecotoxicological aspects of anaerobic fermented sludges. Based on the standardized root elongation test of *Sinapis alba* (white mustard) the microelement amended sludges from anaerobic digestion of sugar beet pressed pulp and maize silage stimulated the growing, while increase of dilution ratio showed the decrease of stimulation effect. On the biomass production of *Hordeum vulgare* L. (spring barley) by the microelement amended sludge

from maize silage inhibitory effect (23.8 IC%) was found, till microelement and iron supplementation had stimulation with -89.4 IC%.

Publications related to the topic of dissertation

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