

## Supplementary Figures and Tables

### Evaluation of *Aurantiochytrium mangrovei* Biomass Grown on Digestate as a Feed Ingredient of Sea Bass, *Dicentrarchus labrax*, Juveniles and Larvae

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**Table S1:** Chemical characterisation of digestate from COOPERL Organic biological waste (pig manure), Brittany (FR) <https://www.nweurope.eu/projects/project-search/alg-ad-creating-value-from-waste-nutrients-by-integrating-algal-and-anaerobic-digestion-technology/publications/the-alg-ad-project-reports-and-deliverables/>. **Table S2.** Cellular parameters (concentration, size, complexity, lipid content) as measured by flow cytometry on two 500 L culture tanks. **Table S3.** Fatty acid composition (expressed as % of total fatty acids in the fraction; n=2 batches) in neutral lipids (NL) and polar lipids (PL) and percentage of total lipids per dry weight of *Aurantiochytrium mangrovei* cultivated on Yeast Extract Peptone medium enriched with liquid effluent from anaerobic digestion. **Figure S1.** Percentages of the major PUFA, DHA (A) and DPA (B), expressed as percentage of total fatty acids in polar, neutral, and total lipids of *Aurantiochytrium mangrovei* biomass collected every hours during 5 hours of cross filtration. **Table S4.** Amino acids and fatty acids profiles of the dried microalgae biomass included in the experimental microalgae diet for Juveniles and micro-diet for larvae. **Table S5.** Fatty acid composition expressed in percentage of the control, and hydrolyzed micro-diets for sea bass larvae. **Table S6.** Fatty acid composition of neutral lipids (expressed as % of total fatty acids of the fraction) of sea bass (*Dicentrarchus labrax*) larvae fed control, and hydrolyzed microalgae diets 41 days after hatching. **Table S7.** Fatty acid composition of polar lipids (expressed as % of total fatty acids of the fraction) of sea bass (*Dicentrarchus labrax*) larvae fed control, and hydrolyzed microalgae diets 41 days after hatching. **Table S8.** Comparison of the amino acid profile (expressed in % of total amino acids) of *Aurantiochytrium mangrovei* with this of fish meal. **Table S9.** Lipid class composition of sea bass (*Dicentrarchus labrax*) larvae fed control and hydrolyzed microalgae diets 41 days after hatching.

**Table S1:** Chemical characterisation of digestate from COOPERL Organic biological waste (pig manure), Brittany (FR) [27]

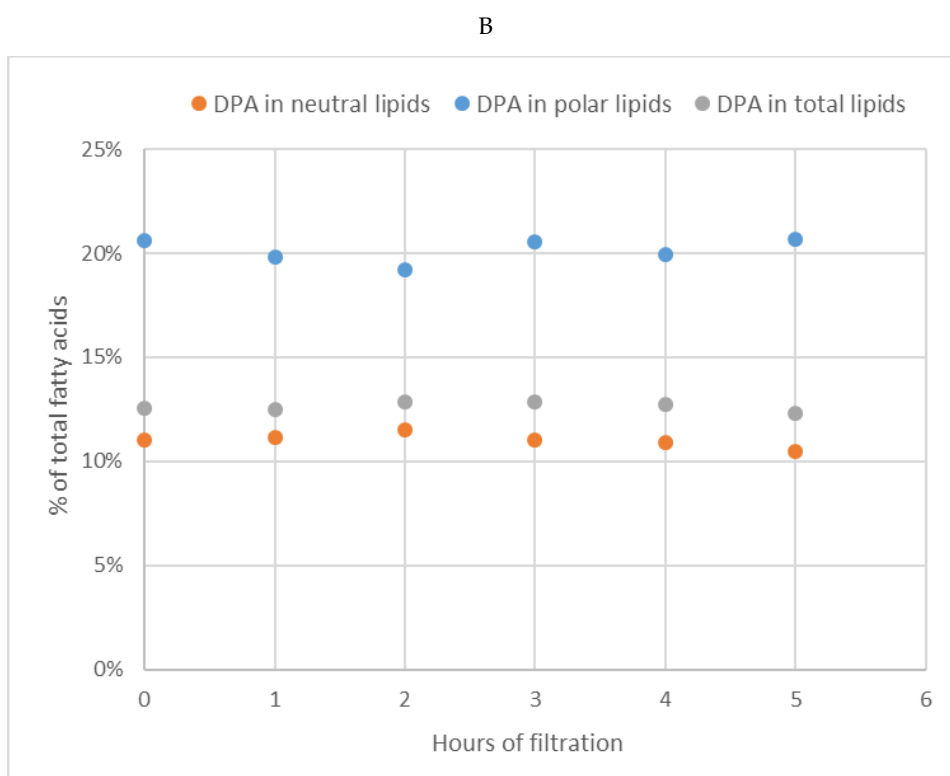
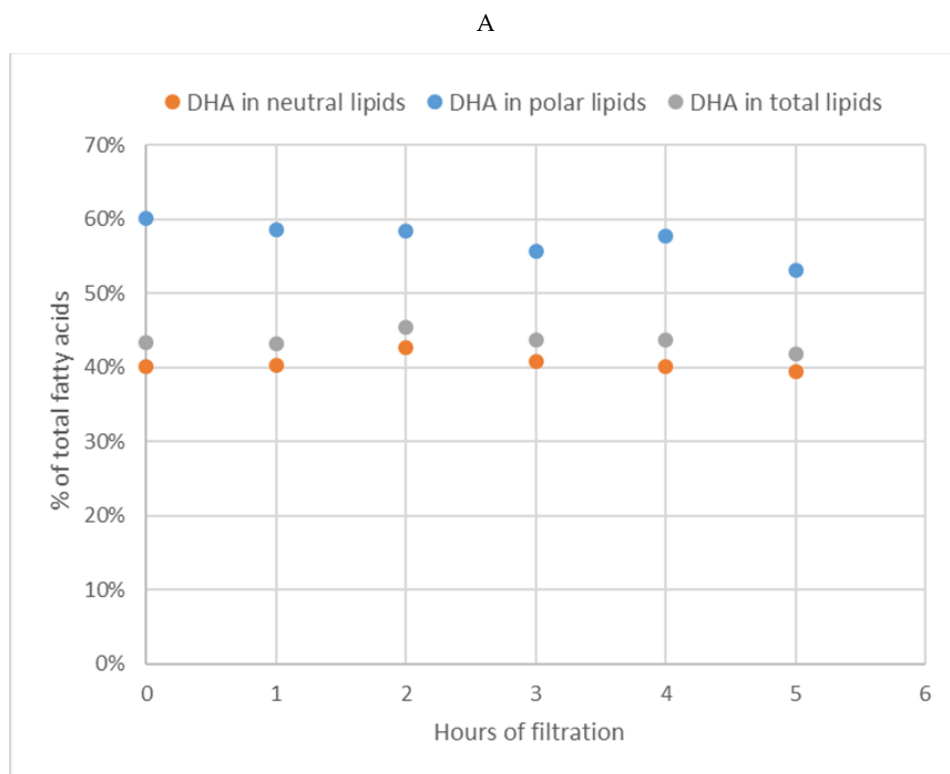
Compound/Unit	Values
Digestate Origin	
Dry Matter (%)	0.56
Dry organic matter (% DM)	28.36
Acetic acid (mg/kg)	< 35
Propionic acid (mg/kg)	< 16
Isobutyric acid (mg/kg)	< 9
Butyric acid (mg/kg)	< 7
Isovaleric acid (mg/kg)	< 11
Valeric acid (mg/kg)	< 12
Caproic acid (mg/kg)	< 24
pH	8.21
Conductivity (mS/cm)	17.92
Elemental analysis (mg/l)	B 0.51; As <0.05; Ca 47.5; Cd < 0.005; Co 0.018; Cr < 0.025; Cu < 0.050; Fe 2.16; Hg < 0.005; K 1600; Mg 8.7; Mn 0.050; Mo 0.012; Na 690; Ni 0.072; P 34.4; Pb < 0.050; S 22.8; Se < 0.005; Si 27.4; Sn < 0.050; Zn 0.250 and Cl 875
Ammoniacal nitrogen (g N/kg)	1.93
Total nitrogen (kg/1000kg FM)	1.98
Phosphate (mg/l)	103
Salinity (g/kg)	2.96

**Table S2.** Cellular parameters (concentration, size, complexity, lipid content) as measured by flow cytometry on two 500 L culture tanks.

Hours of Cultivation	Dry Weight (g.L <sup>-1</sup> )		Concentration (cells.mL <sup>-1</sup> )		Size (A.U)		Complexity (A.U)		Lipid Content (A.U)	
	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D	Mean	S.D
0	N.D.		7.5E+05	1.5E+05	113.6	7.3	181.9	15.1	416.9	55.8
14	1.4	0.1	1.3E+08	1.9E+06	95.9	0.5	163.8	0.4	374.3	35.0
19	2.9		1.8E+08	2.0E+06	120.9	1.1	182.0	2.2	595.7	7.3
23	3.2	0.1	2.4E+08	1.6E+07	101.4	1.4	227.1	49.5	532.0	0.2
38	8.7	0.4	3.0E+08	5.4E+06	97.3	0.6	346.3	2.7	2555.6	48.5
46	9.3	0.3	3.2E+08	1.1E+07	84.2	0.6	372.0	4.7	3790.8	25.3
62	10.5	0.6	3.3E+08	7.4E+05	76.1	0.2	298.5	1.2	2591.3	83.9

**Table S3.** Fatty acid composition (expressed as % of total fatty acids in the fraction; n=2 batches) in neutral lipids (NL) and polar lipids(PL) and percentage of total lipids per dry weight of *Aurantiochytrium mangrovei* cultivated on Yeast Extract Peptone medium enriched with liquid effluent from anaerobic digestion.

Hours of culture	14		19		23		38		46		62	
Fractions	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL	NL	PL
14:0	2.0	1.0	4.1	1.6	4.0	1.3	3.8	1.1	4.0	0.8	3.5	0.4
15:0	1.5	0.9	3.9	1.9	6.6	3.9	4.7	1.8	3.7	1.3	3.2	0.9
16:0	38.1	23.0	42.1	23.5	36.5	21.1	37.4	25.6	37.5	23.5	35.0	21.0
17:0	0.8	0.3	1.6	0.4	2.3	0.7	1.1	0.4	0.9	0.3	0.8	0.2
18:0	2.4	0.4	2.4	0.2	2.0	0.2	1.3	0.2	1.2	0.2	1.3	0.2
19:0	0.0	0.0	0.2	0.0	0.3	0.0	0.1	0.0	0.1	0.0	0.1	0.0
20:0	0.4	0.0	0.5	0.0	0.3	0.0	0.3	0.0	0.3	0.0	0.3	0.0
22:0	0.2	0.0	0.3	0.0	0.2	0.0	0.1	0.0	0.1	0.0	0.1	0.0
16:1n-7	0.0	1.1	0.0	0.5	0.0	0.4	0.0	0.2	0.0	0.2	0.0	0.5
18:1n-7	0.1	0.8	0.0	0.3	0.0	0.3	0.0	0.1	0.0	0.1	0.0	0.6
18:1n-9	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:4n-3	0.4	0.1	0.4	0.1	0.4	0.1	0.2	0.1	0.2	0.1	0.2	0.0
20:3n-6	0.3	0.1	0.3	0.1	0.3	0.1	0.2	0.1	0.2	0.1	0.2	0.1
20:4n-3	0.9	0.4	0.8	0.4	0.8	0.4	0.7	0.3	0.7	0.3	0.7	0.2
20:4n-6	1.1	0.5	1.0	0.6	1.1	0.7	0.8	0.5	0.8	0.6	1.0	0.8
20:5n-3	1.5	2.0	1.0	2.0	1.1	1.9	0.7	0.9	0.7	0.9	1.0	3.5
22:5n-3	0.5	0.2	0.4	0.3	0.4	0.3	0.2	0.2	0.2	0.2	0.2	0.2
22:5n-6	7.4	11.8	6.6	13.3	7.3	14.6	10.0	16.6	10.2	17.0	11.2	17.1
22:6n-3	41.9	57.2	33.7	54.3	35.8	53.7	37.8	51.7	38.5	54.3	40.5	54.0
Total mg.L <sup>-1</sup>	28	79	55	145	281	386	1920	420	1388	282	1388	219
% neutral lipids	26		27		42		82		83		86	
Lipid % of dry weight	7.6		6.8		21.0		27.1		23.4		15.3	



**Figure S1.** Percentages of the major PUFA, DHA (A) and DPA (B), expressed as percentage of total fatty acids in polar, neutral, and total lipids of *Auranthiochytrium mangrovei* biomass collected every hours during 5 hours of cross filtration.

**Table S4.** Amino acids and fatty acids profiles of the dried microalgae biomass included in the experimental microalgae diet for Juveniles and micro-diet for larvae.

Dried microalgae biomass					
Amino Acids (AA)	Total AA %		Fatty acids (FA)	Total FA %	
	Mean	S.D		Mean	S.D
acide aspartique	10.8	± 0.87	14:0	3.3	± 0.17
threonine	5.7	± 0.45	15:0	2.9	± 0.17
serine	5.7	± 0.45	16:0	34.3	± 2.17
acide glutamique	16.9	± 1.36	17:0	0.8	± 0.05
proline	4.0	± 0.31	18:0	1.2	± 0.07
glycine	5.3	± 0.42	16:1n-7	0.1	± 0.06
alanine	6.5	± 0.52	18:1n-7	0.1	± 0.01
cystine	1.7	± 0.14	18:1n-9	0.0	± 0.00
valine	5.4	± 0.42	18:3n-6	0.1	± 0.01
methionine	2.0	± 0.14	18:4n-3	0.1	± 0.01
isoleucine	4.4	± 0.35	20:3n-6	0.2	± 0.01
leucine	7.2	± 0.59	20:4n-3	0.6	± 0.04
tyrosine	3.7	± 0.28	20:4n-6	0.9	± 0.06
phenylalanine	4.3	± 0.35	20:5n-3	1.2	± 0.09
histidine	2.3	± 0.17	22:5n-3	0.0	± 0.00
lysine	6.6	± 0.52	22:5n-6	12.3	± 0.76
arginine	7.6	± 0.63	22:6n-3	40.5	± 2.68
Total g / 100g	28.66	± 2.29	Total	8.31	± 0.53

**Table S5.** Fatty acid composition expressed in percentage of the control, and hydrolyzed micro-diets for Sea bass larvae.

Fatty Acids (%)	Control		Hydrolyzed Microalgae	
	Mean	S.D.	Mean	S.D.
14:0	2.2	0.0	2.6	0.0
15:0	0.4	0.0	0.7	0.0
16:0	20.1	0.1	24.5	0.1
17:0	0.4	0.0	0.4	0.0
18:0	3.5	0.0	3.3	0.0
20:0	0.2	0.0	0.2	0.0
16:1n-7	2.9	0.0	2.7	0.0
18:1n-7	2.3	0.0	2.0	0.0
18:1n-9	17.0	0.0	15.1	0.0
20:1n-11	0.3	0.0	0.3	0.0
20:1n-7	0.1	0.0	0.1	0.0
20:1n-9	1.8	0.0	1.6	0.0
22:1n-11	2.5	0.0	2.5	0.0
22:1n-9	0.2	0.0	0.2	0.0
24:1n-9	0.7	0.0	0.7	0.1
16:2n-4	0.2	0.0	0.2	0.0
16:3n-4	0.1	0.0	0.1	0.0
16:4n-1	0.1	0.0	0.1	0.0
18:2n-4	0.1	0.0	0.1	0.0
18:2n-6	24.6	0.0	24.4	0.2
18:3n-3	2.5	0.0	2.3	0.0
18:4n-3	1.0	0.0	1.0	0.0
20:4n-3	0.3	0.0	0.3	0.0
20:4n-6	0.5	0.0	0.3	0.0
20:5n-3	4.2	0.0	4.0	0.0
22:5n-3	0.6	0.0	0.5	0.0
22:5n-6	0.3	0.0	0.4	0.0
22:6n-3	8.1	0.1	7.0	0.1
Total Branched	0.5	0.0	0.5	0.0
Total SFA	27.3	0.1	32.4	0.1
Total MUFA	28.5	0.1	25.7	0.1
Total PUFA	43.7	0.1	41.5	0.2

**Table S6.** Fatty acid composition of neutral lipids (expressed as % of total fatty acids of the fraction) of Sea bass (*Dicentrarchus labrax*) larvae fed control, and hydrolyzed microalgae diets 41 days after hatching.

Fatty Acids (%)	Control		Hydrolyzed Microalgae		T-Test
14:0	2.4	0.2	2.3	0.3	
15:0	0.6	0.0	0.8	0.1	*
16:0	19.9	0.4	22.2	1.5	
17:0	0.6	0.0	0.6	0.1	
18:0	4.4	0.1	5.6	1.4	
16:1n-7	3.8	0.2	3.2	0.4	
18:1n-7	2.8	0.1	2.9	0.1	
16:1n-9	0.5	0.0	0.4	0.0	
17:1n-7	0.5	0.0	0.5	0.1	
18:1n-9	19.3	0.5	16.3	1.5	
20:1n-11	0.4	0.1	0.5	0.0	
20:1n-9	2.2	0.4	2.2	0.1	
16:2n-4	0.6	0.1	0.8	0.7	
16:4n-3	0.2	0.0	0.3	0.1	
18:2n-6	25.9	0.3	23.9	2.2	
20:2n-6	1.1	0.1	1.3	0.1	
20:4n-3	0.5	0.0	0.7	0.2	
20:4n-6	0.7	0.1	0.8	0.3	
20:5n-3	4.1	0.3	4.4	0.6	
22:5n-6	0.3	0.0	0.5	0.1	
22:6n-3	9.2	1.2	9.9	2.6	
SAFA	27.9	0.5	31.5	0.4	*
MUFA	29.5	1.0	25.9	1.9	
PUFA	42.6	1.4	42.6	2.3	
% LN	46.7	2.6	33.1	3.6	*
Total mg/g	33.8	6.2	22.9	8.9	
DHA mg/g	3.1	0.2	2.0	0.5	*



**Table S7.** Fatty acid composition of polar lipids (expressed as % of total fatty acids of the fraction) of Sea bass (*Dicentrarchus labrax*) larvae fed control, and hydrolyzed microalgae diets 41 days after hatching.

Fatty Acids (%)	Control		Hydrolyzed Microalgae		T-Test
14:0	0.5	0.0	0.6	0.0	*
15:0	0.3	0.0	0.4	0.0	
16:0	23.7	0.4	23.8	0.2	
17:0	0.4	0.0	0.5	0.1	
18:0	6.5	0.3	6.9	0.5	
16:1n-7	1.0	0.0	1.1	0.1	
18:1n-7	2.1	0.1	2.1	0.1	
16:1n-9	0.3	0.0	0.3	0.0	
17:1n-7	0.2	0.0	0.3	0.1	
18:1n-9	12.4	0.2	11.9	0.3	
20:1n-11	0.1	0.0	0.1	0.0	
20:1n-9	0.9	0.0	0.9	0.0	
16:2n-4	0.3	0.0	0.3	0.0	
16:4n-3	0.3	0.1	0.4	0.0	
18:2n-6	17.6	0.9	16.7	2.1	*
20:2n-6	1.3	0.0	1.4	0.0	
20:4n-3	0.2	0.0	0.2	0.0	
20:4n-6	1.8	0.1	2.0	0.3	
20:5n-3	7.7	0.3	7.3	0.4	*
22:5n-6	0.7	0.0	1.0	0.0	
22:6n-3	21.6	0.5	21.6	1.9	
SAFA	31.4	0.6	32.2	0.4	
MUFA	17.0	0.2	16.8	0.2	
PUFA	51.6	0.8	51.0	0.3	
% polar lipids	53.3	2.6	66.9	3.6	
Total mg/g	38.2	4.0	44.9	15.6	
DHA mg/g	8.2	0.7	9.4	2.7	

**Table S8.** Comparison of the amino acid profile (expressed in % of total amino acids) of *Aurantiochytrium mangrovei* with this of fish meal

% of Total Amino Acids (AA)	Dried Microalgae Biomass	Type 62 Fish Meal
<b>Essential AA</b>		
Threonine	5.6	4.6
Valine	6.6	5.5
Methionine	2.2	2.9
Isoleucine	5.3	4.5
Leucine	8.3	7.9
Phenylalanine	4.6	4.3
Histidine	2.1	2.9
Lysine	7.3	8.3
Arginine	4.4	6.6
<b>Non essential AA</b>		
Aspartic acid	11.2	10.1
Tyrosine	3.9	3.3
Serine	5.1	4.3
Glutamic acid	13.8	13.8
Proline	5.0	5.1
Glycine	5.3	7.7
Alanine	7.9	7.0
Cystine	1.3	1.0

**Table S9.** Lipid class composition of Sea bass (*Dicentrarchus labrax*) larvae fed control and hydrolyzed microalgae diets 41 days after hatching.

Lipid Classes in %	Control		Hydrolyzed Microalgae	
Steryl ester	1.5	0.5	1.6	0.2
Free fatty acids	2.7	1.5	1.9	0.3
Free sterols	6.7	0.9	7.1	0.4
Triacylglycerol	21.3	1.8	14.2	2.9
Phosphatidylcholine	35.4	4.7	38.2	1.6
Sphingomyeline	2.0	0.2	2.2	0.3
PE+CL*	25.3	1.8	29.4	2.4
Phosphatidylserine	2.6	0.4	3.0	0.7
phosphatidylinositol	2.2	0.2	2.4	0.1

\* PE+CL= Phosphatidylethanolamine and cardiolipin