Graphics

Figures and Tables

Publishable Summary

B A M M B O

Table 1. Some of the bottlenecks encountered in culture and extraction of marine organisms for HVAB production.

Bottleneck	BAMMBO's Address	Synergies
Macro-algae: Fragile, seasonal, wild harvest with low abundance of HVABs,	•Macro-algal Photo-bioreactor Tissue Culturing •Multi-bioactive HVAB Screening from Source Organism •Epiphyte culturing & screening •Life Cycle Analysis/Cost Benefit Analyses	IPL – UCL + LIT +UNS +UNIGE
Micro-algae: ↑ Biomass ↓ HVAB yield Problems with Gas Balance & light	•Novel Bioreactor Design, Control, Monitoring & Simulation. •Uniform light, nutrient, gas, pH and heat distribution •Use of Environmental Growth Chamber Technology - Recreation •Proprietary Reactor Systems/Modular design capabilities •Auto-, hetero- and mixo-trophic culturing •LCA/CBA	UGent, UCL, UNICAMP, USC, IPL, GreenSea, Algae Health, LIT,
Sponge: Slow growth. Difficulty recreating natural environment. Low yield HVAB in wild harvest. Sustainable supply of sponge & HVAB	•Aquarium culturing •Suspended Seabed Farmed Sponges •Non-sponge destructive HVAB collection & extraction mechanisms •Epiphyte culturing and screening for HVABs •LCA/CBA	UNS, UNIGE + All Partners Contributing
Process: ↓Extraction Efficiency, Product Stability, Safety, Scalability, Practicality.	•Supercritical fluid CO_2 Extraction compared to homogenisation and solvent based extractions. •↑ Products application (thru SCFE) •↓ Residues & Chem. Waste (thru SCFE)	All Partners Contributing
Cost effective production of HVABs from marine sources	•LCA/CBA - All aspects [Energy, Solvents, Footprint, Environ, Yields..] •Multi-bioactive extraction from Source Organisms •Better Waste Utilisation – Screen for HVABs •Seek alternative HVAB sources [Fungi/Protists] - PUFAs, Carotenoids •Reduce\Eliminate Solvent Use => Supercritical Fluid CO_2 Extraction •Aim for More Efficient GREENER processes – 'Organic' label	USC + All Partners Contributing

Table 2. Initial marine organisms and bioactivities screened in the BAMMBO project.

Marine Organism	Initial Bioactive Compound of Interest
Sponges:: *Sarcotragus spinosulus, Crambe crambe*, Mediterranean Sponge Bio-bank	• Terpenoids • Antioxidants • Antimicrobials • Cytotoxic Alkaloids • Antifouling Hydroquinone Compounds
Microalgae: *Haematococcus pluvialis, Phaeodactylum tricornutum, Cylindrotheca closterium, Gambierdiscus toxicus, Scenedesmus obliquus,* 20 Other lesser known microalgal species	• Carotenoids, Astaxanthin, Lutein • Polyunsaturated Fatty Acids • Phycobilins • Fucoxanthin • Toxins (Maitotoxin/Ciguatoxin)
Macroalgae: *Fucus spiralis*, *Sphaerococcus coronopifolius, 12 Lesser known macroalgal species*	• Antioxidants • Polyphenolics/Phlorotannins • Anti-bacterial bromoditerpenes, • Anti-fungal bromoditerpenes • Anti-tumoral bromoditerpenes • Fluorescent Compounds
Epiphytic bacteria: Epiphytes associated with sponges and macroalgae and other selected marine life forms	• Terpenoids • Alkaloids • Anti-bacterial • Bromoditerpenes • Anti-fungals • Hydroquinones • Ubiquinone Q_{10} • Polyunsaturated Fatty Acids
Fungi, Yeast and Bacteria: From the Antarctic marine fungi bio-bank and from White Sea Coast (Arctic) Expedition.	• Lipases • Lignin degrading enzymes • Carotenoids • Polyunsaturated Fatty Acids • Phytases

Table 3: Selected organisms from Workpackage 2 identified for further research in Workpackages 3 (**Sustainable culture**), Workpackage 4 (**Extraction and Purification**), Workpackage 5 (**Analysis of high value added molecules and bioactives**). All organisms can be sustainably cultured with the exception of the macroalgae for which stable cultures were established. In some instances transgenic bacterial expression systems for lipase and phytase genes were sourced from the detailed target organisms below. Specific details of organism identification and extraction method and conditions have been omitted for IP reasons.

HVAB	Organism Selected	Location/Tax	Main Partner(s)
Ubiquinone Q_{10}	Paracoccus sp *Rhodobacter sp.*	Arctic bacteria	Genetika
Ubiquinone Q_{10}	F40/F52 *Fucus spiralis* associated producing Q_{10} at comparative levels to *Paracoccus* sp.	Atlantic bacteria	IPL/LIT
Ubiquinone Q_{10}	SS-BE/CC30 Sponge associated producing Q_{10} at comparative levels to *Paracoccus sp*.	Atlantic bacteria	UNIGE/IPL/LIT
Phytase	*Shewanella sp.*	Arctic bacteria	Genetika
Lipase	*G. pannorum*	Antarctic filamentous fungus macroalga associated bacteria	UNICAMP
Lipase	*C. laurentii*	Antarctic Sea Urchin associated yeast	UNICAMP
Ligninases	*Cadophora luteo-olivaceae* P1	Antarctic filamentous marine sediment fungus	UNICAMP
DHA (and EPA)	*Ulkenia sp.*	Arctic Protist	Genetika
DHA (and EPA)	*P. tricornutum**	Microalga	UGent
Astaxanthin	*H. pluvialis*	Microalga	LIT/UGent Greensea Algae Health
Ciguatoxins	*G. toxicus*	Dinoflagellate	USC
Polyphenols	*F. spiralis*	Atlantic macroalga	IPL
Halogenated Terpenes	*S. coronopifolius*	Atlantic macroalga	IPL
Guanidine Alkaloids	*C. crambe*	Sponge	UNS

Terpenhydroquinones	*S. spinosulus*	Sponge	UNIGE
B-Phycoerythrin	*O. secundiramea*	Atlantic macroalga	UCLouvain/IPL/LIT
Antioxidants	*Rhodobacter sp.*	Arctic bacteria	Genetika
Anti-elastase	*C. crambe***	Sponge	UNS/LIT
Anti-elastase	*F. spiralis*	Atlantic macroalga	IPL/LIT
Anti-elastase	*S. coronopifolius*	Atlantic macroalga	IPL/LIT
Anti-hyaluronidase	*C. crambe***	Sponge	UNS/LIT
Anti-hyaluronidase	*F. spiralis*	Atlantic macroalga	IPL/LIT
Anti-hyaluronidase	*S. coronopifolius*	Atlantic macroalga	IPL/LIT
Anti-microbial	*C. crambe*	Sponge	UNS/LIT
Anti-microbial	*S. coronopifolius*	Atlantic macroalga	IPL
Anti-tumour	*S. coronopifolius*	Atlantic macroalga	IPL

Table 4. Commercial viability analysis for astaxanthin production at different scales and processes.

Company	Volume (L)	TDC (€)	DW (Kg)	Cost/Kg Biomass	Astaxanthin Yield (%)	Astaxanthin (Kg)	Market Value (€)	Cost/Kg Astaxanthin	Margin/Kg Astaxanthin (€)
Greensea	2,500	713	1.08	660	2.1	0.0227	227	31,416	-21,416
Algae Health	20,000	2,277	30	76	4.0	1.2	12,000	1,898	8,103

TDC, Total Direct Cost; DW, Dry weight;

Table 5. Global inventory: Pilot-scale cultivation of *H. pluvialis* to obtain 1 g astaxanthin.

INPUTS from TECHNOSPHERE			
Materials			
Cleaning of the reactor			
Tap water	7.5088 L	Sodium hypochlorite (NaClO)	0.0375 g
Preparation of the culture medium			
Deonized water	31.4681 L	$Na_2C_{10}H_{14}N_2O_8 \cdot 2H_2O$	0.0315 g
KNO_3	6.2936 g	$CuCl_2 \cdot 6H_2O$	0.0006 g
Na_2CO_3	0.1573 g	$ZnSO_4 \cdot 7H_2O$	0.0006 g
$NaHCO_3$	1.5734 g	$CaCl_2 \cdot 6H_2O$	0.0003 g
K_2HPO_4	0.3934 g	$MnCl_2 \cdot 4H_2O$	0.0126 g
$MgSO_4 \cdot 7H_2O$	0.7867 g	H_3BO_3	0.00003 g
Cultivation			
Compressed air for 4 L flask (enriched 1% CO_2)	8.175 kg	Compressed air for 80 L reactor (enriched 0.5% CO_2)	56.809 kg
Fluorescent lamps (58 W)	6.740 g	Polyvinylchloride (PVC)	19.811 g
Energy			
TOTAL ENERGY CONSUMPTION	***196.357 kWh***		
Preparation of the culture medium			
Autoclaving	1.246 kWh		
Cultivation			
Incubation chamber for 10 mL tube	10.266 kWh	Incubation chamber for 0.2 Lflask	10.266 kWh
Lighting for 4 L flask	7.317 kWh	Lighting for 80 L PBR	45.990 kWh
Temperature control for 4 L flask	4.352 kWh	Temperature control for 80 L PBR	27.356 kWh
Aeration for 4 L flask (compressor)	47.936 kWh	Aeration for 80 L PBR (compressor)	41.629 kWh
Transport			
Cleaning of the reactor		***Preparation of the culture medium***	
Truck, 3.5-7.5 t, Euro 4 (Chemicals)	0.200 kg·km	Truck, 3.5-7.5 t, Euro 4 (Chemicals)	7.400 kg·km
Cultivation			
Truck, 3.5-7.5 t, Euro 4 (Equipments)	21.240 kg·km		
Truck, 3.5-7.5 t, Euro 4 (Waste)	1.328 kg·km		
INPUTS from ENVIRONMENT			
Materials			
Inoculum	1 mL		
OUTPUTS to TECHNOSPHERE			
Product			
Culture medium to harvesting, containing:			
Haematococcus pluvialis biomass	27.79 g (1 g astaxanthin)	Nutrient solution	29.97 L
Waste treatment			
Cultivation			
Disposal, PVC, to sanitary landfill	19.811 g	Disposal, lamps, to specific treatment for electronics waste	6.740 g
OUTPUTS to ENVIRONMENT			
Water emissions			
Cleaning of the reactor			
Wastewater	7.5090 L	NaClO (bleach)	0.0375 g

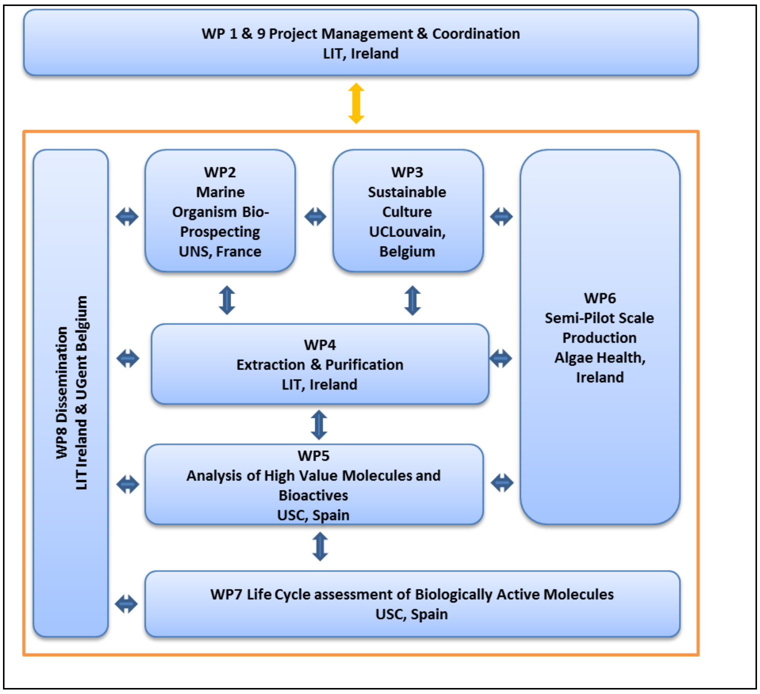


Figure 1. Interaction between BAMMBO workpackages.

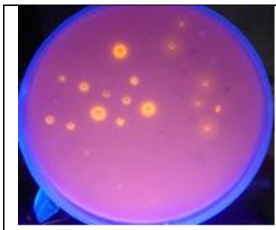


Figure 2. Selection of Antarctic marine derived lipolytic fungi at 15°C (HTS method).

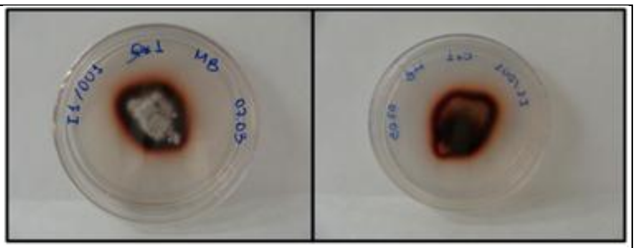


Figure 3. Selection of Antarctic marine derived ligninolytic fungi at 15°C (one by one method).

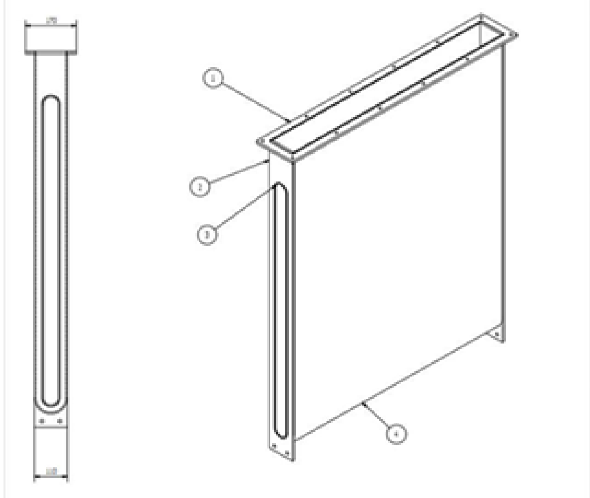


Figure 4: Schematic of the microalgal 60 L APAPBR and the engineering design schematics. The flat plate reactor is annular with the illumination source within the annulus. The front channel of the panel is the airlift riser and the back channel of the panel is the down-comer.

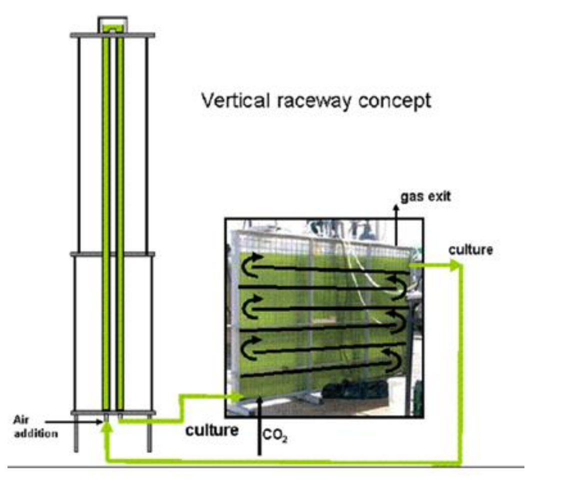


Figure 5: Schematic of the microalgal VRPBR. An airlift pump drives the culture media through a serpentine light exchange unit.

Figure 6: Batch bioreactors for the cultivation of *Gambierdiscus* sp.

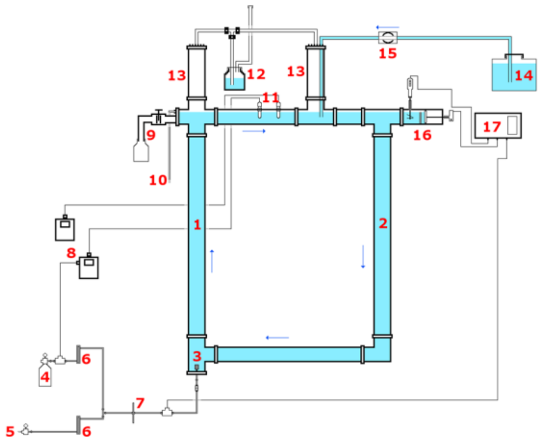


Figure 7: Schematic of the automated, continuous macro plantlet photobioreactor. 1,2) Vertical columns; 3) Sparger; 4) CO_2 inlet; 5) Air inlet; 6) Flow meters; 7) Filter; 8) pH meter; 9) Sampling bottle; 10) Medium outlet; 11) pH and O_2 sensors; 12) Sterile bottle; 13) Gas outlet; 14) Sterile medium storage tank; 15) Medium inlet; 16) Piston and mixer; 17) Timer.

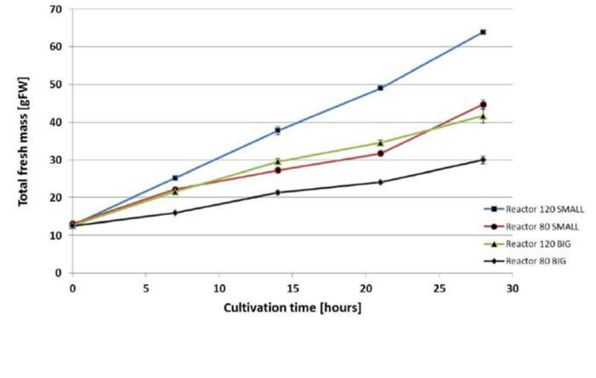


Figure 8. Evolution of the total fresh mass of algal tissues of different initial masses as a function of time in 1L stirred tank photobioreactors operating at 80 or 120 rpm PBR cultivations starting with both small (2 < diameter < 5 mm) and large (diameter > 8 mm) plantlets. Phototrophic cell culture densities were observed of up to 65 g FW L^{-1}, equivalent to 13g dry weight L^{-1}. Cell densities of this magnitude are seldom reported in the literature.

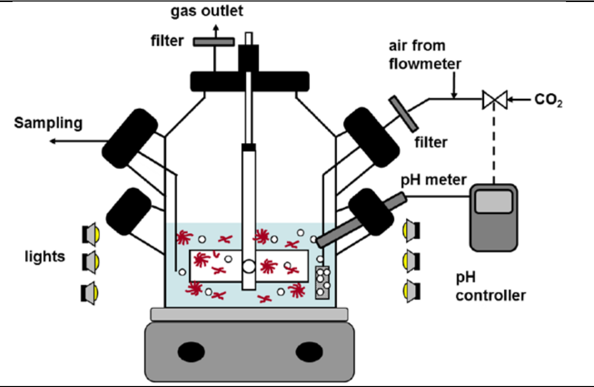


Figure 9. Schematic of the 1 L spinner flask PBR used to study macroalgal growth and breakage in stirred tank photobioreactors.

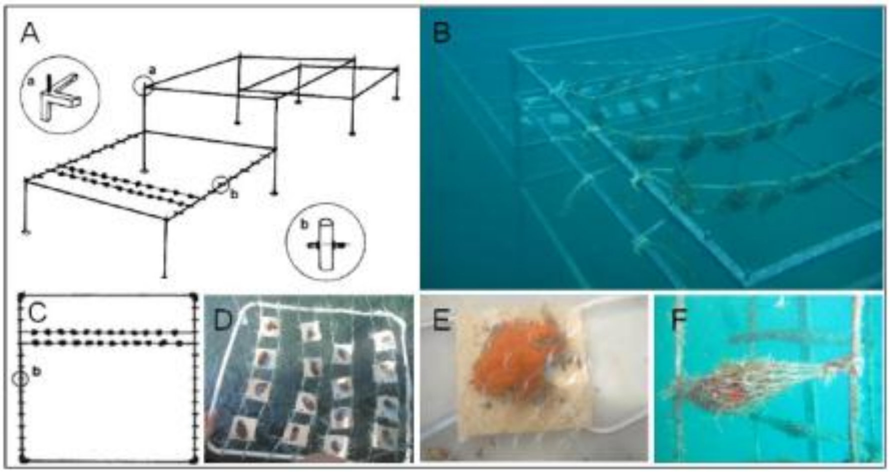


Figure 10. Sponge farming plant modules. A, B) Stainless structure, scheme and *in situ*; C, D) PVC structure, scheme and *in situ*; E) Travertine Tile; F) Nylon Mesh.

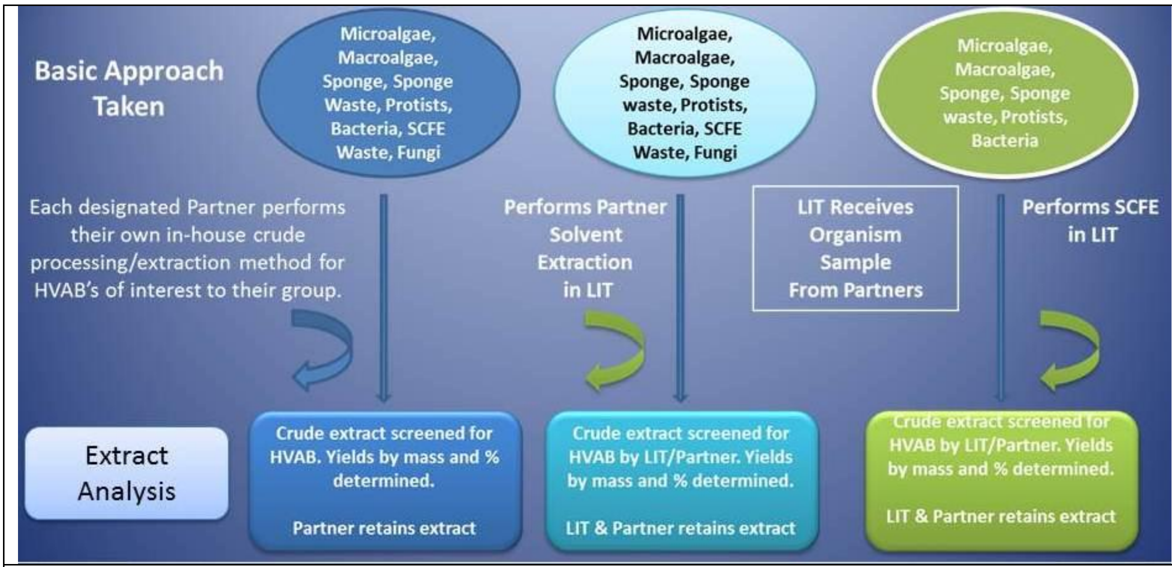
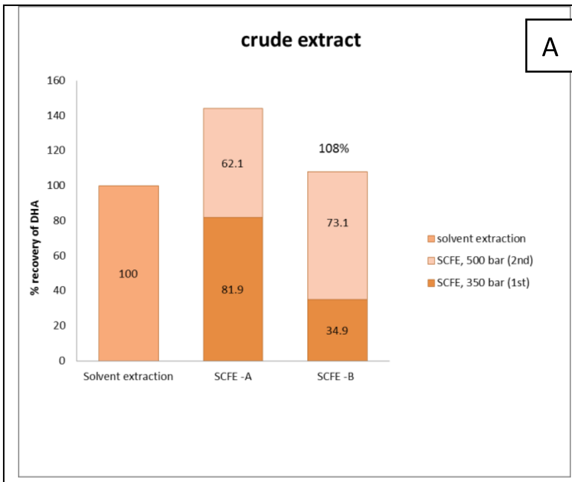
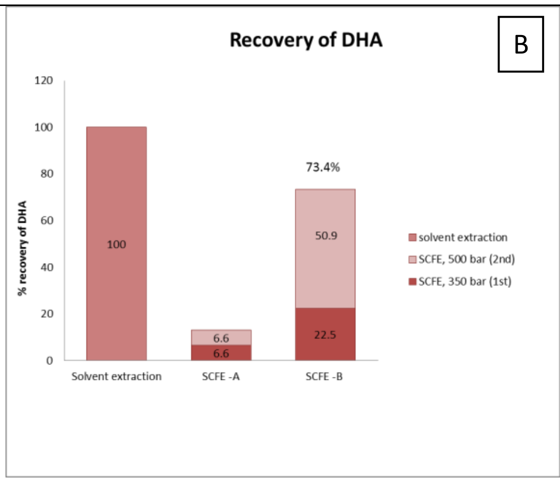


Figure 11. Overview of extraction processes applied to BAMMBO biomass.

	(1)	(2)	(3)	=(1)*(2)*(3)	
	Nb of sponges milked and size	Weight of each major compound purified by sponge	Number of milking per year	Weight of each major compound per year	Nb of major compound per extract
Aquarium based milking	10 sponges of 50 cm^2	0.12 mg	100	120 mg	5
Sea-based milking	5 sponges of 500 cm^2	6 mg	100	3 000 mg	5

Figure 12. The sponge milking factory





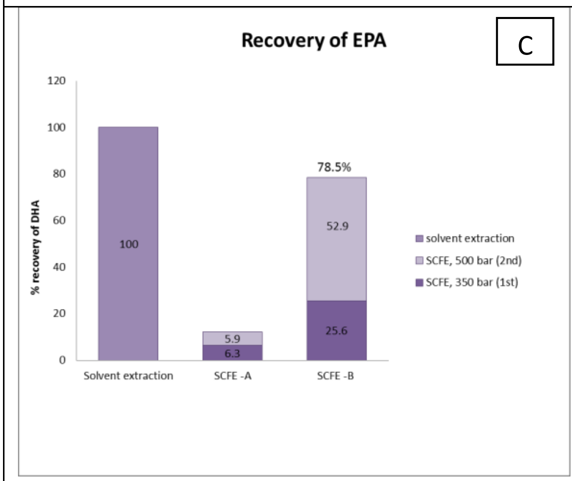


Figure 13. The recovery of crude lipids (A), DHA (B) and EPA (C) by SCFE from *U. visurgensis* F-1157.

Figure 14. Two stage culture (green cells in air lift bioreactor and red cells in tubular bioreactor).

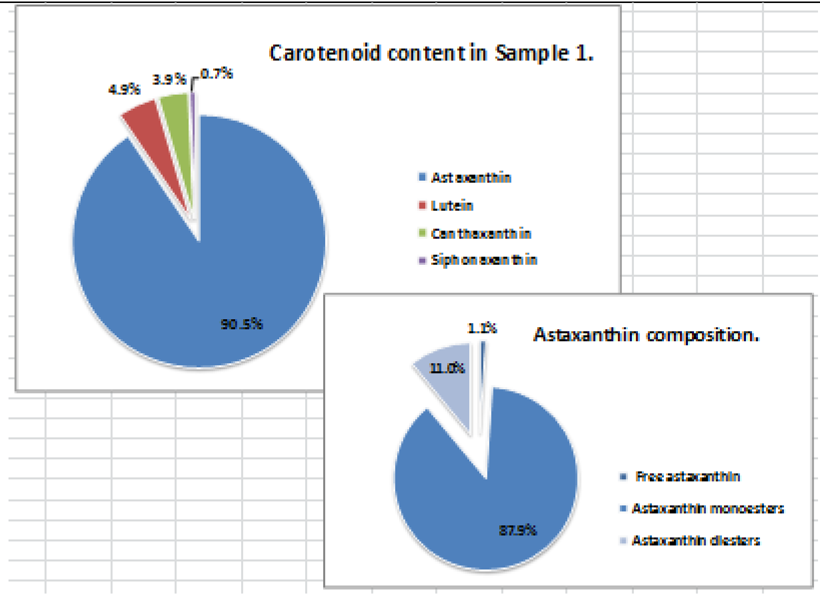


Figure 15. Carotenoid composition an astaxanthin composition

Figure 16. Three dimensions of sustainability according to the concept of 'Triple Bottom line'

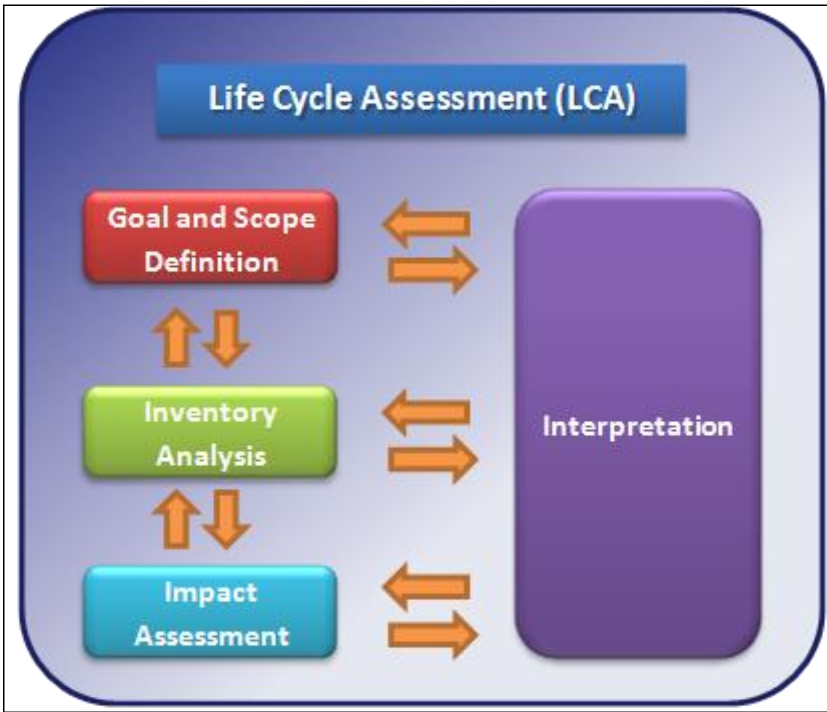


Figure 17. Stages of LCA methodology according to ISO 14040 standards.

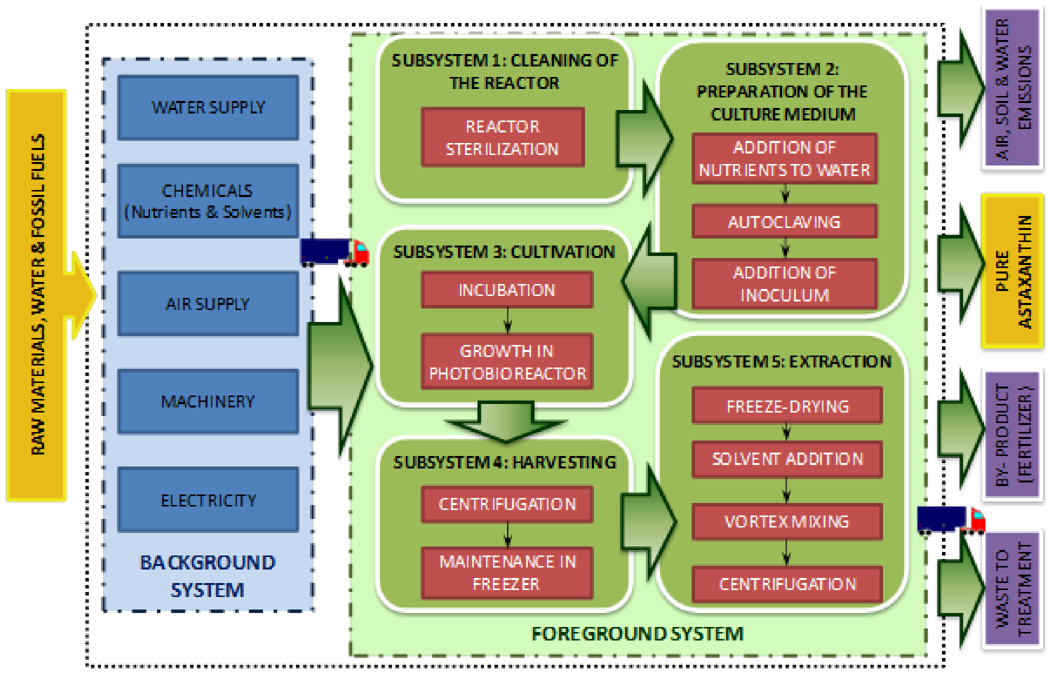


Figure 18. Example of a process flow diagram where system boundaries have been defined for astaxanthin production by microalga *Haematococcus pluvialis.*

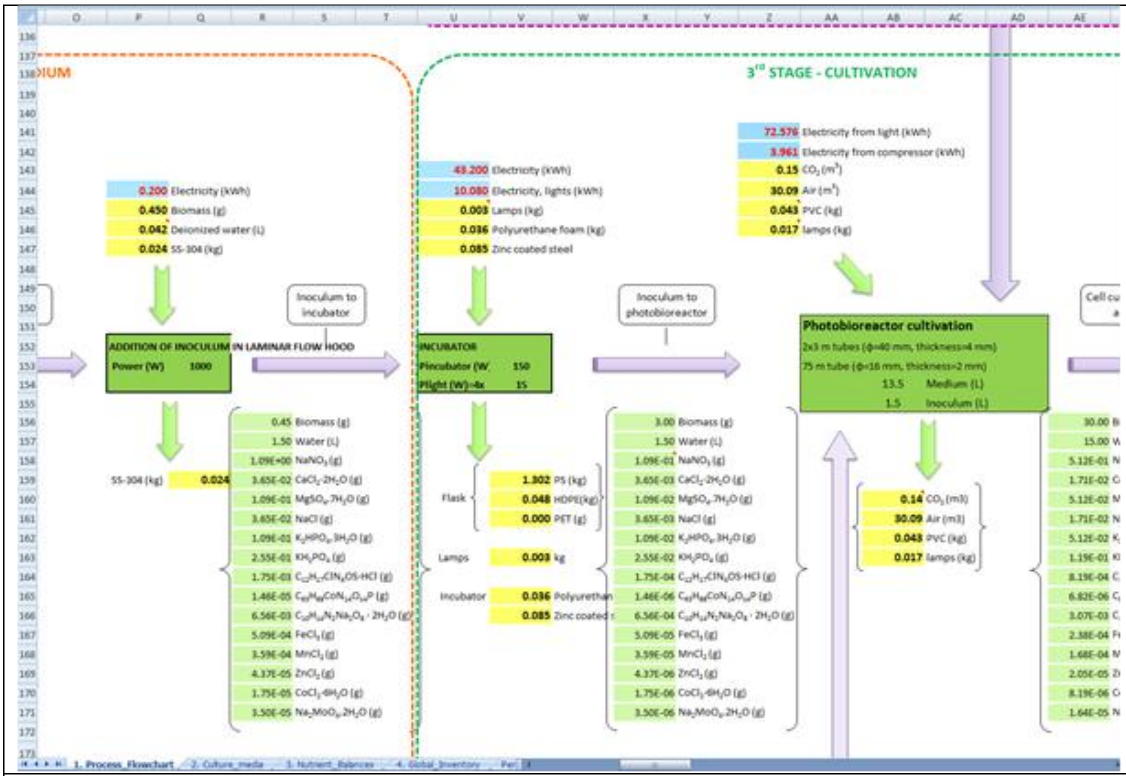


Figure 19. Extract of an Excel simulator modelling astaxanthin production by microalga *Haematococcus pluvialis.*

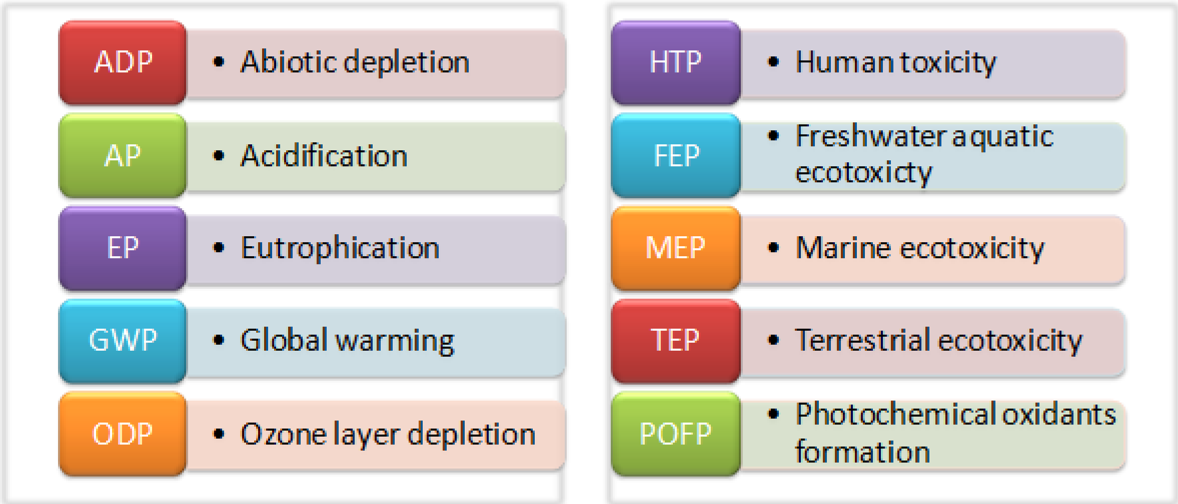


Figure 20. Impact categories evaluated in the LCA of BAMMBO processes according to CML 2001 methodology

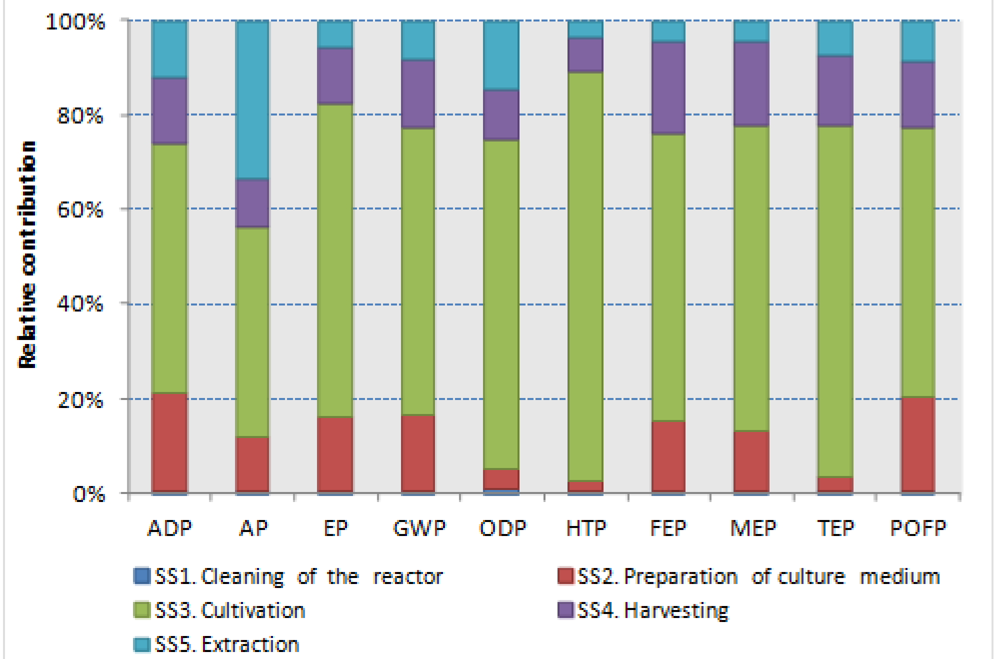


Figure 21. Relative contributions (%) per subsystem to the potential environmental impacts: Example of astaxanthin production by microalga *H. pluvialis*.

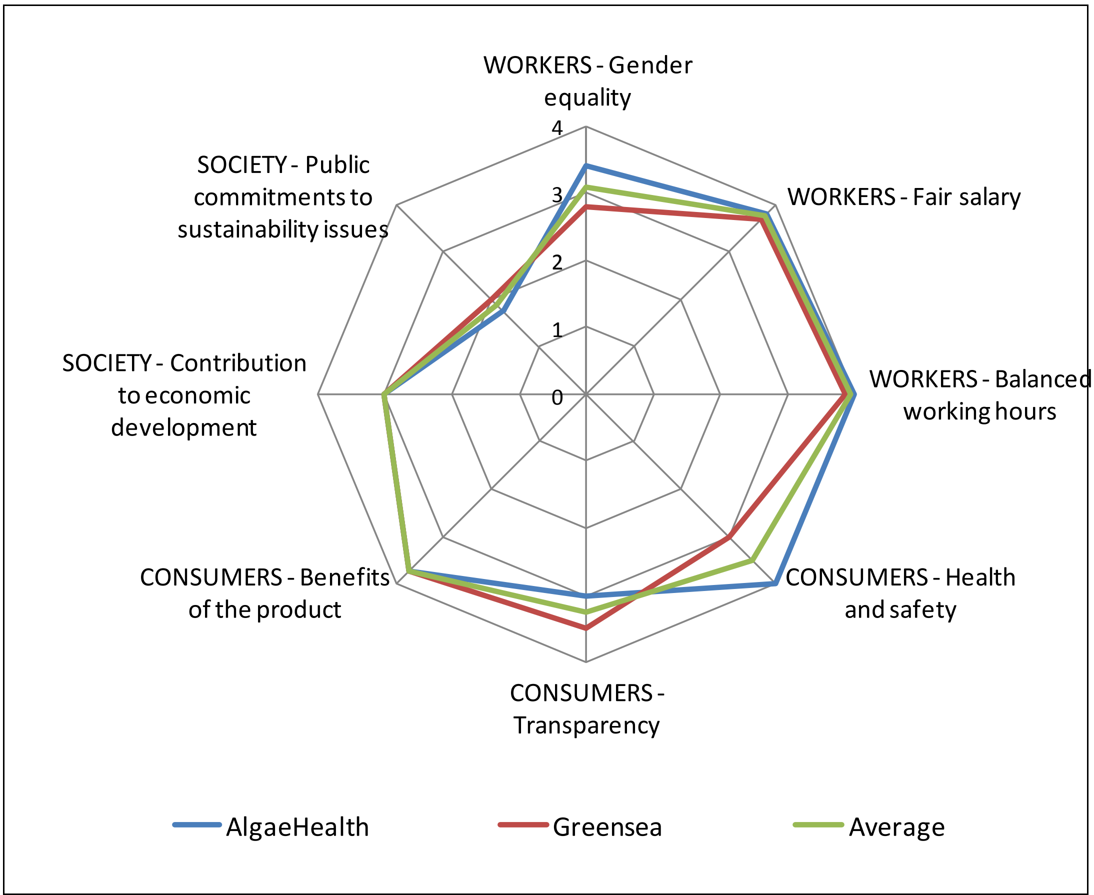


Figure 22. Radar chart representing prominent social issues of the corporate strategy of the two SMEs involved in BAMMBO project.

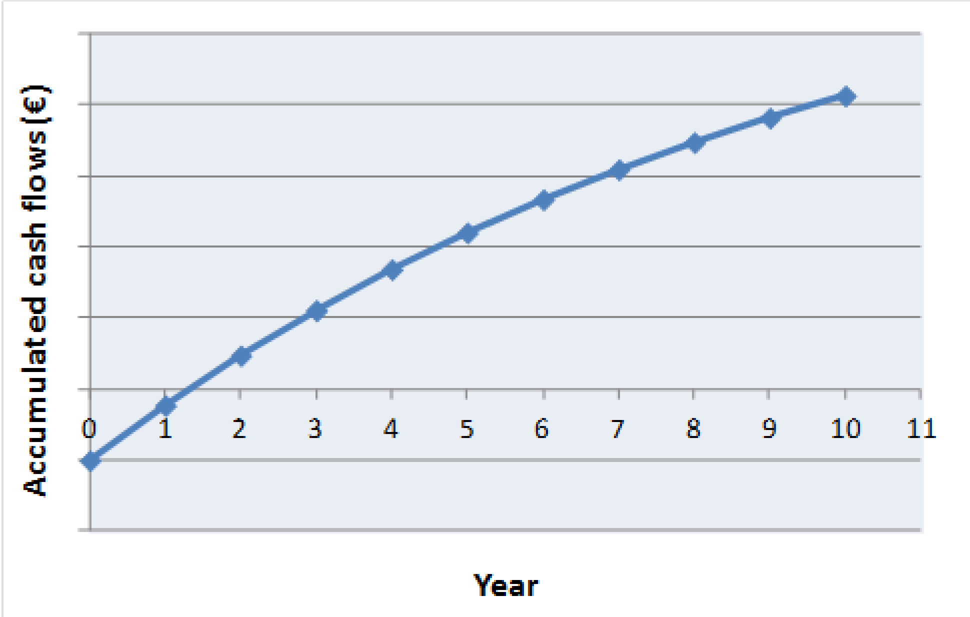


Figure 23. Determination of the Pay-back of astaxanthin production process.

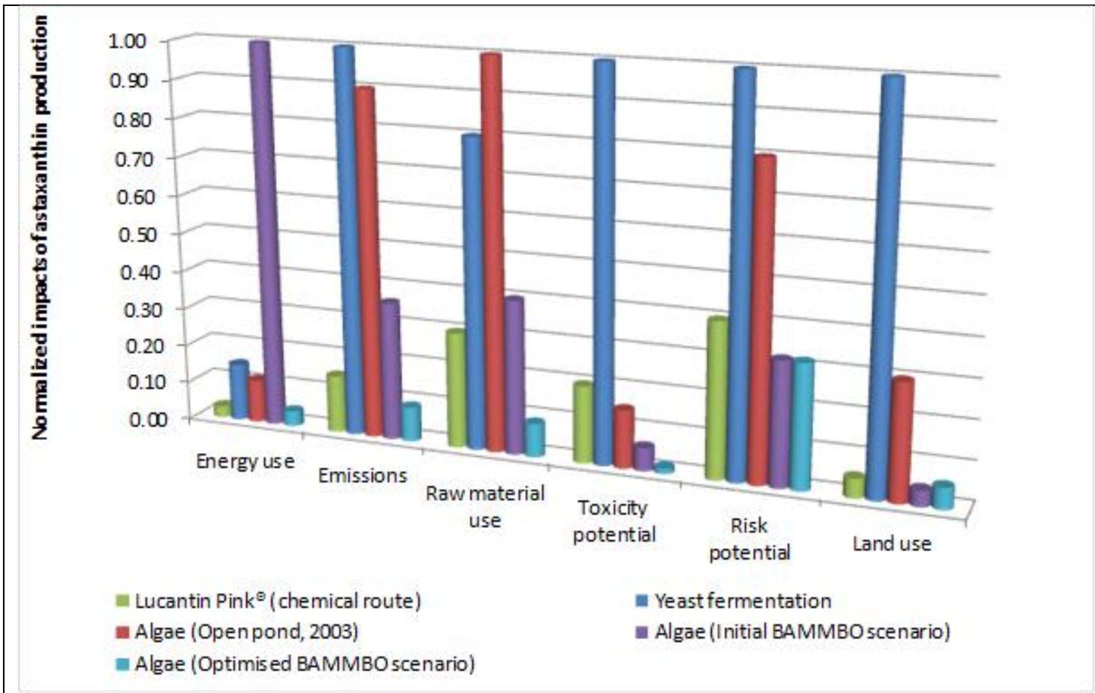


Figure 24. Environmental profiles of conventional and BAMMBO systems for astaxanthin production.

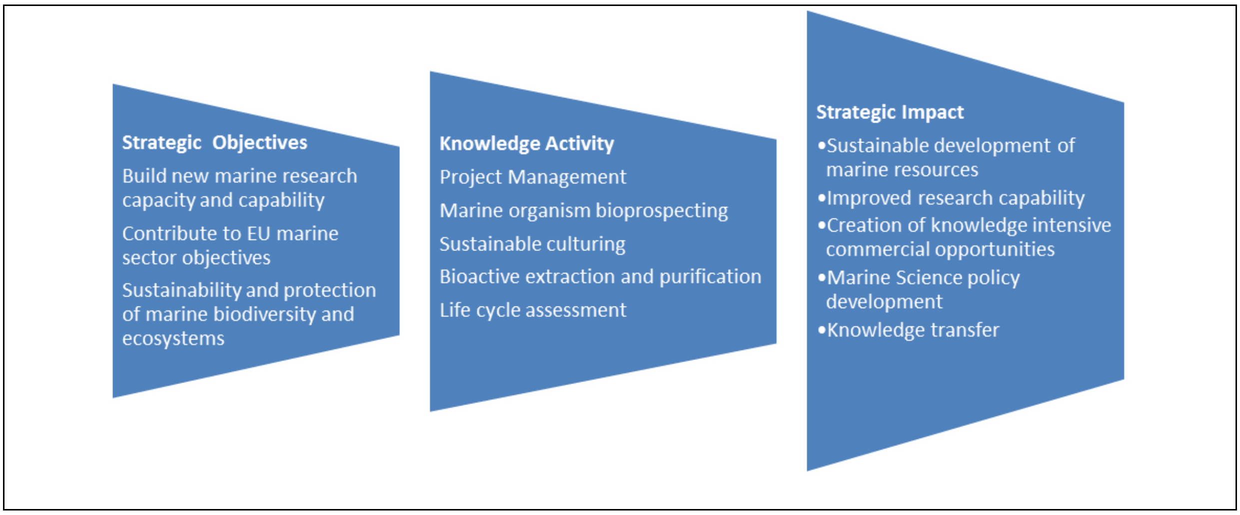


Figure 25. Graphical illustration of BAMMBO's approach to maximize both human and economic benefits from the marine environment while creating new knowledge, processes, products and employment.