



NEW MICROALGAE BIOREFINERY CONCEPT

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Introduction

Table 3
Different culture methods for various microalgae strains.

Strain	Nutrients	Culture condition	Specific growth rate (day ⁻¹)	Biomass productivity (g/L/day)	Biomass yield (g/L)	Lipid productivity (g/L/day)	Total lipid extracted (wt.% of biomass)	Comment	Reference
<i>Botryococcus braunii</i>	Secondary treated sewage	Air flowrate of 0.5 v/v/min Air flowrate of 1 v/v/min	0.13 0.14	0.288 0.346	- -	- -	17.85 17.85	High potential of using secondary treated sewage from domestic wastewater to grow microalgae. Microalgae biodiesel production coupled with waste water treatment appears as a good opportunity to commercialize the process. The organism exhibited wide range of pH adaptability. High lipid content and biomass productivity can be attained through biocoil due to greater light exposure and intensity inside the polyvinyl tubing. Higher lipid content was observed if microalgae cultured in heterotrophic condition.	Órpez et al., 2009 Sydney et al., 2011
<i>Botryococcus braunii</i>	Secondary domestic wastewater	100% of wastewater medium was used without dilution	0.11	0.034	0.48	-	36.14		
<i>Botryococcus braunii</i>	Modified Chu 13	With supplement of 2% (v/v) CO ₂	-	0.043	0.9	-	22		
<i>Chlamydomonas reinhardtii</i>	Wastewater	Microalgae was cultured in biocoil	0.564	2	-	0.505	25.25	Jerusalem artichoke appears as a low cost carbon source for heterotrophic microalgae culture. Anaerobic digested manure served as a low cost nutrients source to culture microalgae while at the same time, microalgae provide a valuable solution to refractory dairy waste. Urea is relatively low cost compare to other inorganic nitrogen sources.	Dayananda et al., 2007 Kong et al., 2010
<i>Chlorella protothecoides</i>	Inorganic basal medium	Heterotrophic culture with corn powder hydrolysate as carbon source	-	2.02	15.5	-	55.2		
<i>Chlorella protothecoides</i>	Basal Medium	Heterotrophic growth with glucose (24 g/L) as carbon source and yeast (4 g/L) as fermentation media	-	7.3	51.2	-	50.3		
<i>Chlorella protothecoides</i>	Basal medium	Heterotrophic culture with Jerusalem artichoke (30 g/L) as carbon source	-	4.1	17	1.7	43	Anaerobic digested manure served as a low cost nutrients source to culture microalgae while at the same time, microalgae provide a valuable solution to refractory dairy waste. Urea is relatively low cost compare to other inorganic nitrogen sources.	Cheng et al., 2009 Wang et al., 2010
<i>Chlorella</i> sp.	Anaerobic digested dairy manure	25 × diluted digested dairy manure	0.409	-	-	-	13.7		
<i>Chlorella</i> sp.	Walne medium with urea as nitrogen source	Urea concentration of 0.025 g/L and microalgae was cultured in batch culture mode for 6 days Urea concentration of 0.20 g/L and microalgae was cultured in batch culture mode for 6 days	0.86 1.42	- -	0.464 2.027	0.051 0.11	66.1 32.6		
<i>Chlorella vulgaris</i>	Modified Fitzgerald medium	Normal nutrients condition (20 days) 20 days of normal nutrients condition followed by 17 days of nitrogen limited condition	- -	0.043 -	0.86 -	0.0128 -	29.5 44		Hsieh and Wu, 2009

Lam and Lee, 2012

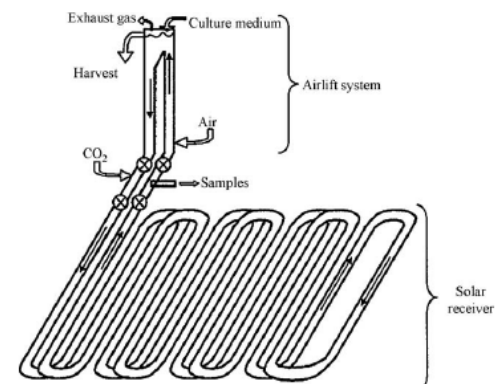


Fig. 2. Basic design of a horizontal tubular photobioreactor (adapted from Becker)

Brennan and Owende, 2010

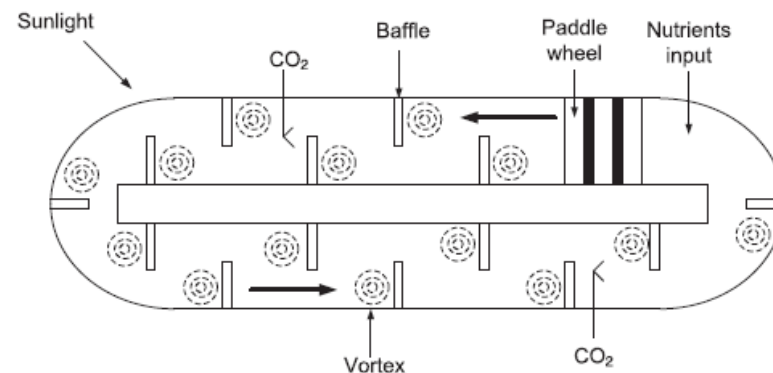


Fig. 2. Conceptual baffled system in raceway pond to culture microalgae. Modified from Chisti (2007).

Downstream

Mata et al. 2012

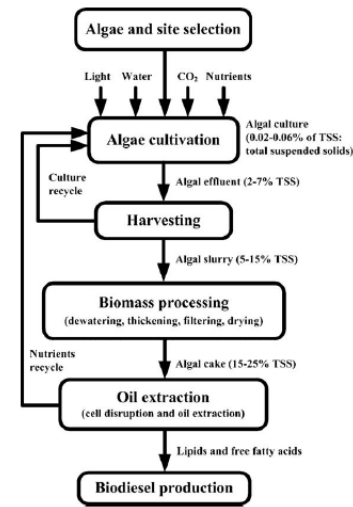


Fig. 1. Microalgae biodiesel value chain stages.

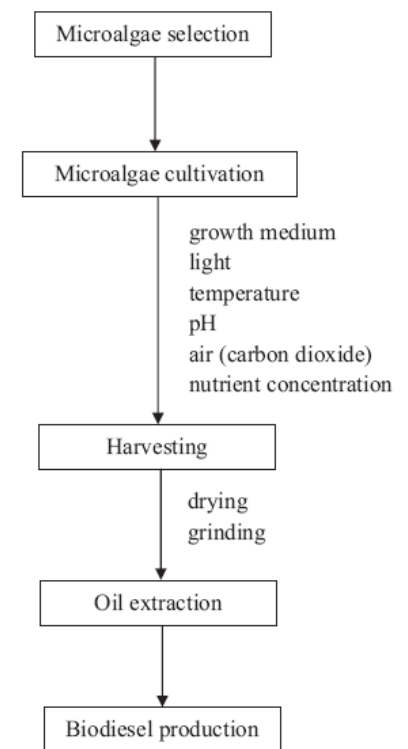


Fig. 6. Production of biodiesel from microalgae.

Ahmad et al. 2012

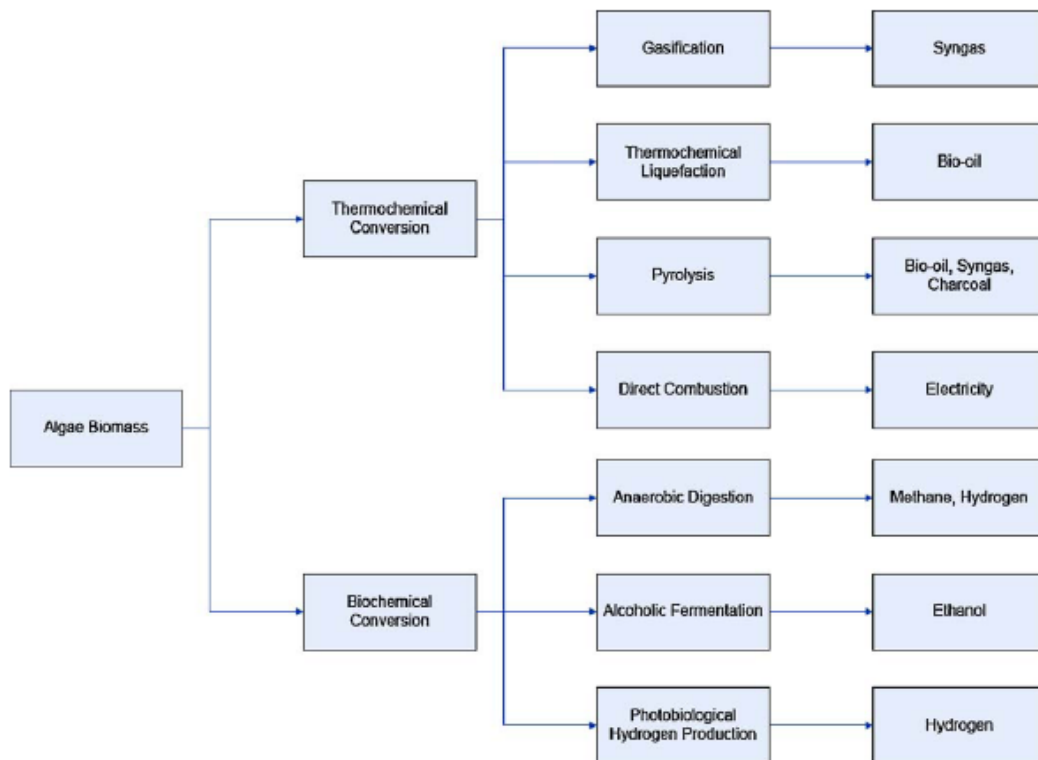
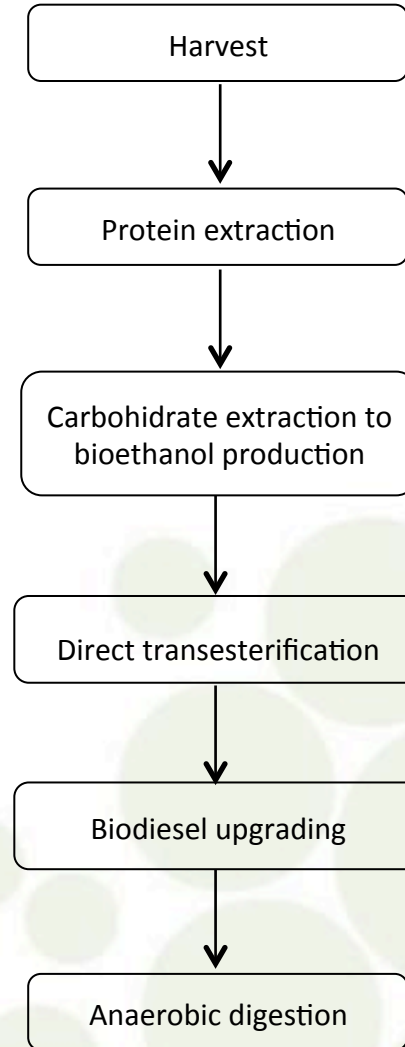
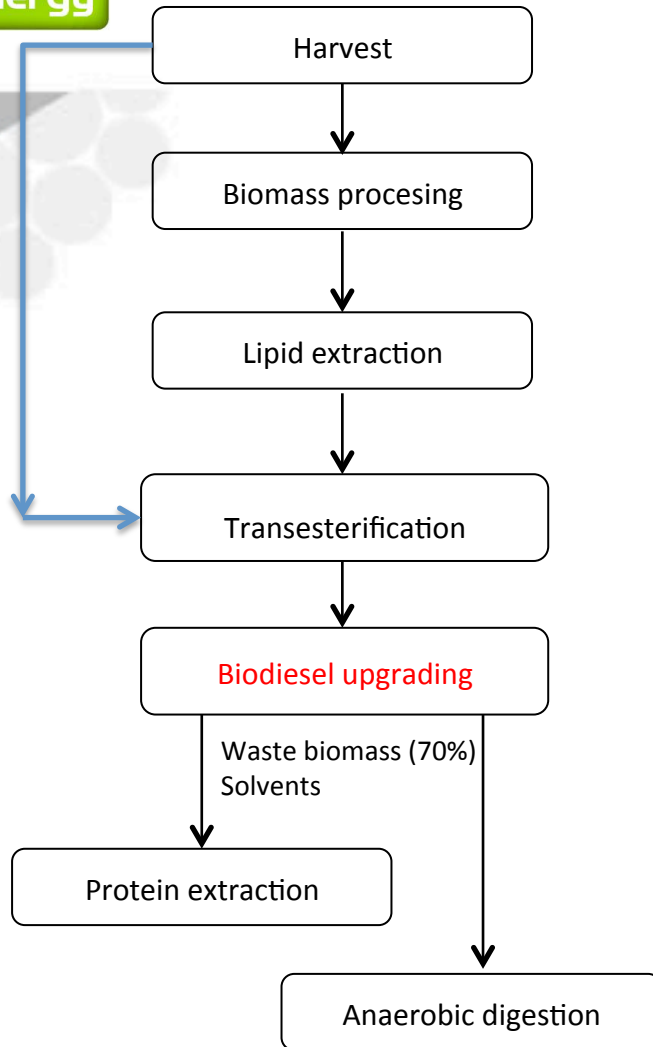


Fig. 3. Potential algal biomass conversion processes (adapted from Tsukahara and Sawayama [162]).

Brennan and Owende, 2010



Downstream



PROTEIN EXTRACTION

Chemical proteins solubilization & Enzymatic hydrolysis

CHEMICAL PROTEIN SOLUBILIZATION

Sequential optimization

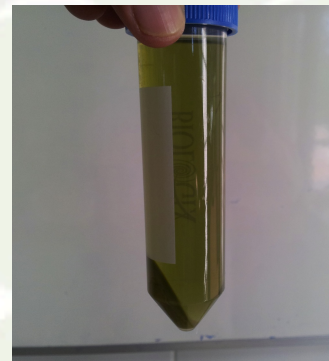
Variables

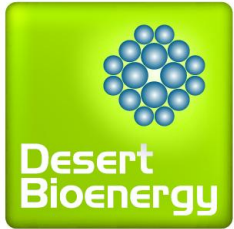
pH= 9-10-11- 12-13

Temperature (°C)= 30-50-70

time (min)= 10- 20- 30

Stir (rpm)= 100- 200- 300





Results

- ✓ 50°C
- ✓ 20min
- ✓ 200 rpm

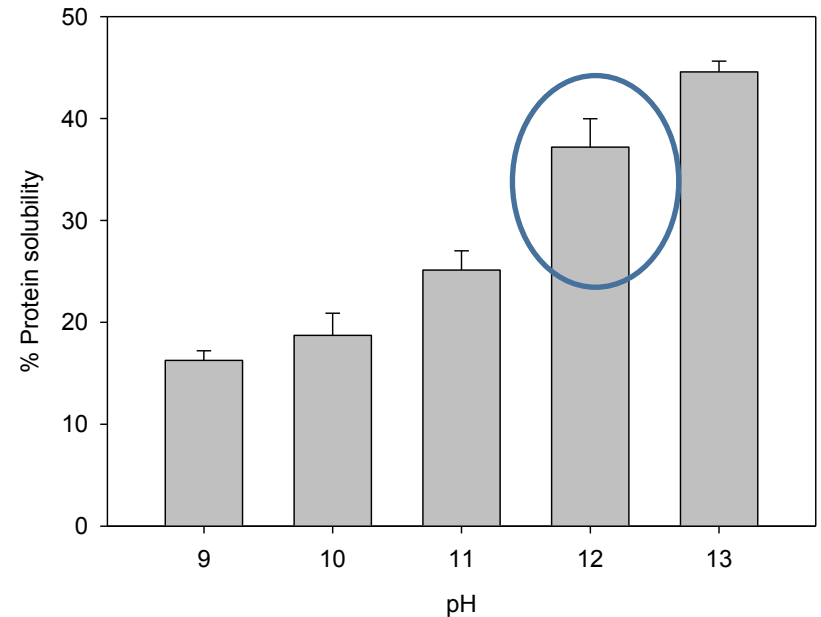


Figure 1. % Protein solubility from microalgae biomass to different pH conditions

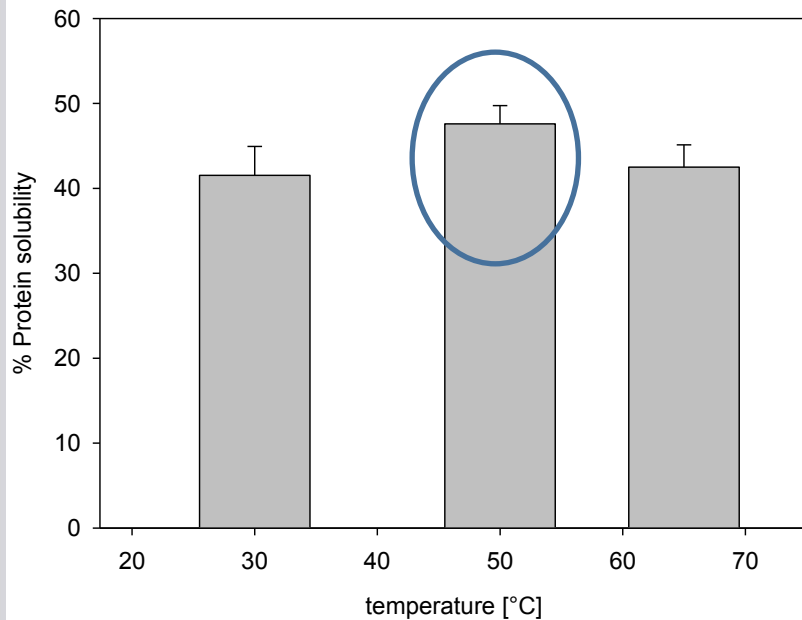
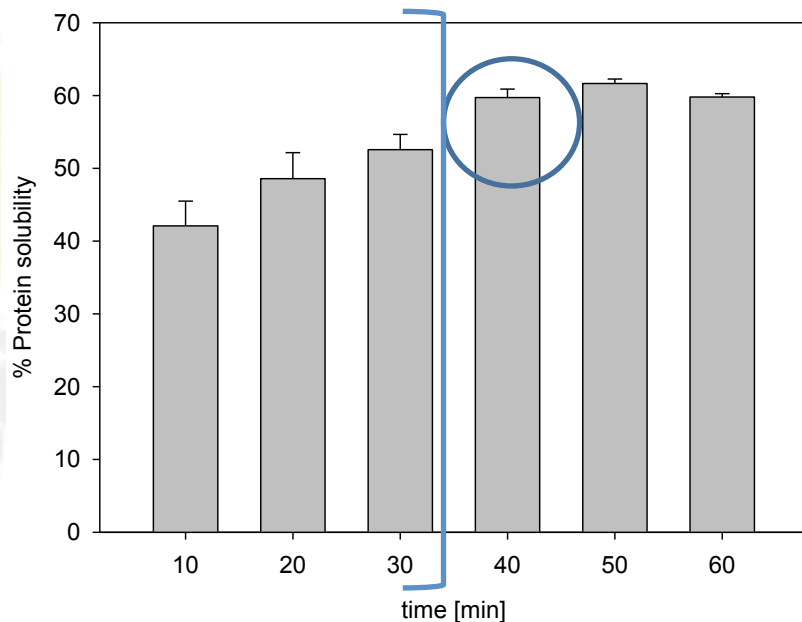
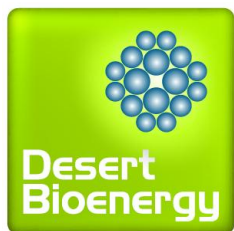


Figure 2. % Protein solubility from microalgae biomass to different reaction temperature

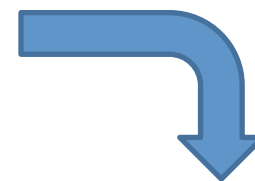
- ✓ pH 12
- ✓ 20min
- ✓ 200 rpm





✓ pH 12
✓ 50°C
✓ 40 min

Figure 3. % Protein solubility from microalgae biomass to different reaction time



Optimal conditions:
pH 12
50°C
40 min
200rpm

Yield: 64%

✓ pH 12
✓ 50°C
✓ 200 rpm

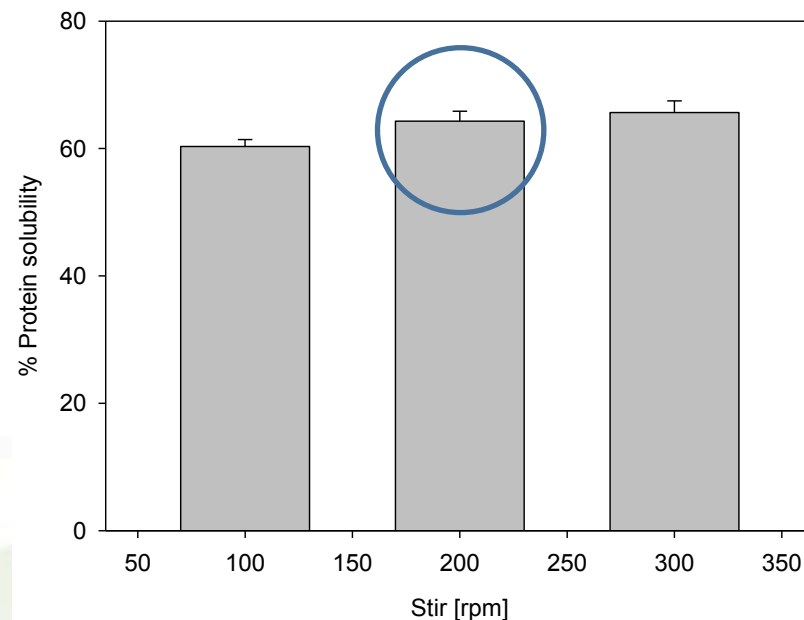


Figure 4. % Protein solubility from microalgae biomass to different stir conditions

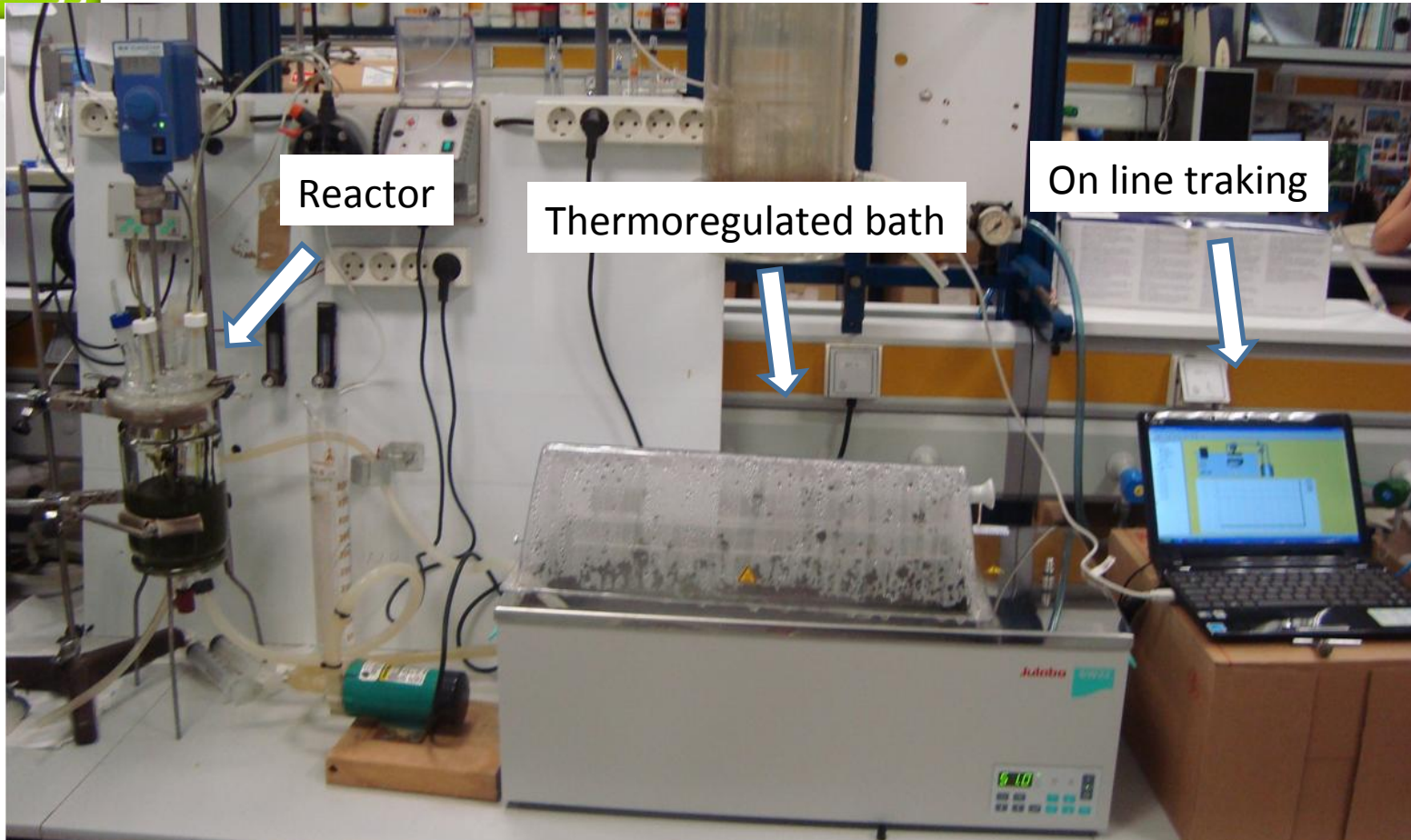
ENZYMATIC HYDROLYSIS

- 1) Enzymatic hydrolysis of microalgae biomass through successive steps addition of: Viscozyme, Alcalasa and Flavourzyme
- 2) Enzymatic hydrolysis of solubilized protein by chemical treatment by using Alcalasa.
- 3) Enzymatic hydrolysis of solubilized protein by chemical treatment by using Flavourzyme.

Raw material

- 1) Pretreated *Microalgae*
150g/L (similar to biomass post harvest)

Set up



Enzymatic hydrolysis of microalgae biomass

Hydrolysis degree: 84%

Disadvantage
Carbohydrates loss
(65%)

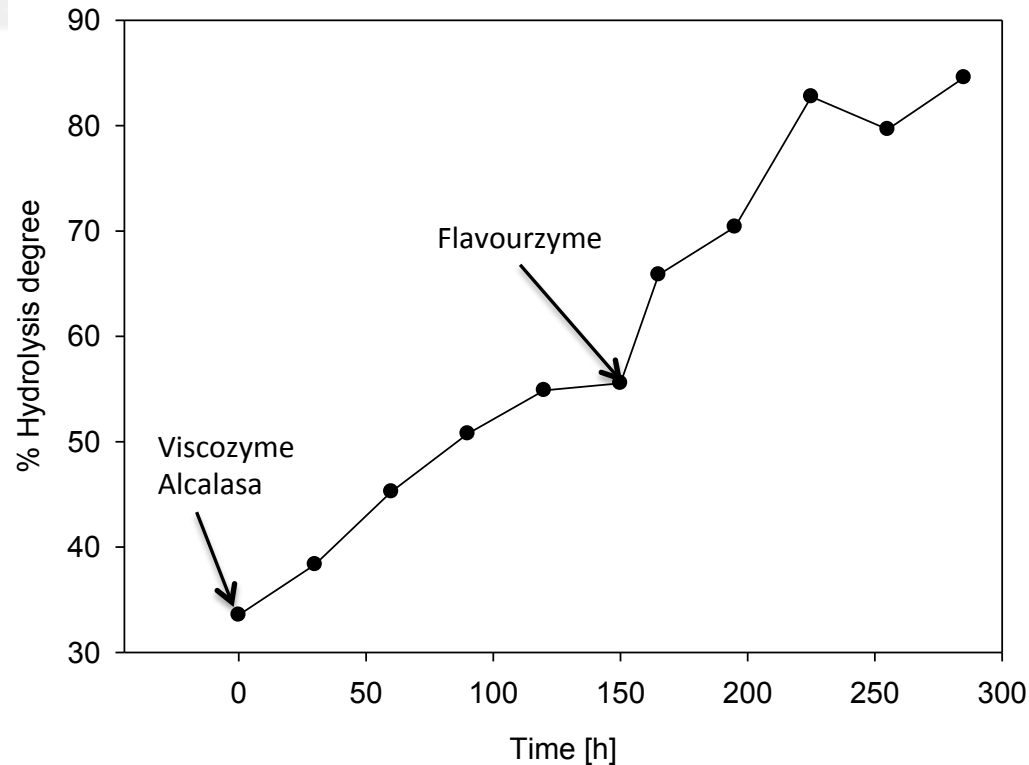


Figure 5. Hydrolysis degree after enzymatic hydrolysis of microalgae biomass



Enzymatic hydrolysis of previously solubilized protein by chemical process

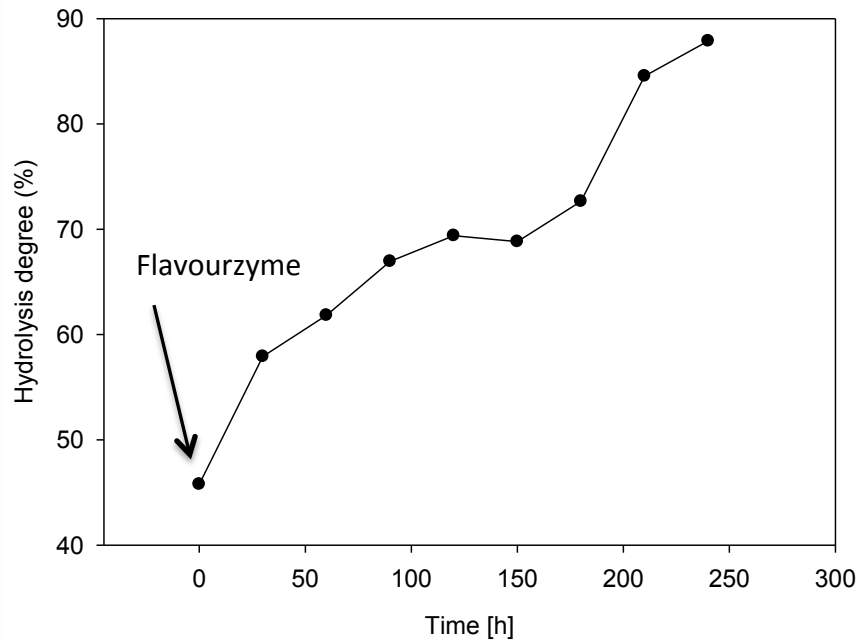


Figure 7. Hydrolysis degree after enzymatic hydrolysis of solubilized protein by chemical process

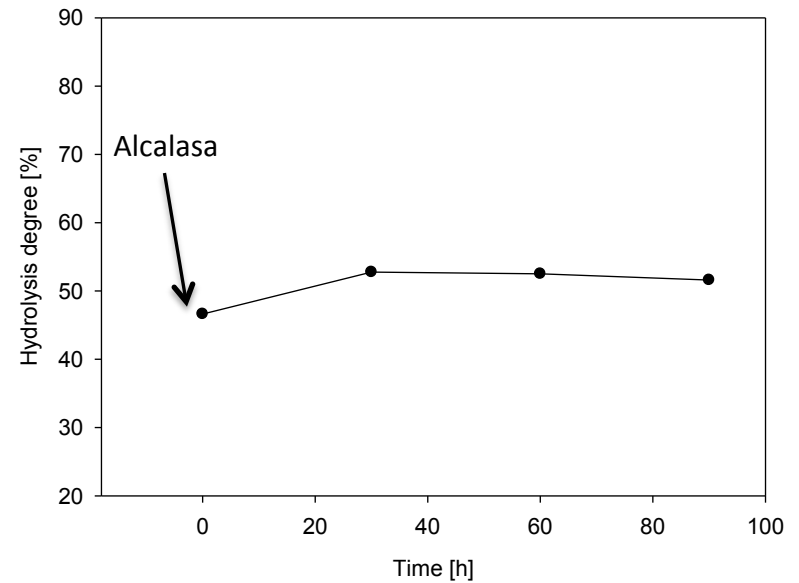


Figure 6. Hydrolysis degree after enzymatic hydrolysis of solubilized protein by chemical process

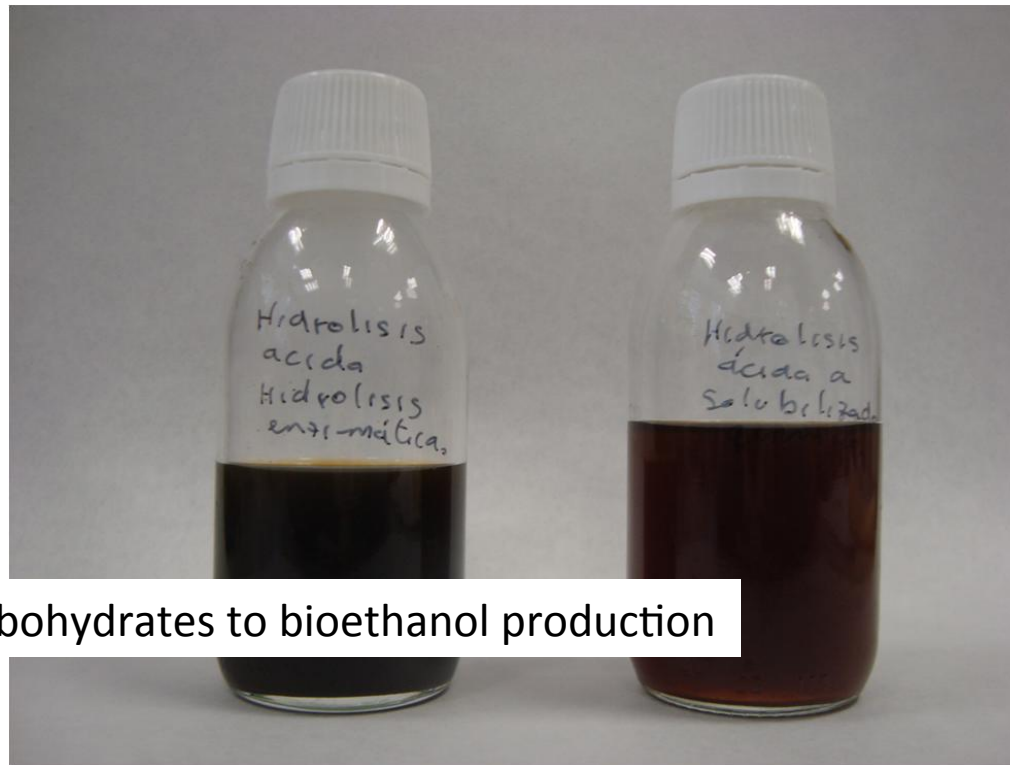
Total yield: 88%

Low carbohydrates loss

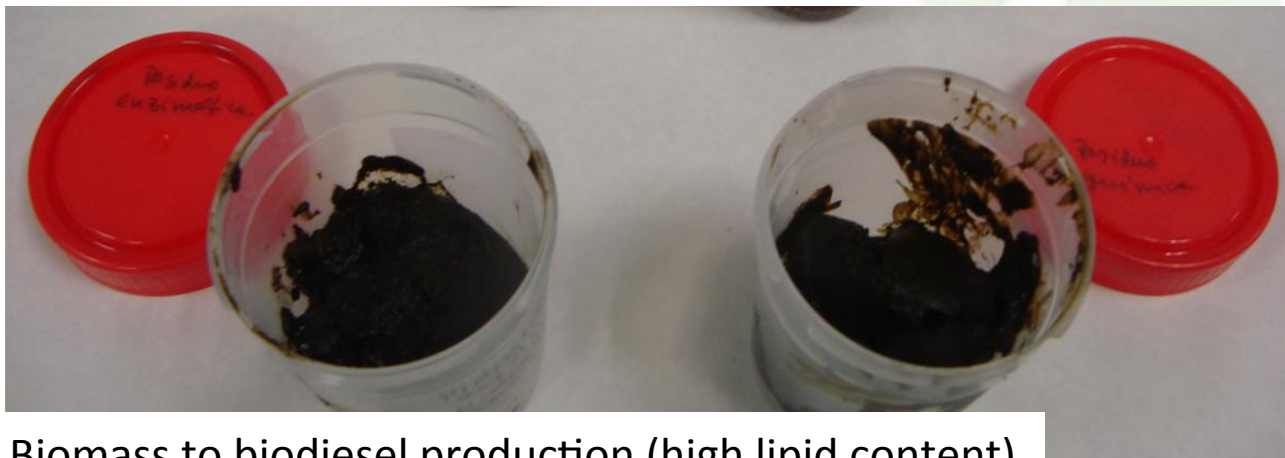


Carbohydrates extraction to bioethanol production???

CARBOHYDRATES EXTRACTION TO BIOETHANOL PRODUCTION



Carbohydrates to bioethanol production



Biomass to biodiesel production (high lipid content)

BIODIESEL PRODUCTION

Lipids extraction and transesterification

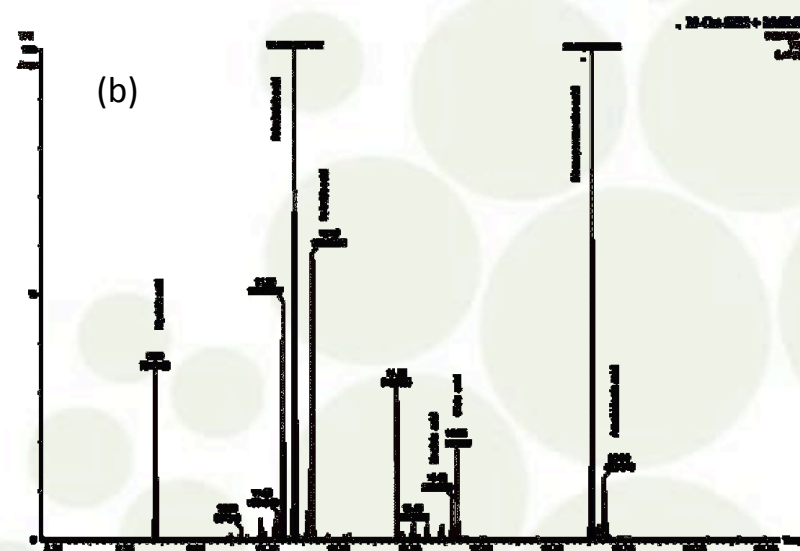
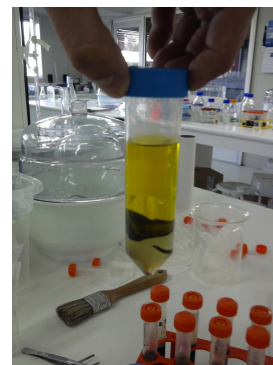
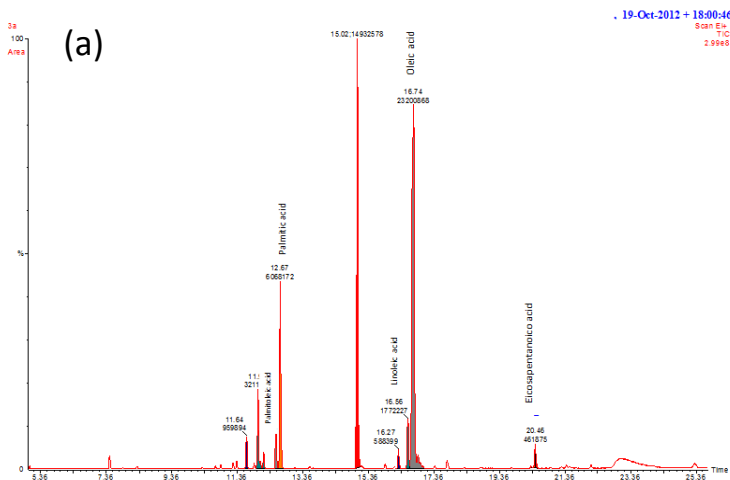


Figure 8. Chromatogram fatty acid profile (a) microalgae 1 and (b) microalgae 2

BIODIESEL PRODUCTION

Direct transesterification

Conditions investigated RSM

- ✓ Methanol to oil molar ratio
- ✓ Catalyst
- ✓ Time
- ✓ Temperature

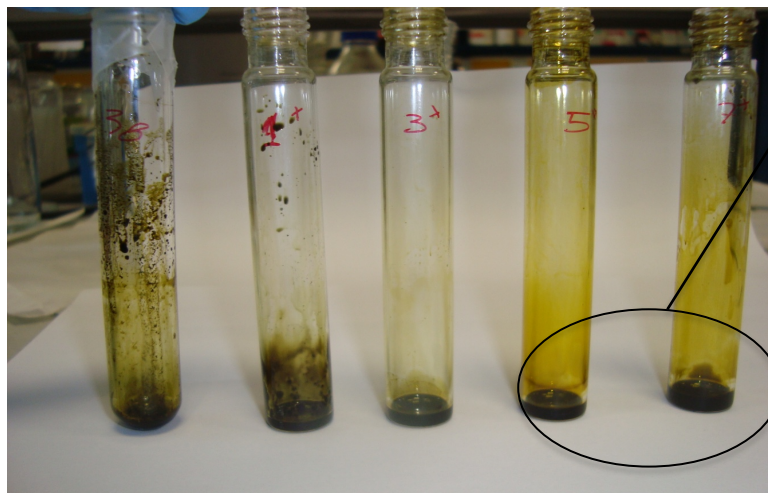
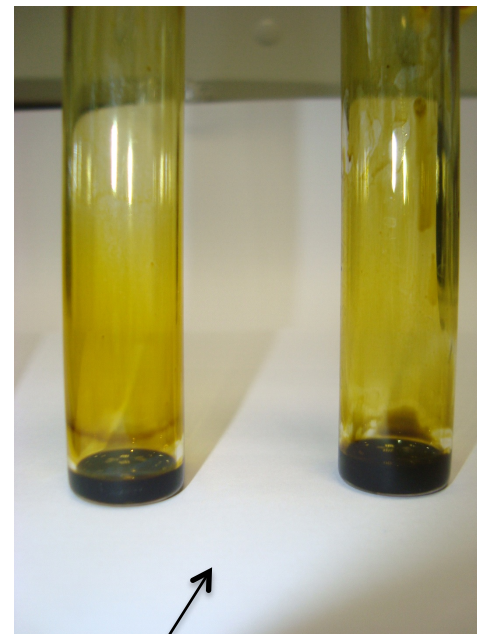


Figure 9. Biodiesel produced by direct transesterification

BIOGAS PRODUCTION

Biochemical methane potential by using microalgae biomass after lipids extraction

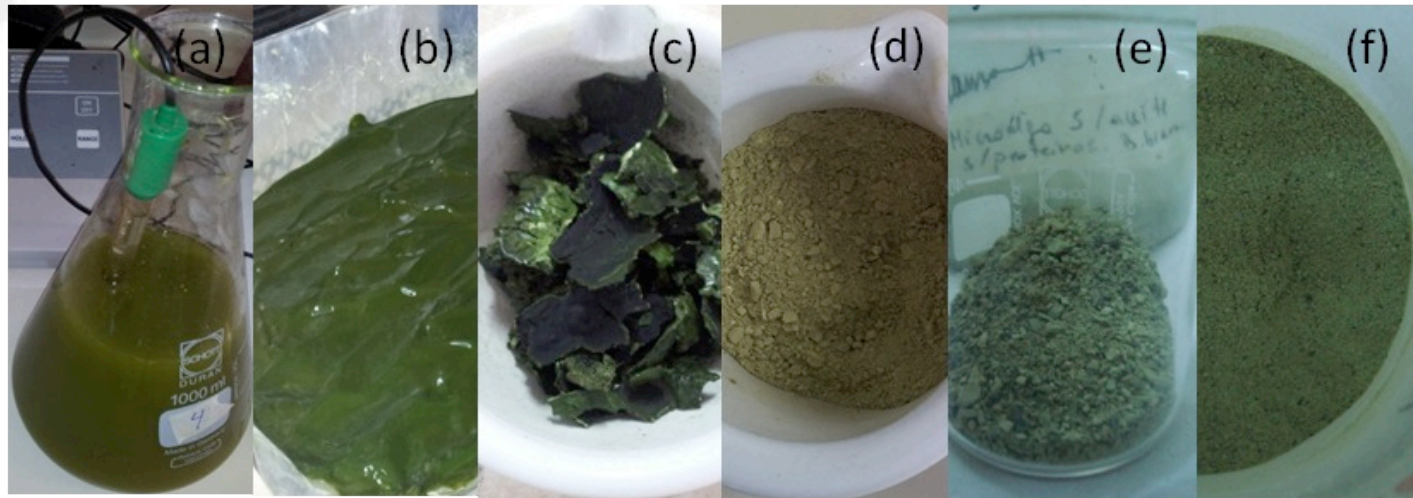
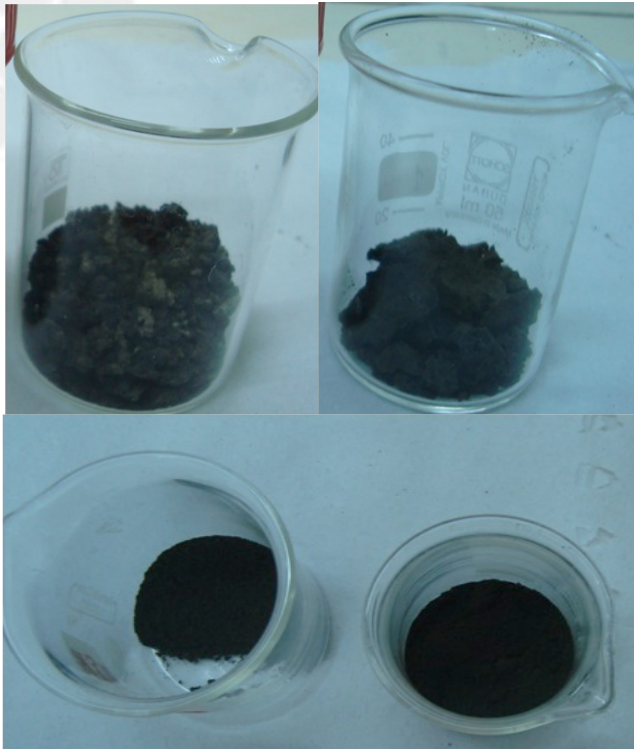


Figure 10. Biomass during microalgae biorefinery process (a) Protein extraction, (b) Drying, (c) Dry biomass, (d) Biomass milled to the lipids extraction, (e) Biomass after lipids extraction, (f) Biomass milled to biogas production

BIOGAS PRODUCTION

Biochemical methane potential by using microalgae biomass after transesterification reaction



Assays

117 serum bottles

50 mL reaction

Nutrients

Bicarbonate

35°C



Figure 10. Biomass after transesterification reaction

BIOGAS PRODUCTION

Table 1. Microalgae characterization

Parámetro	<i>Microalgae 1</i>	<i>Spent microalgae 1</i>	<i>Spent microalgae 2</i>	<i>Spent microalgae 2</i>
Humidity content [%]	79,58	7,66	3,99	3,13
Lipids [%]*	19,20	5,23	9,61	7,4
Protein [%]*	33,00	22,80	39,42	41
Fiber [%]*	3,33	7,94	4,34	3,54
Ash [%]*	31,00	40,13	10,34	13,75
Carbohidrates [%]	13,47	16,24	32,3	31,18
C/N [gC/gN]	5.85	6.97	-	-

*dry basis

BIOGAS PRODUCTION

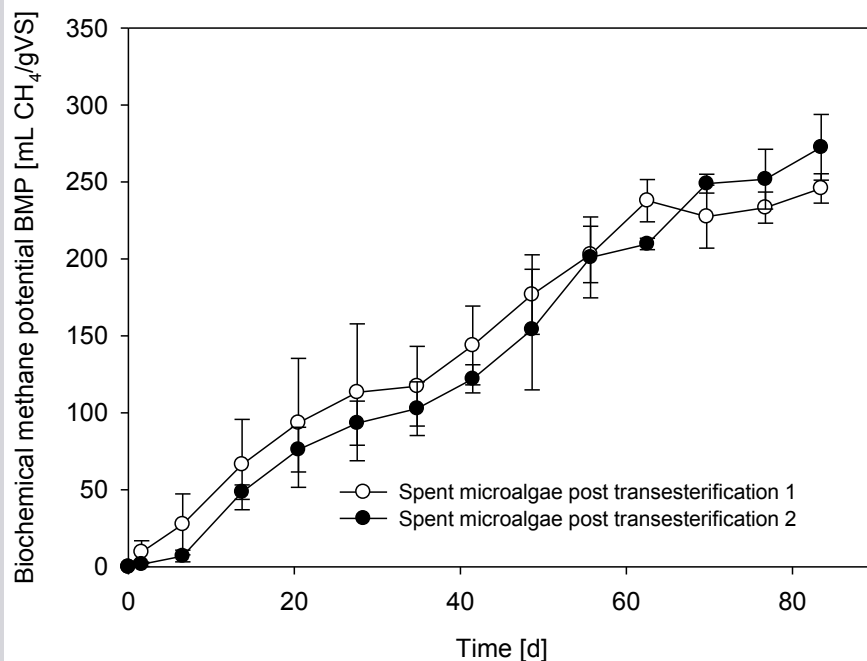


Figure 12. BMP using spent microalgae after direct transesterification

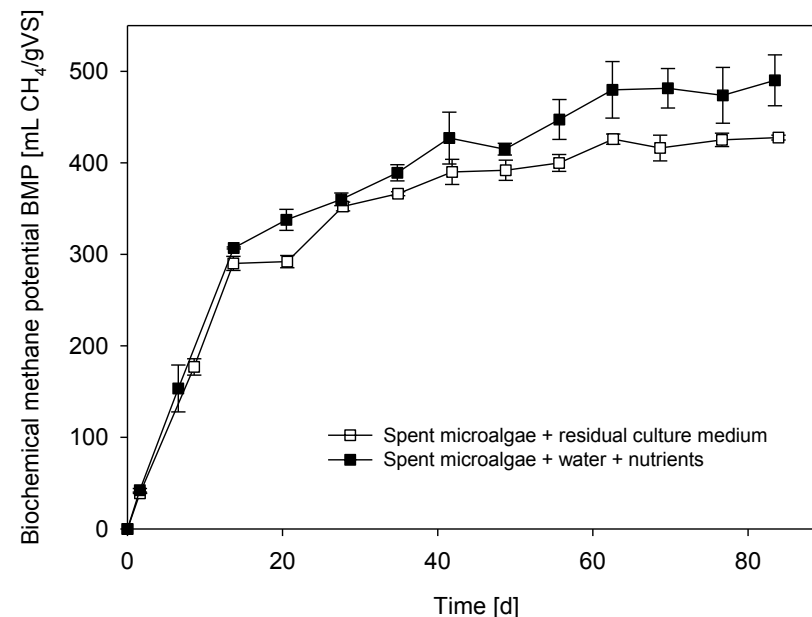


Figure 11. BMP using spent microalgae after protein and lipids extraction

BIOGAS PRODUCTION

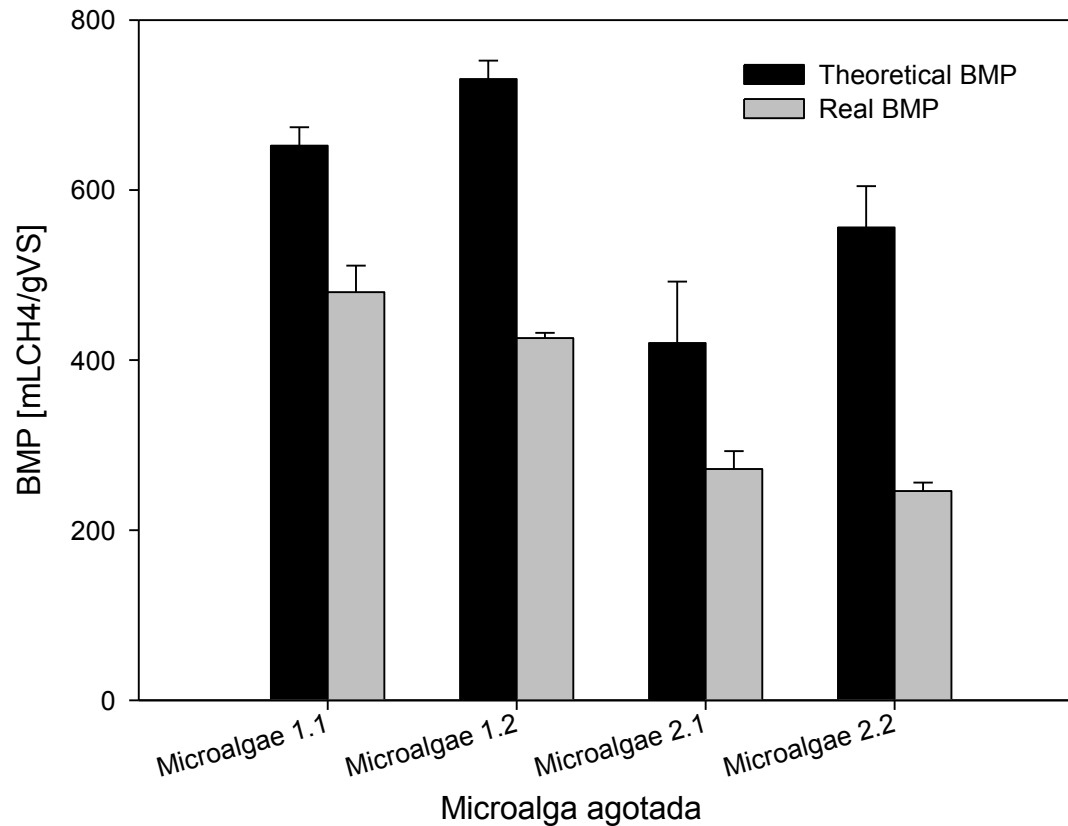
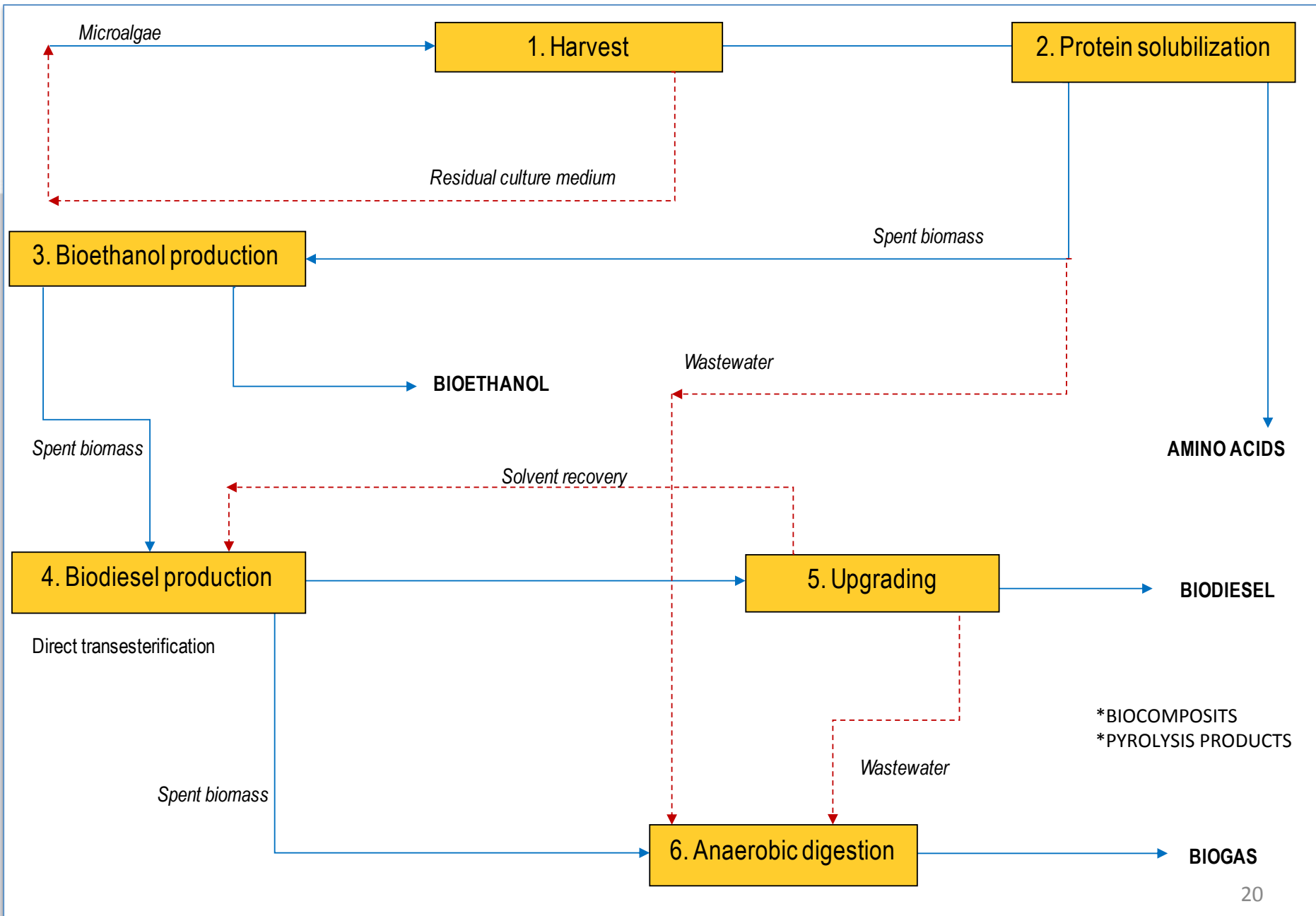


Figure 13. Theoretical and real BMP using spent microalgae

NEW MICROALGAE BIORREFINERY CONCEPT





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Center of Waste Management and Bioenergy
Scientific and Technological Bioresource Nucleus



BIOREN-UFRO





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