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Characterisation of Congolese Aquatic Biomass and Their Potential as a Source of Bioenergy

Tania S. Mayala [1,2], Maryse D. Nkoua Ngavouka [1,2], Dick H. Douma [1,2], James M. Hammerton [3], Andrew B. Ross [3,*], Aaron E. Brown [3], Bernard M'Passi-Mabiala [1,2] and Jon C. Lovett [4]

1 Faculté des Sciences et Techniques, Université Marien Ngouabi, Brazzaville BP.69, Congo; mayalatania@gmail.com (T.S.M.); maryse.dadina@gmail.com (M.D.N.N.); dick.douma@umng.cg (D.H.D.); bmpassimabiala@gmail.com (B.M.-M.)
2 Institut National de Recherche en Sciences Exactes et Naturelles, Unité de Recherche en Matériaux et Energies, Brazzaville BP.2400, Congo
3 School of Chemical and Process Engineering, University of Leeds, Leeds LS2 9JT, UK; j.m.hammerton@leeds.ac.uk (J.M.H.); a.e.brown@leeds.ac.uk (A.E.B.)
4 School of Geography, University of Leeds, Leeds LS2 9JT, UK; j.lovett@leeds.ac.uk
* Correspondence: a.b.ross@leeds.ac.uk; Tel.: +44-(0)113-343-1017

Abstract: This study assesses the bioenergy potential of two types of aquatic biomass found in the Republic of Congo: the green macroalgae *Ulva lactuca* (UL) and *Ledermanniella schlechteri* (LS). Their combustion behaviour was assessed using elemental and biochemical analysis, TGA, bomb calorimetry and metal analysis. Their anaerobic digestion behaviour was determined using biochemical methane potential (BMP) tests. The average HHV for LS is 14.1 MJ kg^{-1}, whereas UL is lower (10.5 MJ kg^{-1}). Both biomasses have high ash contents and would be problematic during thermal conversion due to unfavourable ash behaviour. Biochemical analysis indicated high levels of carbohydrate and protein and low levels of lipids and lignin. Although the lipid profile is desirable for biodiesel production, the levels are too low for feasible extraction. High levels of carbohydrates and protein make both biomasses suitable for anaerobic digestion. BMP tests showed that LS and UL have an average of 262 and 161 mL CH$_4$ gVS^{-1}, respectively. The biodegradability (BI) of LS and UL had an average value of 76.5% and 43.5%, respectively. The analysis indicated that these aquatic biomasses are unsuitable for thermal conversion and lipid extraction; however, conversion through anaerobic digestion is promising.

Keywords: macroalgae; aquatic biomass; *Ledermanniella schlechteri*; *Ulva lactuca*; characterisation; bioenergy; thermochemical; anaerobic digestion

1. Introduction

Biomass is the main source of energy in the rural areas of most developing countries, and globally biomass contributes approximately 14% of the world's energy supply [1,2]. International commitments to Sustainable Development Goal 7, which is to "Ensure access to affordable, reliable, sustainable and modern energy for all", combined with a continued shift away from fossil fuels and increasing populations, ensure that biomass will remain a major source of energy in the future. Biomass and charcoal are used extensively for cooking in the Republic of Congo, resulting in poor air quality and associated health impacts. This firewood and charcoal is often unsustainably sourced from the Congo Basin forest, resulting in deforestation and reduced biodiversity and carbon storage capacity [3]. The identification of alternative biomass feedstocks can relieve the pressure on these forests and may also promote the development of alternative conversion routes such as using biogas for cooking.

The physio-chemical properties which significantly affect the choice of biomass for conversion to bioenergy include biochemical composition (carbohydrate, protein and lignin

content), elemental content (C, H, N, S, O), calorific value (HHV) and proximate analysis (moisture, volatile, fixed carbon and ash content) [2]. These properties not only determine the suitability of a conversion process, but also influence the cost of the conversion technology. Characterisation of biomass is thus a crucial step when assessing new feedstocks for bioenergy production. The following tools are routinely used for its characterisation: elemental analysis to determine the ultimate analysis (C, H, N, S, O), proximate analysis to determine its moisture, volatile fixed carbon and ash content determination, X-ray fluorescence (XRF) spectroscopy, AAS or ICP-MS to determine metals and mineral matter and gas chromatography mass spectroscopy (GC-MS) to determine lipid profiles. Another important technique used to predict the thermal behaviour of biomass during pyrolysis or combustion is thermogravimetric analysis (TGA), in which the mass loss of the feedstock is determined as a function of temperature, under controlled heating rates and atmospheric conditions [4].

Among the most popular biomass feedstocks used for bioenergy production are woods, agricultural wastes, industrial residues, municipal solid waste, and sawdust [5]. Aquatic plants such as algae have recently emerged as potential feedstocks for biofuel production, largely due to their high lipid and carbohydrate contents [6–8]. For large-scale production purposes, algae can be cultivated in both freshwater and marine environments, in either open culture systems such as lakes, ponds or basin raceways; or in highly controlled closed culture systems called photobioreactors [5]. Focus has mainly been directed towards the use of microalgae species; however, macroalgae and other freshwater aquatic macrophytes have also received attention in recent years [9,10]. One such macroalgae is *Ulva* spp., including *Ulva lactuca*, which can be found throughout the marine environments of the Republic of Congo. *Ulva lactuca* is the most abundant macroalgae in the coastal waters of Alexandria, Egypt [11], and represents an unexplored natural resource with potential economic value for use in human and animal nutrition and as a potential biofuel resource [12].

U. lactuca has many potential applications, for instance, it is edible and is a source of essential amino acids; it is also a source of bioactive compounds [13]. The utilisation of *Ulva lactuca* as a feedstock for bioenergy has also been investigated widely by a number of researchers. Bikker et al. [14] presented a biorefinery approach utilising *Ulva lactuca* for the production of animal feed, chemical and biofuels. A sugar-rich hydrolysate containing 38.8 gL^{-1} sugars and a protein-enriched fraction containing 343 g/kgDM^{-1} protein was obtained following hot water treatment and enzymatic hydrolysis. The sugar fraction can be fermented to produce bioethanol and the protein fraction was proposed as a promising source of essential amino acids. From an energy perspective, direct combustion of *U. lactuca* appears an unsuitable conversion route for the generation of bioenergy, due to the inherent high moisture and ash contents of the biomass. In particular, high concentrations of alkali metals can prove to be problematic in the thermal conversion of *U. lactuca* [15], resulting in a severe risk of slagging and fouling. As a result, biological processing has been identified as a more suitable conversion route, due to an increased tolerance for high moisture and high ash feedstocks [15]. The production of both bioethanol and biogas have been investigated for *Ulva lactuca* [16]. However, biogas production is considered a more feasible conversion route due to the complete degradation of macromolecular structures (carbohydrates, lipids and proteins), rather than carbohydrates only, providing a greater energy output [17]. The biomethane yields obtained from *U. lactuca* range from 157–271 mL CH$_4$ gVS^{-1} [15,18]; although the biodegradability is typically low (38%–43%) [18], potentially linked to its low C:N and high sulphur contents, causing an inhibitory effect for anaerobic digestion [19].

Another green aquatic plant with the potential for biofuel production is *Ledermanniella schlechteri* (LS); belonging to the *Podostemaceae* plant family [20]. This green aquatic plant grows abundantly in the falls of the river Djoué, one of the tributaries of the Congo River. This aquatic plant is commonly called Michiélé [21] and is currently used by a minority of the population as a food in the Southwest population of Brazzaville. It is rich in nutrients and is commonly eaten in tropical Africa. Mata et al. [20] have evaluated the concentrations of toxic metals in *Ledermanniella schlechteri* and their potential health risks

to consumers. Metal levels in *Ledermanniella schlechteri* were compared with international regulations for human consumption set by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) and were found to exceed permissible limits for human consumption. Mata et al. [20] demonstrated that metal concentrations in *Ledermanniella schlechteri* varied significantly across different sampling sites. The average values (in mg/kg) ranged from 0.5–9.0 (Cr), 0.2–4.5 (Ni), 5.5–78.4 (Cu), 336–1520 (Zn), 0.1–0.5 (As), 0.25–0.8 (Cd), 0.4–11.8 (Pb) and 0.02–0.24 (Hg). Across all sampling sites, the average concentration of Zn, As, Cd and Hg exceed the FAO/WHO's permissible limits for human consumption. The consumption of plants contaminated by heavy metals may lead to cancer, anemia and male infertility, as well as cardiovascular, nervous and lung diseases [20]. If biomass is contaminated and unfit for human consumption, it may still be possible to use it as a feedstock for bioenergy, exploiting the value of this natural resource. However, little is currently known about the behaviour of *Ledermanniella schlechteri* as a feedstock for bioenergy generation.

For the sustainable production of biofuels it is important to take into account the availability and suitability of potential biomass feedstock resources in a regional context. Potential aquatic biomass in the Congo region, such as UL and LS, has not yet been characterised in the literature. Both UL and LS are abundant in tropical Africa, so this work could have wider implications for identifying future feedstocks for generating bioenergy. The present work is primarily focused on determining the physio-chemical composition of *Ulva lactuca* macroalgae and the aquatic macrophyte *Ledermanniella schlechteri*, which are abundant, respectively, in the coastal marine environment and the rivers of the Republic of Congo. Secondly, this study evaluates the use of these alternative biomass resources as a possible feedstock for the production of bioenergy in the Congo region, by assessing both thermochemical and biological conversion routes.

2. Materials and Methods

The marine macroalgae identified as *Ulva lactuca* (UL) was collected directly from the Ocean at Pointe Noire (Pointe Indienne and Matombi; nomenclated UL1 and UL2, respectively). The freshwater aquatic macrophyte *Ledermanniella schlechteri* (LS) biomass samples were collected in Brazzaville (in two different sites of the Djoué River for LS1 and LS2, respectively). The physical appearances of UL and LS are shown in Figure 1. The sampling dates and grid references of the sampling sites are listed in Table 1.
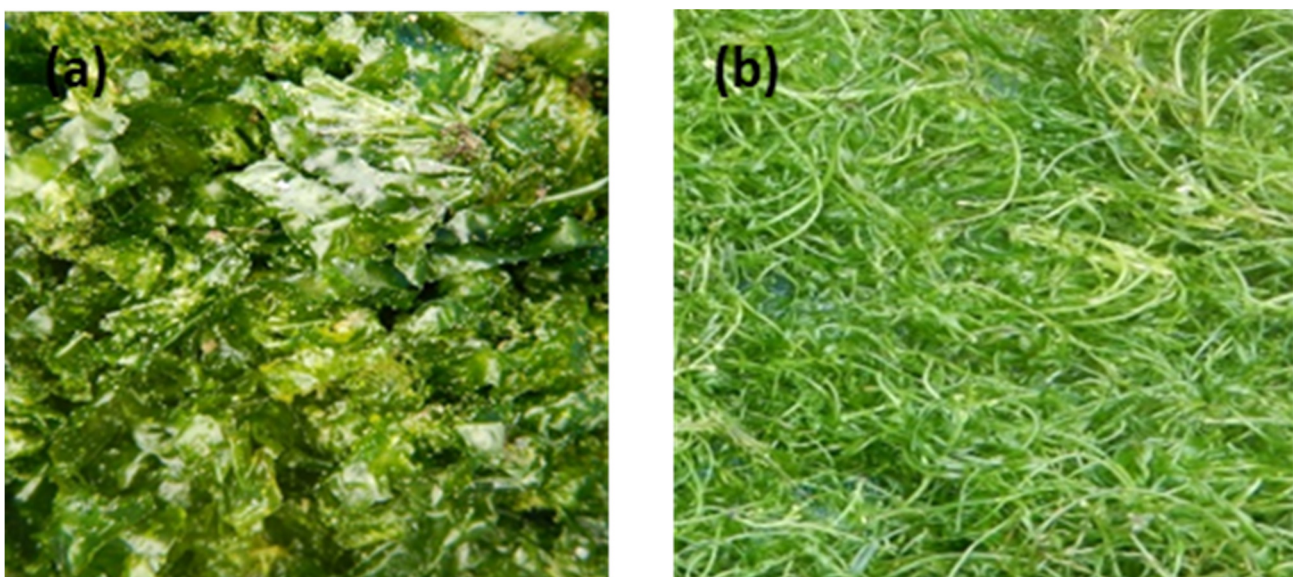

Figure 1. Photographs of (**a**) *Ulva lactuca* (UL) and (**b**) *Ledermanniella schlechteri* (LS).

Table 1. Sampling information of aquatic biomass.

Code	Biomass	Date	Sampling Site	Grid Reference
UL1	*Ulva lactuca*	March 2019	Pointe Indienne	04°38.159′ S 011°49.220′ E
UL2	*Ulva lactuca*	March 2019	Matombi	04°37.733′ S 011°49.472′ E
LS1	*Ledermanniella schlechteri*	April 2019	Djoué, Point A	04°18.940′ S 015°13.176′ E
LS2	*Ledermanniella schlechteri*	March 2019	Djoué, Point B	04°18.946′ S 015°13.141′ E

Each biomass was dried using a solar-dryer and oven for three days at approximately 70 °C. After the drying process, samples were ground using two steps, (i) a common blender and (ii) a Retsch CryoMill (Retch, Haan, Germany), to obtain a fine powder. The ground samples were sieved to obtain particle size of <100 μm and stored in the dark until further characterisation.

Ultimate analysis was performed to determine the elemental (C, H, N, S) content of the biomass using a CHNS Elemental Analyser (Flash 2000, Thermo Fisher Scientific, Waltham, MA, USA) following the protocols of Thermo Fisher Scientific [7]. Oxygen content was calculated via the difference method. Proximate analysis was performed to determine the moisture, fixed carbon, volatile matter and ash content using a METTLER TOLEDO TGA/DSC 1 (Mettler Toledo, Columbus, OH, USA). A 10 mg sample of each feedstock was heated up to 105 °C at a rate of 10 °C min^{-1}, held isothermally for 9 min under an N_2 atmosphere to determine the volatile matter content. Finally, the atmosphere was changed to air to burn off the samples for the determination of the fixed carbon and ash content. Total solids (TS) and volatile solids (VS) contents were determined gravimetrically via drying at 105 °C and subsequently ashing at 550 °C [22].

Biochemical analysis was performed using a modified Van Soest method [23] to determine the cellulose and lignin content. Lipid analysis was determined via Soxhlet extraction in hexane following the method described by Bi and He [6], followed by evaporation of the solvent using a Vacuum Controller V-800 (BÜCHI Rotavapor R-205, BÜCHI, Flawil, Switzerland). Protein content was determined using the DUMAS method using a nitrogen-to-protein conversion factor of 5.13 [24]. The total carbohydrate content was determined by the difference between 100-ash + protein + moisture + lignin. The higher heating value (HHV) was determined using bomb calorimetry (Parr Model 6200, Parr Instrument Company, IL, USA) according to BS ISO 1928:2009.

Inorganic analysis was performed using XRF spectroscopy (ZSX Primus II, Rigaku, Tokyo, Japan), operating at a 4.0 kW Rh anode (50 kV, 50 mA). Then, 2.7 g of each sample in powder form and 0.3 g of binder (BM-0002-1 CEREOX, Fluxana, Bedburg-Hau, Germany) was mixed in a plastic container using a vortex mixer for 4–6 min and sieved for making press pellets. The slagging and fouling behaviour of the biomass was determined according to predictive indices: the alkali index (AI), bed agglomeration index (BAI), acid–base ratio (Rb/a), slagging index (SI), fouling index (FI) and slag viscosity index (SVI); as described previously. Indices were calculated based on the inorganic oxide content of the biomass, determined via XRF analysis; further details on the calculations can be found here [10,25]. The interpretation of each index is described in Table 2.

Table 2. Interpretation of slagging and fouling indices, adapted from [25].

Colour Key	Interpretation	Slagging and Fouling Indices					
		AI	BAI	Rb/a	SI	FI	SVI
🟩	Low/safe	<0.17	>0.15	<0.5	<0.6	<0.6	>72
🟨	Medium/likely	>0.17 < 0.34	<0.15	-	>0.6 < 2.0	>0.6 < 40.0	>65 < 72
🟥	High/certain	>0.34	-	>0.5	>2.0	>40.0	<65

AI = alkali index. BAI = bed agglomeration index. Rb/a = acid–base ratio. SI = slagging index. FI = fouling index. SVI = slag viscosity index.

Fatty acid methyl ester (FAME) analysis was performed on the extracted lipid from both LS and UL, following derivatization using a GC-MSQP2010 SE (Shimadzu, Kyoto, Japan). Derivatization was performed via the addition of 200 µL 2:1 chloroform/methanol and 300 µL 0.6 M HCl in methanol to 5–30 mg of extracted lipid in a 2 mL vial. The vials were sealed and placed on a hot plate for one hour at 70 °C. Once cooled, 1 mL hexane was added to each vial and after vigorous shaking, the two solvent layers formed were allowed to separate. Fifty microliters of the top (organic) layer was added to 950 µL hexane and 20 µL international standard (C:17, 16.3 mg/mL in hexane).

Theoretical biochemical methane potential (^{T}BMP) was calculated stoichiometrically, based on the elemental composition (C, H, N and O) of the biomass, which was applied to Boyle's Equation [26]. Equation (1) describes Boyle's Equation, where coefficients *a*, *b*, *c* and *d* represent the molar fractions of C, H, O and N, respectively.

$$\text{Theoretical BMP} = \frac{22,400\left(\frac{a}{2} + \frac{b}{8} - \frac{c}{4} - \frac{3d}{8}\right)}{12a + b + 16c + 14d} \tag{1}$$

Experimental biochemical methane potential (^{E}BMP), measured using an AMPTS II (Bioprocess Control, Lund, Sweden), was maintained at 37 °C for a 30-day incubation period. A 2:1 inoculum-to-substrate ratio was used by diluting samples to 10 gVSL^{-1} and inoculum to 20 gVSL^{-1}, using distilled water. Two hundred milliliters of each was added to the reactors, leaving a 100 mL headspace. Blank reactors containing only inoculum (200 mL, 20 gVSL^{-1}) and 200 mL distilled water were run simultaneously, to account for the residual methane emissions from the inoculum. ^{E}BMP values were expressed as (mL CH$_4$ gVS^{-1}). More details on the methodology can be found here [10]. The headspaces of ^{E}BMP reactors were flushed with nitrogen before starting the test, to ensure anaerobic conditions. Inoculum was collected from an active digester (Esholt WWTP, Yorkshire, UK), during steady-state operation. The inoculum was passed through a 2-mm screen to remove large particulates and stored at 4 °C, until required. The inoculum was pre-incubated at 37 °C for approximately 2 days before the test, to reduce enteric methane emissions. A particle size of <1 mm was used for each biomass during the ^{E}BMP tests.

The biodegradability index (BI) was determined according to Equation (2) [27]. The digestion kinetics of the ^{E}BMP curves were described using the modified Gompertz model [28] described in Equation (3). Here, H_m is the maximum biomethane yield (mL CH$_4$ gVS^{-1}), R_m is the peak biomethane production rate (mL CH$_4$ gVSd^{-1}), λ is the lag-phase time (d), *t* is time (d) and *e* = 2.71828. H_m, R_m and λ were estimated using the Solver Function in Microsoft Excel, via the least-squares method [29]. The accuracy of the modified Gompertz model was determined through a squared correlation coefficient (R^2), comparing experimental and model data. The peak time of fermentation (T_m), Equation (4) [28], was predicted using parameters from the modified Gompertz model. Finally, the technical digestion time (T^{80}) was used to describe the time taken to generate 80% of the total ^{E}BMP [30].

$$\text{BI(\%)} = \frac{\text{Experimental Biomethane Potential}}{\text{Theoretical Biomethane Potential}} \times 100 \tag{2}$$

$$\text{H} = H_m \exp\left[-exp\frac{R_m e}{H_m}(\lambda - t) + 1\right] \tag{3}$$

$$T_m = \frac{H_m}{R_m e} + \lambda \tag{4}$$

3. Results and Discussion

3.1. Proximate and Ultimate Composition

The ultimate and proximate analysis, higher heating value (HHV) and C:N ratio of the biomass samples are presented in Table 3. The results indicate that LS contains a higher carbon content than UL, with a carbon content ranging between 36.2–40.0% and 19.1–30.9% for LS and UL, respectively. The levels of nitrogen in LS ranged between 2.1% and 2.7%,

whereas N-content was lower for UL, ranging between 1.5% and 2.2%. The levels of N and S can influence the different bioenergy conversion routes, for instance, an optimum C:N ratio is needed for anaerobic digestion and high levels of N and S can lead to emissions of NO_x and SO_2 during combustion. High levels of sulphur can also lead to H_2S formation during anaerobic digestion, inhibiting biomethane generation and producing a foul-smelling toxic odor [31,32]. UL is known to contain high levels of S due to the presence of sulphated ulvans [13]; these can decompose during anaerobic digestion to produce H_2S. The HHV of the biomass is also related to the ash content. LS was found to have a higher calorific value than UL, largely due to its reduced ash content and increased C content. UL had an average HHV of 10.6 MJ kg^{-1}, which is in agreement with previous reports in the literature [33]. LS has not been reported previously and has an average HHV of 14.2 MJ kg^{-1}.

Table 3. Ultimate and proximate analysis and higher heating values of the macroalgae *Ulva lactuca* (UL) and the aquatic macrophyte *Ledermanniella schlechteri* (LS).

Analysis	UL1	UL2	LS1	LS2
Volatile Matter (%*db*)	46.4	52.1	62.8	60.1
Fixed Carbon (%*db*)	15.7	20.2	19.1	15.7
Ash (%*db*)	38.0	27.7	18.1	24.2
C (%*db*)	23.9	30.9	40.0	36.2
H (%*db*)	4.6	4.3	4.5	4.4
N (%*db*)	1.5	2.2	2.7	2.1
S (%*db*)	ND	ND	ND	ND
O (%*db*)	32.0	34.9	34.7	33.1
C:N	15.9	14.0	14.8	17.2
HHV (MJ/kg) *db*	10.0	11.1	14.3	14.0

db = dry basis. ND = not determined. HHV = higher heating value.

Higher ash-containing biomasses are not desirable for thermochemical conversion due to problems associated with slagging and fouling. However, the ash content can provide useful micronutrients during biological conversion such as anaerobic digestion. The ash content in the UL varies significantly between the two samples: 27.8–38.0%, suggesting that one of the samples may have been compromised with epiphytes. The ash content of the LS samples was significantly lower, ranging between 18.1% and 24.2%. The amounts of fixed carbon and volatile matter are also important for producing fuels via thermochemical conversion, providing insights into its ignition and gasification characteristics [2]. Biomass with higher VM is more reactive and also produces less char upon pyrolysis [2]. LS samples contain a higher volatile matter, within the range 60.1–62.8%, whereas the VM content of UL was lower, at 46.4–52.1%. The level of fixed carbon also differed between 15.7–19.1% and 15.7–20.2% for LS and UL, respectively, which is comparable with the results previously reported in [7].

In summary, the ultimate and proximate analysis and higher heating value (HHV) indicate good agreement for the composition of the two LS samples collected. However, the UL samples showed a larger variation between the two sample sites, suggesting a significant difference in their environments. The ash content of UL was higher than that of LS; however, as they are a marine alga, this is unsurprising.

3.2. Biochemical Composition

Biochemical analysis using the Van Soest method provides information on the levels of total carbohydrates and cellulose, together with the lignin content. The protein content can be estimated based on the nitrogen content of the biomass using the Dumas method [5]. Neutral lipid content was determined via exhaustive Soxhlet extraction in hexane. The results of the biochemical analysis of the two biomasses are summarised in Table 4. LS samples have higher cellulose content, at about 21.9%, whereas the *Ulva* contains a lower cellulose content, in agreement with values previously reported by Yaich et al. [34]. Overall, the total carbohydrate content of LS was higher than that of UL, whereas both contained

similarly low lignin contents. The biochemical compositional analysis of UL samples was in agreement with that published in [35]. Aquatic plants and algae are known to have low lignin contents and often the identification of lignin can be attributed to the polyphenol content in the biomass. Protein content is generally higher in LS than in *Ulva*, which affects the C:N ratio of the biomass. The C:N ratio is lower for UL (14–16) compared to LS (15–17). In general, for efficient biogas production during anaerobic digestion, the C:N ratio in the feedstocks should be maintained between 20:1 and 30:1, which improves LS suitability. However, this can be optimised via co-digestion with other feedstocks.

Table 4. Biochemical analysis of *Ulva lactuca* (UL) and *Ledermanniella schlechteri* (LS).

Analysis	UL1	UL2	LS1	LS2
Moisture	6.5	11.9	7.5	6.1
Ash	35.5	24.4	16.8	22.7
Total Carbohydrate [1]	48.4	51.1	60.6	59.4
Cellulose	10.8	14.1	21.9	21.9
Lignin	1.4	1.9	1.4	0.5
Protein [2]	7.2	9.8	12.8	10.3
Lipids	<1	<1	<1	<1

[1] determined by difference: $100 - $ (Moisture + Ash + Lignin + Protein + Lipids); lipid content is assumed to be 1%.
[2] Protein analysis via the Dumas method using a conversion factor of 5.13. Data presented on an as-received basis.

During thermochemical conversion, the decomposition steps of biomass are dependent upon the biochemical composition of the biomass. During pyrolysis for instance, hemicellulose, cellulose, and lignin decompose at temperatures in the ranges of 220–315 °C, 315–400 °C, and 500–900 °C, respectively [2]. Pyrolysis of cellulose and hemicellulose produces a higher bio-oil yield than the pyrolysis of lignin, which significantly contributes to the formation of residual char. Protein results in N incorporation in the char and the bio-oil, which can result in the release of oxides of nitrogen during combustion. Generally, the higher the nitrogen content, the less suitable the feedstock is for solid fuels and bio-oil production. The levels of protein in the biomass were high and ranged between 6–10% and 10–12% for UL and LS, respectively. These higher levels of protein would likely be detrimental for thermochemical conversion.

3.3. Inorganic Composition

One of the other drawbacks of aquatic biomass, particularly marine biomass, is the high level of ash. The metal contents of ULs and LSs were analysed using XRF analysis; with the results presented in Table 5. The higher levels of ash in the UL samples (28–38 wt%) are reflected in the levels of metals identified via XRF. High levels of Si in UL1 indicate contamination by epiphytes. The LS biomass had a lower ash content, ranging between 18–24 wt%; however, they still contain significantly higher levels of alkali metals and chlorine, which would result in corrosion and fouling issues [25]. The higher levels of alkali metal in LS may be due to the composition of the rock layer crossed by the river where the *Ledermanniella schlechteri* is harvested. The impact of the inorganic content can be assessed by calculating slagging and fouling indices, which indicate the likelihood of problems associated with ash during thermal conversion. The slagging and fouling indices are shown in Table 6 and these indicate that slagging and fouling are extremely likely. The combination of high ash, N and low HHV makes these biomass unfavorable for thermal conversion.

Table 5. XRF analysis results for *Ulva lactuca* (UL) and *Ledermanniella schlechteri* (LS).

Elements (ppm)	UL1	UL2	LS1	LS2
Na	8010	5746	31,769	33,135
Mg	32,411	40,247	3206	2205
Al	1972	1222	1195	615
Si	27,166	6171	4834	2384
P	1843	238	2401	3147
Cl	13,212	9081	53,414	60,032
K	1612	15,041	30,506	36,786
Ca	8803	9774	8913	5418
Ti	298	388	350	ND
Fe	1656	1346	1056	396
Br	227	466	67	90
Sr	79	60	79	51
Zr	138	452	10	70
Zn	ND	15	364	415
Mn	ND	ND	181	224

ND = not determined.

Table 6. Predictive slagging and fouling indices for *Ulva lactuca* (UL) and *Ledermanniella schlechteri* (LS).

Sample	Slagging and Fouling Indices					
	AI	BAI	Rb/a	SI	FI	SVI
UL1	1.28	0.19	1.3	0.0	1.7	45.7
UL2	2.33	0.07	6.8	0.1	17.5	13.7
LS1	5.57	0.02	7.6	0.1	60.2	34.6
LS2	6.36	0.01	16.2	0.2	144.3	29.9

AI = alkali index. BAI = bed agglomeration index. Rb/a = acid–base ratio. SI = slagging index. FI = fouling index. SVI = slag viscosity index.

3.4. Lipid Composition

Another conversion option is the extraction of lipids, for conversion into biodiesel. The fatty acid methyl ester (FAME) profiles of the oils extracted from each biomass in hexane are shown in Table 7. The fatty acid profiles indicate the presence of palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) in both aquatic biomasses. The lipid profiles were similar to that of soybean oil, which is the preferential resource for biodiesel production, and in agreement with previous reports [6,8]. LS contained higher levels of palmitic acid (C16:0) and linoleic acid (C18:2) than UL. The common fatty acids present in feedstocks typically used for biodiesel production are mainly palmitic acid (C16:0), oleic acid (C18:1), linoleic acid (18:2) and linolenic acid (C18:3) [6]. Saturated fatty acids (SFAs) are the predominant lipids in UL, ranging between 41.0% and 47.4% of the total fatty acids (TFAs), which is in agreement with the literature [12]. The monounsaturated fatty acids (MUFAs) of *Ulva lactuca* (UL) ranged from 27.9% to 31.7% of TFAs, which was similar to the range reported for *Ulva lactuca* in [12,36]. The total sum of MUFAs ranged between 39.8% and 42.9%, whereas the total sum of polyunsaturated fatty acids (PUFAs) was 12.9–31.7%. The total PUFA content of *Ulva lactuca*-derived lipids was 12.9%. Important long-chain PUFAs, such as eicosapentaenoic acid (EPA, C20:5 n-3), linoleic acid (LA, C18:2 n-6), α-linolenic acid (C18:3, n-3) and arachidonic acid (AA, C20:4 n-6) were also found in significant levels. However, despite the favorable FAME profile, the yields of lipids extracted from the biomass were very low and less than 1%. This suggests that the extraction of oils for biodiesel would not be feasible.

Table 7. Fatty acid methyl ester profiles in wt% of *Ulva lactuca* (UL) and *Ledermanniella schlechteri* (LS).

FAME	UL1	UL2	LS1	LS2
C16:0	13.1	11.3	11.3	16.3
C16:1	0.4	0.6	1.0	0.6
C18:1	0.9	1.0	0.2	2.3
C18:2	2.5	2.7	0.5	1.3
C18:3	2.9	2.8	8.2	14.4
C20:0	4.4	4.2	8.0	ND
C21:5	2.6	3.4	ND	ND
C22:0	1.4	1.2	0.6	1.5
C23:6	2.0	2.6	ND	ND
Total FAs	30.2	31.2	46.7	82.5
SFA	14.5	13.9	13.0	43.2
MUFA	3.8	4.3	1.7	4.2
PUFA	11.9	13.0	32.0	35.1

ND = not determined.

3.5. Biomethane Potential

The biological conversion via anaerobic digestion is likely to be the most suitable conversion approach for these types of biomass, based on their analysed composition. The accumulative methane production for the two different biomasses are shown, based on the BMP test, in Figure 2. The LS biomass had a considerably higher BMP (247–276 mL CH_4 gVS^{-1}) compared to UL (159–161 mL CH_4 gVS^{-1}), suggesting that LS is more accessible to anaerobic digestion. The levels of biogas from UL are in agreement with previous reports from the literature [15,18], whereas it is the authors' belief that these are the first reported data for the BMP of LS. The theoretical biochemical methane potential (^{T}BMP) for each biomass can be predicted from the elemental composition of the biomass. The biodegradability index (BI) can be calculated from the experimental methane potential (^{E}BMP) divided by the (^{T}BMP). ^{T}BMP is the maximum that can be achieved and is typically much lower than 100%. Table 8 indicates that UL had a BI of 43%, much lower than the BI of LS, which ranged between 56–63%. Despite the high levels of alkali metals and chlorine in the LS sample, the yields and biogas were relatively high compared to UL. Optimisation of the C/N ratio via co-digestion with other carbon-rich feedstocks is likely to improve biogas production further. Anaerobic digestion is a relatively simple technology that is widely used in developing regions and can be used to replace the burning of wood for cooking. Based on the composition of the biomass and our assessment of its chemical and physical attributes, the use of anaerobic digestion for producing biogas is the most viable technology for the extraction of bioenergy from these feedstocks.
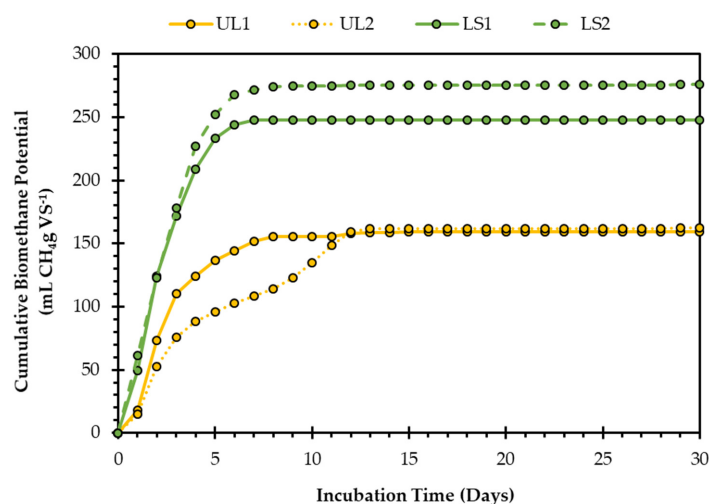

Figure 2. Biomethane potential of *Ulva lactuca* (UL) and *Ledermanniella schlechteri* (LS).

Table 8. Digestion kinetics of *Ulva lactuca* and *Ledermanniella schlechteri*.

Digestion Kinetics	UL1	UL2	LS1	LS2
Experimental Data				
^{T}BMP (mLCH$_4$gVS^{-1})	372.4	378.2	441.6	438.8
^{E}BMP (mLCH$_4$gVS^{-1})	159.5	162.3	247.6	275.7
BI (%)	43	43	56	63
Modified Gompertz Model				
H_m (mLCH$_4$gVS^{-1})	158.1	162.3	248.0	275.9
R_m (mLCH$_4$gVSd^{-1})	41.3	18.5	70.0	69.3
λ (d)	0.4	0.0	0.3	0.2
R^2	0.99	0.97	0.99	0.99
T_m (d)	1.8	3.2	1.6	1.7
T^{80} (mLCH$_4$gVS^{-1})	127.9	129.9	198.0	220.6
T^{80} (d)	5	10	4	4

^{T}BMP = theoretical BMP, calculated using Boyle's Equation. ^{E}BMP = experimental biomethane potential. BI = biodegradability index. H_m = maximum biomethane yield. R_m = peak biomethane production rate. λ = lag phase. T_m = peak time of fermentation. T^{80} = technical digestion time.

3.6. Discussion

Firewood and charcoal is the principle source of energy in the Republic of Congo for households and industry, with 90% of the population using firewood and charcoal for cooking [3,37]. The usage of charcoal in the Republic of Congo is large and is resulting in deforestation of the Congo basin. The amounts of macroalgae and aquatic macrophytes such as *Ulva lactuca* and *Ledermanniella schlechteri* are small in comparison. However, under certain circumstances, they may provide a sustainable source of energy for rural communities. UL is most abundant in the autumn months through to the spring and is found all along the Atlantic Coast of the Congo, particularly around Pointe Indienne and Matombi. *Ulva* is well known to form blooms in different regions of the globe [38], which is thought to be largely a consequence of fertilisers and human waste [39]. The utilisation of UL in the Republic of Congo is not widespread, it is not used as a food and its availability has not been well reported. UL has a high growth rate and has been estimated to yield 56 t^{-1} ha^{-1} year^{-1} (dry weight) under optimum growth conditions [40]. In this study, the levels are lower, and it is estimated to be feasible to harvest around 1000 kg per hectare per day on a wet basis. This represents approx. 100 kg per hectare per day (dry weight), which, if converted into biogas, represents 0.38 GJ energy/day, which potentially produces enough biogas to provide 39 households with energy for cooking (assuming a typical energy consumption per capita of 2.16 MJ per day for cooking and an average household number of 4.5 persons). When considering the use of UL, the feedstock is effectively free and collection of the biomass is beneficial due to its natural decomposition, resulting in fugitive emissions of methane and noxious odours.

LS is a freshwater aquatic macrophyte and is largely prevalent in the rainy season, compared to the dry season. It is estimated to have a higher productivity than that of UL and is abundant inland in many locations, along the Djoué River, the Congo River and the Loufoulakari River. It is estimated that the availability of the LS resource is five times higher than that of UL, at 5000 kg hectare per day on a wet basis, which represent approx. 500 kg per hectare per day (dry weight). If converted into biogas, this represents 4.29 GJ energy/day, which potentially produces enough biogas to provide the energy required by 440 households for cooking.

The use of biogas for cooking is a clean-burning alternative to using firewood, which has the potential to reduce indoor air pollution and improve health in rural communities. In the case of UL, it would only be suitable for coastal communities and the feedstock has seasonal availability. However, there does not appear to be any competing uses of UL in the Congo and so it represents a sustainable, although localised resource that has potential for generating bioenergy and reducing charcoal usage. LS, on the other hand, has

a higher availability and results in higher biogas yields; however, it does have competing applications and is sometimes used as food in this region. Despite this, LS represents a potential alternative feedstock that may be used for producing clean-burning biogas.

4. Conclusions

This study has demonstrated that anaerobic digestion is likely to be the most suitable technology for converting macroalgae and aquatic macrophytes such as UL and LS into bioenergy. However, in order to optimise conditions, such as the C:N ratio, it would be necessary to co-digest these feedstocks with other higher-carbon-containing biomass, such as animal manure, human faecal matter or agricultural wastes. This would increase biogas production and may overcome seasonal variation. An added benefit for converting high nutrient-containing biomass such as UL and LS via anaerobic digestion is the production of organic fertiliser from the digestate residue. Therefore, the potential utilisation of these aquatic biomasses has multiple benefits, including the potential for substituting charcoal and firewood usage with clean-burning biogas for cooking, the reduction of fugitive emissions and the production of organic fertiliser for agriculture. However, the amount of bioenergy that can be produced from the harvesting of wild natural populations is small, compared to the amounts of charcoal currently being produced. One potential solution for increasing biomass availability could be the artificial cultivation of these biomasses in large-scale 'capture to culture' systems, increasing the availability of these resources where demand requires.

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