ALGAE BIOMASS SUMMIT 2020
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Culture media for the production of biomass of green microalgae (Chlorophyta) at low cost

Dágon Manoel Ribeiro
Phd in Biotechnology and Biodiversity
Chief Executive Officer of Biotecland
Visiting Professor at Unizambeze - Mozambique
www.biotecland.com

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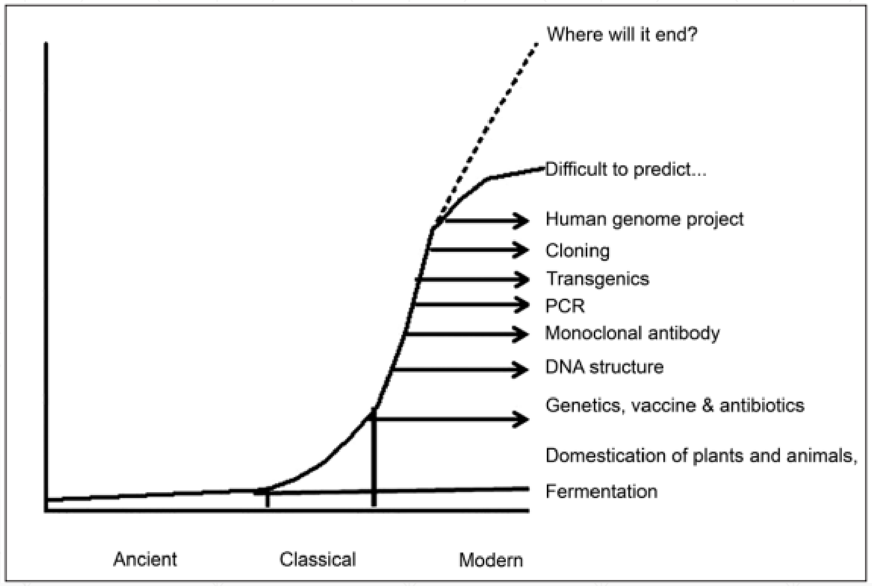
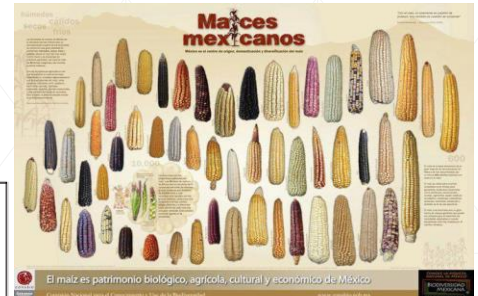
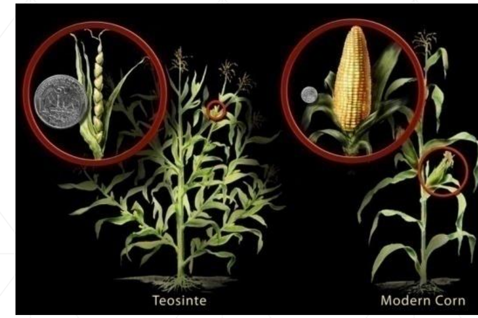
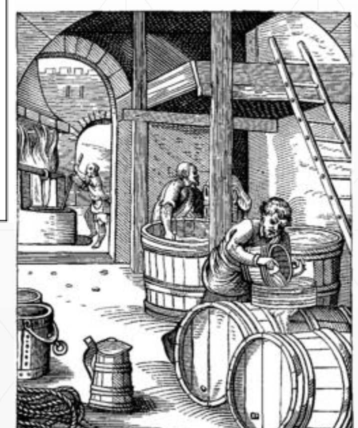
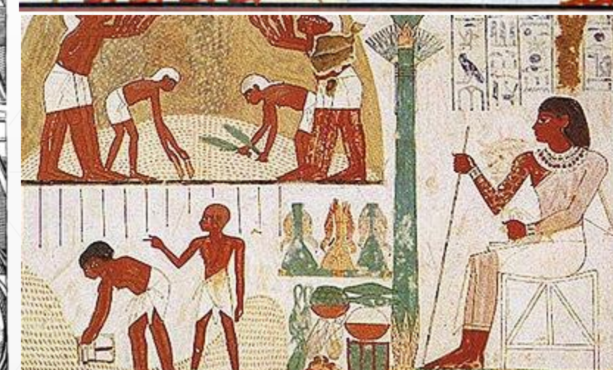
Biotechnology and the 21st Century



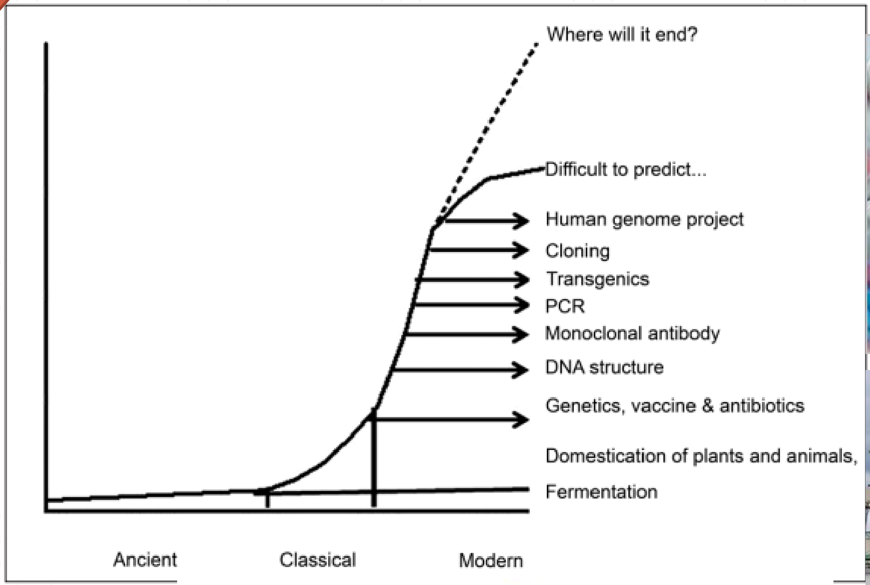


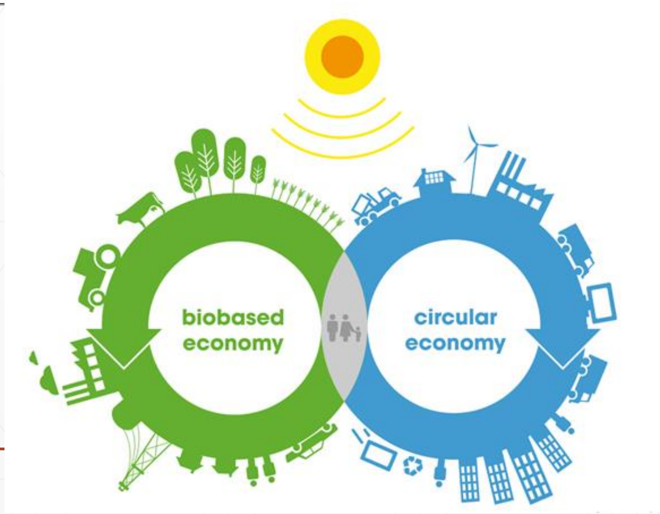






Biotechnology and the 21st Century



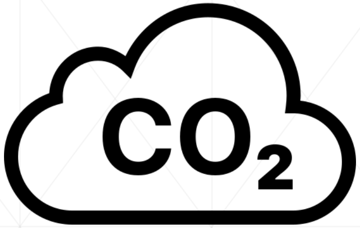


1.1. Introduction

The Advantages of Algal Biomass

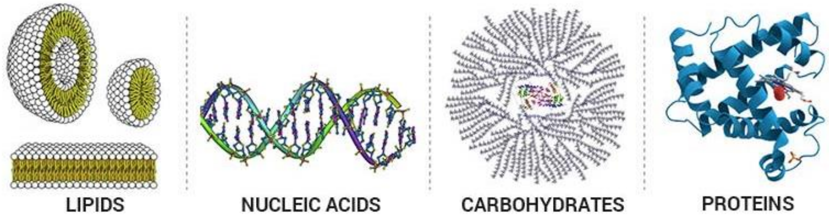
Growth capacity in saline waters and effluents

High CO2 absorption



Need for little area compared to traditional cultures

High content of compounds of interest



(Chisti, 2008; Oncel, 2013; Raheem et al., 2018)

1.1. Introduction

MICROALGAS: ALTERNATIVAS PROMISSORAS PARA A INDÚSTRIA. Brasil, B. S. A. F. & Garcia, L. C. **Agroenergia em revista: microalgas.** Ano IV, nº 10, dezembro de 2016.

Constant challenges in algae biomass production

- Selection of the most productive strains
- Low-cost culture media
- Photobioreactors
- Harvest
- Bioproducts
- Comercialization

Hypotheses

I. Replacing the standard medium with **fertilizers** can **reduce production costs** without reducing productivity,

II. **Different nitrogen sources** alter the composition of metabolites present in algal biomass,

III. Grow microalgae in a chemically **indefinite medium, as industrial effluents (POME),** at low cost,

IV. The growth of microalgae in industrial effluents is capable of **promoting their bioremediation**.

Chemically definite medium

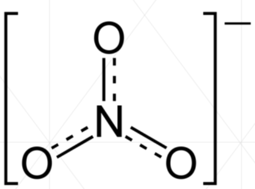
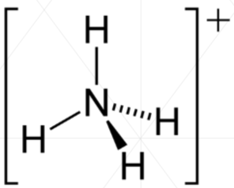
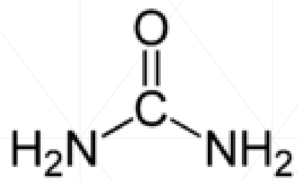
A low-cost approach for *Chlorella sorokiniana* production through combined use of urea , ammonia and nitrate based fertilizers.

Bioresour Technol Reports 9.

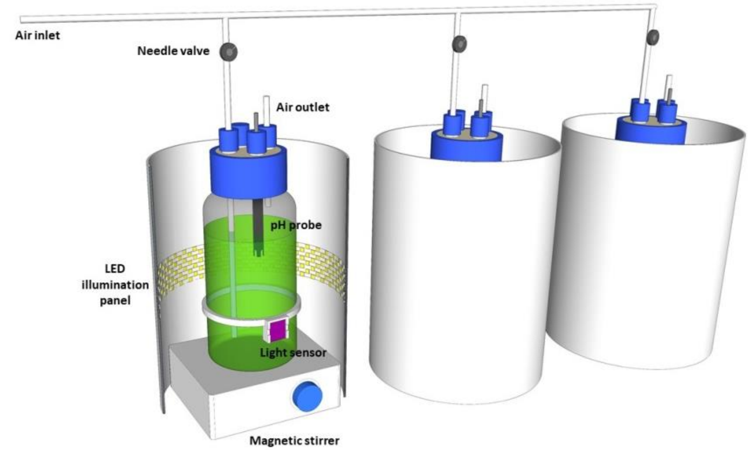
Ribeiro DM, Fernando L, Cunha G, Costa L, Jungmann L, Christopher T, Williams R, Alves S, Brasil F (2020)

Specific objectives – Experimentation

Formulation of medium

Nitrate	Ammonium	Urea
		

Automated photobioreactor



Characterization of biomass

Carbon, nitrogen and hydrogen and proteins	→	Elementary analyzer CHNS / O
Soluble, structural and reserve sugars	→	Van Wychen e Laurens, (2016)
FAME	→	Van Wychen et al., (2013)
Pigments (chlorophyll a and b, carotenoids)	→	Lichtenthaler e Wellburn (1983).
Amino acids and organic acids	→	Lisec et al., 2006

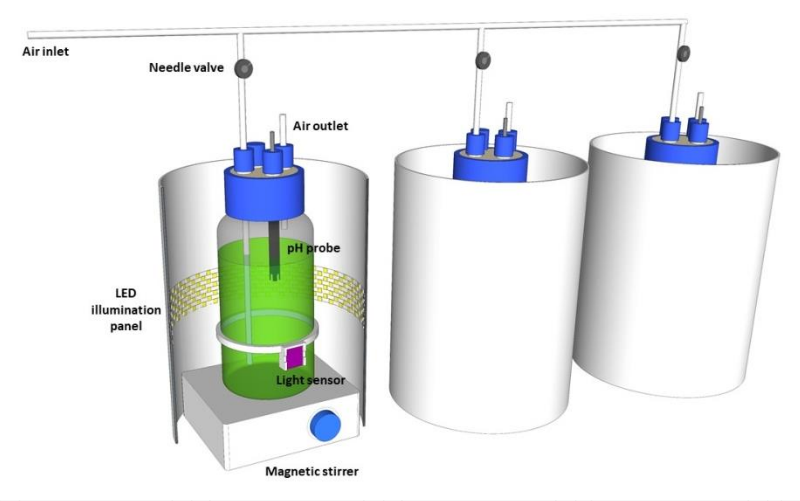
Estimation of biomass production costs

Chlorella sorokiniana LBA # 39

Cell concentration - **2 x 10^5 cel / ml**
pH 6,8, Light/dark - **16h / 8h (1500 lux)**
Temperature - **28 ± 1 ° C**
Air flow - **2 L / min**
with **0,1% (v / v) of CO_2** (1000 ppm).

Every 5 minutes, the system recorded the microalgae density and pH in each reactor during 8 days.

Collection of microorganisms from Embrapa Agroenergy (Laboratório de Biotecnologia Algal - LBA). Collected at Chapada Imperial, Brasília / DF



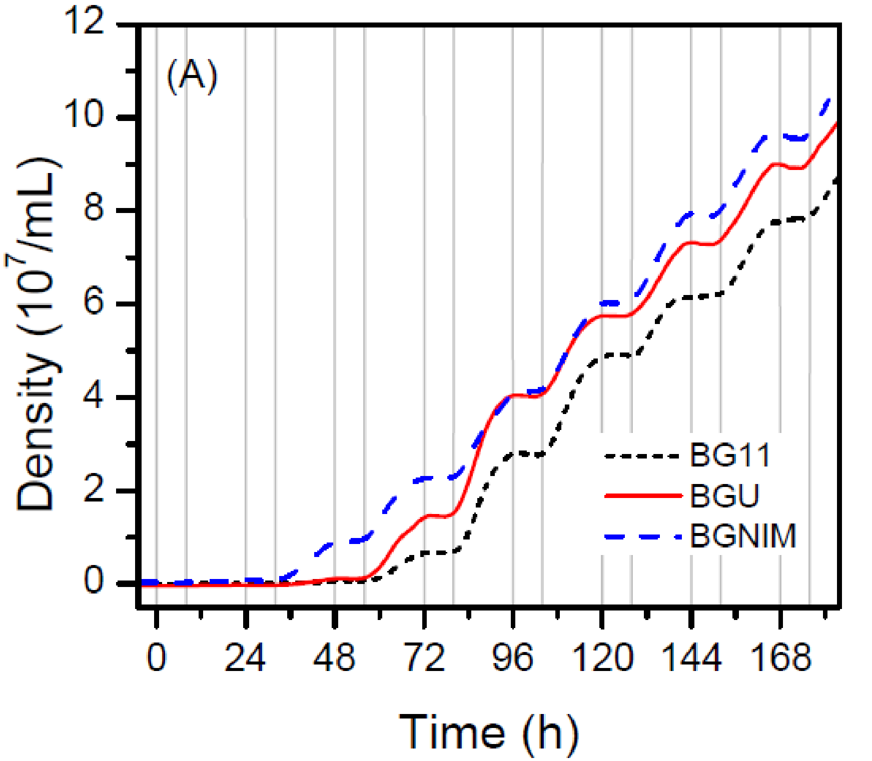
- ➢**BLUE GREEN 11 (BG11)**
- ➢**BLUE GREEN UREA (BGU)**
- ➢**BLUE GREEN NITROGEN MIX (BGNIM)**

Elementos	Formulation BG 11 mg/L	BGU mg/L	BGNIM mg/L
N	247.59	247.62	249.00
H	6.60	42.17	40.37
C	6.49	112.49	102.00
O	946.00	240.12	232.30
S	9.79	9.79	11.04
Fe	1.28	1.28	2.66
Cl	7.12	7.12	-
K	11.49	11.49	11.60
Mg	7.40	7.40	7.40
Na	252.60	5.40	-
P	9.10	9.10	9.42
Zn	0.05	0.05	3.38
Co	0.01	0.01	-
Cu	0.02	0.02	0.36
Mn	0.50	0.50	2.48
Mo	0.15	0.15	0.04
Ca	9.81	9.81	9.77
B	0.50	0.50	2.10

Formulation	BG 11 (mg/L)	BGU (mg/L)	BGNIM (mg/L)
$NaNO_3$	1500	-	-
CH_4N_2O	-	530	-
CH_4N_2O	-	-	510
KH_2PO_4	40	40	
$NH_4H_2PO_4$	-	-	35
$MgSO_4 \cdot 7H_2O$	75	75	-
$MgSO_4 \cdot 7H_2O$	-	-	75
$CaCl_2.2H_2O$	36	36	
$Ca(NO_3)_2$	-	-	40
Na_2CO_3	20	20	-
EDTA NA_2	1	1	-
$Co(NO_3)_2.6H_2O$	0,05	0,05	-
$Na_2MoO_4 \cdot 2H_2O$	0,39	0,39	-
H_3BO_3	2,86	2,86	-
$CuSO_4.5H_2O$	0,08	0,08	-
$MnCl_2.4H_2O$	1,81	1,81	-
$ZnSO_4.7H_2O$	0,22	0,22	-
$C_6H_8FeNO_7$	6	6	-
$C_6H_8O_7$	6	6	-
Micronutrientes	-	-	100

Growth dynamics of Chlorella sorokiniana in the three formulations of culture media

Microalgae density

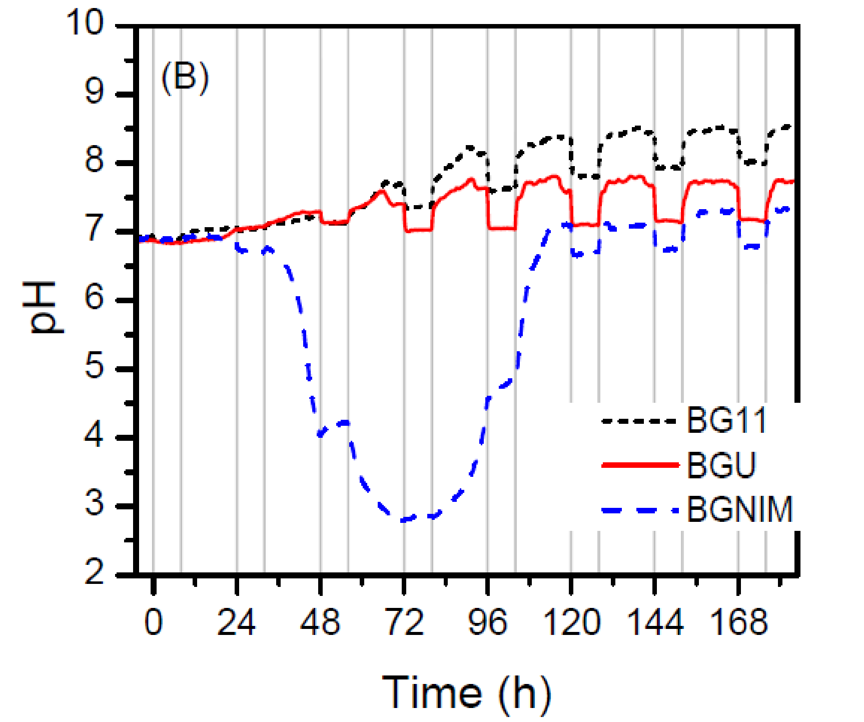


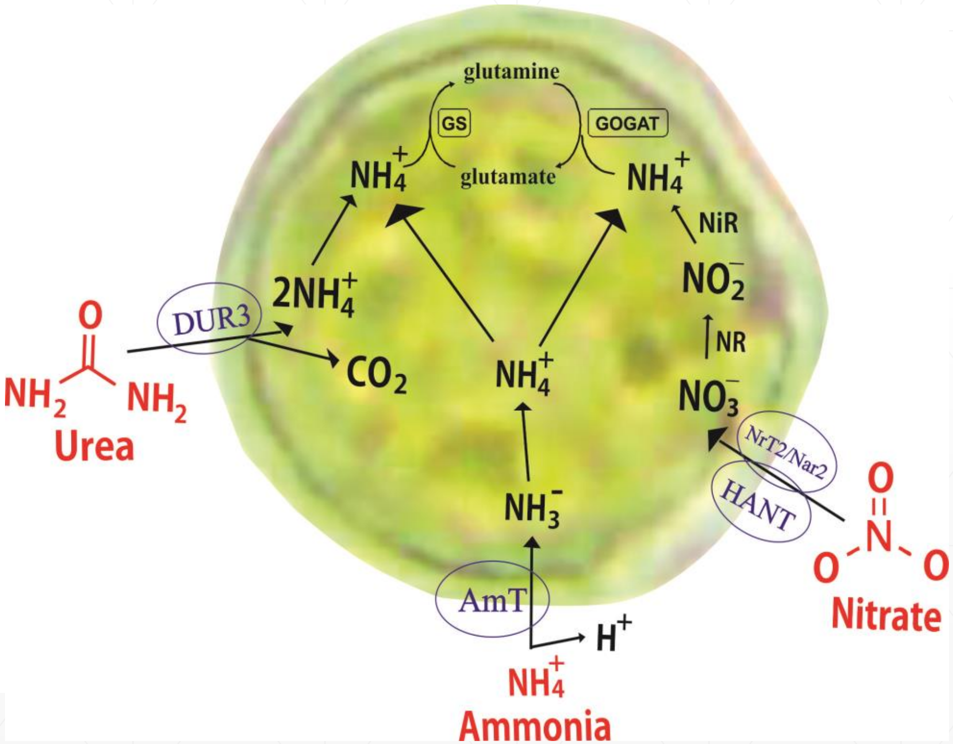
Biomass productivity varied between 47 and 50 mg • L-1 • d-1, No statistical difference was observed in the rate of growth and productivity of biomass between the three media tested

Result obtained by optical density sensor TCS34725

Growth dynamics of Chlorella sorokiniana in the three formulations of culture media

Culture pH value.





Resultado obtido por sonda de pH

Chlorella sorokiniana|LBA#39	BG11	BGU	BGNIM
Protein (%)	41.67 [b]	44.57 [a]	44.63 [a]

Increase of approximately 7% of proteins

Proteins are one of the most valuable fractions of algae biomass when considering use as animal feed and human food (Odjadjare et al., 2017).

Result obtained by CHNS / O elementary analyzer - PE2400 series II PerkinElmer

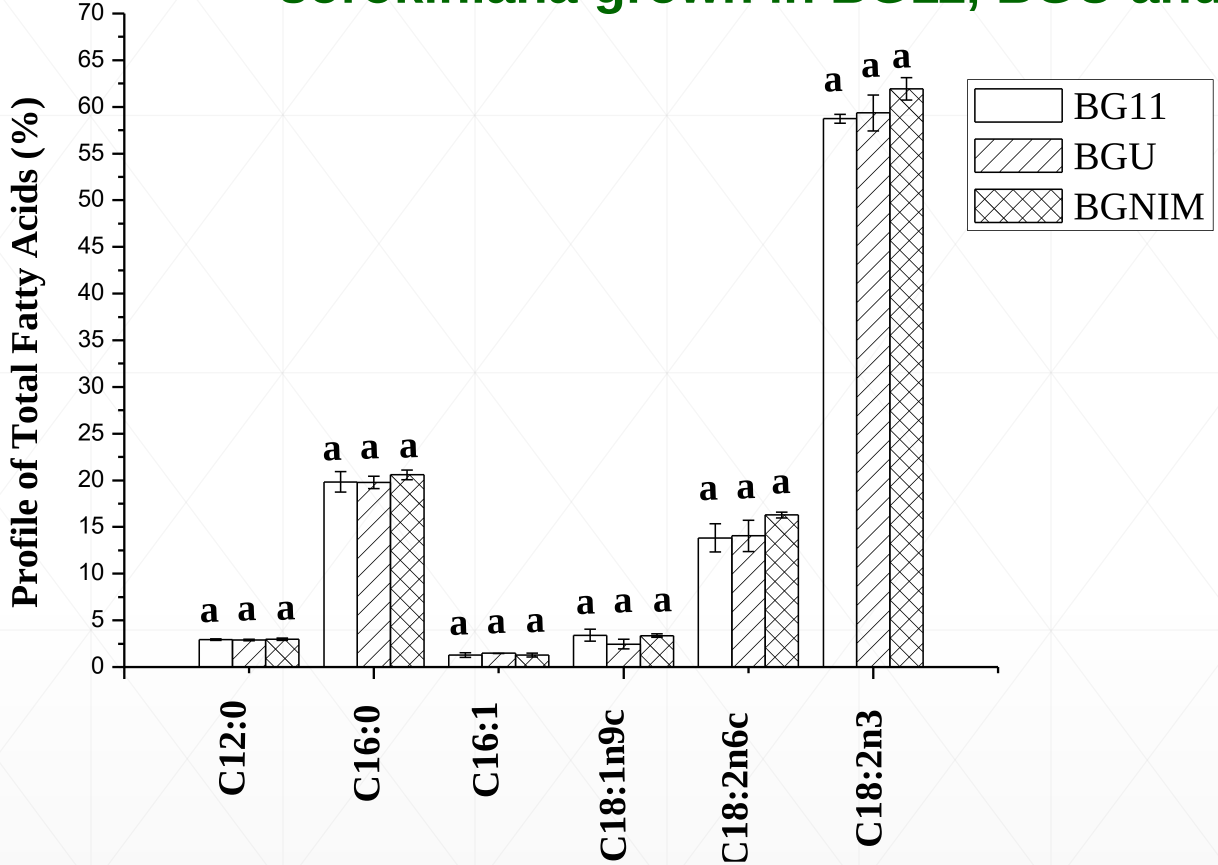
Chlorella sorokiniana|LBA#39	BG11	BGU	BGNIM
Chlorophyll a (%)	2.30 [b]	2.60 [a]	1.70 [c]
Chlorophyll b (%)	1.10 [b]	1.30 [a]	0.60 [c]
Carotenoids (%)	0.43 [b]	0.58 [a]	0.61 [a]

Approximately 41% increase in carotenoids

Chlorella species represent a strong candidate for commercial production of algae carotenoids and, in addition to their economic value, carotenoids play an important role in the robustness of microalgae to photo-oxidation (Patias et al., 2017).

Result obtained by extraction of acetone and reading in a spectrophotometer

Profile of fatty acid methyl esters (FAMEs) of Chlorella sorokiniana grown in BG11, BGU and BGNIM culture media



Profile of fatty acid methyl esters (FAMEs) from Chlorella sorokiniana grown on BG11, BGU and BGNIM culture media. All the experiments were conducted in three independent replicates (n=3). The results are presented as means of the replicates ± error bars show the standard deviation.

* Means followed by the same letter do not differ by Tukey test at the 5% probability level.

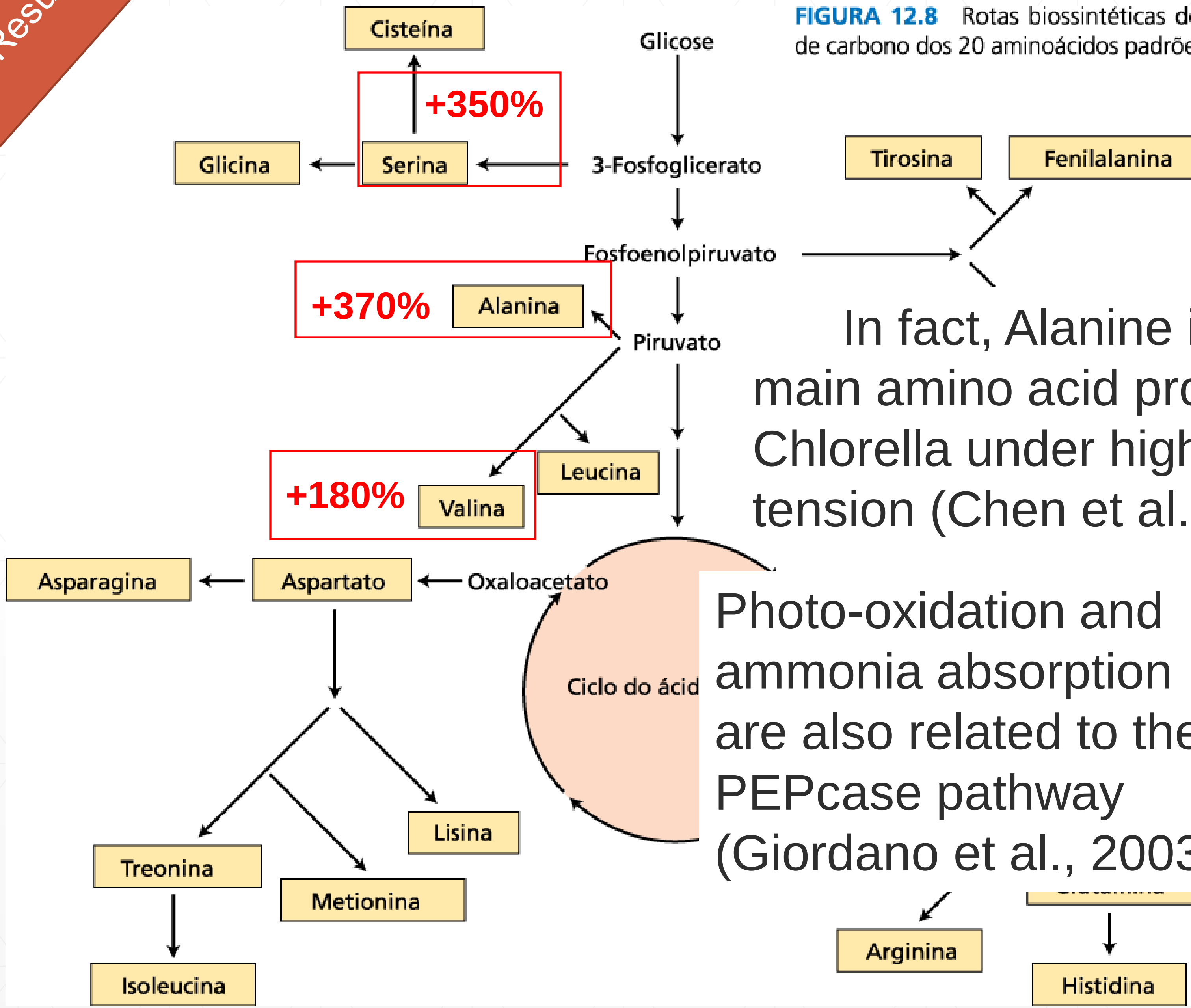


FIGURA 12.8 Rotas biossintéticas dos esqueletos de carbono dos 20 aminoácidos padrões.

In fact, Alanine is the main amino acid produced in Chlorella under high oxygen tension (Chen et al., 2017)

Photo-oxidation and ammonia absorption are also related to the PEPcase pathway (Giordano et al., 2003)

Resultado obtido por cromatógrafo gasosa acoplado a espectrometria de massa (GC/MC)

Comparison between productivity and costs of tested media

Estimates	BG11	BGU	BGNIM
Biomass Productivity ($mgDW \cdot L^{-1} \cdot d^{-1}$)	50.93	48.81	47.93
Biomass Productivity ($ton \cdot year^{-1}$)[a]	2.40	2.30	2.30
Total Protein Productivity ($ton \cdot year^{-1}$)	1.01	1.04	1.02
Total Carbohydrates Productivity ($ton \cdot year^{-1}$)	0.54	0.53	0.56
Total FA Productivity ($ton \cdot year^{-1}$)	0.15	0.15	0.14
Bio-ethanol Productivity ($L \cdot year^{-1}$)[b]	328.70	318.30	338.90
Biodiesel from FA Productivity ($kg \cdot year^{-1}$)[c]	158.90	152.30	142.60
Cost 1 L of Medium (USD)[d]	0.17	0.06	0.01
Cost for 1kg Biomass (USD)[d]	470.41	171.22	19.37

[a]Year round estimates considering 200 $m^3.d^{-1}$ with 240 working days per year, [b]Estimated based on the conversion rate of 0.6 L per kg of total carbohydrates, [c]Estimated based on the conversion rate of 1 kg of fatty acid to 1 kg of biodiesel (Cabanelas et al., 2013; Santana et al., 2017), [d]USD = United States Dollar

Cost analyzes of the different media indicate that

Replacing the nitrogen source with urea (BG11 → BGU) has the potential to **reduce the cost of the medium by 65%**

Formulation with commercial fertilizers (BG11 → BGNIM), the cost is **reduced by approximately 95%**

Chemically indefinite medium

Selection and cultivation of microalgae in palm oil industry effluent for nutrient removal and algal biomass production.

Ribeiro D.M.; Cereijo, C.R.; Santana H.; Nascimento, R.C.; Calsing, L.C.G.; Siqueira, F.G.; Brasil, B.S.A.F.

Specific objective

- Select high strain growth rate in effluent (POME),
- Cultivate in a 15 L photobioreactor,
- Evaluate your bioremediation potential
- Characterize the biomass produced

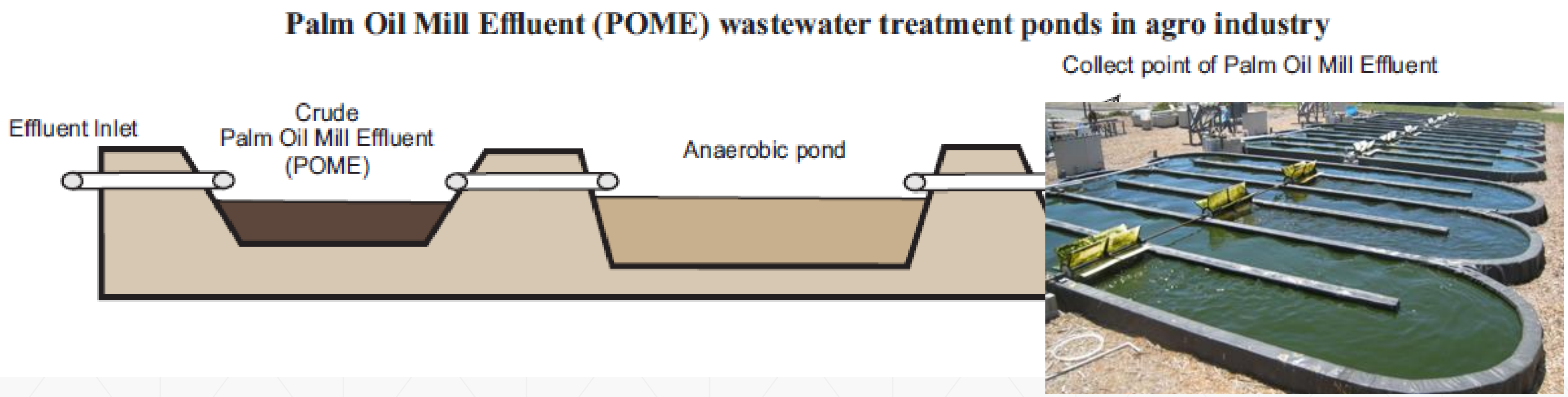
- World palm oil production exceeds 55 million tons annually,
- Market over $ 34 billion a year,
- Industrial production of 1 ton of crude palm oil requires about 5 to 7.5 tons of water,
- More than 200 million tons of effluent from the palm oil mill (POME).

(Center For International Development At Harvard University, 2019; Food and Agriculture Organization of the United Nations, 2019)

Denpasa – Dendê do Pará S/A

Óleo de Palma

Palm oil mill (POME) effluent treatment ponds in the agro-industry (DENPASA - Dendê do Pará S / A)

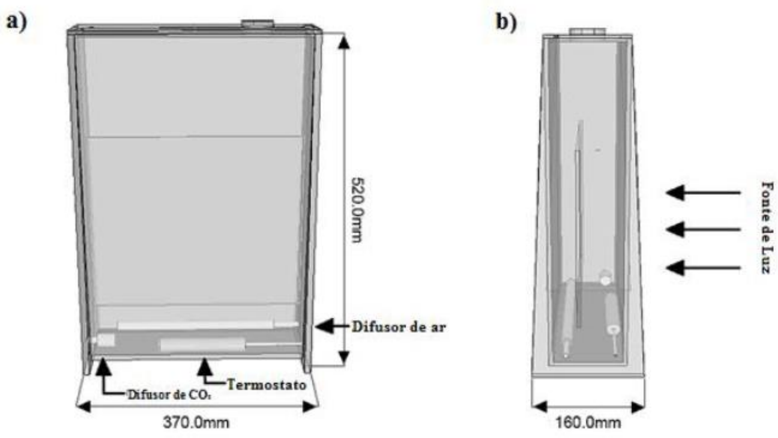


Chlorophyta

Embrapa|LBA#8
Embrapa|LBA#13
Embrapa|LBA#32
Embrapa|LBA#35
Embrapa|LBA#36
Embrapa|LBA#37
Embrapa|LBA#38
Embrapa|LBA#39
Embrapa|LBA#40
Embrapa|LBA#41
Embrapa|LBA#45
Embrapa|LBA#46
Embrapa|LBA#47
Embrapa|LBA#48
Embrapa|LBA#49
Embrapa|LBA#50
Embrapa|LBA#51
Embrapa|LBA#52

18 Microalgae strains from the Collection of Microorganisms and Microalgae for Agroenergy and Biorefineries of the Brazilian Agricultural Research Corporation - Embrapa (Brasília-DF)

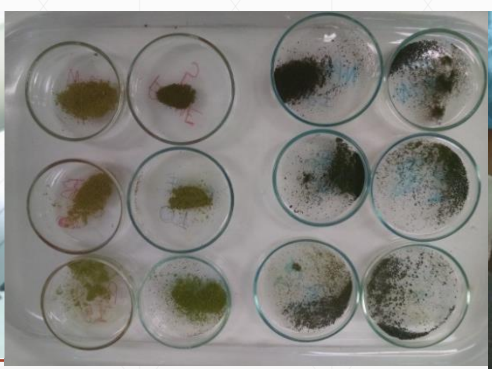
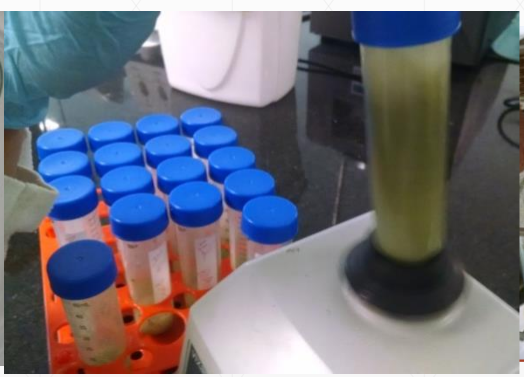
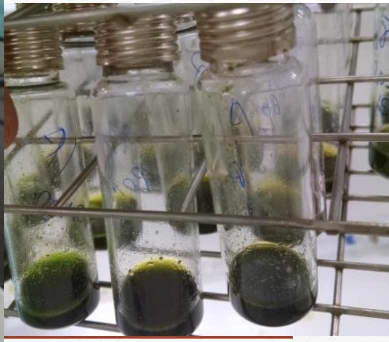
Photobioreactor and culture condition



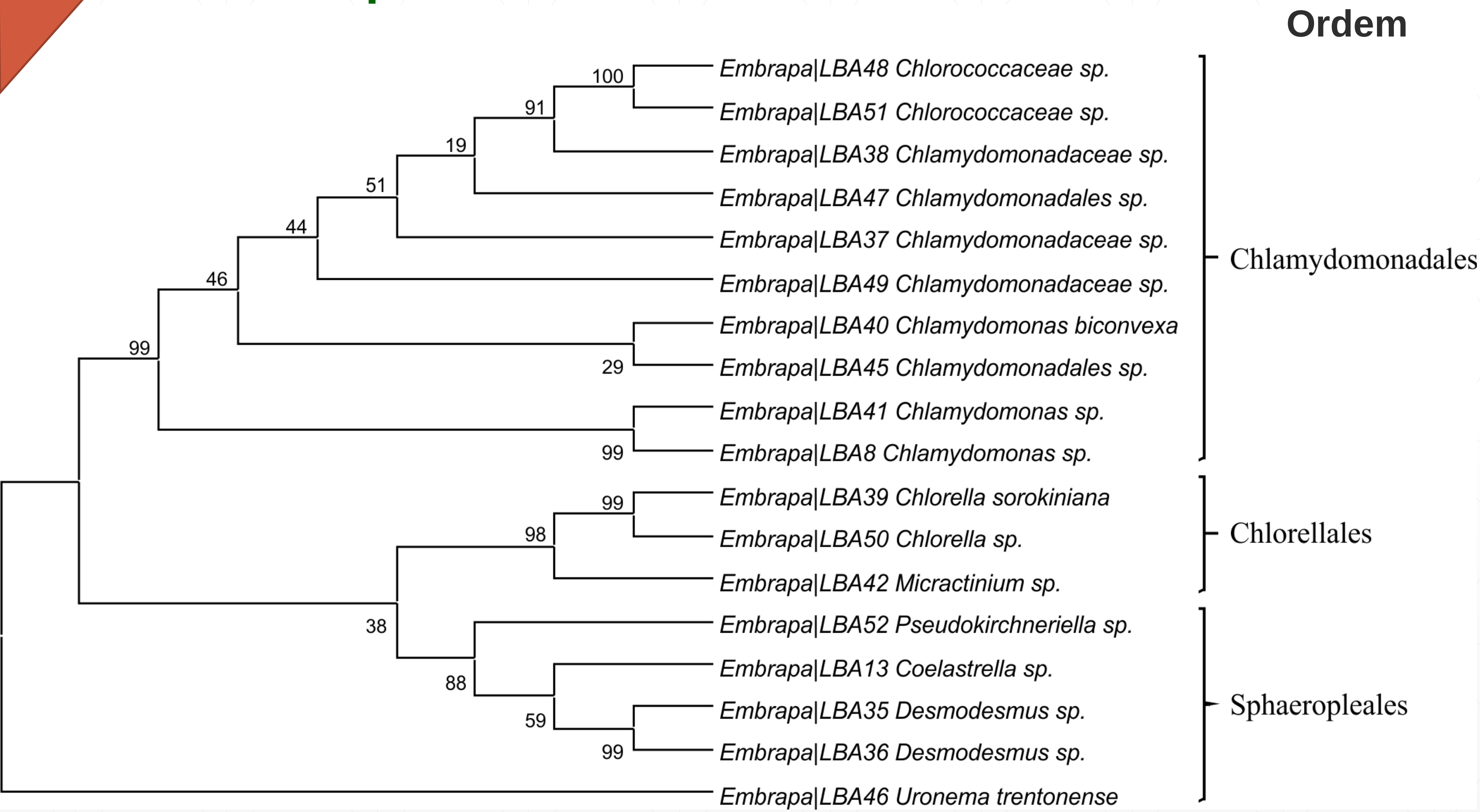
Photobioreactor - **15L air lift**
Working volume - 13L
Aeration 60 Lh^{-1}
CO2 (v/v) - 5%
12h / 12h photoperiod
Luminous intensity 35 klux
Temperature
25 ° C in the dark and
35 ° C in the clear

Análise da composição bioquímica da biomassa

- Total solids and ashes - Wychen and Laurens (2015)
- Total proteins – MicroKjeldahl
- Carbohydrate content and profile - Wychen and Laurens (2015)
- Lipid content - Ankom XT15
- Lipid profile - Wychen, Ramirez and Laurens (2015)
- Carotenoids by Lichtenthaler, H., Wellburn, A., 1983

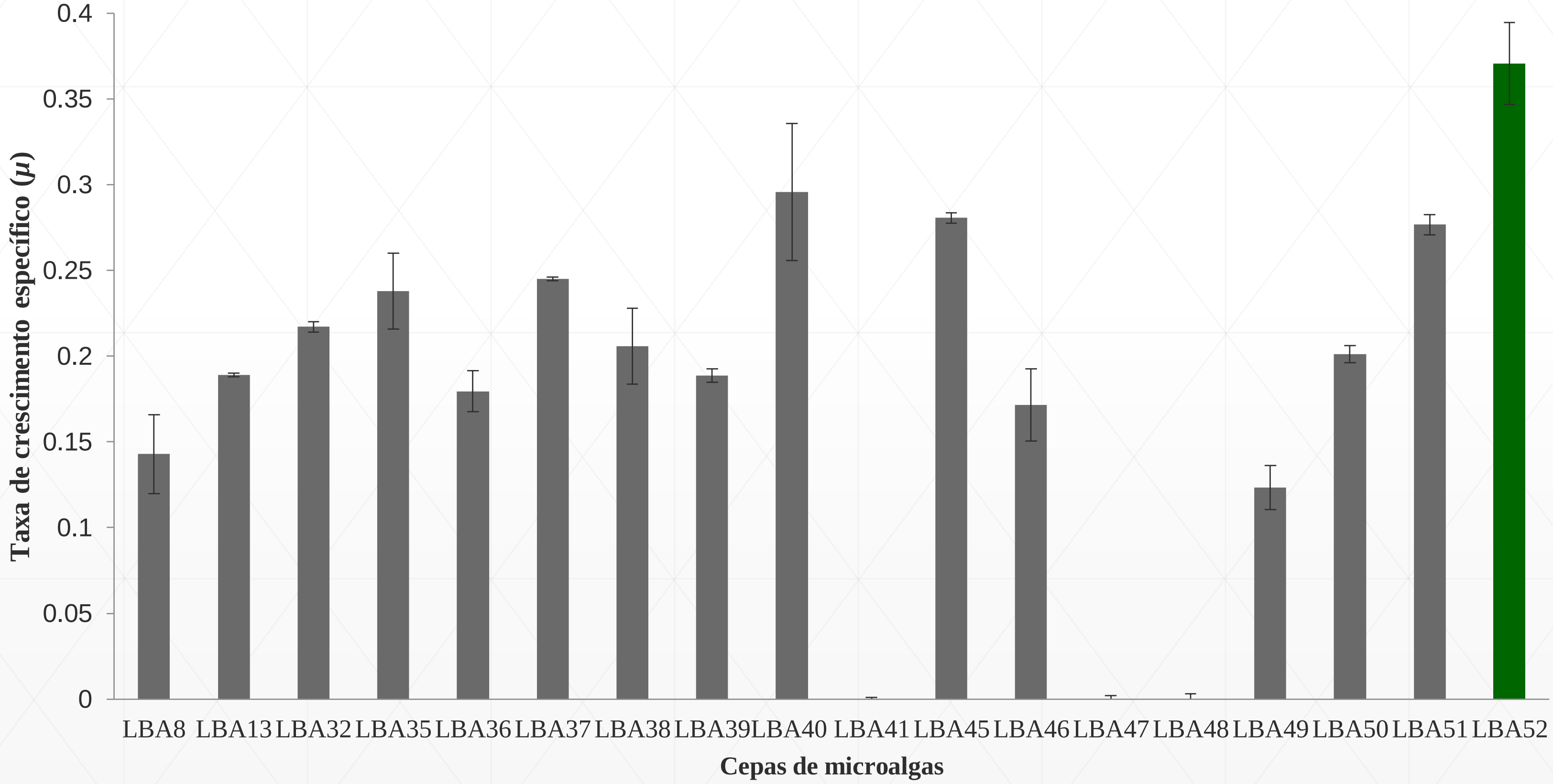





The 18 green microalgae strains of the Phylum Chlorophyta used in this study based on the sequence of the nuITS2 marker



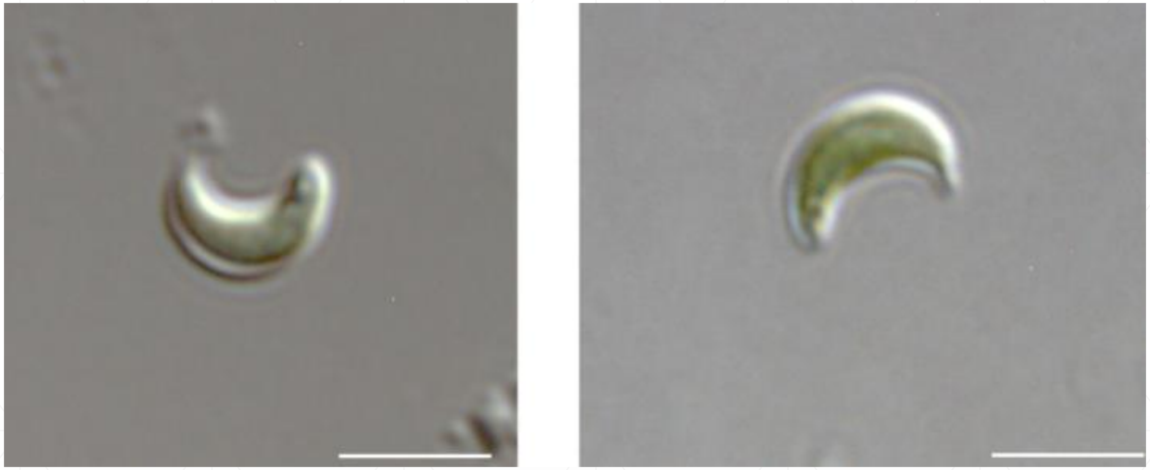
Screening of microalgae for growth in POME

Specific growth rate of 18 microalgae strains grown in 500 ml Erlenmeyer flasks containing 250 ml of POME for 10 days



Resultado obtido por contagem de células em câmera de Neubauer

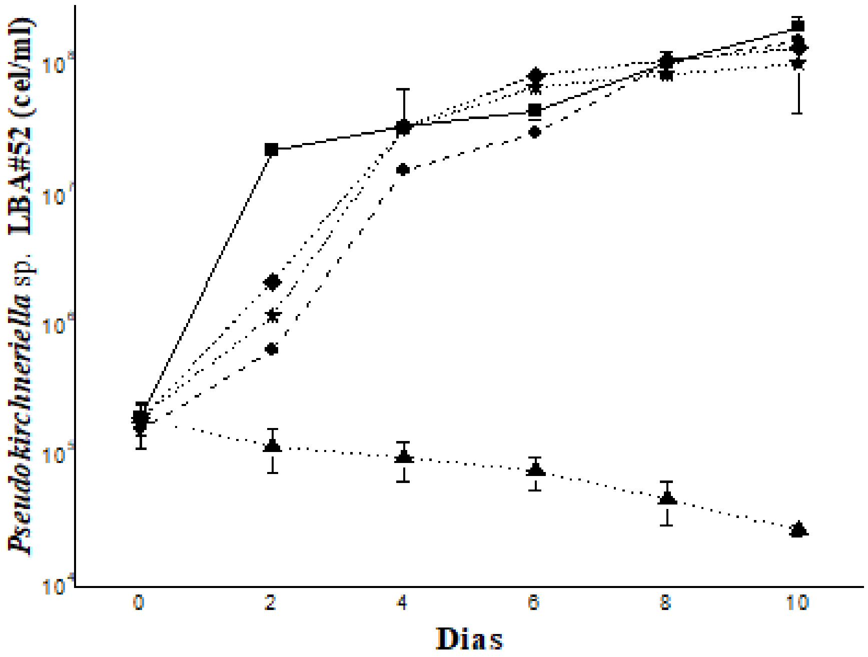
Pseudokirchneriella sp. LBA # 52



Collection of microorganisms from Embrapa Agroenergy

Collected in the POME stabilization pond, in Santo Antônio Tauá / PA. Therefore, an indigenous strain

Growth dynamics of *Pseudokirchneriella* sp. LBA # 52



Bold Basal Medium (- ■ -)

Axenic POME (autoclaved before the experiment) 12h / 12h light / dark (- ● -)

Axenic POME in dark regime (·· ▲ ··)

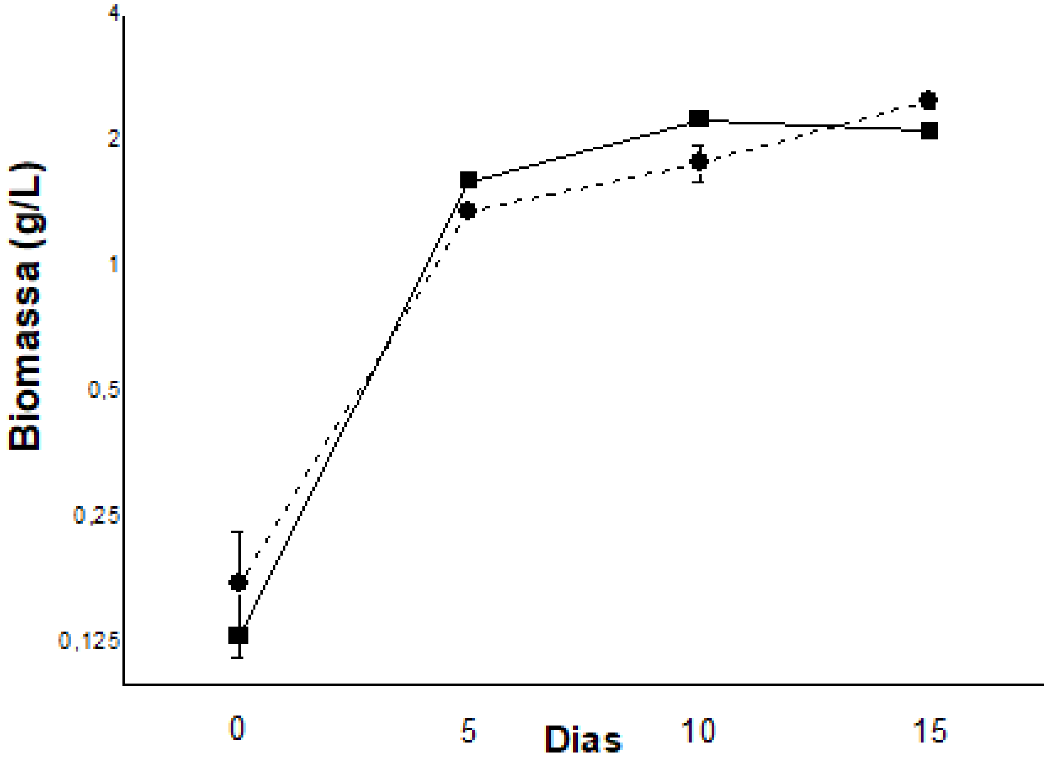
Non-axenic POME 12h / 12h light / dark (- ♦ -)

50% non-axenic POME, 12h / 12h light / dark(··★··)

Resultado obtido por contagem de células em câmera de Neubauer

2.3. Results

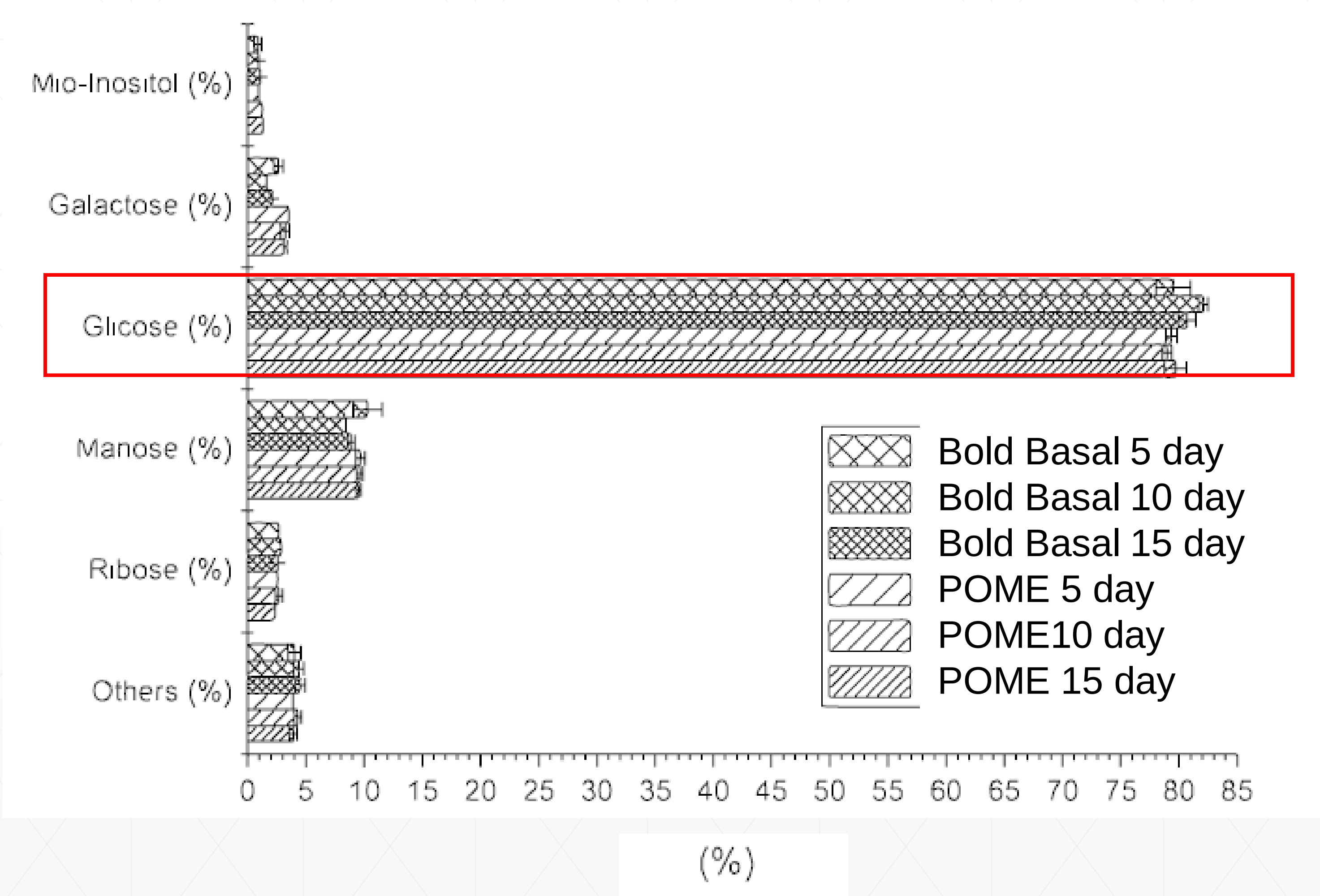
Dynamics of biomass production (dry) from Pseudokirchneriella sp. LBA # 52 15 L flat plate photobioreactor



Bold Basal (−■−)
POME (--●--)

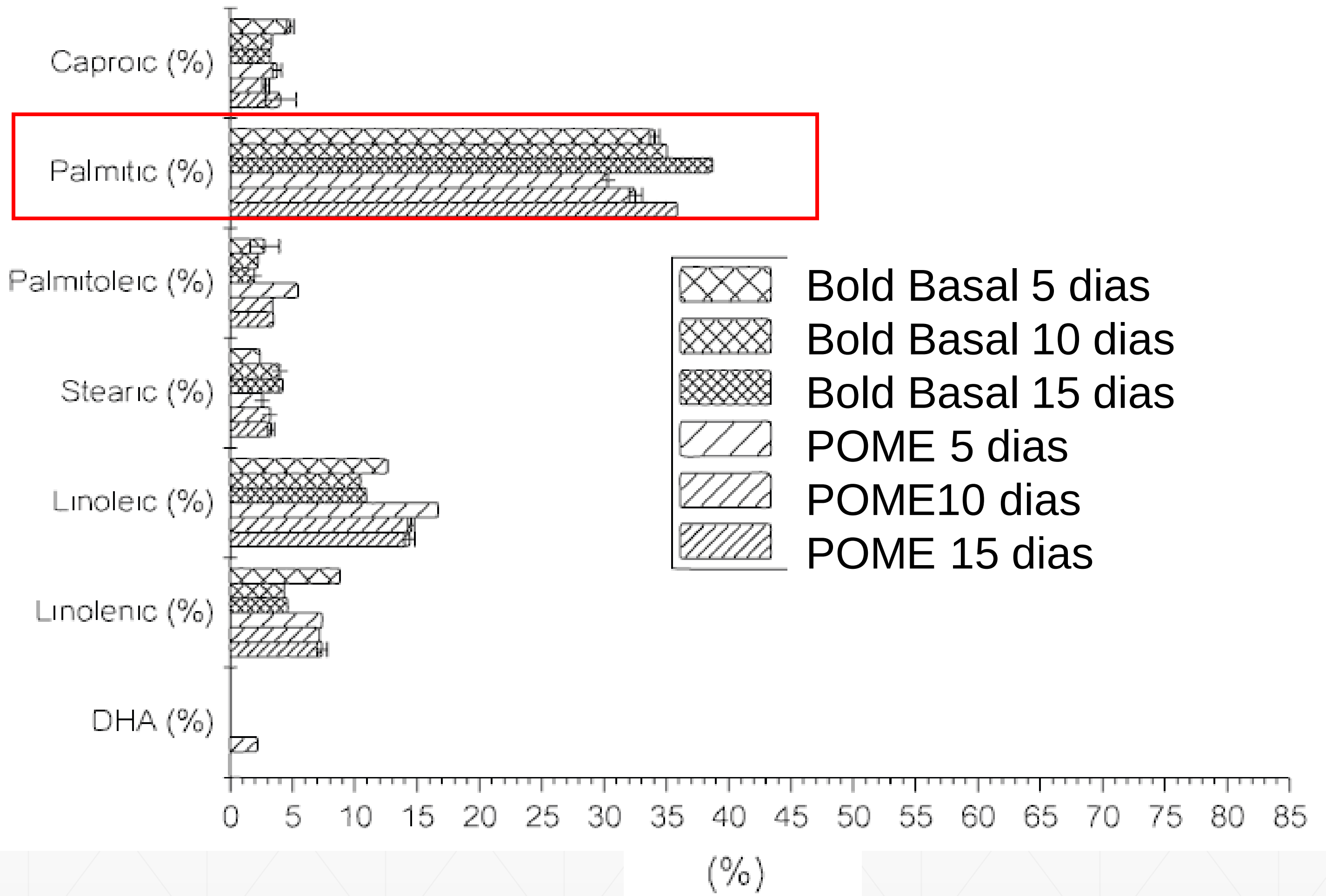
After five days of cultivation, Pseudokirchneriella sp. showed productivity of 320 mgDW • L^{-1} • d^{-1} in BBM and 272 mgDW • L^{-1} • d^{-1} in 100% POME

Profile of carbohydrates accumulated in *Pseudokirchneriella* sp. LBA # 52 grown in different media



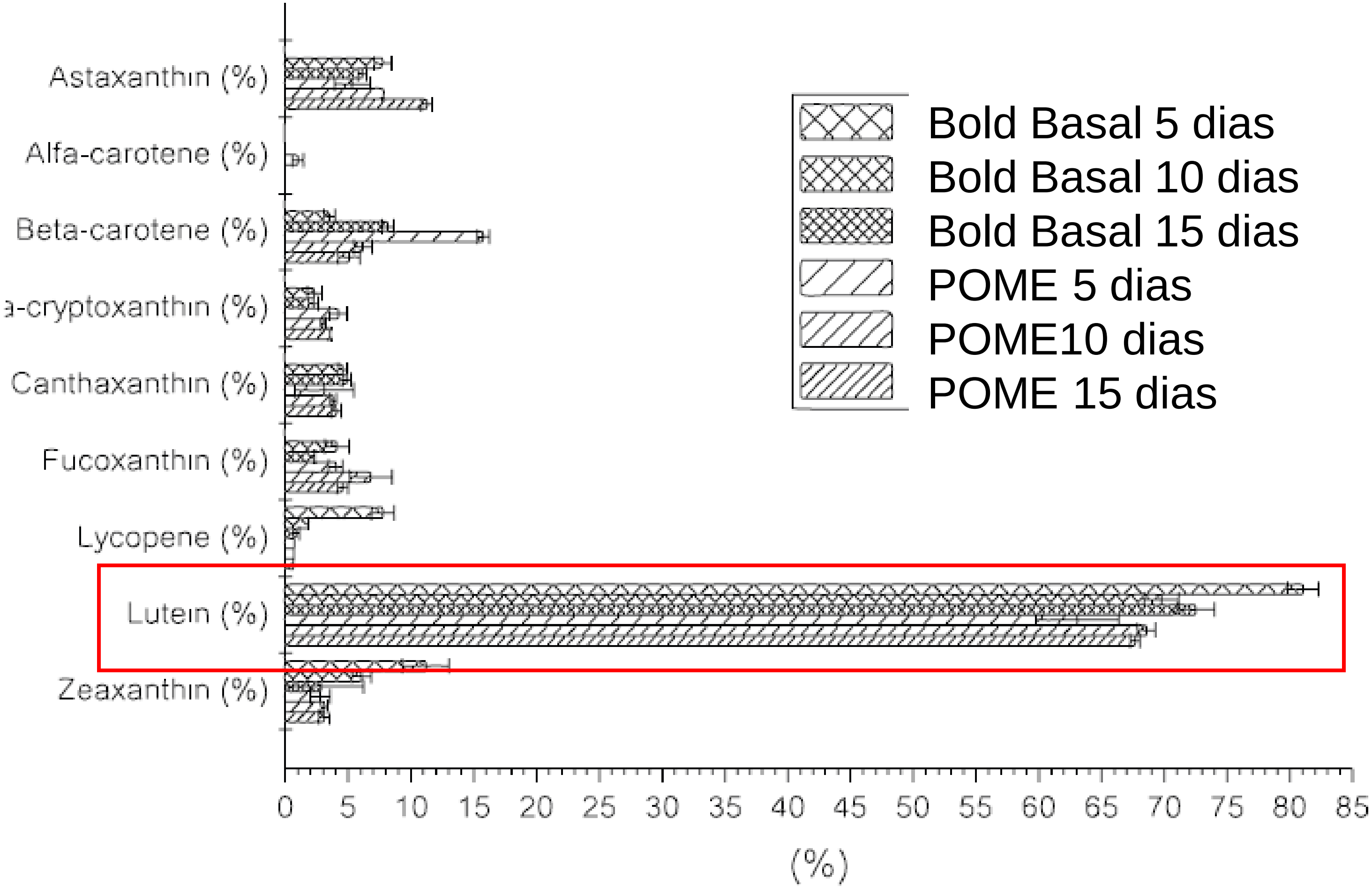
Resultado obtido por cromatografia líquida de alta performance (HPLC)

Profile of fatty acid methyl esters (FAME) accumulated in Pseudokirchneriella sp. LBA # 52 grown in different media



Resultado obtido por cromatógrafo gasosa acoplado a espectrometria de massa (GC/MC)

Profile of accumulated carotenoids in Pseudokirchneriella sp. LBA # 52 grown in different media



Estimated productivity between Bold Basal and POME at different times

Estimates	Bold Basal 5 days	Bold Basal 10 days	Bold Basal 15 days	POME 5 days	POME 10 days	POME 15 days
Biomass Productivity (mgDW • L^{-1} • d^{-1})	320,60	224,90	140,68	272,13	177,23	166,9
Biomass Productivity (ton.year^{1})	15,38	10,79	6,75	13,06	8,5	8,01
Total Protein (ton.year^{1})	3,01	1,67	1,14	3,12	1,85	1,59
Total Carbohydrates (ton.year^{1})	6,71	4,43	2,6	5,18	3,08	2,52
Total FA (ton.year^{1})	1,49	1,19	0,57	1,11	1,06	1,09
Bio-ethanol (L.year^{1})	4028,5	2659,5	1562,3	3113,77	1848,74	1515,07
Biodiesel from FA (Kg.year^{1})	1486,6	1190,7	566,5	1111,59	1060,82	1090,32

Year round estimates considering 200 m3.d-1 with 240 working days per year (Cabanelas et al., 2013; Ribeiro et al., 2020)
Estimated based on the conversion rate of 0.6 L per kg of total carbohydrates (Cabanelas et al., 2013; Ribeiro et al., 2020)
Estimated based on the conversion rate of 1 kg of fatty acid to 1 kg of biodiesel (Cabanelas et al., 2013; Ribeiro et al., 2020)
POME: Palm Oil Mill Effluent.

Physico-chemical characterization of crude POME and POME before and after the cultivation of Pseudokirchneriella sp. LBA # 52

Reduction

63.86% of COD

99.99% of Ammonium

96.72% of Phosphate

It has been reported that the cultivation of ***Chlorella sorokiniana*** in POME can **lead to a reduction of 93.36% in ammonium and 94.50% in phosphate** (Khalid et al., 2019)

The growth of **Nannochloropsis sp. promoted a 71% reduction in COD** (Emparan et al., 2020). However, both studies achieved lower algal biomass productivity than those reported in this work.

General conclusions

The low-cost chemically defined medium (BGNIM)

Increased protein and carotenoid buildup compared to standard BG11 medium

Acidification observed during the initial cultivation phase

Native strain *Pseudokirchneriella* sp. LBA # 52

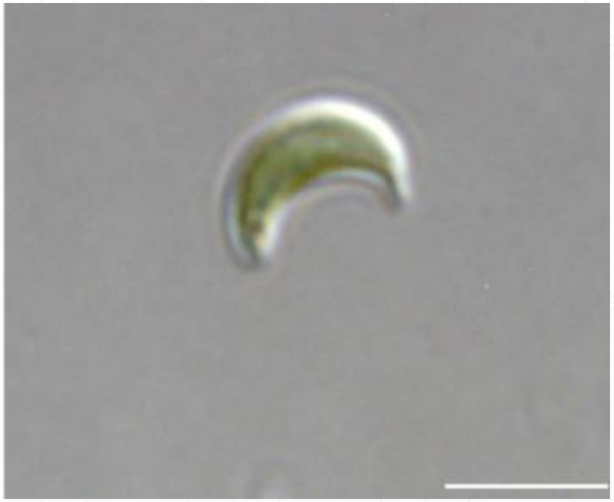
Bioremediation and value-added biomass production

Opens opportunities for industrial application of microalgae and integration of algae biorefineries in industrial palm oil plants

Superior strains for algal biomass production

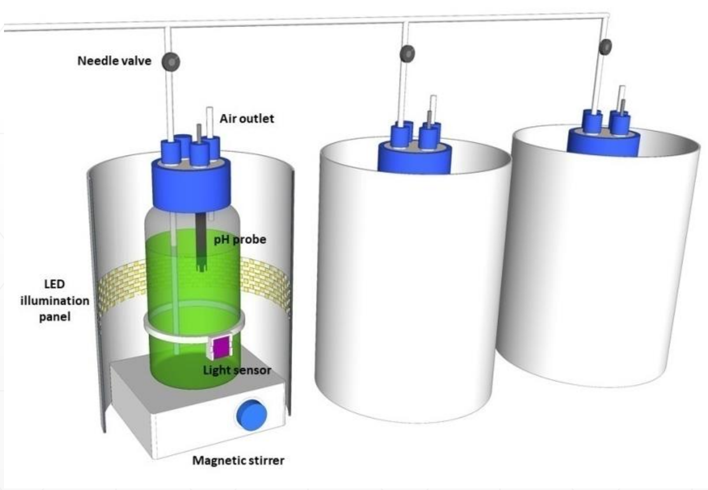
Chlorella sorokiniana LBA#39

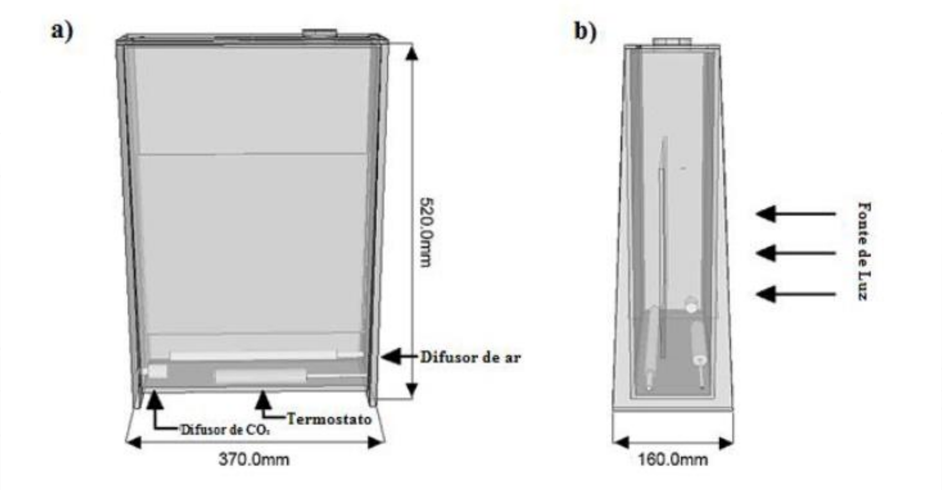
Pseudokirchneriella sp. LBA#52



Closed photobioreactor configurations in air lift cultivation systems

i) 1L tubular photobioreactor with a refined automated system
ii) 15L pilot scale photobioreactor Flate Plate



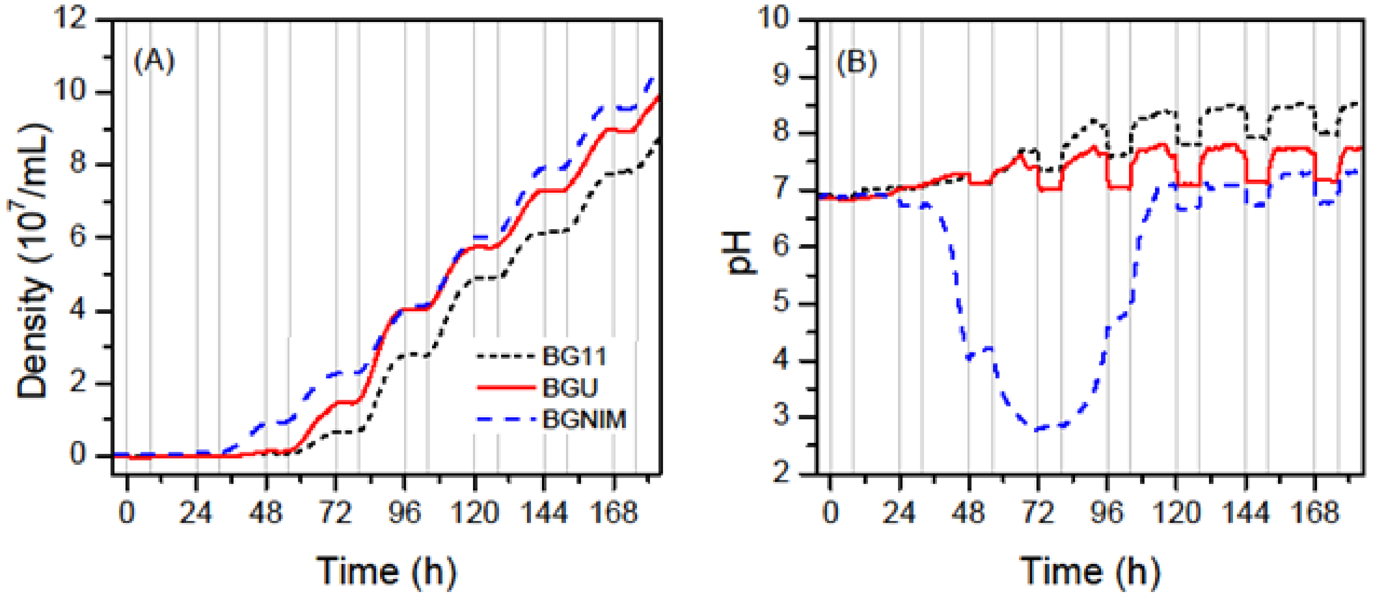


Final Considerations

This work presents the formulation of the

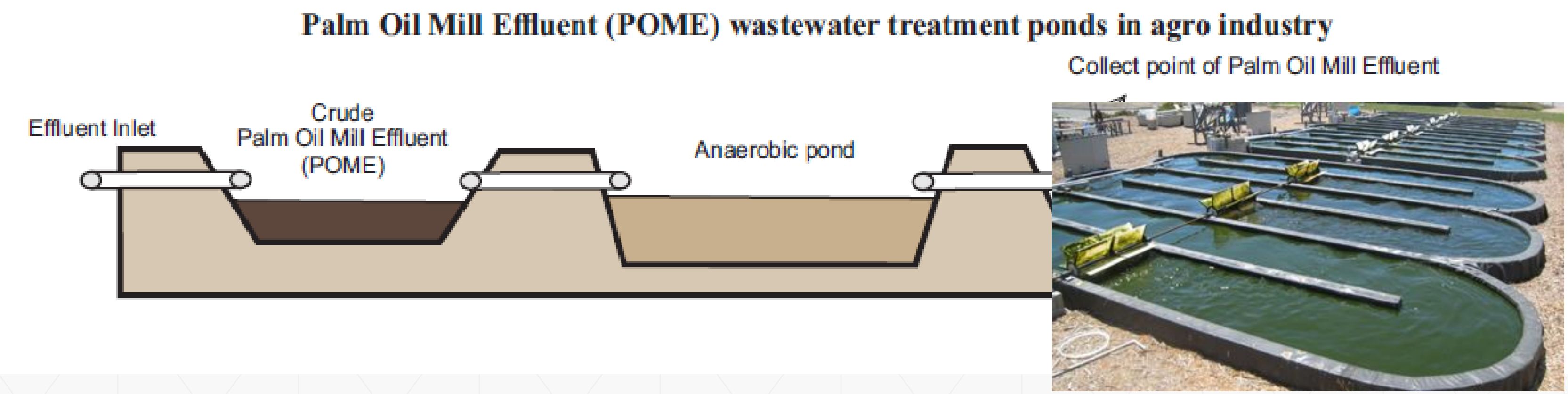
Blue Green Nitrogen Mix (BGNIM)

for cultivation of *Chlorella sorokiniana* LBA # 39



Bioremediation and adding value to the palm oil industry

Indication to the palm oil industry, use its own effluent, POME, and produce biomass from the native microalgae strain *Pseudokirchneriella* sp. LBA # 52.



Thank you very much for your attention

Dágon Manoel Ribeiro
dagonribeiro@biotecland,com
Phd in Biotechnology and Biodiversity
Chief Executive Officer of Biotecland
Visiting Professor at Unizambeze - Mozambique
www.biotecland.com