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Investigating the Anaerobic Digestion of Water Hyacinth (*Eichhornia crassipes*) Sourced from Hartbeespoort Dam in South Africa

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Abstract: The biodegradability of water hyacinth for biogas and biofertilizer production was studied under mesophilic conditions. The effects of water hyacinth pretreatments were also included in this investigation. It was found that water hyacinth has a low biodegradability of 27% when monodigested, while in a 3:1 ratio with cow manure, the biodegradability increases to 46%. At this elevated biodegradability, the water hyacinth biomethane potential was 185 LCH_4/kgVS, while that of cow manure was 216 LCH_4/kgVS. The Gompertz kinetic model had superior parameters than the logistic model for most of the water hyacinth–cow manure combined substrate digestion. Based on the Gompertz model, the lag phase and daily maximum methane production rate were 5.5 days and 22.9 mL/day, respectively, for the 3:1 codigestion (R^2 of 0.99). These values were 6.7 days and 15.2 mL/day, respectively, in the case of water hyacinth monodigestion (R^2 = 0.996). The dominant microbial species detected in the digestates were Bacteroidetes and Proteobacteria. A few microbial species were indigenous to water hyacinth, but more diverse consortia, which are key to efficient substrate biodegradation, came from cow manure. The digestate contained ammonium nitrogen at 68 mg/kg with phosphorous and potassium at 73 and 424 mg/kg, respectively. Nitrogen was lower but phosphorous and potassium were comparable to previously studied digestates of other substrates. Only water hyacinth pretreated by aerobic composting was proven to unlock a higher methane yield that matched the 3:1 codigestion with cow manure. Other pretreatments induced better biodegradation performance than that observed in untreated water hyacinth but these improvements were not as good as that of the 3:1 codigestion scheme. It was concluded that water hyacinth sourced from the Hartbeespoort Dam could be treated by anaerobic digestion to recover biogas and biofertilizer. However, more experiments are required to fully understand and harness the optimisation opportunities available in applying this technology to manage water hyacinths.

Keywords: water hyacinth; cow manure; codigestion; biomethane potential; biofertilizer manure

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1. Introduction

Water hyacinth (WH) infestation on water bodies is an expanding global challenge that threatens the availability of fresh water for domestic and industrial uses as well as disturbs biodiversity in these water bodies [1]. One of the interventions widely investigated for managing this rapidly growing biomass is anaerobic digestion (AD) for biogas production. However, there are still technological and site-specific knowledge gaps that are yet to be

closed for widespread commercial adoption of AD in water hyacinth management [2]. The methane component of biogas infers the gas combustibility attributes and the subsequent use of biogas as an energy carrier gas. The substrate's capacity to produce biomethane under anaerobic conditions is called its biomethane potential (BMP). Among the current challenges surrounding the AD of water hyacinth are the wide variations of BMPs reported in literature ranging from 75 to 360 L/kgTS [3]. A number of investigations on the effects of pretreatments on water hyacinth's BMP have been conducted and there is no agreement between the published BMP results. Biogas production increases of 143% and 23% in separate studies of water hyacinth digestions after biological pretreatments have been reported [4,5]. Codigestion has also been extensively studied as a strategy for improving water hyacinth biomethanation. The selection of an appropriate cosubstrate is important to realize the benefits of this strategy. Cow manure (CM) is the most popular AD substrate because it is a rich source of methanogens as well as due to its availability in huge quantities in most places. It is therefore a popular cosubstrate for many codigestion studies and industrial AD practices. Ali et al. (2022) [6] studied different CM:WH codigestions and reported that a 1:1 ratio gave the highest biogas and methane production increases of 111.3% and 173.6%, respectively. Other ratios gave increases in gas production, but the increases were not as high as those achieved with the 1:1 ratio. These findings by Ali et al. (2022) were in sharp contrast to those reported by Dolle and Hughes (2020) [7], where a WH:CM ratio of 3:1 gave the highest BMP and the 1:1 ratio produced the poorest gas yield results. Other water hyacinth pretreatments to improve biomethanation, such as steam explosion, ionic liquid pretreatment, acid or alkali hydrolysis, and microwave irradiation, have also been applied to other lignocellulosic biomass pretreatments for bioenergy and biofuel recovery. Unfortunately, most of these strategies are either still at the conceptual, laboratory, and pilot stages or are not viable from a techno-economic standpoint, and hence they are not yet widely commercialized [8,9].

It is widely agreed among researchers across the globe that in AD studies, the substrate's BMP is proportional to its volatile matter content, which is a function of the substrate's organic elemental (CHNO) analysis [10]. There are several methods of estimating a substrate's BMP based on its elemental analysis results [11]. One common calculation approach is the Buswell equation, which gives a theoretically determined BMP. The theoretical BMP results are in most cases overstated because, in such calculations, 100% organic matter degradation to biogas is assumed, which in real AD systems is impracticable as some of the organics are diverted towards cell growth while others are locked up in lignocellulosic structures and hence are not available for degradation. The standard BMP determination method is to set up experimental AD equipment, incubate the substrate under conducive AD conditions, and then measure the methane gas produced from a known initial amount of volatile solids (VS) in the substrate. The incubation runs until daily gas production becomes insignificant (less than 1% of accumulated volume) [12].

Hartbeesport Dam in South Africa is currently heavily polluted with water hyacinth, which can be harvested for biogas recovery while cleaning this important water resource. Based on the dam coverage, which can sometimes reach as high as 80% [13], and a conservative yield of 20 tonnes DM/ha/year, despite the literature reports of a wide yield range of 20–230 tonnes DM/ha/year [14,15], the Hartbeespoort Dam, which covers approximately 2000 ha, has the potential to produce at least 32,000 tonnes DM of water hyacinth per year. Unfortunately, there are limited data in the literature on the biogas producing capacity, patterns, and/or AD problems associated with this biomass. The only data that may be available are limited to a narrow scope of study at a laboratory scale with limitations for use in optimizing commercial scale operations [2]. Given the variability in BMPs reported for water hyacinth sourced from different places, including inconsistent results from pretreated water hyacinth or codigestion systems, it is prudent to perform independent BMP assays for the water hyacinth sourced specifically from the Hartbeespoort Dam. The results from this study are so specific to this substrate that they can be used to inform strategies for implementing the AD of this specific biomass. In addition to tracking the BMP for water

hyacinth, understanding the microbial profiles in the digesters as well as the digestate's biofertilizer quality will also yield important information for both optimization and better resource recovery [16–18]. The current study therefore seeks to unpack knowledge about the Hartbeespoort Dam's water hyacinth biomethanation potential and evaluate the resulting digestate (potential biofertilizer) attributes. The original contribution of this study is in the collection of all the biomethanation performance data (BMP, codigestion effects, pretreatment effects, kinetics, digestate quality, and microbial profiles) from a single substrate source. This facilitates process optimization on a site-specific biomass. The data on WH biomethanation currently available in the literature complicates optimization because AD aspects have been investigated in isolation and/or substrates sourced from different sources have been investigated for similar AD performance aspects.

2. Materials and Methods

2.1. Materials and Preparation

Water hyacinth was harvested from Coves Estate East Hartbeespoort Dam (25°44′51″ S 27°52′1″ E) in the North West Province of South Africa. Figure 1 shows the Hartbeespoort Dam with approximately 50% of its surface covered with water hyacinth at the time of sampling, although in the 1990s, the whole dam surface was clear with no cover at all. Three different locations on the dam that were accessible to the researchers were considered for sampling and the three samples were blended into a single composite sample at the laboratory prior to further processing. Samples were collected in 5 L sealed containers and transported to the University of South Africa (UNISA), Florida Science campus. The water hyacinths were rinsed under running water to remove any foreign loose materials such as soil. The cleaned water hyacinth was knife-chopped and then reduced further in size to 2 mm, which was achieved through maceration in a blender. The final water hyacinth pulp was preserved at 4 °C until further use. Proximate and ultimate analyses were performed on the water hyacinth according to the APHA/AWWA/WEF (2012) methods. This was followed by performing biomethane potential (BMP) tests. Portions of the water hyacinth were subjected to different pretreatments prior to BMP testing for the water hyacinth's anaerobic degradability. The five pretreatments investigated were aerobic decomposition (DA), oven drying (OD), autoclave drying (AC), sun drying (SD), and microwave heating (MW) as described by different researchers [19–21]. The pretreatments were chosen on the basis of the non-usage of expensive and toxic chemicals.



Figure 1. The current extent of water hyacinth cover over the Hartbeespoort Dam (picture taken by the authors).

All tests were performed within 5 days of sample collection. Fresh cow manure was used as both inoculum and cosubstrate in the experiments. Cow manure was collected from a nearby farm in Muldersdrift (26.0330 S 27.850 E). Analytical grade chemicals used in the analysis were sourced from Merck Chemicals (Merck Group, Darmstadt, Germany).

2.2. Experimental Set-Up for Biomethane Potential Assays

The volatile solids for water hyacinth and CM were determined based on the method in [22]. Using the setup shown in Figure 2, three differently formulated substrates were tested for biomethane potential (BMP) based on the volatile solids in the CM and water hyacinth. The BMP testing methods were adapted from the procedures outlined by Selvankumar et al. (2017) [23]. The substrates and reactors were allocated codes as follows: R1 (CM only), R2 (WH only), R3 (WH and CM in the ratio 1:1), R4 (WH and CM in the ratio 2:1), and R5 (WH and CM in the ratio 3:1). Reactors (50 mL) were used with a reaction volume of 40 mL and a headspace of 10 mL. Each reactor contained 3 g total VS for the total substrate. In each reactor, 6 g VS of CM was added as inoculum with any excess above the 6 g in the codigestion reactors being considered as a cosubstrate. As a result, an I/S ratio of 2 was maintained across all reactors. Experiments were performed in triplicate. The gas produced was collected and measured in inverted measuring cylinders (Figure 2). Before recording, the collected volumes were corrected to normal volumes (NmL) at standard temperature and pressure (STP) conditions. In addition, the endogenous gas production from the inoculum was also subtracted from the final reported values.

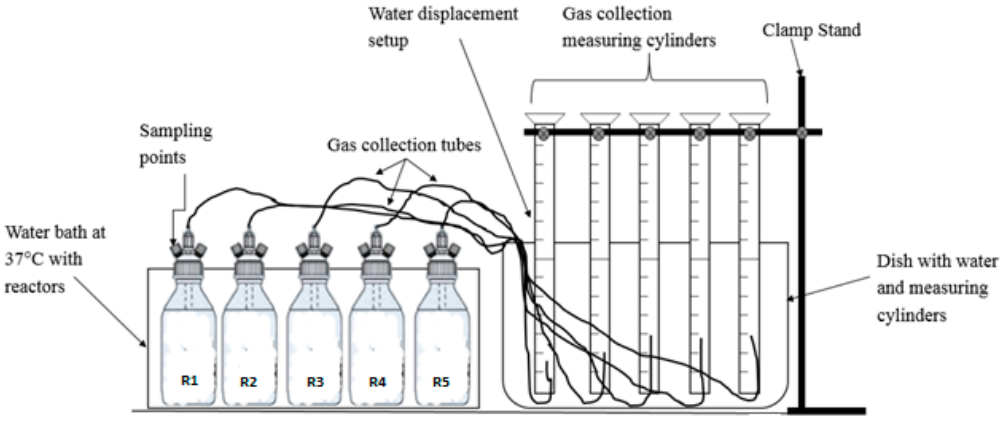


Figure 2. Schematic of the BMP testing experimental setup. R1 (CM only), R2 (WH only), R3 (1WH:1CM), R4 (2WH:1CM), and R5 (3WH:1CM). Drawing by the authors.

2.3. Analytical Methods and Data Analysis

2.3.1. Proximate and Ultimate Analyses

The water hyacinth and cow manure were subjected to proximate and ultimate analyses at the University of South Africa Chemical Engineering and IDEAS laboratory. Standard APHA 2540 E and G methods were used in these analyses [22]. The organic elemental (CHNS) analysis was carried out at the University of Johannesburg Chemistry Department laboratory. The British standard method for elemental analysis, EN15104:2011 was followed and a Thermo Scientific unit model Flash 2000 was employed for this analysis.

2.3.2. Biogas Analysis

During the batch laboratory AD experiments, biogas composition was monitored using a gas chromatography Agilent 7890B system (Agilent Technologies, Santa Clara, CA, USA). The gas chromatography (GC) consisted of a front thermal conductivity detector (TCD) and a back flame ionizing detector (FID) with an Agilent J&W GC column (CP-Sil SCB, length 25 m and diameter 0.15 mm). The FID was operated at 250 °C with helium used as carrier gas flowing at 25 mL/min. The heating program started with an oven temperature of 80 °C being held for 4.5 min, thereafter rising in 20 °C increments targeting

a maximum temperature of 250 °C. The calibration standard gas mixture was supplied by Afrox South Africa and consisted of 5% nitrogen, 30% carbon dioxide, and 65% methane.

2.3.3. Biomethane Potential Analysis

Buswell's approach was used to determine the biomethane potential (BMP). This approach assumes 100% substrate degradation in the stoichiometric ratios represented by Reaction (R1). Methane amounts can be calculated using Equation (1) for an organic compound model with the empirical formula $C_aH_bO_cN_dS_e$ [24].

$$C_aH_bO_cN_dS_e + 1/4(4a - b - 2c + 3d + 2e)H_2O \rightarrow 1/8(4a - b + 2c + 3d + 2e)CO_2 + 1/8(4a + b - 2c - 3d - 2e)CH_4 + dNH_3 + eH_2S \quad \text{(R1)}$$

$$tBMP = \left(\left\{ \frac{\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8} - \frac{e}{4}\right)22400}{12a + b + 16c + 14d + 32e} \right\} \right) \quad (1)$$

where *tBMP* is the theoretical biomethane potential.

To get the actual or practical *BMP* from the *BMP* assay experiments, the *BMP* of the water hyacinth in the combined feed was obtained by subtracting the *BMP* contribution of cow manure which was added as inoculum in the tests. The practical *BMP* and the theoretical biomethane (*tBMP*) of water hyacinth were used in computing the substrate's biodegradability (*BD*) using Equation (2).

$$BD = \frac{BMP}{tBMP} \times 100\% \quad (2)$$

Substrate and inoculum characterisation tests were carried out in triplicate. The results were averaged and reported as means plus/minus standard deviations. These averages were used in the computations of theoretical and practical BMPs. The BMP testing data were fitted to the logistic and modified Gompertz models, which are the most popular kinetic models for anaerobic digestion for biomethane production [25,26]. Equations (3) and (4) were used in the Gompertz and logistic modelling respectively.

$$B = B_\infty . exp\left\{ -exp\left[\frac{R_m . e}{B_\infty}(\lambda - t) + 1 \right] \right\} \quad (3)$$

$$B = \frac{B_\infty}{\left\{ 1 + exp\left[\frac{4R_m . (\lambda - t)}{B_\infty} + 2 \right] \right\}} \quad (4)$$

where

B = Cumulative biomethane potential (mL/gVS);
B_∞ = Substrate final biomethane potential (mL/gVS);
R_m = Maximum specific methane production rates (mL/day);
λ = lag phase period (time before methane production starts) (days);
e = the mathematical constant (2.718282).

2.3.4. Microbial Analysis

Microbial profiling within the digesters was carried out using DNA extraction, purification, amplification, and sequencing as outlined by [27]. Briefly, the DNeasy PowerSoil kit (QIAGEN, Hilden, Germany) was used while amplification used the polymerase chain reaction that targeted the 16S rRNA gene. The purification of PCR products employed the DNA Clean & Concentrator Kit (ZYMO RESEARCH, Irvin, CA, USA). Sequencing was performed on the Illumina platform. The sequenced DNA data were analysed using FastQC software version 0.11.5 (Babraham Institute, Babraham, UK) and trimmed to eliminate reads with an average quality score (Phred Q score) lower than 20 using the same software. The resultant quality-filtered forward reads were subjected to further bioinformatics on the Quantitative Insights into Microbial Ecology (version 2) (QIIME2) software. In this

analysis, the Deblur denoiser was used to obtain amplicon sequence variants (ASVs) and these ASVs were further binned into operational taxonomic units (OTUs) at 97% similarity using the "open reference". The SILVA version 132 database trained using the QIIME2 feature-classifier plugin was used for the OUT classifications. The OUT count tables were further sub-sampled (rarefied) to even depths of 5228 sequences, before computing the alpha and beta diversities (in QIIME2). The OUT table was analysed further using the R-studio software version 1.1.463, microbiome Analyst tool and STAMP. The R packages used included vegan, ape, labdsy, and ggplot.

3. Results and Discussion

3.1. Substrate and Inoculum Characteristics

The physicochemical properties of water hyacinth and CM are presented in Table 1. The lower total solid (TS) and CHN values of water hyacinth with respect to CM indicate that the biomethanation of WH may produce less biogas than that of CM. The water hyacinth C/N ratio was suboptimal at 12.44 compared to the recommended optimum (20–30) for methane-producing microorganisms as reported in other studies [28,29]. The nutritional composition of WH also reflects a low content of protein, and no fats were present. Generally, proteins and fats degrade more rapidly, yielding more methane than their lignocellulosic counterparts [11]. Naturally, carbohydrates are more oxygenated than fats and this oxygen takes up the space of carbon which is the source of methane [30]. While these inferences emanating from substrate characterization may be made regarding the expected biomethanation performance of water hyacinth, the extent of actual impacts on physicochemical properties is only confirmed through biomethane potential assays. The properties of CM in terms of C/N ratio as well as the content of degradables are more favourable towards higher and faster methane production than those of water hyacinth. Theoretical biomethane potential calculations assume 100% organic elemental conversions to methane and carbon dioxide. However, such an approach to BMP calculation in situations where the C/N ratio is not within the generally recommended ranges and the nutritional balances are also biased in favour of slow or non-degradables causes significant estimation errors. In the present study, the ratio of degradables to total VS is lower at 2 for water hyacinth than for CM, which is at 4. This indicates that BMPs for water hyacinth are better determined through the standard BMP tests than through the theoretical estimation routes.

Table 1. Water hyacinth and cow manure physicochemical properties. (mean values ± SD).

Parameter	Units	WH	CM
Moisture content	% of TS	95 ± 2.3	83 ± 3
Volatile solids (VS)	% of TS	88 ± 2	77 ± 1.6
Carbon (C)	% of TS	31 ± 1	48 ± 1
Hydrogen (H)	% of TS	19 ± 0.7	7 ± 0.8
Nitrogen (N)	% of TS	3 ± 1	3 ± 0.6
C:N	% of TS	10	16
Protein	% of TS	1.76 ± 0.12	2.3 ± 0.09
Fats	% of TS	0	0.75 ± 0.01
Cellulose	% of TS	0.98 ± 0.03	3.34 ± 0.02
Hemicellulose	% of TS	1.33 ± 0.02	4.21 ± 0.04
Lignin	% of TS	0.6 ± 0.01	2.55 ± 0.02
Degradables:VS	%	2	4
pH	-	6 ± 0.1	7.8 ± 0.1

3.2. Water Hyacinth Biomethanation and Codigestion

3.2.1. Biomethane Potential Tests

The cumulative biomethane produced from water hyacinth biomethanation over the experimental period as described in this study is shown in Figure 3. The experimental data were fitted to the two most common kinetic models, the modified Gompertz model

and the logistic model to derive kinetic parameters for water hyacinth biomethanation. The kinetic modelling results are displayed in Table 2. The cow manure monodigestion produced a total methane volume of 620 mL, which equates to a BMP of 216 L/kgVS. Among the codigested (WH:CM) substrates, the ratios of 3:1 and 2:1 obtained an equal BMP of 185 L/kgVS. These results corroborate with those of Dolle and Hughes (2020) who also found that the ratio of 3:1 (WH:CM) produced more gas than the other ratios in the codigestion of water hyacinth roots and plants with CM. The ratio of 1:1 in the current study gave the lowest BMP of 131 L/kgVS, although this is 19% higher than that of water hyacinth monodigestion, which produced 110 L/kgVS. These results clearly demonstrate the benefits of codigesting the Hartbeespoort water hyacinth as opposed to monodigestion. The theoretical biomethane potential values of CM and water hyacinth calculated using the Buswell method were 397 and 406 L/kgVS, respectively. These values combined with experimental BMPs achieved in the current study indicate biodegradabilities (BD) of 55% and 46% for monodigestions of CM and water hyacinth, respectively. These BD values for CM and water hyacinth indicate that there is a large error introduced in estimating the BMP from the Buswell formula of water hyacinth compared to applying the same estimation methods on CW. The margin of error could be higher in water hyacinth calculations because of the high content of lignocellulosics in water hyacinth, which reduces the bioavailability of degradables.

3.2.2. Biomethanation Kinetic Studies

The biomethanation experimental data were fitted to the two most popular kinetic models for anaerobic digestion systems. The reported R^2 values indicate a better fit with the Gompertz than with the logistic model (Table 2). These findings corroborate those of Paritosh et al. (2018) and Sunwanee and Chairat (2017), and here the Gompertz model also gave a better fit than the logistic model. Additionally, the model parameters are more realistic in the Gompertz model where the ultimate methane yield values, production rates, and phase lag are close to those that can be read from the graphs (Figure 3). The corresponding values in the logistic model diverge significantly. It may be concluded that plant-based substrate biomethanation is better modelled by the Gompertz model.

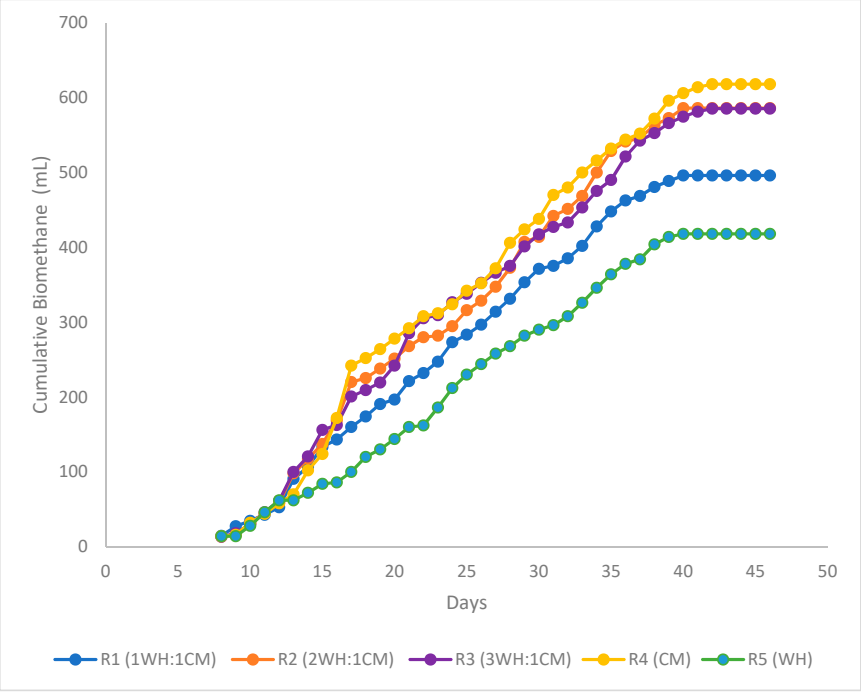


Figure 3. Cumulative biomethane production from BMP assays of WH and CM during mono- and codigestions. Reported gas volumes were normalized to STP conditions.

Table 2. Kinetic modelling parameters for water hyacinth biomethanation.

Kinetic Model	Parameter	R1	R2	R3	R4	R5
Modified Gompertz	B_∞ ($mLCH_4$)	549.47	622.81	526.05	690.53	501.56
	R_m (mL/day)	18.23	21.11	22.75	22.90	15.34
	λ (days)	5.24	5.15	5.28	5.47	6.68
	R^2	0.995	0.990	0.986	0.990	0.996
Logistic	B_∞ ($mLCH_4$)	1377.81	1860.23	1763.66	1907.55	1333.72
	R_m (mL/day)	60.77	69.06	67.46	73.34	51.51
	λ (days)	23.99	27.56	26.36	26.72	28.31
	R^2	0.987	0.985	0.987	0.983	0.995

3.3. Effects of Pretreatment Methods on Water Hyacinth

The biomethanation results of differently pretreated water hyacinths are reported in Figure 4. As displayed in Figure 4, the highest methane yield from pretreated water hyacinth was achieved in the case of aerobic decomposition followed by the oven-dried substrate. Aerobic decomposition resulted in higher water hyacinth biomethane potentials (294 L/kgVS) compared to the optimal 3:1 ratio (WH:CM) codigestion scenario (185 L/kgVS). The oven-dried substrate had a BMP of 217 L/kgVS that was also well above the 3:1 codigestion scheme. However, all other pretreatments could not push BMPs higher to match or exceed that of the 3:1 codigestion. The BMPs for microwave-dried, sun-dried, and autoclave-dried water hyacinth were in the range that matched a 1:1 codigestion. Though these results were higher than the BMPs for untreated water hyacinth, the extra effort and cost of drying equipment and activities for achieving this in the case of microwave, autoclave, and sun drying operations may not justify the small improvements in BMP registered in these cases. It can also be assumed that the low improvements in BMP in the three last pretreatments are a result of the destruction of previously identified microorganisms that are indigenous to water hyacinth roots. Methanogens are known to be sensitive to oxygen and may therefore have been exposed to air during the pretreatment process. Combining a 3:1 codigestion with previously aerobically decomposed water hyacinth may be the best biomethanation strategy for water hyacinth sourced from the Hartbeespoort Dam.

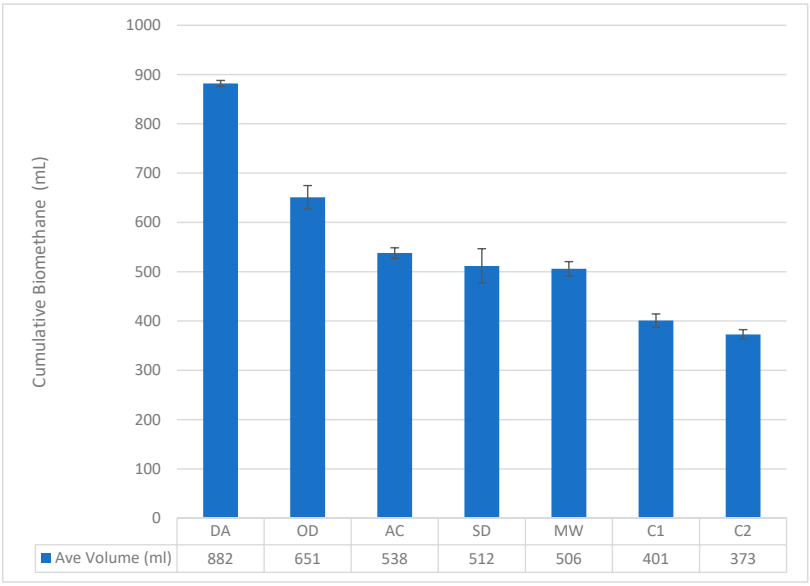
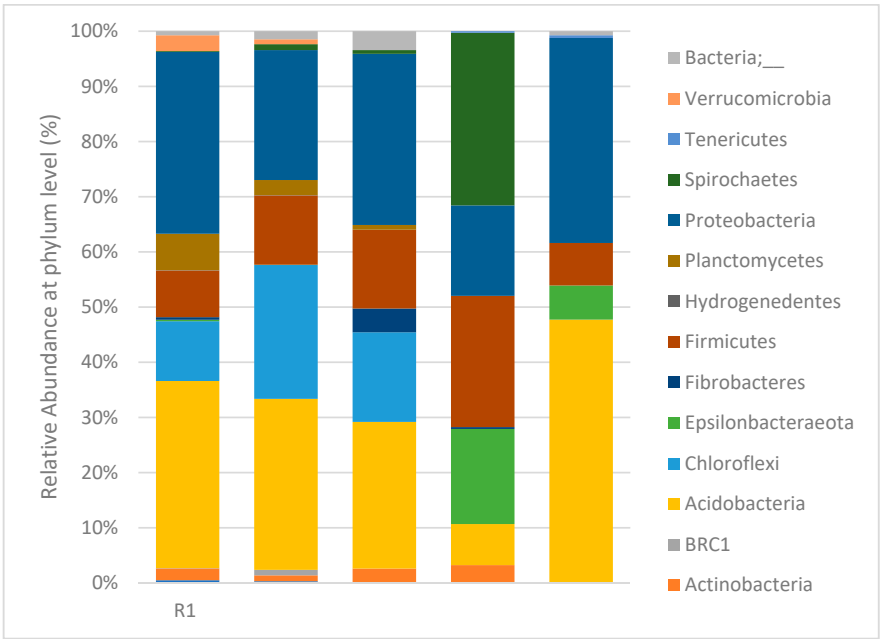


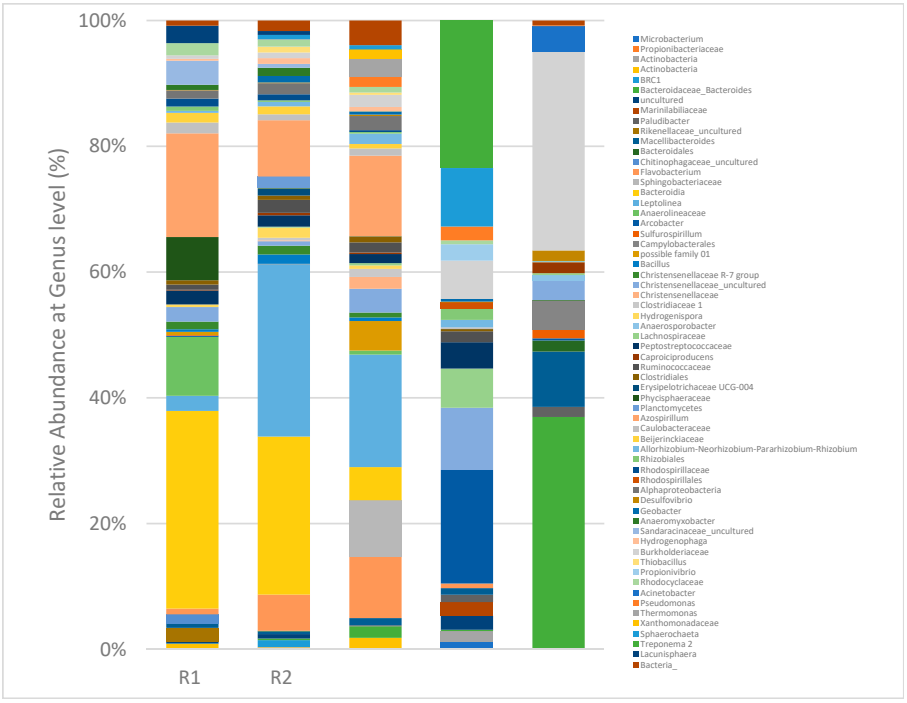
Figure 4. Cumulative biomethane production from the BMP tests of pretreated WH:CM mixtures at a 3:1 ratio. Pretreatments were as follows: C1 and C2 are controls with untreated substrates; aerobic decomposition (DA); oven drying (OD); autoclave (AC); sun drying (SD); microwave (MW). Gas volumes were normalized to STP conditions.

3.4. Digester Microbial Dynamics

The microbial species' diversity and abundances in the digesters at the end of the incubation period are shown in Figure 5a (phylum level) and Figure 5b (genus level). It was evident that the CM:WH digestate contained more diverse microbial consortia arising from both the CM and the water hyacinth. Separately, water hyacinth contained indigenous microorganisms predominantly comprised of Bacteroidetes and Proteobacteria, while low quantities of Firmicutes, Episilonbacteraeota, and Tenericutes were identified. Bacteroidetes promote cellulose hydrolysis and are thus ideal for highly lignocellulosic substrates [31]. Proteobacteria have diverse metabolic functions. Firmicutes, Proteobacteria, and Bacteroidetes are typically the dominant players for the majority of substrates [32,33]. As a result, a low quantity of Firmicutes in water hyacinth may indicate a need for bioaugmentation or supplementation through an inoculum to facilitate efficient biodegradation. On the other hand, CM has more diverse and abundant microbial consortia, comprising all species found in water hyacinth except Tenericutes. In addition to these, CM also showed a high relative abundance of Spirochaetes and low quantities of Fibrobacteres and Actinobacteria. Actinobacteria participate in acidogenesis while Chloroflexi, which are present in both substrates, are responsible for hydrolysis [6]. The combined feed digestates in which water hyacinth was inoculated with CM contained almost all of the microbial species initially detected separately in CM and water hyacinth, though no Episilonbacteria could be found in these combined feed digestates. The relative abundances for all species in the combined feed digestates shifted from those in the separate substrates. The relative abundance of Spirochaetes decreased significantly in the combined substrate digestate when compared to the levels detected in CM. This phylum is rarely mentioned or detected in high abundance in most digester studies, indicating that their role may not be so significant in anaerobic digestion [33,34]. The combined feed digestate also witnessed the emergence of a new species, planctomycetes, which had not been detected in either CM or water hyacinth. The Firmicutes from both CM and water hyacinth survived the combined feed digester conditions and maintained a visible relative abundance in the digesters. This also applies to the Proteobacteria and Bacteroidetes. This is a typical trend that has been observed in many digesters, indicating that these phyla are central to the biogas-producing processes in the digesters [32,33]. The Actinobacteria were introduced into the combined feed digester through CM, and though they survived, their abundance was quite low in the digestates. When the analysis zoomed in to the genus level, it was discovered that the combined feed of water hyacinth and CM digestates had a more diverse culture. This could imply that the combined feed digester conditions were more conducive to microbial proliferation, allowing other species that were dormant in the separate monodigestions to be revived and thrive in the codigestion environment. Normally, this is attributed to a balanced C/N ratio as well as conducive pH/alkalinity controls introduced by codigestion. The majority of microbial species originated from the cow stomach, which is already an anaerobic environment, hence its use as an inoculum and the consequent improvement in the BMP of water hyacinth. To have a better insight into the microbial dynamics involved in WH digestion, and hence be able to optimize processes based on this information, it is recommended that samples for microbial analysis be taken at more points along the incubation duration. Other omics science tools should also be incorporated into the study in future.



(**a**)



(**b**)

Figure 5. Microbial profiles in reactors at the end of the substrate incubation period. (**a**) Phylum level. (**b**) Genus level.

3.5. Digestate Potential as Biofertilizer

The resulting digestates from both mono and codigestion of water hyacinth were combined and analysed for potential use as biofertilizers by analysing the nitrogen (N) as ammonium nitrogen, phosphorous (P), and potassium (K) content. The result was as follows: 68, 73, and 424 mg/kg for nitrogen, phosphorous, and potassium, respectively. Although the nitrogen assay of the digestate in this study was generally low compared to that detected in other digestates from independent studies, which ranged above 2100 mg/kg, the P and K levels are comparable [35,36]. The anomaly picked in nitrogen content can actually depend on many factors, including the levels of that nutrient in the dam water. The

process conditions and microbial profiles existing in the laboratory scale digesters also affect the extent of nutrient release from the substrate into the solution. These laboratory-scale conditions may not be the same as real-life digester conditions. However, based on the NPK results obtained in this study, it can be concluded that water hyacinth digestates can be used for soil conditioning depending on the nutritional demands of crops to be grown as well as compliance with other digestate quality assessments, including pathogenicity, heavy metal toxicity potential, etc. [37]. A full characterization of the digestates to understand these other parameters, including impacts on soil water holding capacity, soil aeration, germination tests, and soil organic content, needs to be carried out in future investigations. These detailed digestate assessments shall be performed in pilot-scale studies as this research advances towards commercialization. Apart from the digestate being used as agricultural fertilizer, other non-conventional applications exist, including growing media for microbes, algae, and plants (biopesticides, biopolymers, and bioplastics), production of briquettes/pellets, and producing bioelectricity through microbial fuel cells [38]. The choice of application depends on market forces and technological improvements required on the digestate before it is marketed [36].

4. Conclusions

This study demonstrated that water hyacinths found in the Hartbeespoort Dam are capable of producing reasonable amounts of biogas for possible commercial exploitation. The NPK assays of water hyacinth digestate are high enough to promote its use as a biofertilizer. The slightly lower biogas yields of water hyacinth compared to those of conventional substrates is a characteristic of most water hyacinths sourced from different areas. Biomethanation can be improved by codigesting with CM. The codigestion increases the C/N ratio towards the optimal level of 25 as well as enriches the slurry's microbial diversity and abundance for improved organics degradation efficiency and rates. Depending on the techno-economic evaluation results, additional biomethanation improvements in water hyacinth can be derived from other pretreatments as well, with the most promising and probably cost-effective method being aerobic decomposition. We recommended further investigations be carried out before scaling up water hyacinth biomethanation to commercial levels. These investigations need to unlock more information regarding the microbial profiling of the slurry throughout the AD period rather than just at the end of the incubation period. Other cosubstrates in addition to CM may also be worth investigating. The quality of digestates pertaining to toxicity from heavy metals and pathogens should also be ascertained. Another exciting component of water hyacinth biomethanation worth studying is the tracking of metabolites during AD. This will allow for subsequent application of metabolomics as well as proteomics science tools in identifying any optimisation opportunities.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the UNISA's College of Science, Engineering and Technology (CSET) Ethics Review Committee and approved on 30 August 2021. The approval reference is 2021/CSET/SOE/048.

Conflicts of Interest: The six authors specified are part of the work by consent and participation and there are no conflicts of interest whatsoever.

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