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Crop Characteristics of Aquatic Macrophytes for Use as a Substrate in Anaerobic Digestion Plants—A Study from Germany

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Abstract: Several thousand metric tonnes of aquatic biomass are removed from water bodies every year, so that these waters can continue to be used for ship and boat traffic and for leisure activities. The mowed material is generally disposed off without any further use. Therefore, the crop properties of samples from 39 weed control measures all over Germany were examined to assess the suitability of aquatic plant biomass as a substrate for anaerobic digestion systems. Analysis of the crop samples consisted of the identification of plant species and the evaluation of sediment contents and concentrations of macroelements. The methane yield was determined for selected aquatic plants. Analysis revealed a carbon/nitrogen ratio (C/N) of between 10 and 20 in 74% of samples. The concentrations of nitrogen and phosphorous in the samples were comparable to grass silages. With regard to heavy metal concentrations, the threshold values for biowaste for nickel, zinc, and cadmium were exceeded in three samples. There were no significant seasonal differences in substrate characteristics and qualities. The specific methane yields of individual aquatic plants were between 142 and 372 L_{CH4}/kg volatile solids (VS). The results of this study showed that aquatic macrophytes can be used as substrates in anaerobic digestion plants without any restrictions.

Keywords: aquatic macrophytes; substrate; anaerobic digestion; crop characteristics; biogas; methane; aquatic biomass

1. Introduction

Aquatic plants are valuable components of aquatic ecosystems. They produce oxygen and perform a water-purification function—for example by binding heavy metals [1] and removing nutrient loads [2–4]. Moreover, aquatic macrophytes can also serve as a hiding place and location for binding for aquatic fauna and its offspring. Nonetheless, it can occur that aquatic plants form plant mats in water as a result of overgrowth; these plant mats can impair the use of bodies of water for humans. This can have serious economic consequences for the operators of these bodies of water. Aquatic plants hinder the passage of ships and boats and impact negatively on the usefulness of bodies of water for leisure purposes (bathing, fishing and other sporting activities). Dead plant remnants can also accumulate at narrow points in bodies of water and hinder the flow of water, which can lead to the backing-up of water or even to flooding. For hydroelectric power plants, such accumulations of aquatic plants in front of turbine inlets represent a serious challenge and can result in significant reductions in output.

In addition to the consequences described above, invasive neophytes (very competitive, non-resident plants with high expansion capacity) can also have a negative influence on competing domestic flora, which may even be completely displaced in extreme cases; this leads to a reduction in the domestic population and its biodiversity [5]. The neophytes that are involved in this regard in bodies of fresh water in Germany include: Water fern (*Azolla filiculoides* LAM.), water pennywort (*Hydrocotyle ranunculoides* L. F.), swamp stonecrop (*Crassula helmsii* (*Kirk*) COCKAYNE, waterweed (*Egeria densa* PLANCH., *Elodea canadensis* MICHX., and *Elodea nuttallii* (PLANCH.) H. ST. JOHN), variable-leaf watermilfoil (*Myriophyllum heterophyllum* MICHX.), straight vallisneria (*Vallisneria spiralis* L.) and duckweed (*Lemna* sp.) [6]. strongly affected by *Elodea* are the reservoir lakes along the Ruhr river in North Rhine-Westphalia, which were completely blocked and overgrown in certain cases, due to their low water levels [7,8].

A comprehensive overview of neophytes and the corresponding management and control strategies has recently been put together by Hussner et al. [9]. There is no single combating measure that is applicable for all invasive neophytes in all types of bodies of water [10]. The use of herbicides is forbidden in Germany for controlling overgrowth of aquatic plants, and thus the removal of aquatic plants by cutting using various items of equipment has become established [11]. The measure results in considerable cost factors for the operators of bodies of water. The cost of controlling individual species of invasive aquatic plants in Europe amounts to several million euros per annum [10]. Herbes et al. (2018) [12] estimated total cost of € 31.68 per tonne of fresh mass for an overgrown lake that contains mainly *Elodea nuttallii.* The authors presumed the use of one small weed-cutting boat with front-mounted machinery (Berky 6410) that alternates between using the cutting and collecting tools.

The harvested aquatic biomass is generally disposed of without being put to any further use. Sometimes it is even left to rot on the shores of the lake, which helps to foster the growth of aquatic plants the following year, due to the supply of nutrients from the rotten biomass. As an alternative, the harvested aquatic plants could be used as a substrate for biogas plants. Initial investigations have shown that the potential of aquatic plants for biogas production should not be underestimated. For example, samples of Western waterweed (*E. nuttallii*) from five different lakes in Germany had yields of 415–520 L_{biogas}/kg volatile solids (VS) [8]. A study by Lizasoain et al. [13] described the possibility of using *Phragmites australis* for anaerobic fermentation. After pretreatment by means of steam explosion was carried out, biogas yields of up to 677 L_{biogas}/kg VS were achieved. Two recent studies from India have demonstrated the anaerobic degradability of duckweed (*Lemna* sp.) [14,15]. Yadav et al. [14] tested the co-digestion of diluted shredded duckweed (*Lemna* sp.) with cattle dung in various ratios. In this study, the highest cumulative biogas production of 12 L was achieved by using a ratio of 1:1 (v/v) of duckweed slurry to cattle dung, which showed good potential for the use of duckweed as a co-substrate in anaerobic digestion (AD). Gaur et al. [15] investigated the effect of thermal pre-treatment of duckweed (*Lemna gibba*) on the specific methane yield (SMY). The authors found that it is possible to achieve an SMY that is up to 24 times higher by autoclaving duckweed at 120 °C (1 bar) for 30 min.

Aquatic plants offer certain advantages, such as no necessity for fertilizers, and no use of agricultural land, when compared to plants conventionally used for energy production, such as maize, sugar beet and grain. In addition, aquatic plants do not compete with agriculturally produced foodstuffs for humans or animal feeds in terms of land use.

Alongside biogas yields, other factors are also of importance in the use of aquatic plants for energy production. One disadvantage that is commonly ascribed to aquatic plants is the entry into biogas fermenters of the sediment that is attached to the aquatic plants, which contributes to a reduction of the active fermenter volume in the long term. Another supposed disadvantage is the high water content of many aquatic plants, which leads to dilution of the digestate and also contributes to the low biogas yield of the fresh mass. On the other hand, aquatic plants provide nutrients and trace elements and can thus be beneficial for the substrate composition [8,16]. For example, it is known that waterweed stores phosphorus in its tissue that it takes both from the water and the sediment [3].

For this reason, the suitability of the harvested aquatic plant material as a biogas substrate was tested in greater detail. Over two harvesting periods (autumn 2015 and summer 2016), the sediment and nutrient contents of samples of harvested aquatic plants from all over Germany were investigated. The biogas-production potential of selected aquatic plants was determined. The results have provided findings that had been lacking in previous research on management strategies, which generally concentrate on the removal, but not the subsequent re-use of the aquatic biomass.

2. Materials and Methods

2.1. Origin of Aquatic Plant Material

In 2015/2016, a nationwide inquiry of the responsible water authorities, river managers and users, as well as operators of mowing boats, was carried out. Thereby, it was determined how much biomass was harvested during the weed controls of flowing and still waters. A total of 1123 questionnaires were sent out, of which 408 responses were received. This corresponds to a return rate of 36.3%. On the basis of this data, weeding was determined from 140 bodies of flowing water and 90 bodies of standing water. This corresponds to a ratio of 1.6:1. Taking this into account, 20 bodies of flowing water and 10 bodies of standing water were selected for the further examination of the aquatic plant biomass (at least 10% per water body type). In addition, samples from the summer mowing and the autumn mowing were examined in order to identify any seasonal differences.

Samples of mowed material were collected by water maintenance staff during mowing measures and sent in buckets by post to the Helmholtz Centre for Environmental Research—UFZ. The sample weight was mostly between 1 and 6 kg. Some bodies of water were sampled multiple times.

The first sampling period was in autumn 2015 and lasted two months (September to October 2015). 26 samples were taken during this time; seven samples originated from standing bodies of water (three lakes, two ponds and one dam) and 19 samples came from 15 flowing bodies of water.

The second sampling period was during summer 2016 (June to August 2016). The total of 13 samples consisted of seven samples from flowing bodies of water and six samples from five standing bodies of water (two ponds, two lakes and one lido).

The harvesting of aquatic plants was performed using mowing boats and various items of auxiliary equipment such a mowing basket, weed rake, slope mower, knife mower and drag scythe.

2.2. Sample Treatment and Analyses

The contents of the buckets were washed in order to quantify the content of sediments and impurities after arrival at the UFZ. The separated sediment was weighed and dried for the determination of its total solid content (TS) according to the standard of the german institute for standardization DIN 12880. Samples of the aquatic plants were taken and prepared for herbarium in order to determine the aquatic plant species. The washed material was weighed and further dried at 60 °C for the determination of TS. The dried material was used for the determination of volatile solids (VS) according to DIN 12879 and for the analysis of carbon and nitrogen contents using a TruSpec elemental analyzer (Leco, St Joseph, MI, USA). The elements aluminum, boron, calcium, iron, potassium, magnesium, manganese, phosphorous, sulfur and zinc in the plant samples were measured using the ICP-OES (ARCOS, SPECTRO Analytical Instruments GmbH, Kleve, Germany) according to the US-EPA method 200.7. The elements arsenic, cadmium, cobalt, chrome, copper, molybdenum, nickel and lead were measured with the ICP-MS (ICAPQs, Thermo Fisher Scientific GmbH, Bremen, Germany) according to the US-EPA method 200.8, due to their lower limit of detection.

2.3. Biochemical Methane Potential Test

The methane yield from selected aquatic plants was determined according to the German guideline for the fermentation of organic materials VDI 4630. Aquatic plants from mowing measures (sample nos. 3, 7, 12, 20, 21 and 37; TS and VS see Tables 1 and 2) were chopped into 4 mm pieces and stored at −18 °C until they were used to determine their methane potential. In addition, fresh samples of four aquatic plants (*E. nuttallii* (TS = 6.42% fresh mass (FM); VS = 79.65%TS), *M. heterophyllum* (TS = 5.71% FM; VS = 73.92%TS), *Ranunculus fluitans* (TS = 6.42% FM; VS = 82.21%TS) and *Callitriche* sp. (TS = 6.52% FM; VS = 61.88%TS)) were harvested from flowing bodies of water in Leipzig (the Parthe river and Karl-Heine-Kanal), chopped in a cutting mill (SM2000, Retsch, Haan, Germany) with a milling coarseness of 4 mm and used for the determination of their methane potential without any storage phase.

In the case of aquatic plant sample nos. 3, 12, 20, 21 and 37, the Automatic Methane Potential Test System (AMPTS, Company Bioprocesscontrol, Lund, Schweden) of the German Biomass Research Centre (DBFZ) was used for the determination of biochemical methane potential (BMP). The samples consisted of 400 g inoculum and app. 2.5 g volatile solids (VS) of the sample material, each in 3 replications. The pure inoculum was measured to determine its methane yield and to subtract it from the other samples. The inoculum consisted of digestate of the research biogas plant of the Deutsches Biomasseforschungszentrum (DBFZ) which was operated with cattle manure and corn silage as substrate material. The inoculum was sieved and incubated without feeding for three days at room temperature prior its use. The test was terminated after the daily gas production was below 0.5% of the total gas production for 5 consecutive days. The BMP test was operated under mesophilic conditions (38 °C); microcrystalline cellulose was used as reference substrate. The methane yields were standardized (273.15 K, 1.01325×10^5 Pa).

The methane potential of the remaining sample no. 7 and of aquatic plants harvested from flowing bodies of water in Leipzig was determined in a fermentation batch test system (FBTS) at the Helmholtz Centre for Environmental Research—UFZ as described in Moeller et al. [17]. The digestate used as inoculum originated from the research anaerobic digestion (AD) plant of the DBFZ in all tests. This material was first sieved through a sieve with a mesh size of 5 mm and then incubated anaerobically for one week at 37 °C in 5 L bottles in a tempered incubator for outgassing prior to being used for batch experiments. The digestate had the following characteristics after sieving: TS = 7.2 ± 0.2%; VS = 76.4 ± 0.6% TS; 221 ± 36 mg/L acetate; 19 ± 3 mg/L propionate; <1 mg/L butyrate.

The 500-mL-test bottles containing 400 mL of the mixtures of the inoculum and aquatic plants as substrate were incubated at mesophilic temperature (38 °C). Each variant was performed three times in order to ensure statistical reliability. The methane volume was measured continuously in the case of AMPTS systems. In the case of FBTS, the methane percentage in biogas was determined twice a week by gas chromatography with an Agilent GC 6850 WLD wavelength detector (Agilent Technologies, Santa Clara, CA, USA) using an HP Plot separation column and argon as carrier gas. The normalized volume of the methane was calculated according to the VDI 4630 guideline. The fermentation batch tests lasted until the termination criterion (i.e., daily biogas rate equivalent to less than 1% of the total volume of biogas produced up to that time) was achieved.

Table 1. Properties of plant material in samples from standing bodies of water (samples 8–13 are from summer harvesting).

Sample No.	Federal State	Type of Water Body	Plant Species in the Sample	Sediment Content (% TS)	$TS_{Biomass}$ (%FM)	$VS_{Biomass}$ (% TS)
1	BE	Lake	Water-starwort (*Callitriche* sp.)	0.91	11.3	74.7
2	SX	Lake	Watermilfoil (*Myriophyllum heterophyllum*, *M. spicatum*)	2.21	6.76	81.0
3	BW	Lake	Water horsetail (*Equisetum fluviatile*)	0.90	19.2	82.5
4	SX	Dam	Spiny water nymph (*Najas marina*)	0.44	5.00	77.6
5	BW	Pond	Broadleaf cattail (*Typha latifolia*), Lakeshore bulrush (*Schoenoplectus lacustris*)	0.86	9.20	93.9
6	BW	Pond	Yellow water-lily (*Nuphar lutea*)	3.10	14.4	80.9
7	BW	Pond	Yellow water-lily (*Nuphar lutea*)	2.70	5.90	86.3
8	NRW	Lido	Watermilfoil (*Myriophyllum* sp.)	13.40	19.2	47.2
9	BA	Lake	Bur-reed (*Sparganium erectum*)	0.30	7.6	71.1
10	BA	Lake	Sago pondweed (*Potamogeton pectinatus*)	0.80	24.5	75.8
11	BA	Lake	Sago pondweed (*Potamogeton pectinatus*)	0.52	12.1	66.3
12	BA	Lake	Western waterweed (*Elodea nutallii*)	0.04	8.31	82.4
13	BA	Lake	Floating pondweed (*Potamogeton natans*)	0.40	11.0	53.0

BA—Bavaria, BE—Berlin, BW—Baden-Württemberg, NRW—North Rhine-Westphalia, SX—Saxony.

Table 2. Properties of plant material in samples from flowing bodies of water (samples 33–39 are from summer harvesting).

Sample No.	Federal State	Type of Water Body	Plant Species in the Sample	Sediment Content (% TS)	$TS_{Biomass}$ (% FM)	$VS_{Biomass}$ (% TS)
14	NRW	River	Bur-reed (*Sparganium* sp.)	0.57	11.9	84.2
15	NRW	River	Bur-reed (*Sparganium* sp.)	0.40	8.40	89.3
16	NRW	Ditch	Reed (*Phragmites australis*), Hornwort (*Ceratophyllum* sp.)	0.68	5.62	90.5
17	MWP	Ditch	Floating sweet-grass (*Glyceria fluitans*), Duckweed (*Lemna minor*)	2.00	10.4	87.6
18	BB	Ditch	Bur-reed (*Sparganium* sp.)	0.33	19.6	84.6
19	BB	River	Reed (*Phragmites australis*), Floating sweet-grass (*Glyceria fluitans*)	0.09	10.8	87.5
20	BB	River	Floating sweet-grass (*Glyceria fluitans*)	0.68	6.07	81.6
21	BB	River	Reed (*Phragmites australis*)	8.70	14.0	93.8
22	BW	Channel	Bur-reed (*Sparganium* sp.)	0.73	7.71	84.4
23	BW	Ditch	Western waterweed (*Elodea nutallii*)	0.40	5.45	88.1
24	HB	Channel	Watermilfoil (*Myriophyllum spicatum*)	1.90	10.1	77.9
25	HB	Ditch	Canadian waterweed (*Elodea canadiensis)*, Duckweed (*Lemna minor*)	2.85	9.28	78.6
26	SX	River	Western waterweed (*Elodea nutalii*)	0.18	7.40	78.5
27	LS	Channel	Sedge (*Carex* sp.), Bur-reed (*Sparganium* sp.), Iris (*Iris* sp.)	3.90	13.2	88.7
28	LS	Channel	Sedge (*Carex* sp.), Waterweed (*Elodea nutallii, E. canadensis*)	0.17	9.30	88.9
29	BW	Channel	River water-crowfoot (*Ranunculus fluitans*), Duckweed (*Lemna minor*)	0.94	8.20	85.8
30	BB	River	Reed (*Phragmites australis*)	0.22	22.0	91.6
31	MWP	River	River water-crowfoot (*Ranunculus fluitans*)	0.40	8.45	81.0
32	SA	River	Bur-reed (*Sparganium erectum*), Duckweed (*Lemna minor*)	1.10	7.20	73.2
33	BB	Channel	Watermilfoil (*Myriophyllum* sp.), Duckweed (*Lemna minor*), Reed (*Phragmites australis*)	0.30	10.8	59.0
34	BB	Channel	Watermilfoil (*Myriophyllum* sp.), Duckweed (*Lemna minor*), Water lily (*Nymphaea* sp.)	0.60	8.1	77.7
35	LS	Channel	Reed (*Phragmites australis*), Hedge grasses	0.80	35.8	88.5
36	BA	River	River water-crowfoot (*Ranunculus fluitans*)	0.80	5.0	61.1
37	NRW	River	Bur-reed (*Sparganium* sp.)	0.30	5.2	77.0
38	BB	River	Bur-reed (*Sparganium erectum*), Duckweed (*Lemna minor*), Water lily (*Nymphaea*)	0.22	5.2	80.4
39	SX	River	Duckweed (*Lemna minor*), Reed (*Phragmites australis*)	0.07	6.2	81.2

BA—Bavaria, BB—Brandenburg, BW—Baden-Württemberg, HB—Hanseatic City of Bremen, LS—Lower Saxony, MWP—Mecklenburg-Western Pomerania, NRW—North Rhine-Westphalia, SA—Saxony-Anhalt, SX—Saxony.

3. Results and Discussion

3.1. Aquatic Plant Species

The properties of the individual harvested samples with regard to plant species and sediment contents are summarized in Table 1 for standing bodies of water and in Table 2 for flowing bodies of water. In total, 18 aquatic plant species were identified in the samples. Interestingly, only one mixed sample (sample no. 5) came from standing bodies of water, whereas the other samples all contained just one species. In the case of the flowing bodies of water, the ratio of mixed samples to single-species samples was one to one.

Bur-reed (*Sparganium* sp.), which was present in a total of nine samples, was most commonly represented, followed by duckweed (*L. minor*) in eight samples and reed (*P. australis*) in seven samples (Figure 1). All these aquatic plants were primarily harvested in flowing bodies of water in both summer and autumn. The only clear trend as regards seasonal dependency was identified in the case of the spiny water nymph (*Nymphaea* sp.) and the sago pondweed (*Potamogeton* sp.), as these species were only found in the summer samples.

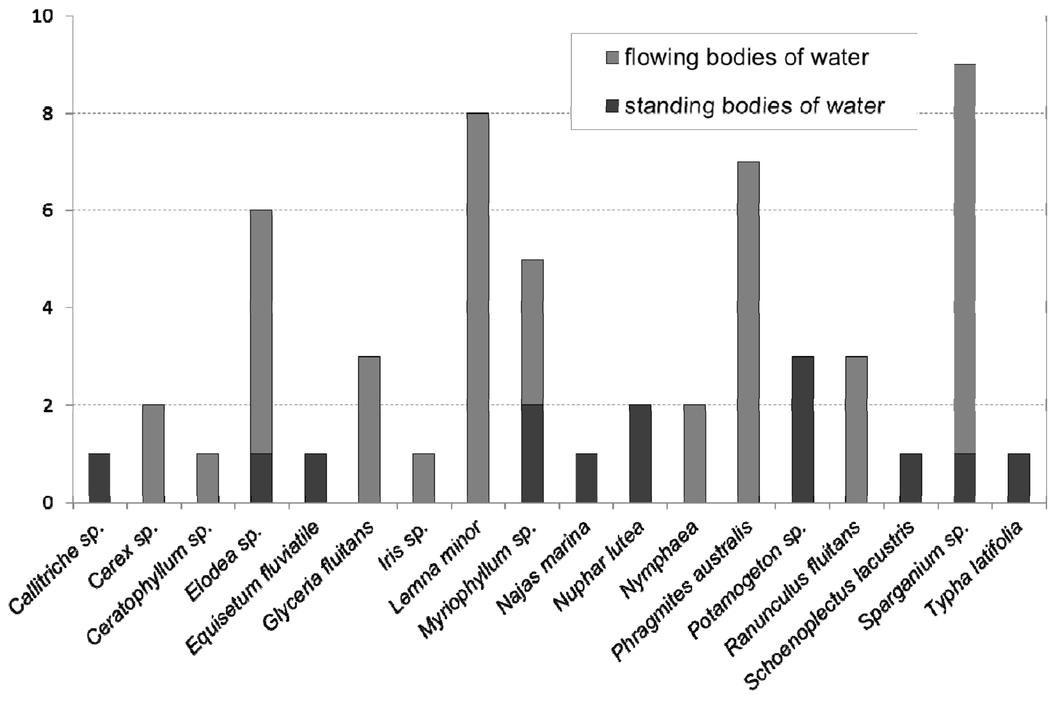

Figure 1. Number of individual plant species in the probes investigated, sorted by type of body of water (standing versus flowing).

Classifying the plant species in terms of their growth forms, ten of them are hydrophytes (aquatic plants that are attached to or rooted in the bottom), seven are helophytes (shore or marsh plants) and one is a pleustophyte (freely floating aquatic plants).

Neophytes were present in 14 of the samples, and a number of neophytes were present together in some samples (see also Tables 1 and 2). Duckweed (*L. minor*) was identified in eight samples, waterweed species (*Elodea* sp.) in six samples and variable-leaf watermilfoil (*M. heterophyllum*) in four samples.

3.2. Sediments and Extraneous Materials

As illustrated by the data in Tables 1 and 2, the sediment content of the harvested material was less than 1% TS in 74% of the samples, and the sediment content was above 4% TS in just two cases (samples 8 and 21). The aquatic plant samples from summer harvesting contained significantly less sediment than the autumn samples: With one exception, the sediment content of the summer samples was less than 1% TS. In general, the sediment most frequently contained sludge (in 33 samples), and also sand (in four samples) and gravel (in one sample).

The most frequently occurring extraneous materials were branches and leaves in three samples from the autumn harvesting. Three samples also each contained shells and plastic components from packaging and bottles.

Before the samples were subjected to further analyses, these materials were removed from the samples and the sediment was washed.

No similar investigations to determine the sediment contents of harvested aquatic plant material can be found in the literature up to now. In order to classify the results presented in Tables 1 and 2, the sediment contents in five substrates from operational agricultural biogas plants—two maize silages and three grass silages—were investigated. All these substrates had a sediment content of greater than 1% TS (see Table 3). In one case, grass silage even had a sediment content of 6.4% TS, i.e., higher than 95% of the aquatic plant samples.

Table 3. Sediment contents in silages from operational biogas plants.

Silage	Sediment Content (%TS)
Maize #1	2.20
Maize #2	1.30
Grass #1	1.10
Grass #2	2.00
Grass #3	6.40

To summarize, it was observed that the sediment content in 37 of 39 samples of aquatic plant material was less than 4% and thus does not represent a hindrance to use as a substrate in biogas plants. However, scientists have reported that unexpected objects, such as plastic bottles, drinks cans, bicycles, car tires and shopping trolleys are often encountered during actual harvesting of aquatic plants from bodies of water (Liegl (Liegl GmbH & Co. KG, Laupheim, Germany) and Podraza (Ruhrverband, Essen, Germany), personal communication). These objects must be removed by hand before the harvested material is put to any subsequent use.

3.3. Properties of the Harvested Material

The TS content of the biomass from aquatic plants after it was washed to remove attached sediment was 10.9 ± 6.37% FM on average (Tables 1 and 2); the average TS contents of 10.3 ± 4.5% FM in summer were lower than the values of 12.2 ± 9.1% FM in autumn. Helophytes in the single-plant samples had a higher average TS content than hydrophytes (12.2 ± 6.2% FM vs. 10.3 ± 5.5% FM, respectively). The high TS content of watermilfoil in sample no. 8 of 19.2% FM can be ascribed to the high sediment content. Separation of the sediment from the plant matter was probably not completely successful in this case. The rather low VS content of this sample of 47.2% also supports this explanation.

The VS content of the aquatic plant biomass (APB) was 79.8 ± 10.6% TS on average. As is the case with TS content, plant-specific differences can also be identified here. Submerged and emergent plants had lower average VS contents of 74.1 ± 12.0% TS than helophytes at 84.0 ± 6.7% TS.

The average C/N ratio was 17.9 ± 6.9 for all aquatic plants (Tables 4 and 5). No sample had a C/N ratio less than 10 or higher than 40. The highest values were measured in samples from autumn harvesting where reed was the main component (34.6, 34.1 and 31.1 in samples 16, 35 and 30, respectively). Kobayashi et al. [18] investigated the characteristics of eight submersed aquatic macrophytes from lakes in Japan (*Hydrilla verticillata* L. F. ROYLE, *Potamogeton x inbaensis* KADONO, *P. dentatus* HAGSTR., *P. malaianus* MIQUEL, *Ceratophyllum demersum* L., *P. perfoliatus* L., *Myriophyllum aquaticum* (VELL.) VERDC. and *E. densa* PLANCH). The C/N ratio varied between 8.5 and 12.9, with an average of 10.3 [18]. However, the optimal C/N ratio of substrates for anaerobic fermentation ranges between 15 and 30 [19]. This applies for 49% of aquatic plant samples investigated in Germany (Tables 4 and 5). 74% of all samples had a C/N ratio in the range 10–20. Co-fermentation with carbon-rich substrates would be beneficial for these samples.

Table 4. Carbon and nitrogen contents in plant material in samples from standing bodies of water (Samples 8–10 are from summer harvesting).

Sample No.	Nitrogen (g/kg TS)	Carbon (g/kg TS)	C/N
1	30.1	354	11.8
2	24.8	360	14.5
3	24.3	385	15.8
4	19.9	367	18.4
5	18.2	398	21.9
6	16.0	397	24.8
7	24.3	385	15.8
8	6.03	172	28.6
9	36.0	396	11.0
10	17.3	371	21.4
11	16.0	325	20.3
12	20.1	283	14.1
13	19.9	363	18.3

Table 5. Carbon and nitrogen contents in plant material in samples from flowing bodies of water (samples 33–39 are from summer harvesting).

Sample No.	Nitrogen (g/kg TS)	Carbon (g/kg TS)	C/N
14	26.6	397	14.9
15	26.2	393	15.0
16	12.2	422	34.6
17	14.2	419	29.5
18	27.3	361	13.2
19	24.1	397	16.5
20	29.3	424	14.5
21	12.8	436	34.1
22	28.8	394	13.7
23	25.9	389	15.0
24	25.1	350	14.0
25	23.5	356	15.1
26	32.8	354	10.8
27	24.2	430	17.7
28	26.7	422	15.8
29	30.4	456	13.7
30	14.0	435	31.1
31	33.9	383	11.3
32	26.2	345	13.2
33	16.2	292	18.1
34	34.9	349	10.0
35	11.7	400	34.1
36	25.9	314	12.1
37	28.4	368	13.0
38	27.7	403	14.6
39	27.2	389	14.3

In the context of the use of aquatic plants as fertilizers or as substrates in biogas plants, it is advantageous to know the concentrations of the most important macroelements and heavy metals. The results of the analyses are summarized in Tables 6 and 7. The concentrations of nitrogen (23.3 ± 7.1 g/kg TS) and phosphorus (2.73 ± 1.41 g/kg TS) were comparable with literature values for grass silages with an N content of 23.5 ± 4.25 g/kg TS and a P content of 2.8 ± 0.50 g/kg TS [20].

In contrast, the potassium concentrations of 12.8 ± 8.9 g/kg TS on average were significantly less than the potassium concentrations of grass silages (23.5 ± 6.01 g/kg TS [20]). With regard to heavy metals, 36 of 39 samples were under the German legal limit values for biogenic waste [21]. The threshold concentration of nickel was exceeded in the case of sample no. 2; the zinc legal limit was exceeded very slightly in the case of sample no. 1. Sample no. 31 showed higher values of two elements–cadmium and zinc. As stated in the literature [16,22,23], the heavy metal contents in the aquatic biomass depend on their concentration in water and sediment, and vary considerably on a regional basis.

Table 6. Concentrations of elements in plant material in samples from standing bodies of water.

Element	Mean Value (mg/kg TS)	Standard Deviation	Minimum (mg/kg TS)	Maximum (mg/kg TS)	Legal Limit [1] (mg/kg TS)
Al	827	1151	13.0	3320	
As	1.47	1.32	0.17	4.35	
B	19.4	10.4	6.11	42.0	
Ca	38,860	31,906	8630	119,650	
Cd	0.17	0.21	0.02	0.47	1
Co	4.44	9.71	0.07	33.0	
Cr	1.84	2.01	0.27	6.44	70
Cu	16.7	21.5	1.6	62.0	70
Fe	2181	2785	61	7880	
K	12,662	11,780	2310	46,330	
Mg	2967	1094	842	4210	
Mn	868	1026	28.8	2810	
Mo	0.34	0.30	0.08	0.68	
Ni	6.46	12.1	0.43	42.0	35
P	1847	908	474	3190	
Pb	2.40	2.81	0.11	7.80	100
S	3065	1441	1270	6570	
Zn	57.5	90.7	8.92	305	300

[1] according to German regulation on the reclamation of bio waste of agricultural, sivicultural or horticultural soils "BioAbfV" [21].

Table 7. Concentrations of elements in plant material in samples from flowing bodies of water.

Element	Mean Value (mg/kg TS)	Standard Deviation	Minimum (mg/kg TS)	Maximum (mg/kg TS)	Legal Limit [1] (mg/kg TS)
Al	844	710	57.5	2690	
As	3.11	4.40	0.10	22.0	
B	121	209	5.01	891	
Ca	24,778	32,865	4220	175,500	
Cd	0.36	0.41	0.06	1.40	1
Co	4.45	6.89	0.09	27.0	
Cr	2.23	1.82	0.19	6.60	70
Cu	17.9	14.2	1.90	54.0	70
Fe	6021	6125	360	24,130	
K	12,247	7180	1960	32,600	
Mg	1935	696	662	3220	
Mn	5320	6237	110	26,020	
Mo	1.01	0.86	0.14	3.80	
Ni	3.72	3.78	0.51	15.0	35
P	3209	1529	932	8320	
Pb	4.80	5.04	0.37	17.0	100
S	3535	1593	1520	6760	
Zn	103	162	15.0	815	300

[1] according to German regulation on the reclamation of bio waste of agricultural, sivicultural or horticultural soils "BioAbfV" [21].

The concentrations of trace elements, such as molybdenum, iron, zinc, nickel and cobalt [24], that are important for anaerobic digestion are shown in Tables 6 and 7. Compared to the trace elements concentrations in maize silage published by Pobeheim et al. [25], lower molybdenum (maize silage (MS): 0.8 g/kg TS versus APB: 0.34 ± 0.30 mg/kg TS), but higher iron (MS: 80 mg/kg TS versus APB: 2181 ± 2781 mg/kg TS), cobalt (MS: <0.05 mg/kg TS versus APB: 4.44 ± 9.71 mg/kg TS), and nickel (MS: 0.7 mg/kg TS versus APB: 6.46 ± 12.1 mg/kg TS) concentrations are present in APB.

3.4. Methane Potential of Aquatic Plants

Determination of the methane yield of aquatic plants is essential in order to evaluate their potential use in biogas plants. The experiments carried out showed that the plants have good digestibility (see Table 8). The highest biogas and methane yields were achieved by floating sweet grass (372 ± 19 L_{CH4}/kg VS). Reed had the lowest specific methane yield (142 ± 4.5 L_{CH4}/kg VS), even though its methane yield per unit fresh mass was the highest at 50.6 ± 4.4 L_{CH4}/kg VS. In addition, reed also had the lowest methane concentration at the end of the experiment at 53%. The poor biogas production when reed is used is mainly due to the high degree of lignification of the blades of reed, as already described by Lizasoain et al. [13]. If the overall analysis results for all aquatic plants from the two harvesting campaigns in Germany are considered, non-pretreated reed can be identified as the least suitable substrate for anaerobic fermentation.

Table 8. Methane yields of selected aquatic plants.

Origin/Sample No.	Aquatic Plant Species	Test System	Methane Yield (L/kg FM)	Specific Methane Yield (SMY) (L/kg VS)
3	Water horsetail (*Equisetum fluviatile*)	AMPTS	20.2 ± 1.1	190 ± 10
7	Yellow water-lily (*Nuphar lutea*)	FBTS	19.9 ± 2.7	202 ± 22
12	Western waterweed (*Elodea nuttallii*)	AMPTS	13.9 ± 0.4	204 ± 6.0
20	Floating sweet-grass (*Glyceria fluitans*)	AMPTS	18.1 ± 0.9	372 ± 19
21	Reed (*Phragmites australis*)	AMPTS	22.2 ± 1.5	169 ± 12
37	Bur-reed (*Sparganium* sp.)	AMPTS	16.6 ± 0.2	223 ± 2.6
	Fresh material			
Parthe	Western waterweed (*Elodea nuttallii*)	FBTS	12.3 ± 0.5	233 ± 11
Karl Heine Canal	Watermilfoil (*Myriophyllum heterophyllum*)	FBTS	8.8 ± 2.2	160 ± 26
Parthe	River water-crowfoot (*Ranunculus fluitans*)	FBTS	15.7 ± 2.4	222 ± 6.0
Parthe	Water-starwort (*Callitriche* sp.)	FBTS	12.0 ± 0.5	292 ± 34

AMPTS—automatic methane potential test system, FBTS—fermentation batch test system.

The calculated specific methane yields (Table 8) are quite low compared to energy crops, but do achieve gas yields similar to those of farm-produced fertilizers, such as cattle slurry, in certain cases at values of 242.7 ± 60.2 L_{CH4}/kg VS [26] or of non-pretreated hay at values of 243 L_{CH4}/kg VS [27]. Relatively low biogas yields are less critical for aquatic plants, as they are a residual material that results from harvesting that would have to be carried out anyway and this material would also have to be disposed of in any case. In addition, aquatic plants contain trace elements that could be significant in the stabilization of the anaerobic biogas process [8].

Labatut et al. [26] determined the methane yields of many different complex substrates, including the four aquatic neophytes of Eurasian milfoil (*Myriophyllum spicatum* L.), frogbit (*Hydrocharis morsus-ranae* L.), water chestnut (*Eleocharis dulcis* (BURM. F.) TRIN. EX HENSCH.) and water celery (*Apium inundatum* (L.) W.D.J.KOCH) from Oneida lake and Oneida river. The methane yields ranged between 279 and 451 L_{CH4}/kg VS, with *M. spicatum* responsible for the lowest value. These methane yields are significantly higher than those achieved in the batch test described here (160 ± 26 L_{CH4}/kg VS in Table 8). However, the value provided by Labatut et al. [26] is the average of two values with a very high standard deviation of around 148 L_{CH4}/kg VS (measured from the graphic in Figure 1 of the article).

Most studies confine themselves to anaerobic fermentation of submersed aquatic plants. Samples of *E. nuttallii* from five different locations in Germany were investigated in Zehnsdorf et al. [8]. The reported methane yields ranged from 261.8 to 301.6 L_{CH4}/kg VS. This is slightly higher than the values of 206 and 233 L_{CH4}/kg VS presented in Table 8. The eight submersed aquatic plants investigated by Kobayashi et al. [18] produced methane yields of 275 (*E. crappipes*) to 418 L_{CH4}/kg VS (*M. aquaticum*). In the case of five submersed macrophytes from Lake Biwa in Japan analysed by Koyama et al. [28] (*Ceratophyllum demersum* L., *E. densa*, *E. nuttallii*, *Potamogeton maackianus* A. BEN and *Potamogeton malaianus* MIQUEL), the methane yields ranged from 161.2 to 360.8 L/kg VS depending on the species being investigated. The highest methane yield was achieved for the fermentation of *E. nuttalii* and was higher than the methane yields presented in Table 8. The lowest methane yield was achieved in the case of fermentation of *P. maackianus*. The reason given for this low digestibility was the high degree of lignification of the tissue, as the lignin content of this plant was up to 20.7% TS. In comparison, *E. nuttallii* contained just 3.2% lignin in its dry matter [28].

Aquatic helophytes, such as reed, also have a high degree of lignification. Lizasoain et al. [13] reported a methane yield of 188 L_{CH4}/kg VS for non-pretreated reed, which is slightly higher than the methane yield of 169 ± 12 L_{CH4}/kg VS presented in Table 8. By employing suitable pre-treatment with the aid of steam explosion (220 °C, 15 min), the authors achieved a specific methane yield that was up to 89% higher than that of untreated reed [13]. Further experiments on lignin pulping were carried out by Koyama et al. [29] using the aquatic plant *P. malaianus*. The methane yield was increased by 66% from 161 L_{CH4}/kg VS for untreated material to 243 L_{CH4}/kg VS after alkaline thermochemical pre-treatment. However, the same authors showed in a different article [30] that the products of this type of pre-treatment have a negative influence on the microbial hydrolysis stage of the biogas process. In addition, it must be considered whether the benefits for anaerobic fermentation justify the increased costs for pre-treatment. It would be a better idea to adapt the use of aquatic macrophytes to suit their properties: Aquatic plants with low lignin contents can be used for biogas production, while plants with a high lignin content are more suitable as a structural material for composting.

4. Conclusions

To conclude, the sediment content of aquatic plant material from two harvesting campaigns from waters in Germany was not greater than in conventional silages. Analyses of macroelements nitrogen and phosphorus showed results similar to those of grass silages. The methane yields between 142 and 372 L_{CH4}/kg VS were comparable to those of farm-produced agricultural residues. These results show the great diversity in the quality of the aquatic biomass, which depends more on the prevailing plant species than on the harvest time or the location and the type of water body. In general, it was shown that the aquatic plants are well suited as a substrate in biogas plants.

This is the first study to investigate the suitability of aquatic plant material for anaerobic fermentation in Germany. For the future, integrating the harvested biomass into the substrate mix in a biogas plant could be a major challenge. For this reason, further investigations into the political framework conditions and national legislature are of great importance.

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