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Potential for valorization of dehydrated paper pulp sludge for biogas production: Addition of selected hydrolytic enzymes in semi-continuous anaerobic digestion assays

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ABSTRACT

The effects of five commercially available hydrolytic enzyme additives on methane yields from dehydrated paper pulp sludge (DPPS) were determined in 5L pilot-scale reactors operated in semi-continuous mode for 60 days. Methane production was 40% and 43% higher in reactors receiving Novozymes and Novalin additives, respectively, compared to controls. Effects of time of DPPS inclusion on bacterial and archaeal microbial communities were many times larger than effects of enzyme type as enzyme addition did not produce rearrangements larger than random fluctuations observed in reactors receiving only DPPS. The ratio between volatile organic acids and alkalinity signified progressive decrease in process stability until day 45 irrespective of enzyme supplementation. Complementation with clarified pig slurry (1.5% vol.) for subsequent 15 days effectively stabilized process parameters and was sufficient for microbial communities to maintain DPPS hydrolytic capacity and process additional carbon flow derived from hydrolytic activity of enzyme additives. Consequently, initially unadapted full-scale biogas plant inoculum was capable of significantly increased methane yields from DPPS. Based on annual DPPS availability in EU the potential for additional energy recovery was estimated to be in the range of nearly 1 TJ.

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1. Introduction

Dehydrated paper pulp sludge (DPPS) represents a solid waste material composed of pulp residues and ash generated from the pulping and paper-making process [1,2]. It is a solid residue recovered from the wastewater and consists primarily of complex plant polysaccharide materials such as cellulose, lignin and hemicelluloses [3]. The effluents from the pulp and paper industry cause considerable damage to the receiving waters if discharged untreated and are characterized by high biochemical oxygen demand (BOD), chemical oxygen demand (COD) and contain chlorinated compounds, suspended solids (i.e. fibers), fatty acids, lignin and sulphur compounds. Most of the solids are removed after the mechanical treatment resulting in a DPPS that contains large quantities of fibers [2,4]. In general, most of DPPS (69%) is landfilled or incinerated (21%) and as such represents measurable financial burden to the industry: 50.000 tons of paper mill sludge are being produced and disposed at the average cost of transportation and landfill disposal of 75€ per tonne by paper industry in Slovenia annually [1]. In addition to its costly disposal for the paper mills its energy potential and nutrient content are lost [5].

Another option for at least partial valorization of these resources is inclusion of DPPS into existing agricultural biogas plant anaerobic digestion (AD) located nearby. Increased methane yields can be attained using enzymatic pre-treatments including the reduction of organic matter, however, without significant installation and construction costs related to enzyme utilization [5,6]. Microorganisms that are present in the anaerobic reactor secrete enzymes to help them enhance the degradation of cellulose, hemicellulose, lipids,

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proteins, starch and other complex plant polymers. Hydrolysis is nevertheless the rate limiting step in the anaerobic digestion [7,8] hence enzymatic pretreatments are introduced to increase the extent of organic matter depolymerization and improved methane yields from anaerobic digestion. Economical feasibility of these approaches depends on performance of enzyme types under conditions close to those utilized at full scale and additive price.

So far, the effects of short-term (60 days) additions of various enzyme additives on DPPS methane yields, process parameters and microbial communities of bacteria and archaea in agricultural biogas plant inoculum have not been assessed comparatively. Agricultural biogas plants encounter substrate shortage due to market fluctuations, differences in annual cycles or unanticipated weather events. The short-term addition of DPPS as a sole substrate was tested in this study as an approach to bridge the time gap between the availability of regular agricultural substrates. In addition, enzyme additives and pig slurry complementation were tested as mitigation strategies to increase methane yields from DPPS and stabilize anaerobic digestion of DPPS. In this study (i) five different enzyme additives available on market were used to increase methane yields from DPPS over 60 days; (ii) methane yields were determined in 5 L semi-continuous reactors and a number of targeted process parameters ($n = 16$) were monitored in all variants over the same period; (iii) rearrangements in bacterial and archaeal community structure were monitored in order to delineate the contribution of stochastic changes over time relative to those potentially related to the type of enzymatic supplementation; (iv) based on annual DPPS availability in EU the potential for additional energy recovery was estimated and sustainability of short-term DPPS inclusion into 1 MW agricultural biogas plant assessed.

2. Materials and methods

2.1. Baseline methane yields from paper pulp

Methane yields relevant for DPPS were obtained from Methane yield Database [9]. Average, quartiles, median, 95% confidence intervals were calculated for paper sludge ($n = 38$ data points). Additional entries ($n = 16$) collected from Kinnunen et al. [10] and Meyer & Edwards [11] were submitted in order to complement the data on methane yields from DPPS and are hereafter part of the freely available Methane yield Database [7]. In addition, the experimental methane yields determined in this study were submitted to Methane Yield Database [7] under the accession numbers mdb2215-mdb2261.

Theoretical methane yield of dehydrated paper pulp sludge (DPPS) was calculated using Bushwell's equation [12] assuming that most of the organically degradable part of the paper sludge was composed of cellulose ($C_6H_{10}O_5$) with an ash content of 22% and 30% for DPPS1 and DPPS2, respectively (Table 1).

Table 1
Characteristics of substrates and inoculum used in assays. BDL- below detection limit.

Parameter	Swine slurry	DPPS 1	DPPS 2	Inoculum
TS (%)	0.33 ± 0.01	16.99 ± 0.02	17.64 ± 0.02	8.53 ± 0.18
VS (% TS)	41.03 ± 1.19	78.37 ± 0.27	69.83 ± 0.12	69.17 ± 0.60
VS (% FM)	0.13 ± 0.01	13.31 ± 0.01	12.32 ± 0.09	5.90 ± 0.16
pH	7.24 ± 0	6.60 ± 0.01	6.54 ± 0.01	7.87 ± 0.01
VOA (mg l^{-1})	1403 ± 61	3549 ± 494	3212 ± 359	5534 ± 247
TIC (mg l^{-1})	2232 ± 55	15087 ± 2957	14932 ± 3247	13275 ± 175
VOA TAC^{-1}	0.69 ± 0.38	0.237 ± 0.01	0.215 ± 0.02	0.417 ± 0.01
N_{total} (mg l^{-1})	2500 ± 25	1055 ± 35	905 ± 40	5230 ± 60
NH_4^+-N (mg l^{-1})	300 ± 12	BDL	BDL	1301 ± 39

2.2. Semi-continuous experiments

2.2.1. Inoculum and paper pulp sludge

Inoculum was collected from a mesophilic agricultural biogas plant (ABGP) Šijanec (Ormož, Slovenia; 1 MW) following the established procedure described before [6]. Two distinct batches of DPPS, (batch one - DPPS 1; batch two - DPPS 2) were collected in the period of 1 month from 2000 PE (population equivalents) paper pulp wastewater treatment plant located at the site of the hygienic paper manufacturer Paloma d.d. (Sladki vrh, Slovenia; http://web.paloma.si/en/). DPPS samples were stored at 2 °C and homogenized by mixing before use in experiments. Hygienic papers of Paloma are produced from pure cellulose, waste paper and board. Flotation and flocculation are used to separate paper pulp from wastewater and further dehydrated by rotating drums to form DPPS (130–210 t daily; 40% TS). Different types of paper that are produced within Paloma d.d. rely on distinct technological approaches and hence yield DPPS batches with distinct characteristics and theoretical methane yields. Characteristics of inoculum and all substrates are listed in Tables 1 and 2.

In order to complement the semi-continuous reactors with sources of trace metals and nutrients during the observed phase of process instability (d_{46-60}; $n = 14$ days) clarified pig slurry (PS) [13], i.e. slurry devoid of particulate material by prolonged settling was collected from a nearby ($d < 20$ km) pig farm Vucja vas, Slovenia (capacity = 250 pigs).

2.2.2. Residual degradation of inoculum by five enzyme brands

To assess whether the existing inoculum contained a fraction of organic matter amenable to further enzymatic degradation by provided additives, five brands of commercially available products were tested (Table S1) in the first experiment. Two Automatic Methane Potential Test unit (AMPTS II, Bioprocess Control, Sweden) upgraded to 5 L scale were used in semi-continuous experiments as detailed before [6]. All anaerobic reactors ($n = 18$) received 4000 mL inoculum from BGP Šijanec. Negative controls represented anaerobic reactors ($n = 3$) that received tap water, while all the others received enzymatic amendments: hydrolytic enzymes Novozymes (Denmark) ($n = 3$), hydrolytic enzymes Novalin (NovaBiotec, Germany) ($n = 3$), Micropan Biogas additive (Eurovix, USA) ($n = 3$), additive BFL 4400AN (Biofuture, Ireland) ($n = 3$) and Zeolit M (Ipus, Austria) ($n = 3$). Daily preparations and additions of enzyme additives into anaerobic digesters were conducted essentially as described before [6]. Before the start up, each reactor was flushed with N_2 for 3 min. Mixing (15 min on-off regime) continued throughout the operation of reactors and temperature in reactor water baths was maintained at 38 ± 2 °C.

2.2.3. Testing the efficiency of five enzyme brands

In the second experiment five commercially available additives of different brands were amended to the anaerobic reactors on daily basis: hydrolytic enzymes Novozymes (Denmark) ($n = 3$), hydrolytic enzymes Novalin (NovaBiotec, Germany) ($n = 3$),

Table 2
Chemical analysis of total metal content in both batches of DPPS substrate introduced to 5l semicontinuous reactors in this study (mg kg^{-1} TS^{-1}).

Metals	DPPS1	DPPS2
Cd	0.5	0.7
Cr	10.2	18.4
Cu	59.8	63.4
Hg	0.003	0.040
Ni	3	9.8
Pb	12	12.8

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