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Potential carbon dioxide fixation by industrially important microalg

Eduardo Bittencourt Sydney [a], Wilerson Sturm [a], Julio Cesar de Carvalho [a], Vanete T. [illegible]naz-Socc[illegible] [a,b], Christian Larroche [c], Ashok Pandey [d], Carlos Ricardo Soccol [a,*]

[a] Bioprocess Engineering and Biotechnoloy Department, Federal University of Parana, Centro Politécnico, Usina Piloto B, CEP 815[illegible]0 Curitiba, Para[illegible]
[b] Industrial Biotechnology, Universidade Positivo, CEP 81280-330 Curitiba, Paraná, Brazil
[c] Biochemical Engineering Department, School of Engineering, Blaise Pascal University, Clermont-Ferrand, France
[d] Biotechnology Division, National Institut for Interdisciplinary Science and Technology, CSIR, Trivandrum 695 019, Indi[illegible]

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ABSTRACT

The present study aimed at investigati[illegible]arbon metab[illegible]n in terms of carbon dioxide fixation and its destination in microalgae cultiv[illegible]s. To this purpose, an[illegible]ysis of growth parameters, media of cultivation, biomass composition and [illegible]oductivity and nutrients balance were performed. Four microalgae suitable for mass cultivation were [illegible]aluated: *Dun[illegible]lla tertiolecta* SAD-13.86, *Chlorella vulgaris* LEB-104, *Spirulina platensis* LEB-52 and *Botry[illegible]ccus braunii* [illegible]G-30.81. Global rates of carbon dioxide and oxygen were determinated by a system deve[illegible]d in our [illegible]oratory. *B. braunii* presented the highest CO_2 fixation rate, followed by *S. pl[illegible]*, *D. tertiolect[illegible]* and *C. vulgaris* (496.98, 318.61, 272.4 and 251.64 mg L^{-1} day^{-1}, respectively). Carbon [illegible]d was mainly used for microalgal biomass production. Nitrogen, phosphorus (calcium for *D. [illegible]tiolecta*), [illegible]ium and magnesium consumption rates (mg gX^{-1}) were evaluated for the four microa[illegible]. Bi[illegible]mass c[illegible]mposition presented a predominance of proteins but also a high amount of [illegible] especia[illegible]. *tertiolecta* and *B. braunii*.

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1. Introduction

As a consequence of globa[illegible] warming [illegible] technologies are being developed for greenh[illegible] gases mitiga[illegible] Chemical reaction-based CO_2 mitigatio[illegible]proach[illegible]s are energy consuming and costly processes (Lin et [illegible]2003)[illegible]d the only economical incentive is the CO_2 credits t[illegible]erated u[illegible]er the Kyoto Protocol (Wang et al., 2008). Biologi[illegible]O_2 mit[illegible]ion through microalgae has attracted [illegible]tion a[illegible]t[illegible]gic alternative that associates both e[illegible]onmen[illegible] and eco[illegible]ical interests.

The rat[illegible]carbo[illegible] is limited by the metabolic activity of microalgae, [illegible]s in ta[illegible]imited by photosynthesis. Several authors have st[illegible]d microalgae CO_2 fixation capacity. In theory microalgae can us[illegible] to 9% of the incoming solar energy to produce 280 tons of d[illegible] biomass ha^{-1} $year^{-1}$ while consuming/sequestering roughly 513 tons of CO_2 (Bilanovic et al., 2009). Hirata et al. (1996) cultivated *Chlorella vulgaris* in 3% CO_2 for 8 days and achieved a carbon dioxide fixation of 865 mg CO_2 L^{-1} day^{-1}. This represents around 1.7 times more carbon dioxide fixated than the estimative of Bilanovic et al. (2009), considering a liquid column depth of 15 cm. Besides the important environmental role against global warming, the carbon dioxide fixation is of industrial interest since carbon papers can now be traded in the international market and used as a marketing move by companies.

Most studies quantify the carbon fixated in terms of biomass production (Chae et al., 2006; Jacob-Lopes et al., 2008; Kajiwara et al., 1997). On the other hand, many several papers describe the production of extracellular compounds by microalgae, including volatile organic compounds (Shaw et al., 2003), organohalogens (Scarrat and Moore, 1996), polysaccharides, hormones and others. This indicates that carbon fate may be quite diverse and that microalgal biomass generation is just a part of the carbon destination. Moreover, little information is available with respect to the simultaneous research of both the global rates of carbon dioxide sequestration and the rates of incorporation of carbon into the microalgae biomass (Chiu et al., 2008).

Microalgae can fix carbon dioxide from different sources including CO_2 from the atmosphere, from industrial exhaust gases and in form of soluble carbonates. The utilization of industrial gaseous and/or liquid residues is becoming a reality in microalgae cultures in view that the major barrier in industrial cultivation of microalgae is the cost of the media for cultivation. Therefore, knowledge of residue composition, microalgae metabolic pathways and nutritional needs plays a central role in processes development.

The aims of this study were to evaluate growth, metabolism, and consumption of nutrients and to quantify the carbon dioxide assimilation of the four widely used microalgae *Dunaliella*

* Corresponding author.
E-mail address: soccol@ufpr.br (C.R. Soccol).

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tertiolecta SAD-13.86, *C. vulgaris* LEB-104, *Spirulina platensis* LEB-52 and *Botryococcus braunii* SAG-30.81.

2. Methods

2.1. Microalgae cultivation

C. vulgaris LEB-104 and *S. platensis* strain LEB-52 were obtained from Federal University of Santa Maria (UFSM, Brazil) and cultivated at 30 °C in Modified Bristol Medium (Watanabe, 1960) and Zarrouk medium, respectively. *D. tertiolecta* SAG-13.86 and *B. braunii* SAG-30.81 were obtained from the Culture Collection of Algae at Gottingen and cultivated at 25 °C in artificial sea water (DUN medium) and 3N-MBM medium, respectively.

The main cultivations were performed in an 11L BioFlo Fermentor (New Brunswick Sci.) with a working volume of 8 L. For pH measurement and control, a sensor was used and the pH controlled by automatic injection of specific acid and/or base as required. All microalgae were cultivated in pH 7.2 ± 0.2, except *S. platensis* LEB-52 (pH 9.0 ± 0.2).

Air enriched with 5% CO_2 (White Martins, Curitiba, Brazil) was sparged through a ring sparger. Illumination of culture was provided by eight cool white 32 W fluorescent lamps (providing 3500 lux) in 12:12 h (light/dark) photoperiod. Temperature was measured by a thermocouple and controlled by a water jacket. Experiments duration were 15 days for all microalgae tested.

2.2. Analysis

Samples were withdrawn daily and centrifuged in a Sorva[illegible] end Mach 1.6 R centrifuge (Sorvall, Germany) at 246*g* for 15 [illegible]n. Cells were washed once and dried at 60 °C until constant we[illegible] while the cell-free medium was used for fur[illegible]nalysis nitrate, alkalinity (carbon solubility), phosp[illegible]us [illegible] cation concentration.

Nitrate determinations were done daily [illegible] th[illegible] method proposed by Cataldo et al. (197[illegible] Alkal[illegible]was measured daily by titration of 10 ml of the cel[illegible]e mediu[illegible]th 0.1 N HCl using phenolphthalein (0.2 g L^{-1} [illegible]anol 95%) [illegible]carbonate and methylorange (0.5 g L^{-1} in [illegible]ater) [illegible]bicarbonate quantification as dye indicators. Phos[illegible]rus consum[illegible]n was assessed during the experiment in 5[illegible]ys intervals by [illegible]quantification of soluble phosphorus b[illegible]e phos[illegible]o-molybdate method. The 15th day cell-free medi[illegible]was [illegible]alyzed for total sugars by the phenol–sulfuric metho[illegible]is et al[illegible]56).

The determ[illegible]n of c[illegible]s w[illegible]one with a 761 Compact IC 817 Biosca[illegible]iron[illegible]graph. [illegible]umn used was METROSEP C3 250/4.0 [illegible]etrohm 250 mL × [illegible]0 mmID. Analytical conditions were: 3[illegible]M [illegible]/min, 40 °C, 20 μL sample volume, 11.2 MPa. A [illegible]ndard chromatogram was prepared with the following salts: [illegible]·2H_2O, $MgCl_2·6H_2O$, KCl, Na_2SO_4, $ZnSO_4·7H_2O$, NH_4Cl e $FeSO_4$·[illegible]. All reagents used were analytical grade (Sigma–Aldrich).

2.3. Carbon dioxide data acquisition

The cultivation vessel was coupled with sensors for the measurement of carbon dioxide and oxygen in the inlet and outlet gases. In the inlet, carbon dioxide flow was monitored by a rotameter and measured by a thermal dispersion mass flow sensor (Aalborg GFM), while oxygen flow was monitored by a rotameter and its concentration in the air measured by an electrochemical sensor (Alphasense O2-A2). In the outlet, total flow was measured by a mass flow sensor (Aalborg model GFM), the percentage of carbon dioxide measured by an infrared sensor (Vaisala GMT) and the percentage of oxygen by an O2-A2 sensor. These sensors were all connected to Novus model N1100 controllers (Sturm et al., 2008). Data acquisition occurred at 15 min intervals by Laquis software (Laquis, 2009). To perform the calculations, this industrial net was connected to a personal computer running the Laquis software.

A blank trial, using only sterile media in the vessel, was run for 5 days with data acquisition in order to define sensors baselines for O_2 and CO_2 be used as basis to further calculations of carbon dioxide consumption.

2.4. Biomass analysis

After 15 days of experiment, the[illegible]s were [illegible]ved by centrifugation (1500*g* at 25 °C), washed[illegible]tilled wate[illegible]ecentrifuged again and dried at 60 °C until[illegible]onsta[illegible]eight. Th[illegible]ried biomass was analyzed for chloroph[illegible], carbohy[illegible]s, p[illegible]ins, lipids and ash.

Chlorophylls were[illegible]cted[illegible]n 90% acet[illegible]ne and the quantification follows the[illegible]uat[illegible]ggested [illegible]Strickland and Parsons (1968). Lipids w[illegible]determi[illegible]by ex[illegible]tion with methanol:chloroform 1:1 ([illegible]and Dyer,[illegible]ollowed by a liquid–liquid extraction[illegible]h [illegible]e. Ash wa[illegible]quantified by the AOAC 941.12 method, while phe[illegible]sulfuric method (Dubois et al., 1956) was used f[illegible]tal carboh[illegible]e and the Lowry method (Lowry, 1951) fo[illegible]otein determinatio[illegible]

3[illegible]esults and [illegible]cussion

3.1. C[illegible]ameters

[illegible]*vulgaris* LEB-104 presented an exponential growth from 96 to [illegible]68 h of experiment. Maximum cell concentration of 1.94 g L^{-1} was reached on the last day (15th) of cultivation. Maximum specific growth rate (μmax) of 0.29 day^{-1} and biomass doubling time (td) of 2.39 days was achieved during exponential growth and the maximum productivity ($P_x$max) was 0.31 g L^{-1} day^{-1}. Morais and Costa (2007) obtained very similar results (μmax = 0.31d^{-1}, td = 2.27 days and $P_x$max = 0.14 g L^{-1} day^{-1}) cultivating the strain of the same group in vertical tubular reactors supplemented with 6% CO_2.

B. braunii SAG-30.81 reached an exponential growth phase during the 15 days of cultivation. Final biomass concentration was 3.11 g L^{-1}. Maximum specific growth rate, productivity and the biomass doubling time were calculated and resulted in 0.24 day^{-1}, 0.61 g L^{-1} and 2.9 days, respectively. These results are in accordance to Vovola et al. (1998), who achieved a biomass concentration of 3.9 g L^{-1}, specific growth rate and generation time of 3–4 days.

For *S. platensis* LEB-52, maximum cell concentration was observed in day 14th (2.18 g L^{-1}). Specific growth rate and doubling time were calculated in the exponential growth phase and resulted in 0.22 day^{-1} and 3.12 days, respectively. Maximum cell productivity was 0.73 g L^{-1} day^{-1}. This data are in accordance with Binaghi et al. (2003) and Morais and Costa (2007).

D. tertiolecta SAG-30.81 also presented an exponential growth during the whole duration of experiment. Maximum cell concentration was observed in day 15th (2.15 g L^{-1}). Specific growth rate and doubling time were calculated at the exponential growth phase and resulted in 0.21 day^{-1} and 3.29 days, respectively. Maximum cell productivity was 0.42 g L^{-1} day^{-1}.

3.2. Biomass composition

Table 1 presents the biomass composition of the four microalgae studied. A high amount of proteins was observed in *C. vulgaris*

Table 1
The biomass composition in terms of proteins, sugars, pigments, lipids and ash of the analyzed microalgae.

Microalgae	Protein (%)	Sugars (%)	Pigments (%)	Lipids (%)	Ash (%)	Total carbon (%)[a]
Chlorella vulgaris LEB-104	40.95 ± 3.0	16.74 ± 1.8	12.41 ± 1.6	9.95 ± 2.1	13.35 ± 0.9	45
Botryococcus braunii SAG-30.81	39.61 ± 3.2	2.38 ± 0.4	13.05 ± 1.5	33 ± 2	7.54 ± 0.5	58
Spirulina platensis LEB-52	42.33 ± 1.9	11 ± 0.88	16.12 ± 2.3	11 ± 2.2	7.11 ± 1.5	50
Dunaliella tertiolecta SAG-13.86	29.41 ± 2.8	13.95 ± 1.2	7.61 ± 1.3	11.44 ± 1.8	33.35 ± 3.5	36

[a] Considerations of carbon composition for the organic compounds: protein = 45%, sugars = 40%, chlorophyll = 74% and lipids = 87%. The values of biomass compositions were corrected to 100% before the estimation of carbon composition.

LEB-104 and *S. platensis* LEB-52, while *B. braunii* SAG-30.81 and *D. tertiolecta* SAG-13.86 accumulated a high amount of lipids. The high amount of ash in *D. tertiolecta* SAG-13.86 biomass might be a consequence of the high salinity of the cultivation media.

Lipid productivity for each microalga was calculated as the ratio of the amount produced by the growth time, considering the composition of the biomass constant over time. *B. braunii* SAG-30.81 presented the higher lipid productivity (61.38 mg L^{-1} day^{-1}), followed by *D. tertiolecta* SAG-13.86, *S. platensis* LEB-52 and *C. vulgaris* LEB-104 (15.25, 14.3 and 11.54 mg L^{-1} day^{-1}, respectively).

3.3. Media analysis and nutrients balance

Carbon solubility (C_{SOL}) was determined daily and compared with the biomass production ($X_t - X_0$, where X_0 is the biomass concentration in the beginning of the experiment and X_t is the concentration in a given time). It can be observed that carbon solubility increased in all cases (Fig. 1). Eriksen et al. (2007) related that the concentration of HCO_3^- in the growth medium increased in p portion to the decrease concentration in nitrogen source, wh was also observed during our experimentations. This is a cons quence of the nitrogen source used, because the reduction o 1 mol of NO_3^- consumes one proton that comes fro ssocia- tion of carbonic acid.

Through the analysis of dissolved cation d anio in the media, it was possible to calculate the ra phosphorus, magnesium, nitrogen and ssium e 2 shows the obtained nutrient specific consum rate for ea icroalga studied. These are very important d dicates spe nutri-

Table 2
The nutrients specific consumption rate in terms of tion for the four microalgae analyzed.

Microalgae	Nitrogen rate (mg gX^{-1})	Magnesium rate (mg gX^{-1})	Potas rate (mg gX^{-1})	Phosphoro (mg gX^{-1})	Calcium (mg gX^{-1})
C. vulgaris LEB-104	49.35	2.85	32.1	31	–
B. braunii SAG-30.81	40.72	.60	15.02	75.9	–
S. platensis LEB-52		17	24	247.4	–
D. tertiolecta SAG-13.86	59.71	–	375.46		26.05

ent requ of each microalga, being very useful in the utiliza- of complex media for cultivation (industrial liquid wastes, for e

Table 3 shows the profile of accumulated nutrient consumption and biomass production during cultivation for each microalga studied. It can be observed that magnesium and nitrogen consumption accompanied biomass production in all cases. It was observed that for *C. vulgaris* LEB-104 the highest potassium consumption was towards the end of the exponential growth. The same behavior observed for phosphorus consumption by *S. platensis* LEB-52. Calcium consumption analysis in *D. tertiolecta*

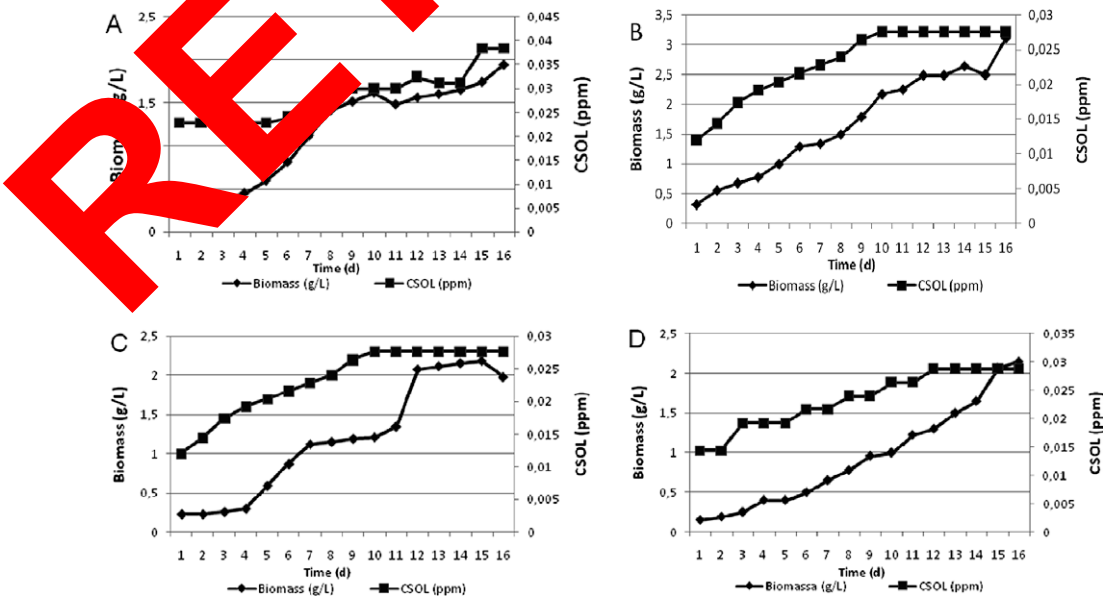

Fig. 1. Profile of dissolved carbon (CSOL) through time in comparison with growth of (A) *C. vulgaris* LEB-104, (B) *B. braunii* SAG-30.81, (C) *S. platensis* LEB-104 and (D) *D. tertiolecta* SAG-13.86.

Table 3
Nutrient accumulated consumption and biomass production during cultivation of each microalga.

Time (days) (mg L^{-1})	Nitrogen (mg L^{-1})	Magnesium (mg L^{-1})	Potassium (mg L^{-1})	Phosphorous (mg L^{-1})	Biomass (mg L^{-1})
Chlorella vulgaris LEB-104					
0	0	0	0	0	0.20
5	34.83	1.90	4.24	193.08	0.81
10	68.29	3.58	17.14	526.96	1.48
15	85.87	4.96	56.00	547.06	1.94
Botryococcus braunii SAG-30.81					
	0	0	0	0	0.32
	42.94	2.60	12.68	128.72	1.29
	78.06	5.68	20.44	305.72	2.25
	78.06	7.26	41.92	490.75	3.11
Spirulina platensis LEB-52					
	0	0	0	0	
	57.10	2.14	21.88	179.65	
	80.41	4.14	68.93	187.	
	120.50	5.45	110.73	1	18
Time (days) (mg L^{-1})	Nitrogen (mg L^{-1})	Magnesium (mg L^{-1})	Potassium (mg L^{-1})	icium (L^{-1})	Biomass (mg L^{-1})
Dunaliella tertiolecta SAG-13.86					
	0	0	0	0	0.15
	33.15	84.16	74.82	534	0.5
	47.46	108.48	99.38	727.21	1.23
	52.10	116.90	119.42	750.92	2.15

SAG-13.86 growth indicates major consumption in the beginning of the growth, which might be a consequence of adsorption to the cell membrane.

Assuming the biomass composition presented in (Table 1) and nutrient consumption data obtained (Table 2), it was possible to determine the destination of the nutrients. Proteins produ n was the main destination of the nitrogen consumed in all as expected. The amount of nitrogen used in protein produ n was 75%, 65%, 91% and 56% for *C. vulgaris* LEB-104, *B. bra i* SAG-30.81, *S. platensis* LEB-52, and *D. tertiol* AG-13. respectively.

Magnesium consumption by all microa was her than the necessary for chlorophyll production, res nutrient play others important roles micro metabolism. In *C. vulgaris* LEB-104 cultivation i as observe extra consumption of 1.38% of magnesi ile for *B. nii* SAG-30.81 and *S. platensis* LEB-52 t s ext nsumption was of 3% and 3.2%, respectively.

Through the biomass position of the oalgae presented in Table 1 it was possi to esti e the amount of carbon in each microalga (Table 1). was de considering the lipid profile of *B. braunii* SAG-30.81 (B da et al 007) and *C. vulgaris* LEB-104 (Petkov a cia, 2) and gars as glucose. For carbon estimation prot , an av as done for all aminoacids.

3.4 fixation and nce

The metabo m of the microalgae was studied during the entire c tion of th xperiment (15 days). As example, the profile of ox and ca n dioxide uptake by *C. vulgaris* LEB-104 is shown in Fig ve that, as oxygen is produced by microalgae, the values of oxygen uptake are negative). It is observed the complementary behavior of photosynthesis and respiration during microalgal growth. Under light regimem the increase in carbon dioxide consumption is simultaneously accompanied by a decrease in oxygen consumption (photosynthesis process); and the opposite was observed under dark regimem (respiration process). The distances between peaks and valleys of carbon dioxide consumption line is approximately 12 h, which is in accordance with the duration of photosynthesis and respiration under the light photoperiodicity. These same characteristics were observed in the other assays with *B. braunii* SAG-30.81, *S. platensis* LEB-52 and *D. tertiolecta* SAG-13.86.

The determination of carbon dioxide fixation by each microalga was done based on the CO_2 consumption profile. The trapezoidal method was used in order to integrate the curves (CO_2 cons g/h and CO_2 base line) (see Fig. 2). The areas obtained was subtracted and the difference between them corresponding to the total amount carbon dioxide consumed.

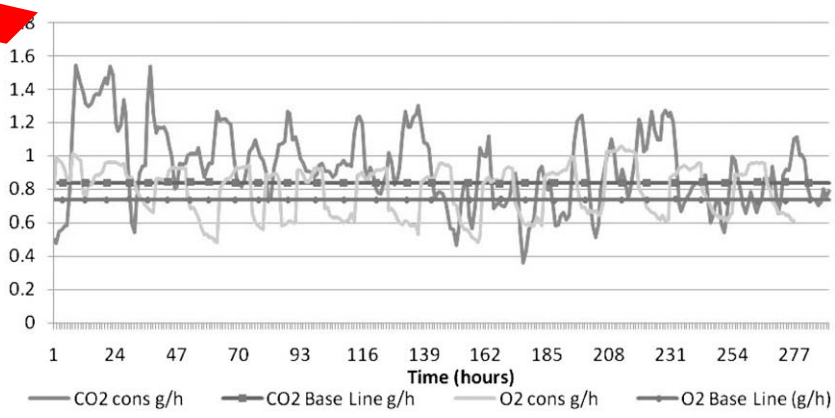

Fig. 2. Carbon dioxide and oxygen consumption profiles (in g h^{-1}) during *C. vulgaris* LEB-104 growth plotted together, presenting symmetry and accordance to photosynthesis and respiration processes.

Table 4
Comparison between carbon fixation rates indicated in the literature and our results. It is also shown the amount of carbon dioxide used in biomass generation.

Microalga	Literature (mg_{CO_2} L^{-1} day^{-1})	Reference	This work (mg_{CO_2} L^{-1} day^{-1})	% To biomass	CO_2 fixated per ton of biomass produced (in kg)
C. vulgaris LEB-104	624	Yun et al. (1997)	251.64	86.68	144.63
B. braunii SAG-30.81	1100	Marukami and Ikenouochi (1997)	496.98	87.96	178.09
S. platensis LEB-104	413[a]	Morais and Costa (2007)	318.16	80.40	182.81
D. tertiolecta SAG-13.86	313[a]	Kishimoto et al. (1994)	272.40	70.42	136.19

[a] Calculated from the biomass productivity (see Wang et al., 2008).

It is important to note that one of the objectives of this work was to obtain the carbon dioxide fixation rate of industrially important microalgae under conditions recommended by the strains suppliers, intenting to serve as basis for carbon dioxide fixation researches.

Carbon dioxide fixation rate (in mg L^{-1} day^{-1}) was equal to 251.64 for *C. vulgaris* LEB-104 , 496.98 for *B. braunii* SAG-30.81, 318.61 for *S. platensis* LEB-52 and 272.4 for *D. tertiolecta* SAG-13.86. Table 4 compares carbon fixation rate obtained in this work and some described in the literature. The amount of carbon used in biomass production is also shown in table.

A good similarity was observed between the data obtained in our work and the literature in the cases of *D. tertiolecta* and *S. platensis*, while some disparities were observed in the cases of *B. braunii* and *C. vulgaris* (Table 4).

These discrepancies are acceptables, once they are a consequence of the conditions to which microalgae are submitted. For example, Marukami and Ikenouochi (1997) used a specific medi for the accumulation of hydrocarbons by *B. braunii* (which can plain the highest carbon fixation) and Scragg et al. (2002) do n use CO_2 enrichment in cultivation of *C. vulgaris* (which can explai such low carbon fixation). These conditions are very t from the ones we used in this work.

On the other hand, the different conditions by us those by Kishimoto et al. (1994) and Morais and Costa tivation of *D. tertiolecta* and *S. platensis*, r ctively not lead to significant changes in the carbon fix rate. Thes ows the metabolic flexibility of microalgae mitted to d ct cultivation conditions.

4. Conclusions

The methodology de e CO_2 q antification was useful in the determinatio of the on fixat capacity and evaluation of microalgal n m bel r.

The dete iation biomas mposition linked carbon and nutrients nces, viding important and rare data in terms of carbon de ti croalgal metabolism. Different biomass comp ns suggests different carbon metabolism in microalgae, wh can also be influenced by nutrients availability.

Biomass production is the main destination of carbon in microalgal cultivation. In association to the determination of its composition and analysis of nutrients uptake, biomass production gives important data of microalgal metabolism and might be considered in industrial applications.

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