

ESTIMATING THE ELEMENTAL BIOMASS COMPOSITION OF *DESMODESMUS* SP. CULTIVATED IN SUGARCANE STILLAGE

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Cultivating algae on various stillage types present an alternative to current stillage application for fertirrigation of the sugarcane. The objective of this study was to determine how algal concentration, nutrient removal efficiency and elemental composition of *Desmodesmus* sp. algae change in response to different (raw and digested) types of stillage. Algal biomass was cultivated and harvested at laboratory scale. Obtained concentrations were 0.5 g/L and 0.3 g/L for *Desmodesmus* sp. grown in raw and digested stillage, respectively. At these concentrations, total carbon (TC) and total nitrogen (TN as Total Kjeldahl Nitrogen or N-TKN) removal from stillage medium accounted 39.78% and 5.58% for raw; 20.83% and 5.43% for digested stillage, respectively. The *Desmodesmus* sp. used in the present study contained high carbon and oxygen content, similar to other algal species. Calcium, phosphorus, and sulfur were the three most abundant inorganic elements. Both biomasses were free of chlorine and with the low content of potassium. The phosphorous content in both biomasses was in the range of 6.03%–6.84%, which could classify these biomasses as useful plant nutrients.

Keywords: Sugarcane stillage, *Desmodesmus* sp., biomass, elemental composition.

Introduction

Sugarcane stillage, also known as vinasse, is a biorefinery by-product. It is obtained from the bottom of the distillation column from 1G (first generation) and 2G (second generation) technologies at relative rates of 6-15 L stillage/L bioethanol [1-3]. While 1G sugarcane stillage disposal is regulated by law due to its toxic environmental impact, 2G sugarcane stillage is used for fertirrigation of the sugarcane field [2]. Fertirrigation, as a simple and inexpensive approach, was reported to have a negative environmental impact which can be more harmful especially concerning high dinitrogen monoxide (N₂O) emissions [4]. Additionally, local communities are facing the problem of a strong unpleasant odor of the stillage. Anaerobic digestion of the stillage can generate a better environmental impact producing a surplus of bioenergy, such as biomethane. However, this comes with an unchanged odor and still relatively high nutrients concentrations in an anaerobic digestion effluent. Cultivating algae on raw or digested stillage intends to show that negative environmental impact and odor of the stillage nutrients can almost be eliminated. It is because algae assimilate nutrients from the stillage for its growth and synthesize cell components.

Currently, the algae used in the cultivation of the stillage medium are: *Chlorella vulgaris* [5-7], *Chlorella* sp. Bejerinck [8],

Chlorella sorokiniana [9], *Chlamydomonas reinhardtii* [10], *Tetraselmis suecica* [8], *Scenedesmus* sp. [11] and *Neochloris oleoabundans* [12]. The influence of nutrients on the algae growth in raw or digested stillage is mostly studied [5,6,8,9,11,12]. However, the influence of chemical oxygen demand (COD) concentration on the algal growth is controversial. For example, Marques et al. [5] show that *Chlorella vulgaris* is not able to grow in a medium of raw stillage with the COD content higher than 4%. Ozturk and Demir [8] showed that a higher content of raw sugarcane stillage (5 and 10%) decreased the cell number, chlorophyll Chl a, Chl b, Chl a/b ratio, carotenoid contents, the biomass yield and the protein content of both *Chlorella* sp. and *Tetraselmis suecica*. Similar results [10] are reported for *Chlamydomonas reinhardtii* cultivated in 1%-5% raw stillage media. Solovchenko et al. [9] reported a high biomass concentration with 20 g/L of COD in stillage at the start of semi-batch *Chlorella sorokiniana* cultivation, while higher raw stillage contents up to 40% added to Guillard modified medium contributed positively to *Scenedesmus* sp. biomass production at a light intensity of 10000 lux [11]. Furthermore, even though digested samples have a significant presence of volatile fatty acids (VFA) which serve as an organic source of carbon [13], the production may need the add-

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on C-source in the form of NaHCO_3 [12] or CO_2 [5]. This phenomenon was not reported for raw stillage samples. The N/P of about 5:1, suggesting N-limitation [14], is the excellent ratio for algae to grow and induce the accumulation of lipids. As it can be seen in Table 1, both raw and diluted stillages show a wide range of N/P ratio and need dilution instead of N, P sources add-in. Dilution can limit the photosynthetic carbon flux which is necessary for the lipid accumulation [15]. However, dilution is also required not only to avoid an excess of N, P sources but also to di-

minish turbidity, reduce the toxicity of some compounds, the limit of photo-oxidative damage and solve the problem of the harvest water management. Since the stillage already has a negative environmental impact, and its dilution could increase the demands of limited clean and fresh water supplies, the use of the stillage for the algae growth must be maintained as undiluted. Only de Mattos and Bastos [16] showed that heterotrophic cultivation of *Desmodesmus* sp. in the undiluted stillage was promising.

Table 1. Sugarcane stillage chemical composition

Parameters, mg/L	[12]	[17]	[18]	[8]	[11]	[9]	[5]	[12]	[17]	[5]	
	Raw			Digested							
pH	4	4	4-4.5 ^a	3.5-4.5 ^b	3.5-4 ^c	4	3-4	3.5±0.1	8.1	6.9	6.7±0.1
COD	124900	29000	37000-57000 ^{a,d}	19000-45000 ^{b,d}	15000-35000 ^{c,d}		20000	23.2±1.16	7944	9000	0.3±0.01
Total N	2876.5	550	750-790 ^{a,e}	330-480 ^{b,e}	260-350 ^{c,e}	4022	245.51 ^e		579.6	600	
Ammonia	752	40						0.01±0.01	473.3	220	0.02±0.01
NO ₃							15±1.3	0.03±0.01			0.01
Total P	102 ^f		100-350 ^{a,g}	90-610 ^{b,g}	90-500 ^{c,g}	24.5	773±73 ^f	0.05 ^f	98.6 ^f	32	0.01 ^f
SO ₄	11360	450	1500 ^a	1600 ^b	2030 ^c		178±18		1331.3	32	
(VFA)	1511.6								697.1		

^a Derived from molasses. ^b Derived from mixed liquors. ^c Derived from the juice. ^d Reported as organic matter. ^e Reported as N. ^f Reported as PO₄. ^g Reported as P₂O₅.

The knowledge of the elemental biomass composition is shown to be of great importance for various calculations in industry. Very often, due to lack of published data, an average and simplified elemental biomass composition of yeast ($\text{CH}_{1.8}\text{O}_{0.5}\text{N}_{0.2}$) is taken into account to calculate elemental balances of various microbial processes. However, the biomass composition varies depending on the type of microorganism [19], growth conditions [20], harvesting conditions and isolation methods [21]. Thus, the elemental biomass composition should be determined for each particular microorganism. However, to the best author's knowledge only Torri et al., [22] reported that elemental composition in (% by weight) of *Desmodesmus* sp. is: carbon (51.96), oxygen (33.87), hydrogen (7.31) and nitrogen (6.86). It is important to note that the elemental composition of microbial biomass has to be improved to contain not only organic but also inorganic elements due to more accuracy in calculations of basic thermodynamic properties (*i.e.*, biomass stoichiometric formula, enthalpy, entropy and density).

The aim of this article is to determine the elemental composition of *Desmodesmus* sp. biomass cultivated in sugarcane stillage. Also, the influence of the growth conditions on the elemental biomass composition is discussed. For that purpose, *Desmodesmus* sp. grown in both raw and digested sugarcane stillage was analyzed for their elemental content.

Materials and methods

Stillage

Raw and digested stillage samples were collected

from the sugarcane processing industry Usina São Martinho in the city of Pradópolis, São Paulo, Brazil and frozen in the laboratory until use.

TC and TN analysis

The measurements of TC and TN in the stillage samples before inoculation and after cultivation were carried out on a carbon analyzer TOC-VCSN (Shimatzu, Kyoto, Japan). Stillage and spent stillage samples were centrifuged and diluted prior analysis.

Microorganisms and growth conditions

The culture bank of green microalgae *Desmodesmus* sp., donated by the Laboratório de Pesquisas com Organismos Aquáticos (LAPOA), Grupo Integrado de Aquicultura e Estudos Ambientais (GIA), Universidade Federal do Paraná (UFPR), Curitiba/Paraná, was maintained in sterilized BG-11 media [23]. *Desmodesmus* sp. inoculum was grown autotrophically in 250 mL Erlenmeyer flask filled with 200 mL (total volume) of cultivating sterilized BG-11 media and 10% of the culture bank inoculum. The experiments were carried out in duplicate under following conditions: pH 7.0; the light flux of 62 $\mu\text{E m}^{-2} \text{s}^{-1}$ for 24 h; shaking rate of 250 rpm; temperature of 26 ± 4 °C; period of 9 days (the end of the exponential growth phase). These cultivated cells were used to inoculate stillage. Heterotrophic cultivation of *Desmodesmus* sp. was carried out in duplicate in 250 mL Erlenmeyer flasks with 200 mL (total volume) of stillage and 20% of the autotrophic inoculum at pH 7.0, 25 °C, 250 rpm for 11 days.

Desmodesmus sp. counting and contamination monitoring

Algal growth was monitored by direct counting of algal cells with a microscope using a Neubauer chamber. Average of the counted values was performed and multiplied by the dilution factor and 50 for the cells per mL concentration. After cultivation, the broth was centrifuged to obtain a residue and spent stillage. The residue (algal biomass) was dried to constant mass at 105 °C. The algae weight was used to calculate the concentration of the final biomass (g/L). TC and TN were determined in the spent stillage. The bacterial and fungal contamination was monitored by counting bacterial and fungal colony-forming units in Luria Bertani and Sabouraud dextrose agars, respectively, applying the Pour plate technique.

Elemental analysis

The samples of *Desmodesmus* sp. dry biomass were analyzed in triplicate by CHN analyzer (Perkin Elmer Corp., USA). Mass contents of carbon, hydrogen, and nitrogen are expressed as mass fractions of the dry sample. The contents of inorganic elements in *Desmodesmus* sp. dry biomass were analyzed by Central Analítica at the Institute of Chemistry (University of Campinas, São Paulo, Brazil) by the scanning electron microscopy with an energy dispersive X-ray analytical system (SEM-EDX).

Results and discussion

During the algal growth, cells absorb nutrients not only to grow but also to synthesize metabolic products such as carbohydrates, proteins, and lipids. The reduction of the presence of nutrients in the culture media, especially nitrogen, stresses the *Desmodesmus* sp., reducing the chlorophyll content and cell growth. Also, the photosynthesis metabolism turns the synthesis of protein to carbohydrate and lipid [24,25]. Table 2 presents the composition of raw and digested stillage before and after the growth of *Desmodesmus* sp. As it can be seen in Table 2, stressing conditions (low TC and TN content) in digested stillage caused less carbon and nitrogen utilization of 20.83% and 5.43%, respectively. As a result of this lower nutrients utilization, the concentrations of *Desmodesmus* sp. biomass in digested stillage (0.3 g/L) was lower compared to the raw one (0.5 g/L).

Table 2. The change of the composition in stillage before and after *Desmodesmus* sp. cultivation

mg/L	Before cultivation		After cultivation	
	Raw stillage	Digested stillage	Raw stillage	Digested stillage
TC	14767.05	8854.58	8892.05	7010.00
TN	4347.16	856.17	4104.55	809.70

In order to achieve a more accurate elemental biomass composition, *Desmodesmus* sp. cultures must be pure. Biomasses obtained after 11 cultivation days in

both raw and digested stillage were without a report of bacterial and fungi contamination. The SEM-EDX analyses carried out on the raw and digested biomass samples were compared with each other, by the appearance in the SEM micrograph. Table 3 and Figure 1 present the results of the SEM-EDX semi-quantitative analyses performed on the major biomass forming elements. Differences among the samples from the two types of biomass mostly reflect the differences in the composition of calcium. Other algal biomass species have relatively high carbon and oxygen content (Table 3). The *Desmodesmus* sp. used in the present study also contained high carbon and oxygen content, 80%-83% and 15%-18%, respectively. Nitrogen and hydrogen contents, determined by CHN analyzer, were 6.03% and 8.62% for raw and 6.47% and 11.01% for digested stillage, respectively. Those values were similar to that reported of *Chlorella vulgaris* of 6.2%–7.7% and 7.0%–10.0% for nitrogen and hydrogen, respectively.

Table 3. The SEM-EDX elemental analysis of the *Desmodesmus* sp. biomass

Element	% by weight			
	Raw stillage	Digested stillage	<i>Nannochloropsis salina</i> [21]	<i>Chlorella vulgaris</i> [26]
Carbon (C)	80.14	83.07	70.43	51.4–72.6
Oxygen (O)	17.68	15.48	22.19	11.6–28.5
Sodium (Na)		0.07	0.74	
Magnesium (Mg)	0.10	0.11	1.16	0.36–0.80
Phosphorous (P)	0.09	0.29	2.42	1.0–2.0
Chlorine (Cl)			0.36	
Potassium (K)	0.09		1.32	0.85–1.62
Calcium (Ca)	1.67	0.68	0.94	0.005–0.08
Sulfur (S)	0.25	0.26	0.34	0.28–0.39
Aluminium (Al)	0.15			
Silicium (Si)	0.18			
Iron (Fe)		0.14		0.04–0.55

The algal biomasses tend to be rich in a relatively similar composition of inorganic elements, *i.e.* calcium, potassium, silicon, phosphorus and magnesium (Table 3, Figure 1). In analyzing the composition of the two *Desmodesmus* sp. biomass samples, it appears that calcium, phosphorus and sulfur were the three most abundant inorganic elements. Although variations are significant, phosphorus is one of the most important indices for evaluating the agricultural value of biomass. The phosphorous content of the biomasses used in the present study was in the range of 6.03%–6.84%. These phosphorous contents are higher than previously reported for various agricultural wastes of 2.2%–2.7% [27] which could classify these biomasses as useful plant nutrients.

Low (0.09%) or no potassium contents in raw and digested biomasses samples, respectively would also be very positive if biomasses were implemented as fuels plant. Both raw and digested biomasses were free of chlorine, but have a high content of sulfur (0.25%). However, chlorine and sulfur are considered to have an increased risk of corrosion.

biomass caused by stressed cultivating conditions. The biomass cultivated in raw stillage showed a higher calcium content comparing to the biomass from digested stillage and other microalgae species. On the other hand, both biomasses contained low amounts of phosphorus, magnesium, and potassium. Sulfur contents were found to be comparable, while the content of iron in a digested biomass was relatively high.

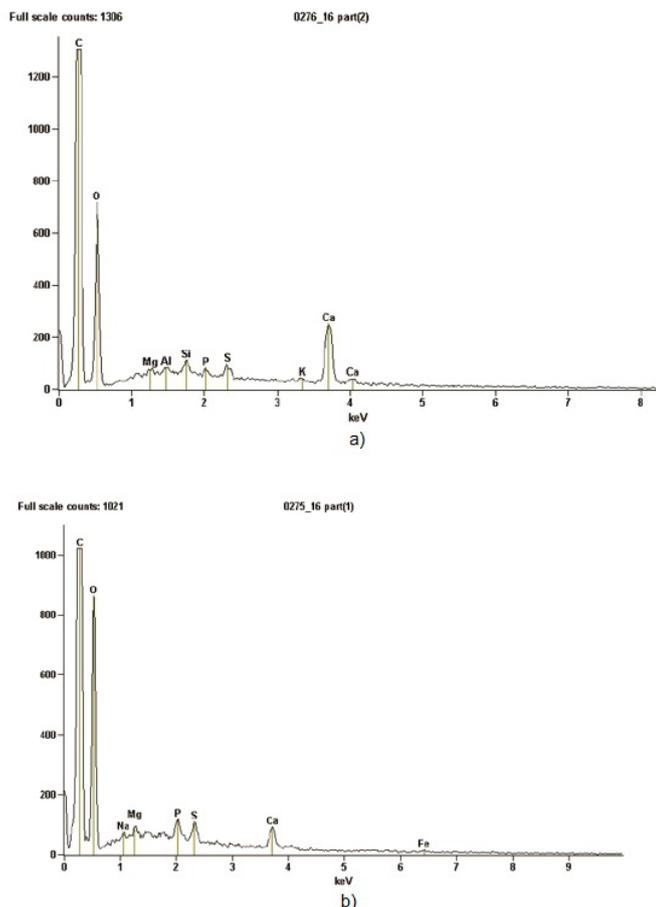


Figure 1. Randomly selected SEM-EDX spectars of *Desmodesmus* sp. biomass cultivated in: a) raw and b) digested stillage

Conclusions

Green algae *Desmodesmus* sp. can be grown in undiluted sugarcane stillage without adding additional sources of nutrients. The TC and TN removal in digested stillage were less than from raw stillage leading to lower digested biomass concentrations. Despite a lower biomass concentration obtained in digested stillage, this medium has been proven as better due to higher carbon, nitrogen and hydrogen contents. However, as discussed earlier, performing a wider elemental analysis of biomass is necessary for more precise calculations of the elemental, enthalpy balances and medium optimization. The SEM-EDX analysis quickly detects and localizes all essential elements in algal biomass from stillage samples. The CHN coupled with SEM-EDX analyses of the samples revealed that there was a substantial increase in carbon and a decrease in the oxygen content in digested

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Izvod

PROCENA ELEMENTARNOG SASTAVA BIOMASE *DESMODESMUS* SP. KULTIVISANE U DŽIBRI ŠEĆERNE TRSKE

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Kultivacija algi na različitim vrstama džibre predstavlja alternativu njenoj trenutnoj primeni za đubrenje šećerne trske. Cilj ovog istraživanja je istražiti kako se koncentracija alge, efikasnost usvajanja hranljivih materija i elementarni sastav alge *Desmodesmus* sp. menja u odnosu na različite vrste džibre (sirova i digestivna). Biomasa alge *Desmodesmus* sp. je gajena i skupljena u laboratorijskim razmerama. Dobijene su koncentracije od 0,5 g/L i 0,3 g/L za *Desmodesmus* sp. gajenim u sirovoj i digestivnoj džibri, respektivno. Pri ovim koncentracijama, efikasnosti usvajanja ukupnog ugljenika i azota iz džibre su: 39,78% i 5,58% za sirovu; 20,83% i 5,43% za digestivnu džibru, respektivno. Alga *Desmodesmus* sp. korišćena u ovom istraživanju sadržala je veliki udeo ugljenika i kiseonika, slično drugim algalnim vrstama. Kalcijum, fosfor i sumpor bila su tri najviše zastupljena neorganska elementa. Obe biomase su imale nizak sadržaj kalijuma i nisu sadržale hlor. Sadržaj fosfora u obe biomase je bio u opsegu 6,03%–6,84%, što bi moglo klasifikovati ove biomase kao korisne biljne hranljive materijale.

Ključne reči: Džibra šećerne trske, *Desmodesmus* sp., biomasa, elementarni sastav.