

BIOAUGMENTATION AS A METHOD LIMITING THE TOXIC EFFECT OF MATURE LANDFILL LEACHATE CO-DIGESTED WITH SEWAGE SLUDGE

Agnieszka MONTUSIEWICZ^{1*}

¹ Lublin University of Technology, Faculty of Environmental Engineering,
Nadbystrzycka 40B, 20-618 Lublin, Poland;

Abstract

The study was aimed at evaluating the influence of bioaugmentation on the limitation of the toxic impact of mature landfill leachate (MLL) in the co-digestion with sewage sludge (SS). The bioaugmenting mixture (BA) comprised wild-living microorganisms at the concentrations of 0.07 and 0.19 g kg⁻¹VS. The study was conducted in semi-flow mesophilic digesters, using different SS:MLL:BA ratios of 90:5:5 and 87:4.3:8.7% v/v at related HRTs of 18.2 and 17.4 d, respectively. The control runs included the sole SS anaerobic digestion, and co-digestion of SS and MLL in a volumetric ratio of 95:5%. Using a sufficiently high BA dose and an adequate inoculum size, bioaugmentation improved the co-digestion efficiency. The methane yields were higher as compared to a non-bioaugmented co-digestion system, reaching 0.24 m³ kg⁻¹VS_{added} and 0.17 m³ kg⁻¹TS_{added}, and the difference was of statistical significance. The same tendency occurred regarding the VS removal. Bioaugmentation seemed to accelerate the metabolic transformations, which was confirmed by the enhanced values representing the rate constant of biogas production. Thus, a beneficial effect of overcoming the toxic impact of MLL on the efficiency of its co-digestion with SS was revealed using bioaugmentation.

Keywords: Landfill leachate management; Renewable energy; Environmental protection; Bioaugmentation; Co-digestion; Sewage sludge; Biogas yields; Kinetics

Introduction

The landfill leachate treatment still constitutes a great challenge, especially its mature form (MLL) generated in the final stages of waste degradation. This is mainly because of the complex, highly contaminated MLL composition characterized by the presence of stable refractory compounds with high molecular weights (i.e. humic and fulvic substances), low biodegradability (BOD₅/COD ratio < 0.1) and leveled concentration of ammonium nitrogen (even 3000-5000 mg L⁻¹), dissolved salts and toxic contaminants [1, 2]. The unique composition and adverse environmental effect of MLL frequently necessitate its multi-stage treatment, involving the physico-chemical and biological methods combined in different orders and configurations [3-6]. The adequate procedure requires at first consideration of their usefulness in increasing the biodegradability and removal efficiency of both humic substances and ammonium nitrogen, although other issues such as the technological and technical limitations, cost-effectiveness and secondary pollution problems should also be considered. Among the methods investigated, much attention is directed to advanced oxidation processes (AOPs), electrochemical oxidation, membrane separation, coagulation-flocculation, chemical

* Corresponding author: a.montusiewicz@pollub.pl

precipitation, activated carbon adsorption, ammonia stripping and ion exchange, often combined with each other or coupled with anaerobic/aerobic treatment [7-15].

It is generally assumed that unassisted biological treatment aimed at MLL is insufficient. This is due to the nature of the leachate, including its resistance to biodegradation and the inhibitory/toxic effect. Interestingly, some promising research was undertaken to change such an approach. Thus, the fungal treatment by white rot fungi and their extracellular enzymes turned out to be efficient in the degradation of recalcitrant organic matter as well as the reduction of the MLL toxicity [16]. The algae-based methods were also recognized as a novel alternative to the conventional biological treatment of MLL, although an adequate choice of the species which could thrive under unfavorable conditions with high content of humic acid and ammonia nitrogen was required. Moreover, an external supplementation with phosphorus was needed to ensure a desirable biomass growth and nutrient removal [17]. Another approach concerned employing bioaugmentation by isolated domesticated strains of *Bacillus cereus* and *Enterococcus casseliflavus* for the efficient biological treating of MLL [18].

Among the different attempts to find a beneficial, environmentally-friendly and cost-effective solution for the MLL treatment, the co-digestion of sewage sludge (SS) and MLL was also involved [19]. Unfortunately, adverse results proved this method inappropriate because of its extremely low efficiency, much worse in comparison to the sole SS anaerobic digestion or its co-digestion with other leachates (categorized into young or intermediate form). This was most likely attributed to the presence of hardly degradable organic matter and toxic compounds, which negatively influenced the process performance and the biogas/methane production. Taking into consideration the possibility of enhancing the metabolic transformations and limiting the toxic effects via bioaugmentation, an idea of using this technique for improving SS and MLL co-digestion was created. Importantly, the bioaugmented co-digestion was conceived as a method that combines two aspects: the management of hardly-biodegradable leachate posing a risk to the environment, and the efficient generation of energy from waste biomass known to be a source of renewable energy. Such an approach seems to be of great importance because it promotes a concept of natural resources conservation and ensures environmental protection against the adverse effect of MLL.

Bioaugmentation is a specialized procedure that involves introduction of pure, enriched or mixed cultures of microorganisms (allochthonous or indigenous) to increase the activity of biological systems, enhancing their efficiency and the process performance [20]. Bioaugmentation was successfully applied in the field of biogas production, although distinct augments were proposed for increasing the process stability and the biogas/methane yields. Their selection depended mainly on the type and specificity of the substrate used. The beneficial bioaugmentation effects concerned: i/ improving the degradation of specific substances such as lignocellulosic, lipid-rich or keratin-rich matter [21-24], ii/ reducing odorous compounds, the digester start-up phase and the recovery time after toxic events [25-28], and iii/ overcoming the transient toxicity induced by the oxygen presence [29]. Moreover, alleviation of the ammonia toxicity effect and, as a result, enhancement of the process efficiency under stress conditions was achieved [30,31]. According to Herrero and Stuckey [32], degradation of a specific contaminant or mixture may only occur along the key metabolic pathways and due to the synergistic cooperation of the microbial consortium involved. Thus, inoculation of the bioaugmentive microorganisms may facilitate the removal of refractory organic matter when indigenous organisms do not have the ability to degrade them.

The present study evaluates the influence of bioaugmentation on the limitation of the toxic impact of mature landfill leachate (MLL) in the co-digestion with sewage sludge (SS). The volatile solids (VS) removal, biogas yields and kinetics were analyzed for comparison the process efficiency in bioaugmented and non-bioaugmented runs. As an augment, a mixture of wild-living Bacteria and Archaea (BA) from Yellowstone National Park, USA, was used. The choice considers the advantages of this product reported by ArchaeaSolutions, Inc., among

these the possibility of degrading complex organic pollutants such as BTX, polyaromatic hydrocarbons, chlorinated organic compounds (including pesticides), essential oils, metal chelates, azo dyes and others.

Experimental part

Materials

Sewage sludge was sampled at the Puławy municipal wastewater treatment plant (WWTP), (Poland). It was taken once a week and comprised primary and secondary sludge (i.e. waste sludge), both being separately thickened. The mixing of these residues was conducted under the laboratory conditions, retaining their stable volumetric ratio of 60:40 (primary: waste sludge) recommended to achieve the optimal biogas production. The mixed samples (SS) were homogenized, manually screened through a 3-mm screen and stored at 4°C in a laboratory fridge up to one week. The adopted SS volumes were supplied to the digester daily. An hour before feeding, the samples were left at 20°C to warm up. The average SS characteristics are presented in Table 1.

The leachate was obtained from the Rokitno sanitary landfill (Lublin, Poland) with over 20 years of age, and thus considered as mature (MLL). An averaged collected sample of 30L was drawn from a storage reservoir ensuring preparation of an appropriate number of MLL portions for experiments. Upon delivery, the MLL was homogenized, analyzed (in triplicate) and partitioned into the volumes of 100 and 200mL, then frozen and stored at -25°C in a laboratory freezer. Before mixing with other feed components, the MLL samples were thawed daily for 6h in laboratory indoor air (at 20°C). The MLL composition is given in Table 1.

Table 1. Composition of SS, MLL and BA (average value ± standard deviation)

Parameter	Unit	SS	MLL	BA phase 1	BA phase 2
Total chemical oxygen demand (COD)	mg L ⁻¹	32067 ± 4620	5605 ± 113	28 ± 9	29 ± 3
Soluble chemical oxygen demand (SCOD)	mg L ⁻¹	1800 ± 950	4430 ± 52	–	–
Biochemical oxygen demand (BOD ₅)	mg L ⁻¹	–	271 ± 41	–	–
BOD ₅ /COD	-	–	0.05 ± 0.008	–	–
Total solids (TS)	g kg ⁻¹	27.3 ± 4.2	25.4 ± 0.04	0.43 ± 0.04	0.47 ± 0.02
Volatile solids (VS)	g kg ⁻¹	17.6 ± 3.4	14.3 ± 0.03	0.07 ± 0.03	0.19 ± 0.07
Alkalinity	mg L ⁻¹	550 ± 325	15010 ± 320	330 ± 1	330 ± 1
pH	-	6.24 ± 0.29	7.95 ± 0.07	7.20 ± 0.01	7.20 ± 0.01
Volatile fatty acids (VFA)	mg L ⁻¹	595 ± 180	960 ± 225	26 ± 9	21 ± 11
Total nitrogen (TN)	mg L ⁻¹	1608 ± 468	7200 ± 1215	75 ± 2	75 ± 2
Ammonium nitrogen (NH ₄ ⁺ -N)	mg L ⁻¹	64 ± 43	6916 ± 337	0.05 ± 0.01	0.05 ± 0.01
Total phosphorus (TP)	mg L ⁻¹	225 ± 101	71 ± 13.5	0.17 ± 0.01	0.17 ± 0.01
Ortho-phosphate phosphorus (PO ₄ ³⁻ -P)	mg L ⁻¹	77 ± 29	39 ± 2.7	0.08 ± 0.01	0.05 ± 0.01

The bioaugment (BA) constitutes a mixture of wild-living microorganisms (Bacteria and Archaea) from Yellowstone National Park (USA). It was prepared in a liquid form by releasing the microorganisms from a peat powdery substrate (closed in a pouch made from vinyl alcohol) upon its contact with dechlorinated tap water flowing through the BA generator. This was in accordance with the procedure given by ArchaeaSolutions Inc. (Evansville, IN, USA). In order to sustain an appropriate microbial content in the bioaugmenting mixture, the pouch was replaced by a new one after each 30 days, while the flow rate was kept at about 0.5L min⁻¹. The BA liquor was directed from a generator into the storage tanks with a total volume of 320L and

stored there at room temperature, ensuring long-term composition averaging. The average values of BA chemical parameters distinctive for two phases of the experiment are presented in Table 1. The microbial composition of the powdery substrate was revealed in the previous study of our team [33]. When the substrate was cultivated in distilled water at 37°C for 1 day under constant mixing conditions, the observed microbial composition comprised 36% of Archaea (*Methanosaeta* being the dominant genus), as well as approximately 59% of Bacteria (predominantly *Acinetobacter*, *Exiguobacterium*, *Janthinobacterium*, and *Stenotrophomonas* genera).

Methods

Experimental set-up

The experiments were conducted using the laboratory installation which included three completely mixed anaerobic reactors, each with an active volume of 40L. The reactors worked in semi-continuous mode under mesophilic conditions (35°C) and were equipped with a heating jacket, a gas installation (i.e. pipelines, gas sampler, pressure equalization unit, valves and mass flow meter), feed/digest storage vessels and a peristaltic pump. The parallel operating of digesters ensured a comparison of the results of sole SS anaerobic digestion as well as its bioaugmented and non- bioaugmented co-digestion with MLL.

The laboratory reactors were inoculated with a digest sampled from full-scale anaerobic reactors operating at the Puławy WWTP at a hydraulic retention time (HRT) of 25 days. The adaptation of such biomass to the experimental conditions was conducted in start-up phase, which lasted 30 days.

Experimental settings

The study was aimed at evaluating the influence of BA bioaugmentation on the limitation of the inhibitory/toxic effect of MLL in the co-digestion with SS. The experiments consisted of two phases which differed in terms of both the BA composition and its doses (Tables 1 and 2). The SS characteristics varied due to its weekly sampling (and thus the separate controls were scheduled), but the MLL composition was unchanged throughout the investigations. Each phase was scheduled for a period of 90 days (30 days for acclimation and 60 days for measurements) and comprised three runs, including control (SS anaerobic digestion), non-bioaugmented and bioaugmented co-digestion of SS and MLL. Phase 1 (runs R 1.1, R 1.2 and R 1.3) was conducted for evaluating the influence of BA at a minor dose and the VS concentration, while phase 2 (runs R 2.1, R 2.2 and R 2.3) referred to higher BA inoculation. The SS:MLL volumetric ratio was stable and adopted in accordance with the previous author's studies, the SS:MLL:BA volumetric ratio differed affecting the HRT due to an increase in the feedstock volume. The detailed experimental settings are given in Table 2 (including organic loading rate – OLR).

Table 2. Experimental settings

Run	Feedstock composition	Component volume			SS:MLL:BA volumetric ratio	HRT	OLR*
		SS	MLL	BA			
		L	L	L	%	d	kg VS m ⁻³ d ⁻¹
Phase 1							
R 1.1	SS (control)	2.0	–	–	100	20	1.27 ± 0.18
R 1.2	SS + MLL	2.0	0.1	–	95:5	19.1	1.30 ± 0.18
R 1.3	SS + MLL + BA	2.0	0.1	0.1	90:5:5	18.2	1.30 ± 0.18
Phase 2							
R 2.1	SS (control)	2.0	–	–	100	20	1.32 ± 0.25
R 2.2	SS + MLL	2.0	0.1	–	95:5	19.1	1.35 ± 0.25
R 2.3	SS + MLL + BA	2.0	0.1	0.1	87:4.3:8.7	17.4	1.33 ± 0.25

* The average value ± standard deviation is reported

The parameters (as listed in Table 1) were analyzed once a week for SS and BA, while twice a week for the digestate. The MLL characteristics were determined once for the overall study. Most parameters were measured with a Hach Lange UV–VIS DR 5000 (Hach, Loveland, CO, USA) using the Hach analytical methods. The supernatant samples required for determining the SCOD, VFA, pH, alkalinity as well as $\text{NH}_4^+\text{-N}$ and $\text{PO}_4^{3-}\text{-P}$ values were achieved via centrifugation at 4000 rpm for 30min. Some analyses (TS, VS, pH) were conducted in accordance with the Standard Methods for the Examination of Water and Wastewater [34].

Biogas measurements

The co-digestion efficiency was controlled on the basis of daily biogas production and its composition. The former was measured using an Aalborg (Orangeburg, NY, USA) digital mass flow meter, the latter – using a ThermoTrace GC-Ultra (Thermo Fisher Scientific, Milan, Italy) gas chromatograph coupled with a conductivity detector fitted with DVB-packed columns (RTQ-Bond). The parameters used for the analysis were 50°C for the injector and 100°C for the detector. The carrier gas was helium with a flux rate of $1.5\text{cm}^3\cdot\text{min}^{-1}$. The peak areas were determined by means of a computer integration program (CHROM-CARD).

Kinetics

The kinetics was based on the biogas production curves created using averaged experimental data (from 30 measurement days). These were collected by the continuous acquisition system via an XFM Control Terminal. A first-order kinetic equation $V_f = V_{\max} [1 - \exp(-k t)]$ was approved for a suitable description of the biogas production in semi-continuous system, where V_f is the biogas volume in time (L), V_{\max} is a constant referring to the maximum theoretical biogas production possible to obtain from a portion of feedstock supplied to the digester daily (L), k is a constant of the biogas production rate (h^{-1}) and t is the operational time (hours).

Statistical analysis

The statistical analysis was performed using ANOVA with Statsoft Statistica software (v 13). The differences were considered statistically significant at $p < 0.05$. The k and V_{\max} constants were calculated by means of a nonlinear regression.

Results and discussion

Characteristics of feedstock and digestate

The chemical composition of MLL seems to indicate that the leachate originated from the sanitary landfill operating under the intermediate phase of stabilization, i.e. remaining between phases IV (methane formation) and V (final maturation) [35]. This is especially seen considering BOD/COD ratio and BOD as the values typical for the final phase of stabilization, and the VFA concentration which should be rather attributed to phase IV.

The characteristics of feedstock and digestate as well as VS removal for the specified runs were presented in Figure 1. While introducing MLL (R 1.2 and R 2.2) and then BA (R 1.3 and R 2.3), a decrease in the feedstock was observed with regard to COD, VS and TS as compared to SS (R 1.1 and R 2.1), although the differences were of no significance. This was accompanied by a several percentage increase in SCOD and thus the enhanced SCOD/COD ratios, from 0.043 and 0.028 (control) to 0.05 and 0.034 (co-digestion) in phases 1 and 2, respectively, indicating better bioavailability of organic compounds (despite the low BOD_5/COD ratio in MLL). Moreover, the pH and alkalinity values grew (the latter being significantly higher) which ensured favorable conditions for stable process performance both in the bioaugmented and non-bioaugmented co-digestion of SS and MLL.

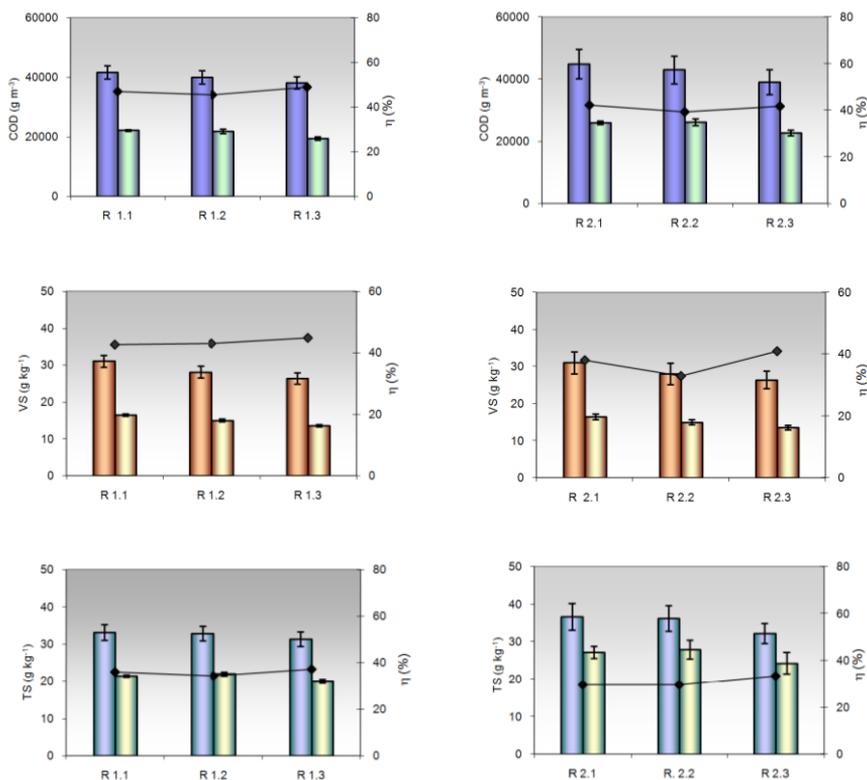


Fig. 1. Concentration of organic compounds (expressed as COD, VS and TS) in feedstock (left bars) and digestate (right bars); black markers denote the VS removal (η), error bars represent the confidence levels at $\alpha = 0.05$

VS removal

Regardless of the BA dose, its introduction turned to be beneficial for the VS removal. In both phases, a related increase was found, especially visible comparing R 2.3 and R 2.2 (Fig. 1 and Table 3). Importantly, this was despite the HRT shortening (Table 2). This observation is consistent with the study by Yu *et al.*[18], who reported that the bioaugmentation by bacterial strains such as *Bacillus cereus* and *Enterococcus casseliflavus* improved the removal efficiency of organic compounds (expressed as COD), ammonia nitrogen and humic acid in mature landfill leachate treatment. On the other hand, the addition of superior mixed microorganisms (consisting of *Coriolus versicolor*, *Phanerochate chrysosporium* and *Azotobacter* sp.) was recognized as useful for shortening the sludge acclimation time and enhancing the treatment efficiency in sequencing batch reactor at high volume loading rate [36].

It is worth noting that there was a distinct tendency of the VS removal in the non-bioaugmented runs. Analogous levels occurred in phase 1, while in phase 2, a visible decrease was observed as compared to the controls. In both cases, the results were much better than the authors' previous findings for the SS and MLL co-digestion under the same operational conditions and using an identical dose of the same-sourced MLL. In that study, an almost 50% decrement in the VS removal was found. The possible explanation of this inconsistency is that in phase 1 the process was performed in a primarily bioaugmented reactor (of three months prior to the co-digestion start-up) and it remained efficient enough because of retaining the favorable effects ensured by the BA pre-bioaugmentation. However, such effects are known to be temporary and tend to fade out after three months [37]. Thus, in phase 2 the SS and MLL co-digestion results were worse due to a longer period of the same reactor operating after pre-

bioaugmentation completion and a time-increasing the negative impact of MLL. This is where the favorable effects of BA bioaugmentation were best seen, being manifested in both an increase in the VS removal and a decrease in the content of organic compounds in digestate (Fig. 1 and Table 3), despite a visible HRT shortening.

Biogas and methane yields

The average biogas and methane yields as well as the kinetics parameters of the biogas production (including coefficient of determination R²) are presented in Table 3. The average biogas volume generated daily in semi-continuous systems and calculated for every run involving 30 measurements day is demonstrated in Fig. 2. A graphical form ensures both the presentation of averaged experimental data and the related function.

Table 3. The VS removal, biogas and methane yields, and methane concentration in biogas (average values are reported ± standard deviation)

Parameter	Unit	Phase 1				Phase 2	
		R 1.1	R 1.2	R 1.3	R 2.1	R 2.2	R 2.3
VS removal	–	0.43 ± 0.09	0.43 ± 0.10	0.45 ± 0.09	0.38 ± 0.06	0.33 ± 0.14	0.41 ± 0.03
Biogas yield	m ³ kg ⁻¹ VS _{add}	0.37 ± 0.06	0.35 ± 0.05	0.34 ± 0.05	0.42 ± 0.05	0.38 ± 0.03	0.44 ± 0.05
	m ³ kg ⁻¹ TS _{add}	0.28 ± 0.04	0.26 ± 0.04	0.25 ± 0.04	0.31 ± 0.05	0.28 ± 0.03	0.32 ± 0.04
	m ³ kg ⁻¹ VS _{rem}	0.94 ± 0.34	0.88 ± 0.31	0.80 ± 0.25	1.16 ± 0.28	1.03 ± 0.15	1.09 ± 0.18
	m ³ kg ⁻¹ TS _{rem}	0.88 ± 0.37	0.87 ± 0.36	0.75 ± 0.28	1.16 ± 0.46	0.99 ± 0.20	1.00 ± 0.20
	m ³ kg ⁻¹ COD _{rem}	0.51 ± 0.14	0.50 ± 0.13	0.42 ± 0.07	0.48 ± 0.06	0.48 ± 0.04	0.51 ± 0.07
	Methane content	%	54.0 ± 1.4	53.4 ± 0.8	54.7 ± 0.8	53.6 ± 1.0	53.0 ± 1.0
Methane yield	m ³ kg ⁻¹ VS _{add}	0.20 ± 0.03	0.19 ± 0.03	0.18 ± 0.03	0.23 ± 0.03	0.20 ± 0.02	0.24 ± 0.03
	m ³ kg ⁻¹ TS _{add}	0.15 ± 0.03	0.14 ± 0.02	0.14 ± 0.02	0.17 ± 0.03	0.15 ± 0.01	0.17 ± 0.02
	m ³ kg ⁻¹ VS _{rem}	0.51 ± 0.18	0.47 ± 0.17	0.43 ± 0.14	0.63 ± 0.15	0.55 ± 0.08	0.59 ± 0.09
	m ³ kg ⁻¹ TS _{rem}	0.48 ± 0.20	0.46 ± 0.19	0.41 ± 0.15	0.63 ± 0.26	0.53 ± 0.12	0.54 ± 0.11
	m ³ kg ⁻¹ COD _{rem}	0.27 ± 0.07	0.27 0.07	0.23 ± 0.04	0.26 ± 0.03	0.26 ± 0.03	0.28 ± 0.04
	Kinetics						
k	h ⁻¹	0.074	0.077	0.085	0.067	0.071	0.076
V _{max}	L	30.10	29.41	27.68	39.09	36.37	39.29
V _{max} -V _f	L	4.99	4.59	2.51	7.33	6.60	5.33
R ²	–	0.9992	0.9994	0.9963	0.9989	0.9992	0.9979

It is noteworthy that in phase 1, the bioaugmentation using BA did not beneficially influence the SS and MLL co-digestion. Although the methane concentration enhanced by 1.3%, the biogas/methane yields were slightly or even clearly lower as compared to a non-bioaugmented system. An analogous trend concerned the control. However, in all cases the differences were of no statistical significance, both regarding the results calculated per mass of the organic compounds added to and removed from the system. Such findings were most likely attributed to the insufficient BA inoculation at shortened HRT of 18.2 d (i.e. both a low BA dose and its VS concentration). When inoculation is too low, it may cause fundamental problems with adaptation and competition of microorganisms introduced into the reactor[38]. This happens because the inoculum size consists a bottleneck for a successful and economically viable bioaugmentation [39, 40].

In contrast, a positive influence of bioaugmentation was revealed in phase 2 with higher BA inoculation. It was despite the further HRT shortening to 17.4 d. There, a visible increment in the biogas and methane yields occurred as compared to non-bioaugmented co-digestion, with the significant difference for yields expressed per the organics added to the system. These seem to indicate that the increased inoculation brought the expected effects in phase 2, counteracting the BA being washed-out of the system too quickly. Such a risk is due to the extremely long generation times ranging for Archaea from 36h to 11d, and the low specific growth rate of $0.35\text{--}0.4\text{d}^{-1}$ [41].

Conversely, such a beneficial effect was much less clear while comparing the results of bioaugmented SS and MLL co-digestion with those achieved for the sole SS digestion. This was probably due to the low biodegradability of organic compounds contained in MLL. However, similar results were maintained, even though the HRT decreased from 20h to 17.4d.

Kinetics

While studying the process kinetics, favourable effects of bioaugmentation were observed for both phases, regardless of the BA inoculation size. The constant of the biogas production rate was significantly enhanced in the BA presence with the related increases of 7-10% and 13-15%, as compared to the non-bioaugmented and control runs, respectively (Table 3). Moreover, the untapped biogas potential (i.e. the difference between the maximum biogas production from the feedstock (V_{\max}) and the actual biogas production achieved after 24h (V_f)) was the smallest when bioaugmentation was applied (Fig. 2), indicating more effective use of available organic compounds and faster substrate assimilation even though a clear HRT shortening took place.

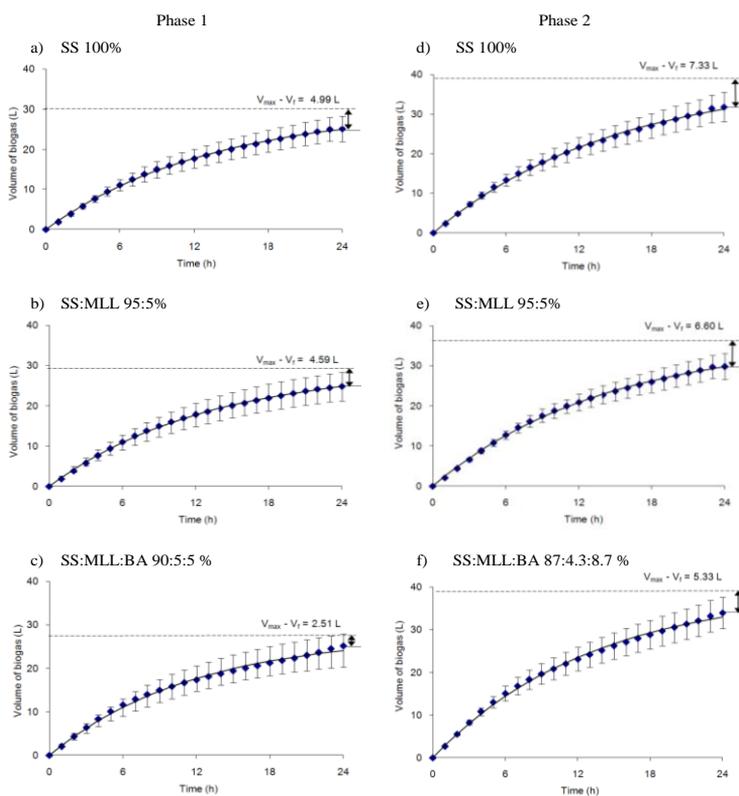


Fig. 2. Biogas production in time through the specified runs; the difference $V_{\max} - V_f$ represents the untapped biogas potential remaining in digestate

This could be attributed both to larger concentration of solutes (SCOD/COD ratio) and greater rate of metabolic transformations, which led to an increase in SS and MLL co-digestion efficiency at sufficiently high BA dose. Moreover, the hydrogenotrophic methanogenesis could prevail, just as it was found in the previous study of our team [33]. It is of great importance, since hydrogenotrophic methanogens are known to be more tolerant to various stress factors [42], and thus the co-digestion results could be much better in the bioaugmented system.

To sum up, using an adequate BA inoculation size, the improved SS and MLL co-digestion efficiency occurred despite the low MLL biodegradability and the decrease in HRT from 19.1h to 17.4d. The methane content in biogas increased, and the methane yields were significantly higher as compared to a non-bioaugmented co-digestion system, reaching $0.24\text{m}^3 \text{kg}^{-1}\text{VS}_{\text{added}}$ and $0.17\text{m}^3 \text{kg}^{-1}\text{TS}_{\text{added}}$, while in the BA absence, it was only 0.20 and 0.15, respectively. Bioaugmentation seems to accelerate the metabolic transformations, which was confirmed by the enhanced values representing the rate constant of biogas production. These were 0.076 and 0.085h^{-1} in the BA presence, while only 0.071 and 0.077h^{-1} in non-bioaugmented co-digestion of SS and MLL. Regarding the control runs, the rate constant was the lowest while the untapped biogas potential the highest (Table 3 and Fig. 2), which indicated that the greater SCOD/COD ratio (typical of SS and MLL co-digestion) as well as the BA introduction influenced the process kinetics beneficially.

Taking the above into consideration, it could be stated that bioaugmentation using a proper BA dose allowed for overcoming the toxic impact of MLL on the co-digestion with SS both in terms of the process performance and its efficiency.

Conclusions

The study revealed a beneficial effect of using a sufficient BA inoculation on the efficiency of the SS and MLL co-digestion. Despite the decrease in HRT from 19.1h to 17.4d, the VS removal enhanced from 32.9 to 41%, the methane concentration rose by 0.7% and the methane yields significantly increased as compared to a non-bioaugmented co-digestion system, with corresponding values of $0.24\text{m}^3 \text{kg}^{-1}\text{VS}_{\text{added}}$ and $0.17\text{m}^3 \text{kg}^{-1}\text{TS}_{\text{added}}$. The same tendency occurred regarding the kinetics, which turned out to be highly accelerated in the BA presence, ensuring minimization of the untapped biogas potential in digestate. These findings seem to indicate the possibility of overcoming the toxic impact of mature landfill leachate on the efficiency of its co-digestion with sewage sludge involving bioaugmentation by the mixture of wild-living Bacteria and Archaea.

The bioaugmented co-digestion of MLL and SS may be recognized as a method which integrates the management of hardly-biodegradable medium with the efficient waste-to energy transformation. This is of great importance considering both the preservation of the environment from the toxic effect of MLL and the natural resources conservation by using waste biomass as a source of renewable energy.

Acknowledgments

Dr. Magdalena Lebiocka and Ms. Iwona Musielewicz are greatly appreciated for their support during the research.

Funding body

The author thanks for the financial support from the National Centre of Science (Poland) No.7405/B/T02/2011/40.

References

- [1] T.A. Kurniawan, W.-H. Lo, G.Y.S. Chan, *Physico-chemical treatments for removal of recalcitrant contaminants from landfill leachate*, **Journal of Hazardous Materials**, **129**, 2006, pp. 80-100.
- [2] T. Eggen, M. Moeder, A. Arukwe, *Municipal landfill leachates: a significant source for new and emerging pollutants*, **Science of the Total Environment**, **408**, 2010, pp. 5147-5157.
- [3] A.A. Abbas, G. Jingsong, L.Z. Ping, P.Y. Ya, W.S. Al-Rekabi, *Review on landfill leachate treatments*, **American Journal of Applied Science**, **6**(4), 2009, pp. 672–684.
- [4] A.R. Abood, J. Bao, J. Du, D. Zheng, Y. Luo, *Non-biodegradable landfill leachate treatment by combined process of agitation, coagulation, SBR and filtration*, **Waste Management**, **34**, 2014, pp. 439-447.
- [5] I.A. Tafałaj, P. Bledka, I. Bartkowska, *Treatment of landfill leachates with biological pretreatments and reverse osmosis*, **Environmental Chemistry Letters**, **17**, 2019, pp. 1177-1193.
- [6] J. Tejera, R. Miranda, D. Hermosilla, I. Urra, C. Negro, Á. Blanco, *Treatment of a Mature Landfill Leachate: Comparison between homogeneous and heterogeneous photo-Fenton with different pretreatments*, **Water**, **11**(9), 2019, p. 1849.
- [7] K.Y. Foo, B.H. Hameed, *An overview of landfill leachate treatment via activated carbon adsorption*, **Journal of Hazardous Materials**, **171**, 2009, pp. 54-60.
- [8] X. Wang, J. Han, Z. Chen, L. Jian, X. Gu, C.J. Lin, *Combined processes of two-stage Fenton-biological anaerobic filter–biological aerated filter for advanced treatment of landfill leachate*, **Waste Management**, **32**, 2012, pp. 2401-2405.
- [9] C. Amor, E. de Torres-Sociás, J.A. Peres, M.I. Maldonado, I. Oller, S. Malato, M.S. Lucas, *Mature landfill leachate treatment by coagulation/flocculation combined with Fenton and solar-Fenton processes*, **Journal of Hazardous Materials**, **286**, 2015, pp. 261-268.
- [10] M.J.K. Bashir, H.A. Aziz, S.S. Abu Amr, S. Sumathi, C.A. Ng, J.W. Lim, *The competency of various applied strategies in treating tropical municipal landfill leachate*, **Desalination and Water Treatment**, **54**, 2015, pp. 2382-2395.
- [11] T.K. Nivya, T. Minimol Pieus, *Comparison of photo electroFenton process (PEF) and combination of PEF process and membrane bioreactor in the treatment of landfill leachate*, **Procedia Technology**, **24**, 2016, pp. 224-231.
- [12] M. Hassan, X. Wang, F. Wang, D. Wu, A. Hussain, B. Xie, *Coupling ARB-based biological and photochemical (UV/TiO₂ and UV/S₂O₈²⁻) techniques to deal with sanitary landfill leachate*, **Waste Management**, **63**, 2017, pp. 292-298.
- [13] P. Mandal, B.K. Dubey, A.K. Gupta, *Review on landfill leachate treatment by electrochemical oxidation: Drawbacks, challenges and future scope*, **Waste Management**, **69**, 2017, pp. 250-273.
- [14] T.F.C.V. Silva, P.A. Soares, D.R. Manenti, A. Fonseca, I. Saraiva, R.A.R. Boaventura, V.J.P. Vilar, *An innovative multistage treatment system for sanitary landfill leachate depuration: studies at pilot-scale*, **Science of the Total Environment**, **576**, 2017, pp. 99-117.
- [15] A. Montusiewicz, M. Bis, S. Pasieczna-Patkowska, D. Majerek, *Mature landfill leachate utilization using a cost-effective hybrid method*, **Waste Management**, **76**, 2018, pp. 652-662.
- [16] G. Kalčíková, J. Babič, A. Pavko, A. Žgajnar Gotvajn, *Fungal and enzymatic treatment of mature municipal landfill leachate*, **Waste Management**, **34**, 2014, pp. 798–803.
- [17] T. Nawaz, A. Rahman, S. Pan, K. Dixon, B. Petri, T. Selvaratnam, *A review of landfill leachate treatment by microalgae: Current status and future directions*, **Processes**, **8**, 2020, p. 384.

- [18] D. Yu, T. Yang, F. Teng, L. Feng, X. Fang, H. Ren, *Bioaugmentation treatment of mature landfill leachate by new isolated ammonia nitrogen and humic acid resistant microorganisms*, **Journal of Microbiology and Biotechnology**, **24**(7), 2014, pp. 987-997.
- [19] M. Lebiocka, A. Montusiewicz, M. Zdeb, *Anaerobic co-digestion of sewage sludge and old landfill leachate*, **Polish Journal of Environmental Studies, Series of Monographs**, **2**, 2010, pp. 141-145.
- [20] J. De Vrieze, W. Verstraete, *Perspectives for microbial community composition in anaerobic digestion: from abundance and activity to connectivity*, **Environmental Microbiology**, **18**(9), 2016, pp. 2797-2809.
- [21] D.G. Cirne, L. Björnsson, M. Alves, B. Mattiasson, *Effects of Bioaugmentation by an anaerobic lipolytic bacterium on anaerobic digestion of lipid-rich waste*, **Journal of Chemical Technology & Biotechnology**, **81**, 2006, pp. 1745-1752.
- [22] J.C. Costa, S.G. Barbosa, D.Z. Sousa, *Effects of pre-treatment and bioaugmentation strategies on the anaerobic digestion of chicken feathers*, **Bioresource Technology**, **120**, 2012, pp. 114–119.
- [23] A. Martin-Ryals, L. Schideman, P. Li, H. Wilkinson, R. Wagner, *Improving anaerobic digestion of a cellulosic waste via routine bioaugmentation with cellulolytic microorganisms*, **Bioresource Technology**, **189**, 2015, pp. 62–70.
- [24] P. Tsapekos, P.G. Kougiyas, S.A. Vasileiou, L. Treu, S. Campanaro, G. Lyberatos, I. Angelidaki, *Bioaugmentation with hydrolytic microbes to improve the anaerobic biodegradability of lignocellulosic agricultural residues*, **Bioresource Technology**, **234**, 2017, pp. 350–359.
- [25] N. Tepe, D. Yurtsever, R.J. Mehta, C. Bruno, V.L. Punzi, M. Duran, *Odor control during post-digestion processing of biosolids through bioaugmentation of anaerobic digestion*, **Water Science and Technology**, **57**(4), 2008, pp. 589-594.
- [26] P. Lins, C. Malin, A.O. Wagner, P. Illmer, *Reduction of accumulated volatile fatty acids by an acetate degrading enrichment culture*, **FEMS Microbiology Ecology**, **71**, 2010, pp. 469-478.
- [27] V.P. Tale, J.S. Maki, D.H. Zitomer, *Bioaugmentation of overloaded anaerobic digesters restores function and archaeal community*, **Water Research**, **70**, 2015, pp. 138-147.
- [28] Y. Li, G. Yang, L. Li, Y. Sun, *Bioaugmentation for overloaded anaerobic digestion recovery with acid-tolerant methanogenic enrichment*, **Waste Management**, **79**, 2018, pp. 744-751.
- [29] A. Schauer-Gimenez, D. Zitomer, J. Maki, C. Struble, *Bioaugmentation for improved recovery of anaerobic digesters after toxicant exposure*, **Water Research**, **44**, 2010, pp. 3555-3564.
- [30] I.A. Fotidis, L. Treu, I. Angelidaki, *Enriched ammonia-tolerant methanogenic cultures as bioaugmentation inocula in continuous biomethanation processes*, **Journal of Cleaner Production**, **166**, 2017, pp. 1305-1313.
- [31] Y. Li, Y. Zhang, Y. Sun, S. Wu, X. Kong, Z. Yuan, R. Dong, *The performance efficiency of bioaugmentation to prevent anaerobic digestion failure from ammonia and propionate inhibition*, **Bioresource Technology**, **231**, 2017, pp. 94-100.
- [32] M. Herrero, D.C. Stuckey, *Bioaugmentation and its application in wastewater treatment: A review*, **Chemosphere**, **140**, 2015, pp. 119-128.
- [33] M. Lebiocka, A. Montusiewicz, A. Cydzik-Kwiatkowska, *Effect of bioaugmentation on biogas yields and kinetics in anaerobic digestion of sewage sludge*, **International Journal of Environmental Research and Public Health**, **15**(1717), 2018, pp. 1-16.
- [34] * * *, *Standard Methods for the Examination of Water & Wastewater*, Centennial Edition 21. ed.; **American Public Health Association**, Washington, DC, USA, 2005.
- [35] D.R. Reinhart, T.G. Townsend, **Landfill Bioreactor Design and Operation**, Lewis Publishers CRC Press LLC, 1998, p. 19.

- [36] W. Hailei, L. Guosheng, L. Ping, P. Feng, *The effect of bioaugmentation on the performance of sequencing batch reactor and sludge characteristics in the treatment process of papermaking wastewater*, **Bioprocess and Biosystem Engineering**, **29**, 2006, pp. 283-289.
- [37] A. Montusiewicz, *Co-digestion of sewage sludge and mature landfill leachate in pre-bioaugmented system*, **Journal of Ecological Engineering**, **15**(4), 2014, pp. 98-104.
- [38] A. Nzila, *Mini review: Update on bioaugmentation in anaerobic processes for biogas production*, **Anaerobe**, **46**, 2017, pp. 3-12.
- [39] I.A. Fotidis, H. Wang, N.R. Fiedel, G. Luo, D.B. Karakashev, I. Angelidaki, *Bioaugmentation as a solution to increase methane production from an ammonia-rich substrate*, **Environmental Science & Technology**, **48**, 2014, pp. 7669-7676.
- [40] A. Nzila, S.A. Razzak, J. Zhu, *Bioaugmentation: an emerging strategy of industrial wastewater treatment for reuse and discharge*, **International Journal of Environmental Research and Public Health**, **13**, 2016, p. 846.
- [41] S. Vieira-Silva, E.P. Rocha, *The systemic imprint of growth and its uses in ecological (meta)genomics*, **PLoS Genetics**, **6**(1), 2010, Article Number: e1000808, Supplementary table S1.
- [42] J. De Vrieze, A.M. Saunders, Y. He, J. Fang, P.H. Nielsen, W. Verstraete, N. Boon, *Ammonia and temperature determine potential clustering in the anaerobic digestion microbiome*, **Water Research**, **75**, 2015, pp. 312-323.

Received: April 19, 2021

Accepted: July 20, 2021