Digestate as a nutrient source for algae cultivation

Final IDEA+ event, September 2023

Alla Silkina, Mohamed Emran – Swansea university (UK)

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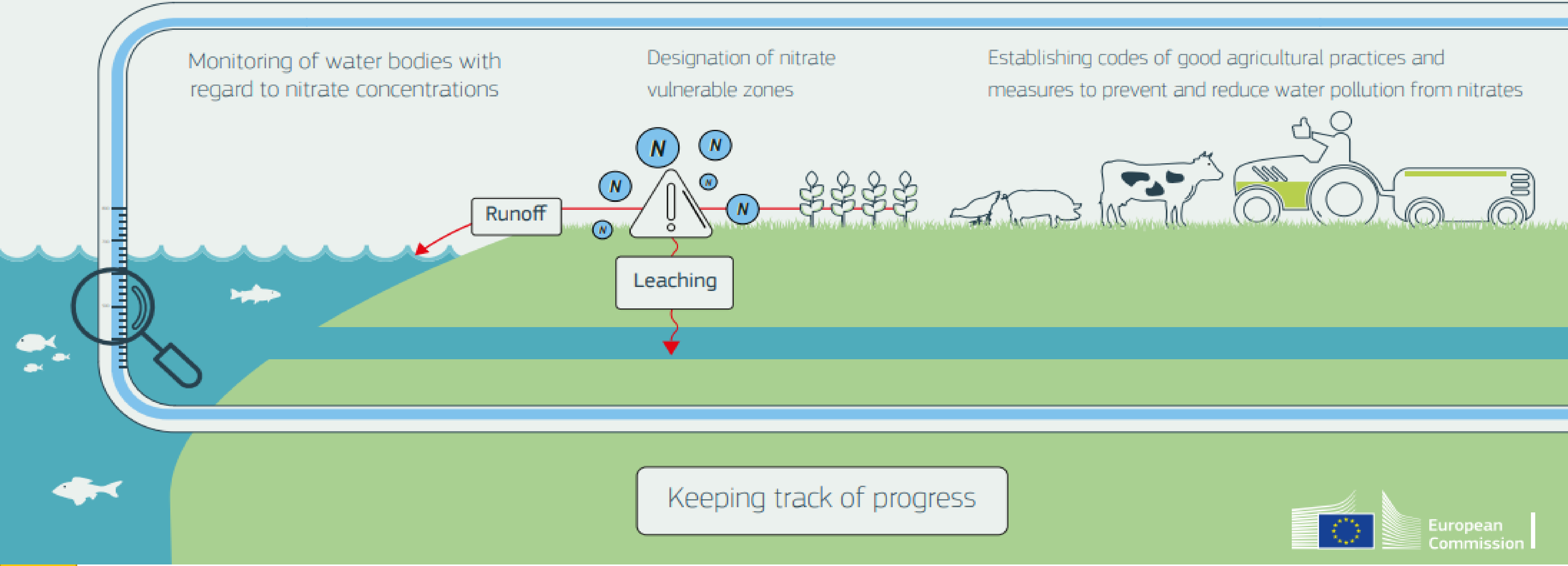
Interreg North-West Europe IDEA

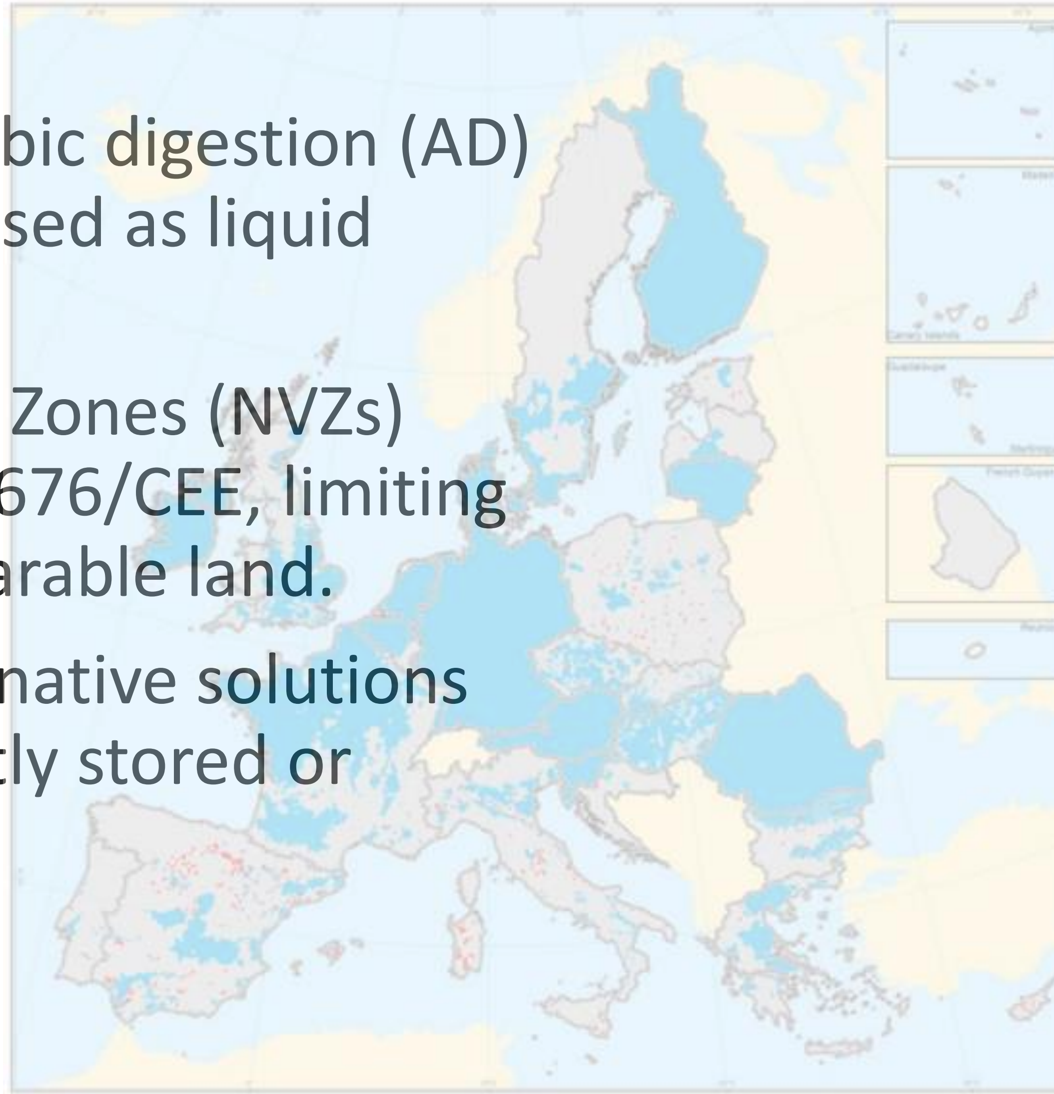
The EU wants to reduce water pollution caused by nitrates used in agriculture and sets out steps for EU countries to take



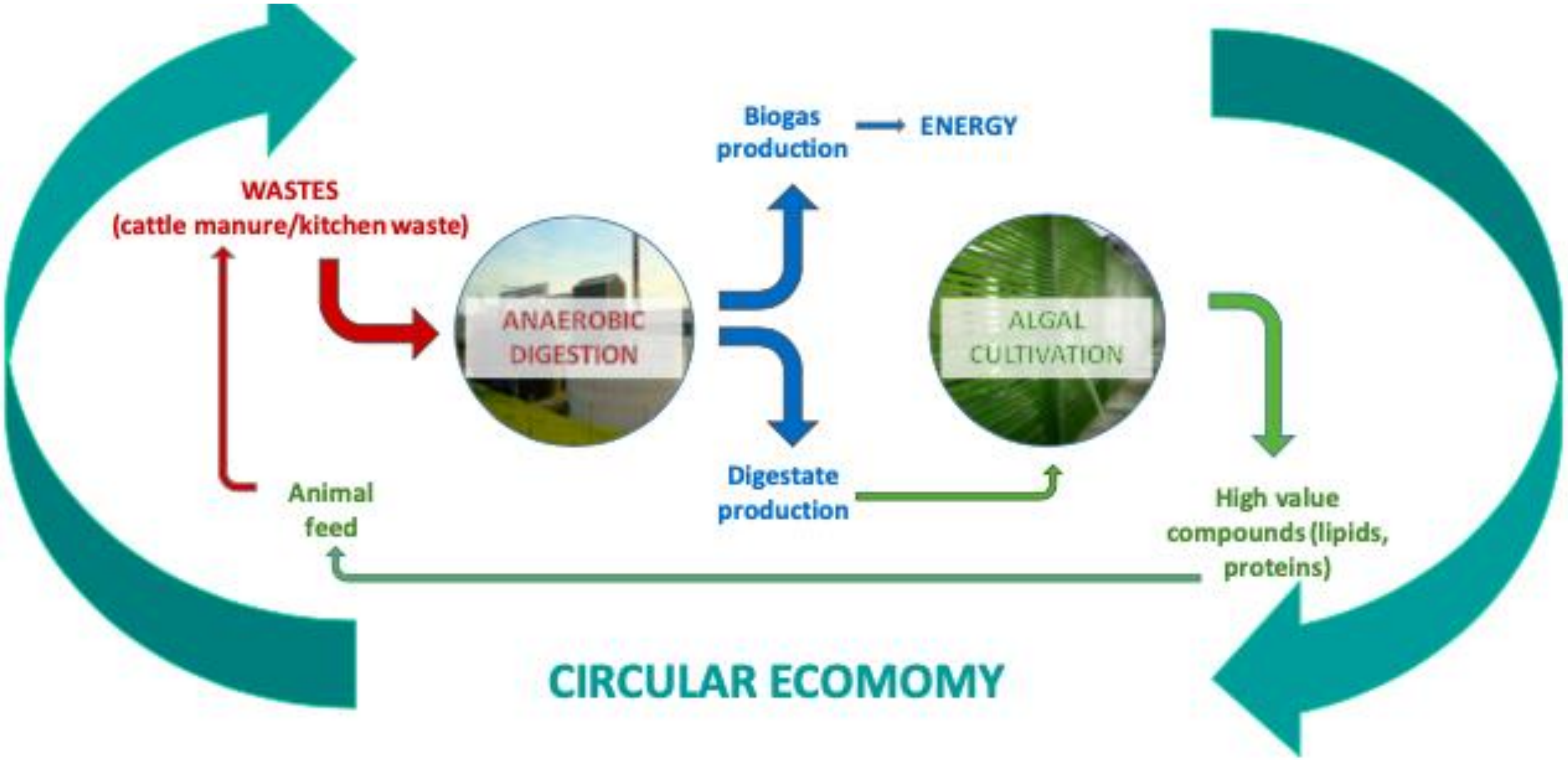
European Commission

Digestate-environmental problems

- Digestate is a by-product from the anaerobic digestion (AD) of food and farm waste, and is currently used as liquid fertiliser across Northwest Europe
- Implementation of the Nitrate Vulnerable Zones (NVZs) policy, the European Nitrate Directive 91/676/CEE, limiting the annual load of nitrogen applied onto arable land.
- AD plants are under pressure to find alternative solutions for their excess digestate, which is currently stored or buried.



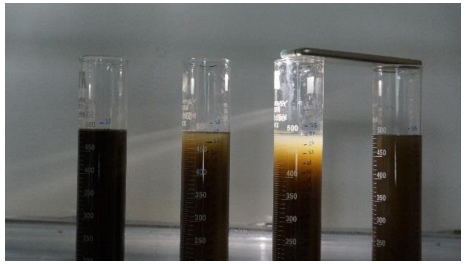
Digestate and microalgae

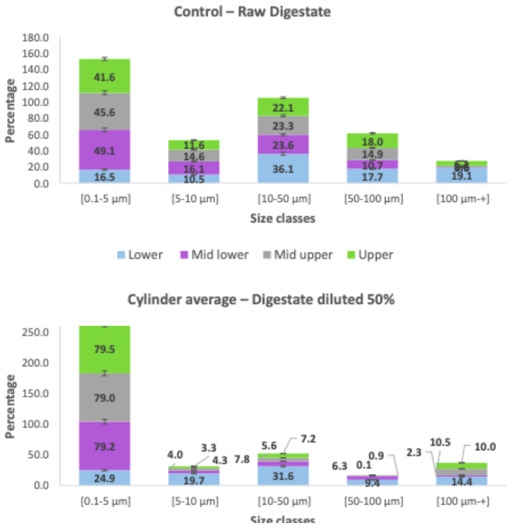


Pre-treatment technologies developed in ALG-AD project

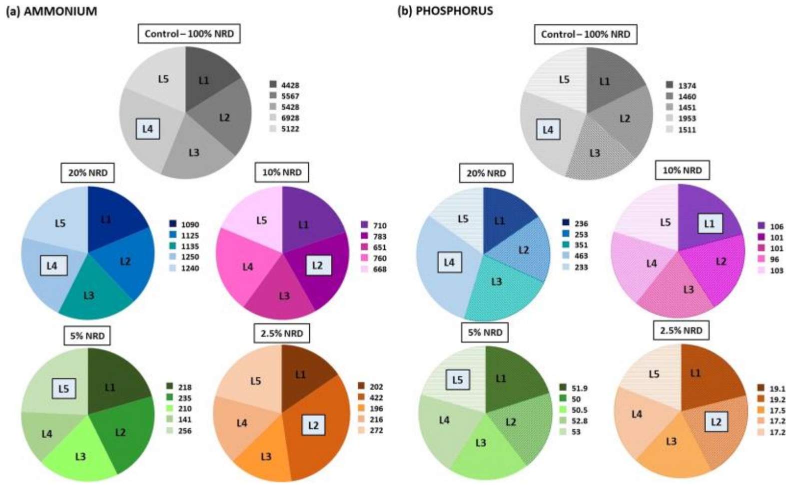
Reduction of particles, contaminants and color
Adjustment of the nutrients

Low cost:
- ✓ **Dilution**
- ✓ **Settlement**





Medium, High cost :
- ✓ **Centrifugation**
- ✓ **Filtration**



Fernandes, Silkina et al, 2020 : https://doi.org/10.1016/j.wasman.2020.08.037

Silkina et al, 2020 : https://doi.org/10.1007/s12649-019-0076

How to use the digestate for algal cultivation

- **Initial: Measurements of N, P and C ; Turbidity, pH**
- **Application of low or medium/high-cost pretreatment methods or combinations**
- **Measurements of nutrients after pre-treatment**
- **Dilution factor application**
- **Small scale adaptation of selected algal species**

Pretreatment requirements of digestate for use in algae cultivation

Final IDEA+ event, September 2023

Alla Silkina, Fleuriane Fernandes, Mohamed Emran– Swansea university (UK)

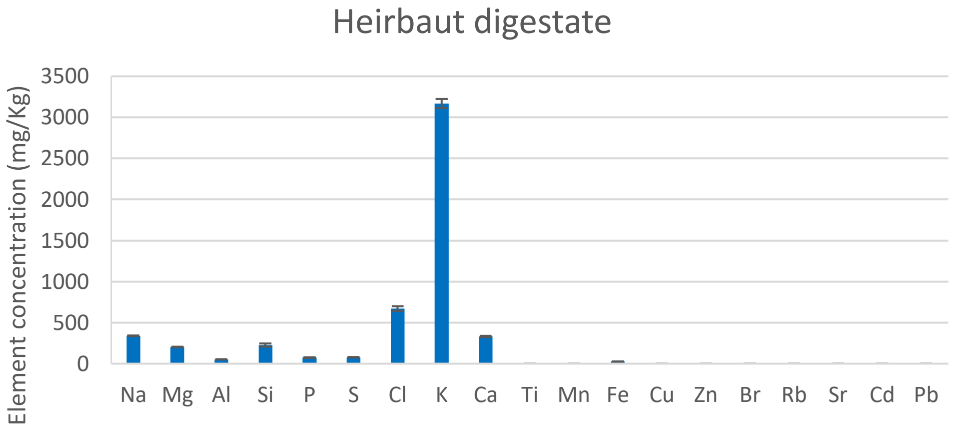
Kris Heirbaut Collaboration with Heirbaut (Belgium)

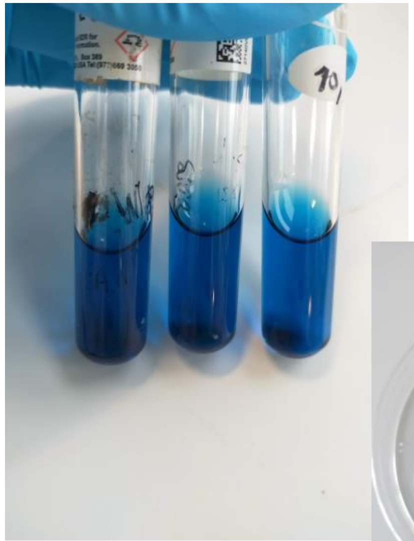
Digestate sources

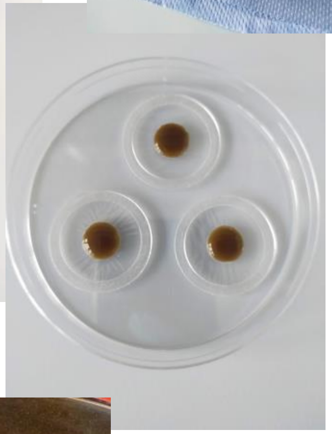
Analysis of **3 digestates**

	Digestate 1	Digestate 2	Digestate 3
Company/Country	Heirbaut Algriculture (BE)	Wrexham (UK)	Asgard (UK)
Feedstock	Cow manure	Broiler manure/sheep intestine content	Kitchen waste

- NH_4^+ & PO_4^{3-}
- Dry weight/Turbidity
- Elemental analysis using XRF (macronutrients N,K; metals Al, Zn, Cu and other elements)







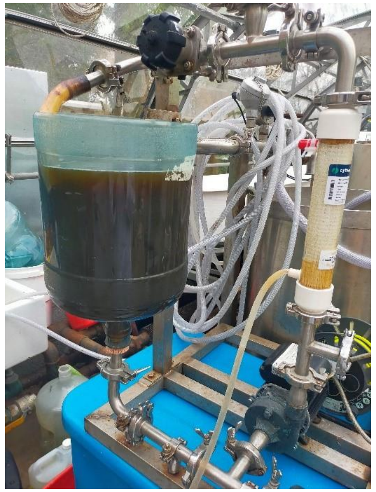
Digestate pre-treatment and composition

	Heirbaut	Wrexham	Asgard
NH_4^+	797.8 ± 18.4	1786.7 ± 108	2406.2 ± 414.9
PO_4^{3-}	622.1 ± 8.8	660.3 ± 9.1	880.4 ± 9.8
DW	7.2 ± 0.7	24.7 ± 0.8	18.2 ± 1.2
N:P	1.3	2.7	2.7
Potential dilution required	8	18	24
Potential percentage used	12.50%	5.50%	4%

Pre-treatment of the digestate by membrane filtration 0.2um pore size

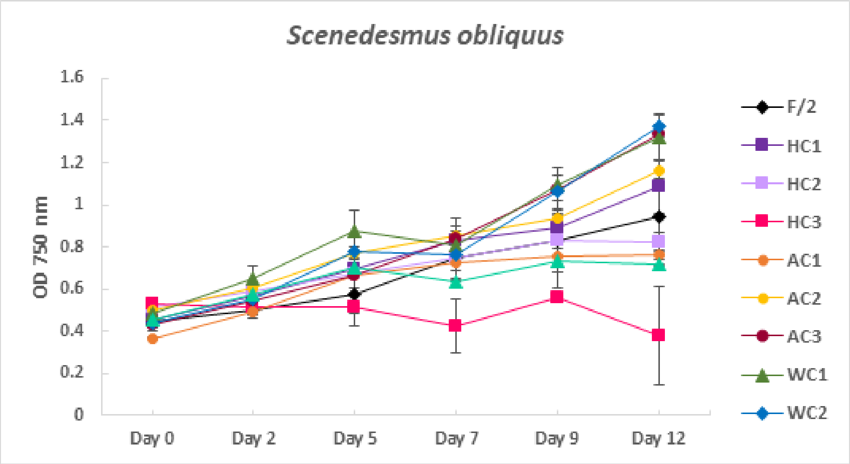
Ammonium and phosphorus analysis of digestates after membrane filtration

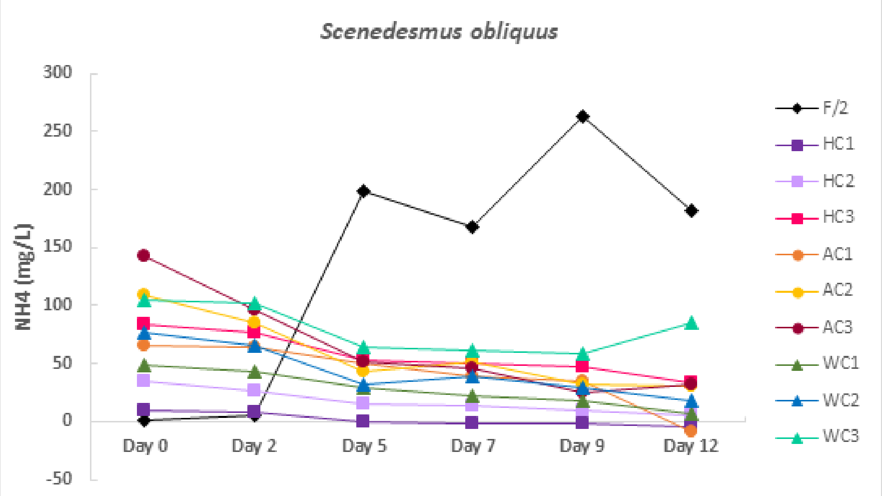
	Heirbaut	Wrexham	Asgard
NH_4^+	**102.62 ± 1.95**	509.56 ± 20.57	1569.83 ± 19.69
PO_4^{3-}	**24.38 ± 0.22**	45.25 ± 0.58	139.49 ± 1.38
N:P Ratio	**4.2**	11.3	11.3



REDUCTION:
- ✓ Colour
- ✓ Particles
- ✓ Adjust N & P concentration

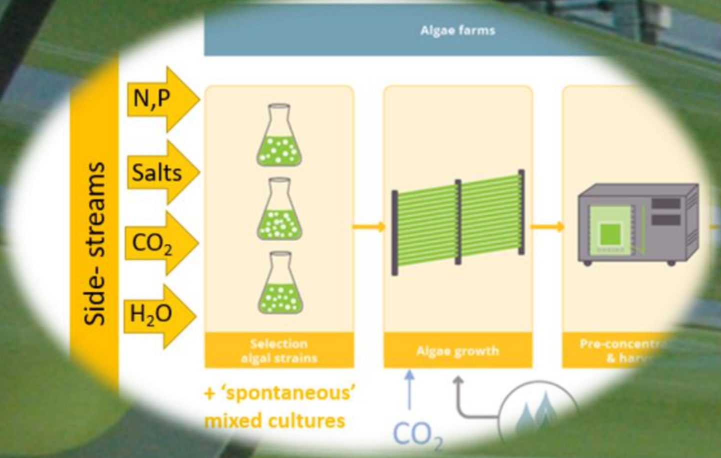
Bioremediation small scale test





From this laboratory-scale study, it is recommended to use *Scenedesmus* for larger applications as it performed efficiently on all tested digestates.

The Heirbaut digestate is chosen to be used at the pilot facility, it is recommended to use up to 5% - 50 mg/L of ammonium concentration of the treated digestate in pilot scale studies.



Establishing growth conditions for algal growth on pre-treated digestate

Final IDEA+ event, 28 September 2023

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Aims

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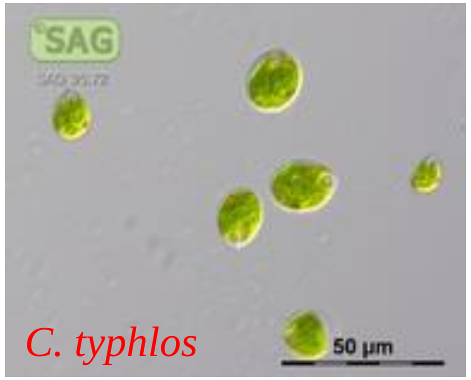
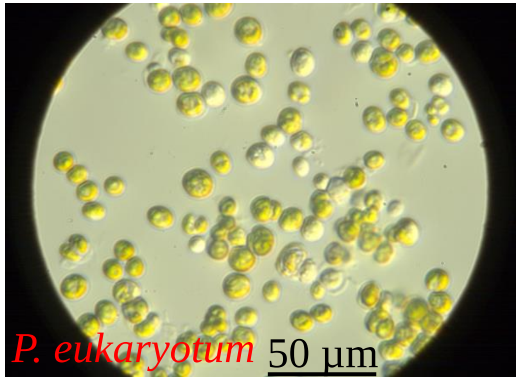
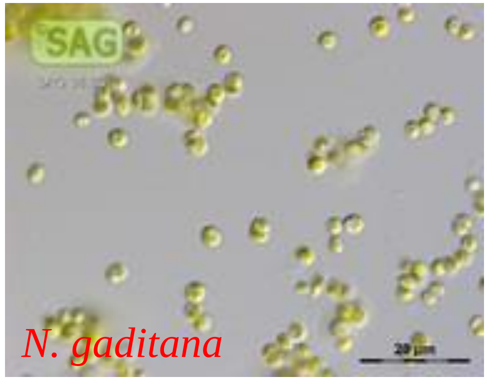
Determine the possibility of pre-treated digestates as nutrients source for microalgae growth and subsequently identify their optimal nutritional conditions

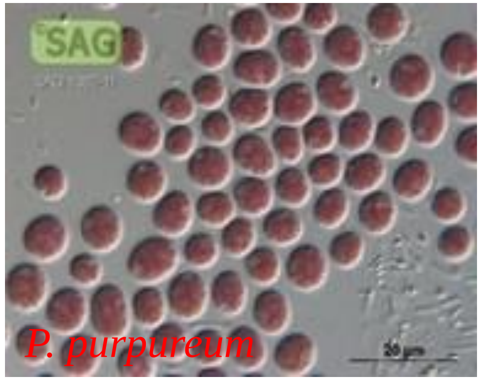
- Anaerobic digestion effluents (3 digestates, from feedstocks)
- Species:
  - *Chloromonas typhlos*
  - *Picochlorum eukaryotum* (Wild population, isolated from open pond)
  - *Nannochloropsis gaditana*
  - *Porphyridium purpureum*





Freshwater algae





Marine algae

Potentials and barriers of digestates for algal cultivation

Table 1: Overview of tested digestates before the experiment.

Digestate	Code	Source	Dilution before experiments	pH	$N\text{-}NH_4^+$ Concentration (mg L^{-1})	$N\text{-}NO_3^-$ Concentration (mg L^{-1})	$P\text{-}PO_4^{3-}$ Concentration (mg L^{-1})
D1	SU-D-1-CS from ASGEARD	Kitchen waste	No dilution	8.51	2033.7 ± 12.4	10.6 ± 0.9	42.7 ± 0.7
D2	SU-D-1-CS from WREXHAM	Broiler manure/sheep intestine content	1:3	8.24	376.3 ± 1.8	11.5 ± 4.8	2.8 ± 0.2
D3	SU-D-1-CS from HERBAUT	Cow manure feedstock	1:5	7.98	137.2 ± 0.5	101.9 ± 4.8	9.4 ± 0.5

Three digestates could be used for feeding microalgae due to their contents of nitrogen and phosphorus.

Main barriers:

1. high turbidity (dark brown color), affecting light availability for algal cells;
2. High NH_4+ concentration, toxic to cells

Optimize dilution rate

Experimental setup

Table 2: Nitrogen and phosphorus concentrations of digestates under different dilution rates before the experiment

Digestate / Dilution rate	D1 (from ASGEARD)				D2 (from WREXHAM)				D3 (from HERBAUT)			
	$N\text{-}NH_4^+$ Concentration (mg L^{-1})	$N\text{-}NO_3^-$ Concentration (mg L^{-1})	$P\text{-}PO_4^{3-}$ Concentration (mg L^{-1})	N:P molar ratio	$N\text{-}NH_4^+$ Concentration (mg L^{-1})	$N\text{-}NO_3^-$ Concentration (mg L^{-1})	$P\text{-}PO_4^{3-}$ Concentration (mg L^{-1})	N:P molar ratio	$N\text{-}NH_4^+$ Concentration (mg L^{-1})	$N\text{-}NO_3^-$ Concentration (mg L^{-1})	$P\text{-}PO_4^{3-}$ Concentration (mg L^{-1})	N:P molar ratio
1	2033.7 ± 12.4	10.6 ± 0.9	42.7 ± 0.7		-	-	-	-	-	-	-	-
3	677.9 ± 4.1	3.5 ± 0.3	14.2 ± 0.2		376.3 ± 1.8	11.5 ± 4.8	2.8 ± 0.2		-	-	-	-
5	406.7 ± 2.5	2.1 ± 0.2	8.5 ± 0.1		225.8 ± 1.1	6.9 ± 2.9	1.7 ± 0.1		137.2 ± 0.5	101.9 ± 4.5	9.4 ± 0.5	
10	203.4 ± 1.2	1.1 ± 0.1	4.3 ± 0.0		112.9 ± 0.5	3.4 ± 1.4	0.8 ± 0.1		68.6 ± 0.3	51.0 ± 2.2	4.7 ± 0.2	
15	135.6 ± 0.8	0.7 ± 0.1	2.8 ± 0.0		75.3 ± 0.4	2.3 ± 1.0	0.6 ± 0.0		45.7 ± 0.2	34.0 ± 1.5	3.1 ± 0.2	
20	101.7 ± 0.6	0.5 ± 0.0	2.1 ± 0.0		56.4 ± 0.3	1.7 ± 0.7	0.4 ± 0.0		34.3 ± 0.1	25.5 ± 1.1	2.4 ± 0.1	
30	67.8 ± 0.4	0.4 ± 0.0	1.4 ± 0.0		37.6 ± 0.2	1.1 ± 0.5	0.3 ± 0.0		22.9 ± 0.1	17.0 ± 0.7	1.6 ± 0.1	
40	50.8 ± 0.3	0.3 ± 0.0	1.1 ± 0.0		28.2 ± 0.1	0.9 ± 0.4	0.2 ± 0.0		17.2 ± 0.1	12.7 ± 0.6	1.2 ± 0.1	
50	40.7 ± 0.2	0.2 ± 0.0	0.9 ± 0.0		22.6 ± 0.1	0.7 ± 0.3	0.2 ± 0.0		13.7 ± 0.1	10.2 ± 0.4	0.9 ± 0.0	
60	33.9 ± 0.2	0.2 ± 0.0	0.7 ± 0.0	106	18.8 ± 0.1	0.6 ± 0.2	0.1 ± 0.0	307	11.4 ± 0.0	8.5 ± 0.4	0.8 ± 0.0	56

Growth condition: 25℃, 120 rpm, 2.0% CO_2 (v/v), Continuous light: 35 µmol photons m^{-2} s^{-1}.

Note: for marine algae, NaCl was added to maintain cellular osmotic equilibrium.

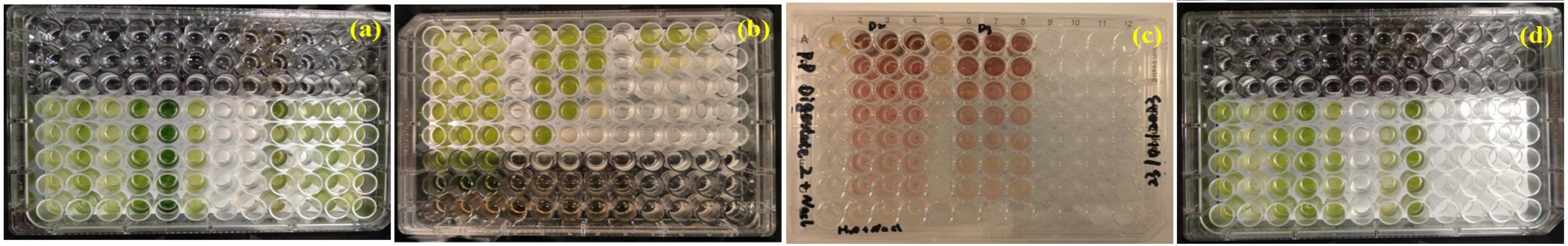
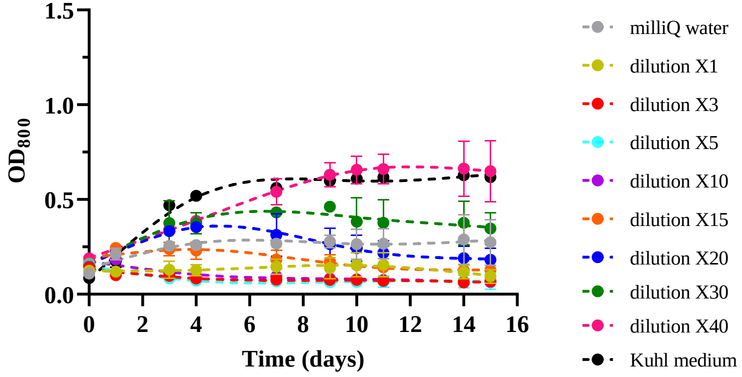


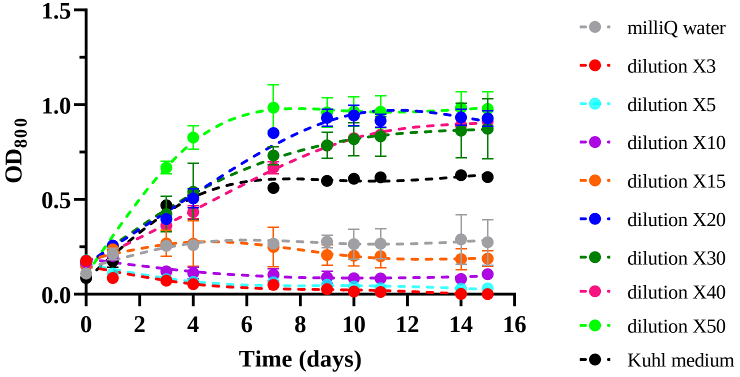
Figure 1. Illustration of algae growth trials performed in 96-well microplates (cultures for *Chloromonas typhos*, a; *Nannochloropsis Gaditana*, b; *Porphyridium purpureum*, c; and wild algae consortium, d).

Growth characteristics of microalgae under different dilution rates- freshwater algae

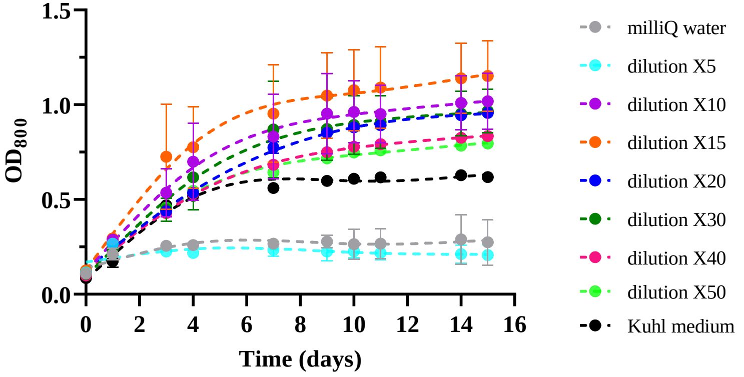
Chloromonas typhlos in Digestate 1



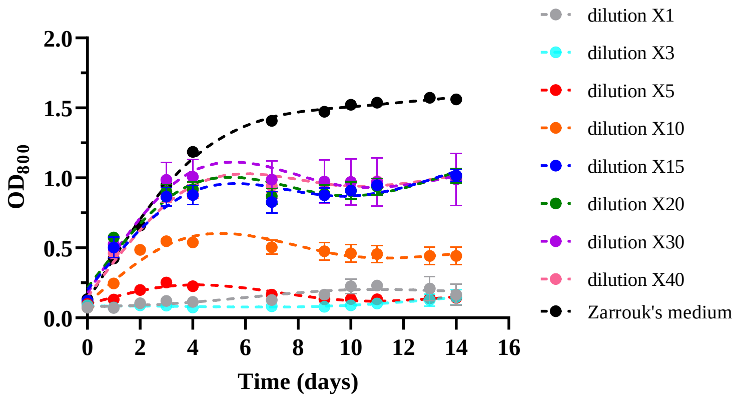
Chloromonas typhlos in Digestate 2



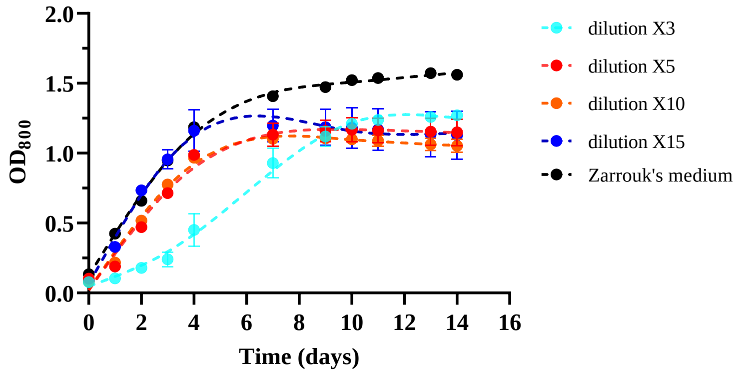
Chloromonas typhlos in Digestate 3



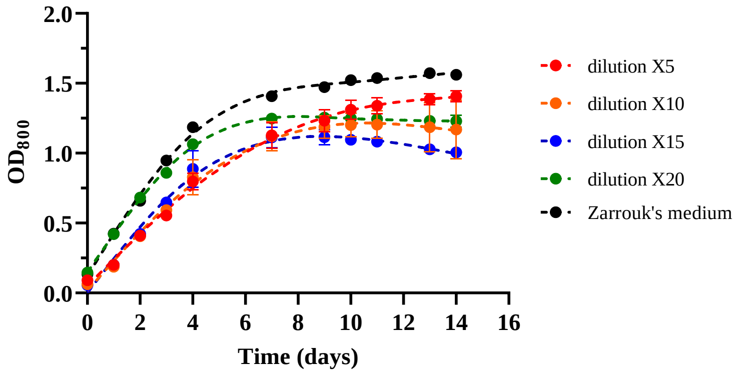
Picochlorum eukaryotum in digestate 1



Picochlorum eukaryotum in digestate 2



Picochlorum eukaryotum in digestate 3



Growth characteristics of microalgae under different dilution rates- freshwater algae

Table 3: Growth date of *Chloromonas Typhlos* in differerent digestates dilutions.

Dilution times	Digestate 1		Digestate 2		Digestate 3	
	Max OD_{800}	μ (d^{-1})	Max OD_{800}	μ (d^{-1})	Max OD_{800}	μ (d^{-1})
dilution X1	0.156	-				
dilution X3	0.145	-	0.177	-		
dilution X5	0.158	-	0.179	-	0.267	-
dilution X10	0.184	-	0.202	-	1.018	0.434
dilution X15	0.244	-	0.269	0.117	1.152	0.455
dilution X20	0.354	0.134	0.944	0.222	0.957	0.351
dilution X30	0.462	0.133	0.874	0.292	0.967	0.413
dilution X40	0.663	0.178	0.912	0.201	0.835	0.387
dilution X50	-	-	0.985	0.443	0.796	0.382
Kuhl medium	0.628	0.558				

Table 4: Growth date of *Picochlorum eukaryotum* in differerent digestates dilutions.

Dilution times	Digestate 1		Digestate 2		Digestate 3	
	Max OD_{800}	μ (d^{-1})	Max OD_{800}	μ (d^{-1})	Max OD_{800}	μ (d^{-1})
dilution X1	0.231	-	-	-	-	-
dilution X3	0.148	-	1.271	0.44	-	-
dilution X5	0.253	-	1.168	0.30	1.407	0.444
dilution X10	0.548	-	1.106	0.19	1.204	0.482
dilution X15	1.017	0.198	1.198	0.15	1.114	0.49
dilution X20	1.015	0.167	-	-	1.255	0.30
dilution X30	1.008	0.231	-	-	-	-
dilution X40	0.997	0.24	-	-	-	-
Zarrouk's medium	1.573	0.30				

Growth characteristics of microalgae under different dilution rates- marine algae

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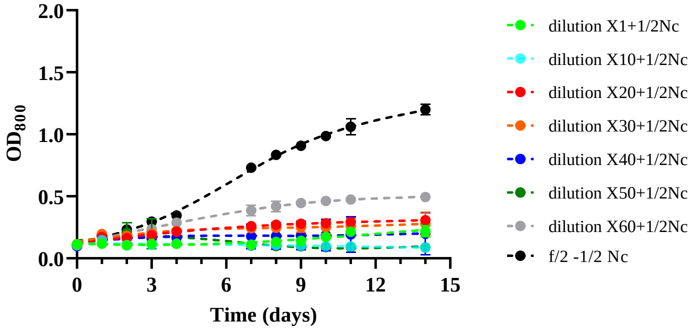
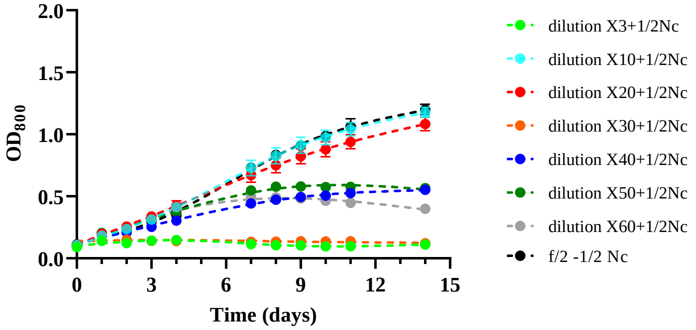
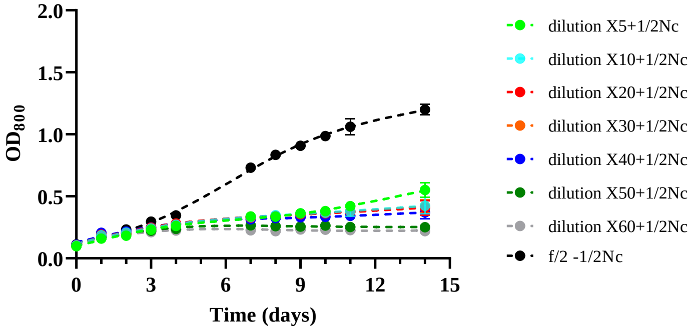
N. gaditana in Digestate 1 + 1/2 NaCl



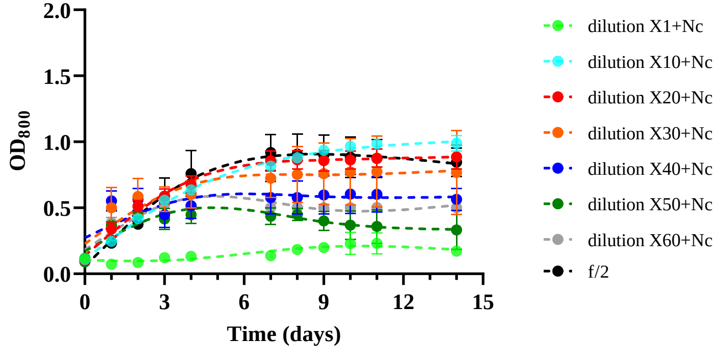
N. gaditana in Digestate 2 + 1/2 NaCl



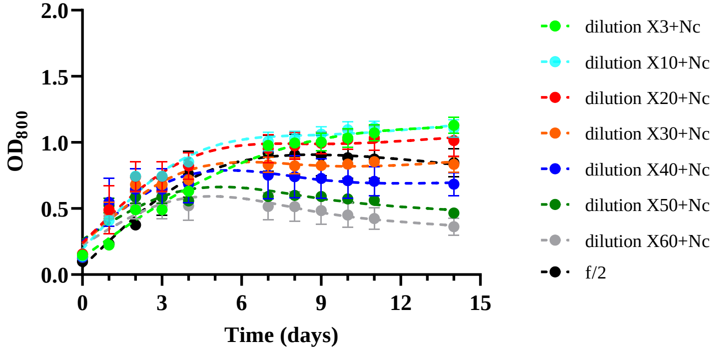
N. gaditana in Digestate 3 + 1/2 NaCl



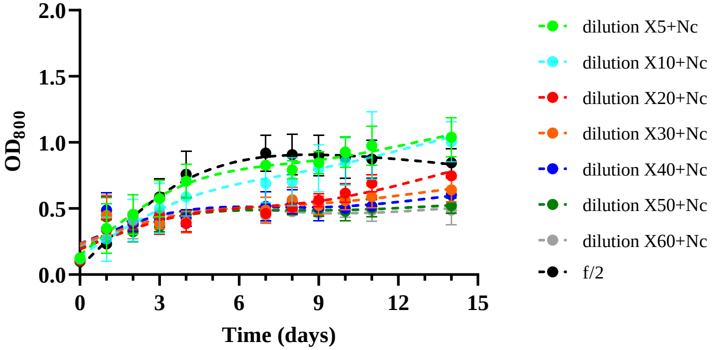
P. purpureum in Digestate 1



P. purpureum in Digestate 2



P. purpureum in Digestate 3



Growth characteristics of microalgae under different dilution rates- marine algae

Table 5: Growth date of *N. Gaditana* in differerent digestates dilutions.

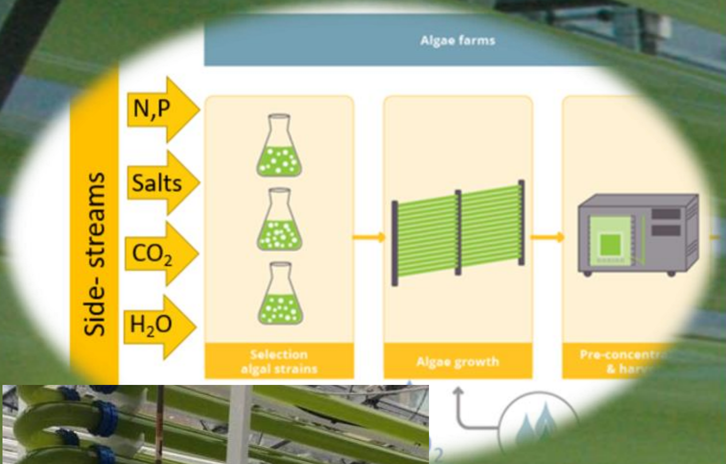
Dilution times	Digestate 1		Digestate 2		Digestate 3	
	Max OD_{800}	μ (d^{-1})	Max OD_{800}	μ (d^{-1})	Max OD_{800}	μ (d^{-1})
dilution X1	0.217	-	-	-	-	-
dilution X3	-	-	0.148	-	-	-
dilution X5	-	-	-	-	0.551	0.12
dilution X10	0.146	-	1.178	0.18	0.424	0.08
dilution X20	0.308	-	1.083	0.18	0.407	0.08
dilution X30	0.277	-	0.190	-	0.419	0.08
dilution X40	0.198	-	0.552	0.11	0.369	0.06
dilution X50	0.206	-	0.579	0.15	0.265	0.03
dilution X60	0.494	0.1	0.495	0.15	0.233	-
f/2 medium - 1/2Nacl	1.199	0.19				

Table 6: Growth date of *P. purpureum* in differerent digestates dilutions.

Dilution times	Digestate 1		Digestate 2		Digestate 3	
	Max OD_{800}	μ (d^{-1})	Max OD_{800}	μ (d^{-1})	Max OD_{800}	μ (d^{-1})
dilution X1	0.230	-	-	-	-	-
dilution X3	-	-	1.130	0.37	-	-
dilution X5	-	-	-	-	1.039	0.24
dilution X10	0.989	0.24	1.113	0.22	1.005	0.25
dilution X20	0.885	0.22	1.032	0.16	0.748	-
dilution X30	0.774	-	0.854	0.12	0.641	-
dilution X40	0.605	-	0.753	0.07	0.602	-
dilution X50	0.449	-	0.599	-	0.522	-
dilution X60	0.526	-	0.521	-	0.494	-
f/2 medium	0.920	0.24				

Conclusions

- 3 digestates could be used for algal growth, and 4 microalgae showed species-specific growth performances in digestates (even under the same dilutions);
- *C. typhlos* appears to be a low-ammonium tolerant strain, while the wastewater-born alga, *Picochlorum eukaryotum*, can grow in digestates with highest NH_4^+ concentration;
- High amount of nitrate present in digestate 3 (from HERBAUT) might alleviate the toxicity of excess NH_4^+ conditions to microalgae;
- Digestates could be adopted for marine algae cultivation, but extra salt should be added in the medium or these species could be cultured at sites situated near the sea.



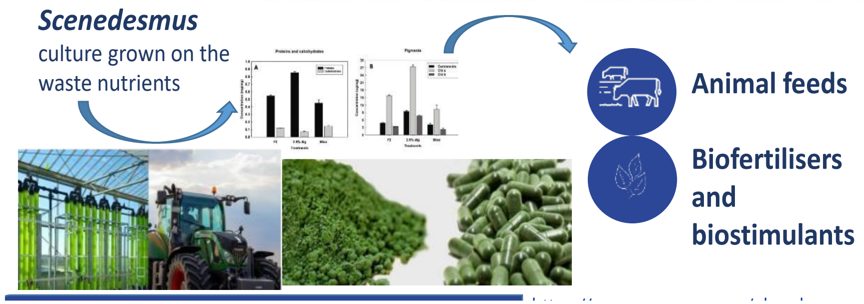

Pilot scale growth of *Scenedesmus* on pre-treated digestate

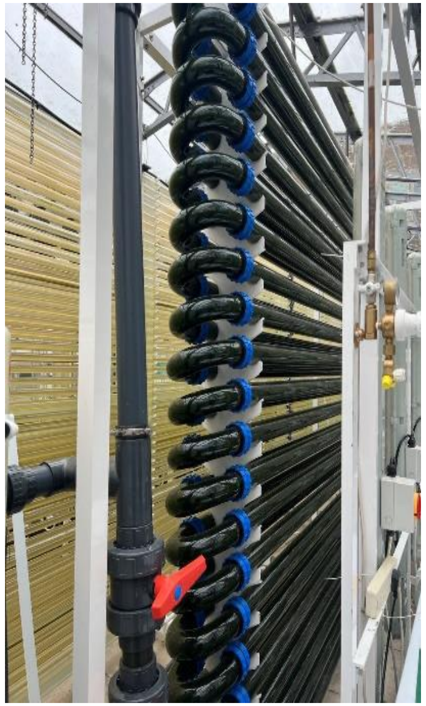
Final IDEA+ event, September 2023

Alla Silkina, Mohamed Emran, Fleuriane Fernandes – Swansea University (UK)

Leen Bastian, Quennie Simons Collaboration with VITO (Belgium)

Pilot scale growth





- Testing the waste remediation in small scale (1L) and cultivation in batch conditions

- *Scenedesmus obliquus* - is a model species
- Identification of optimum waste concentration for bioremediation and biomass production

- The most suitable digestate for PBR -1,000L cultivation- semi continuous
- Cultivation Janury–May 2023 in greenhouse PBR with natural light conditions
- Assessment of abiotic parameters: pH, T ºC and light
- Biotic parameters : cell density, dry weight, nutrients uptake

Initial *Scenedesmus obliquus* cultivation

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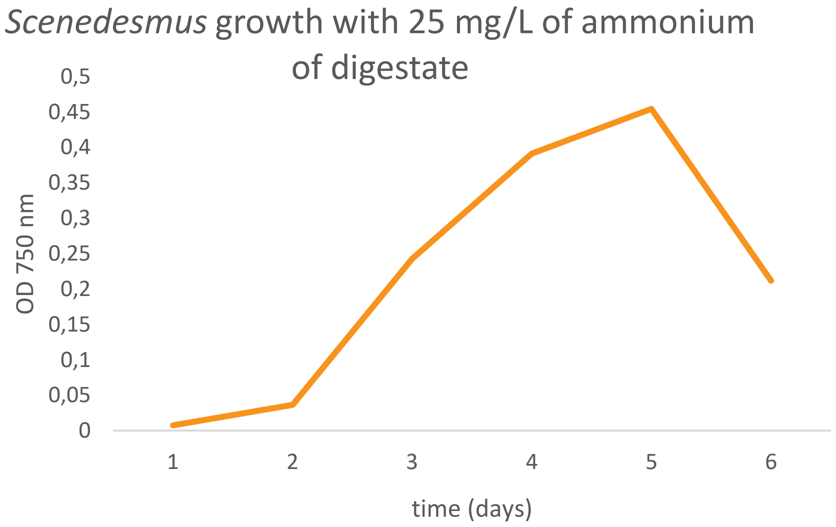
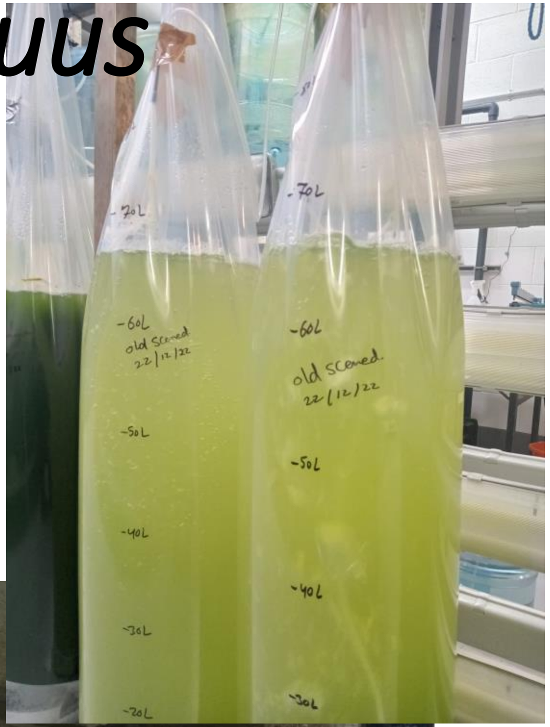
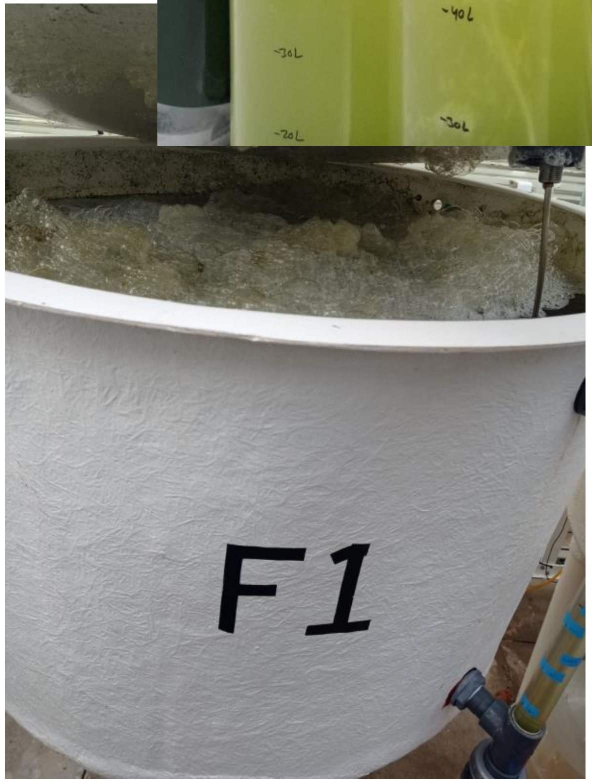
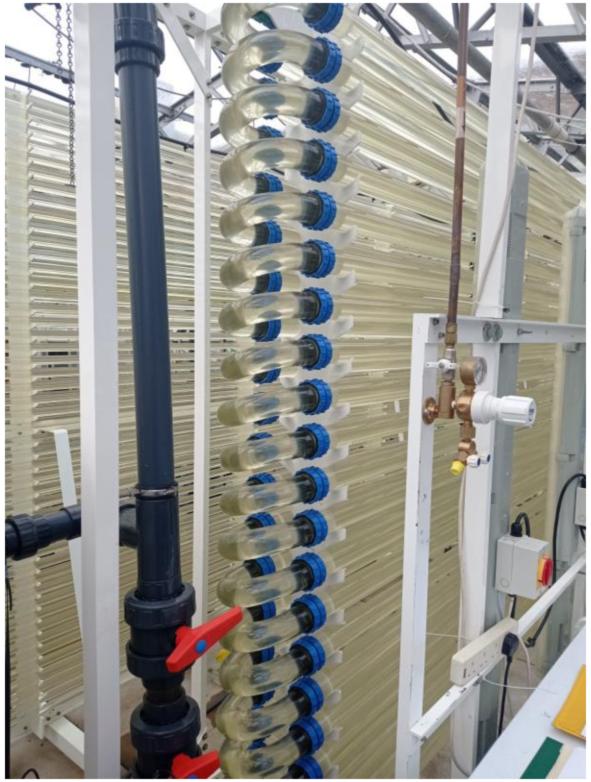
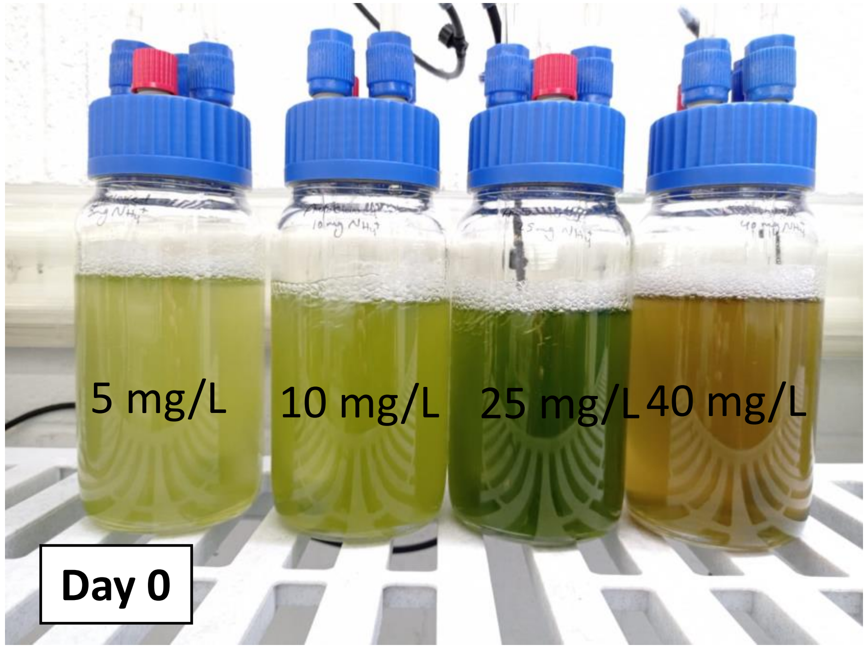


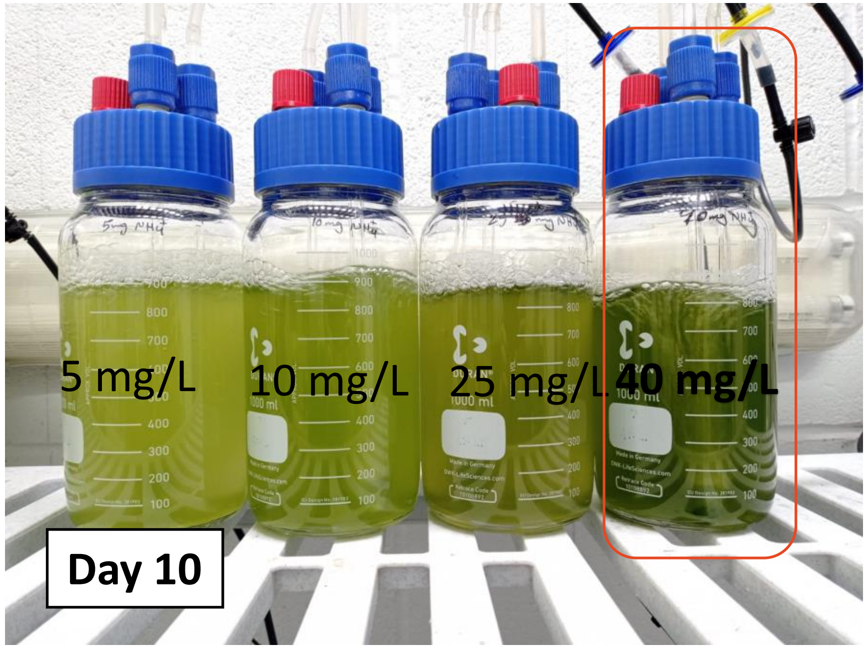






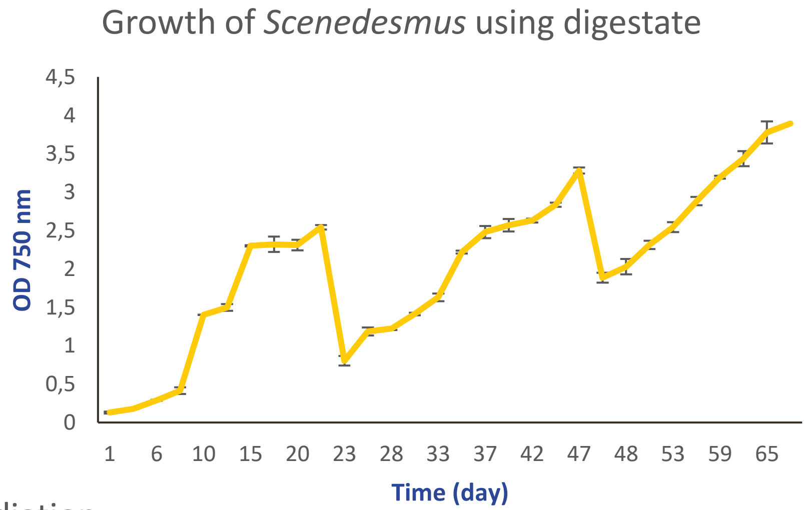
Back to the laboratory scale for the digestate growth and culture acclimation

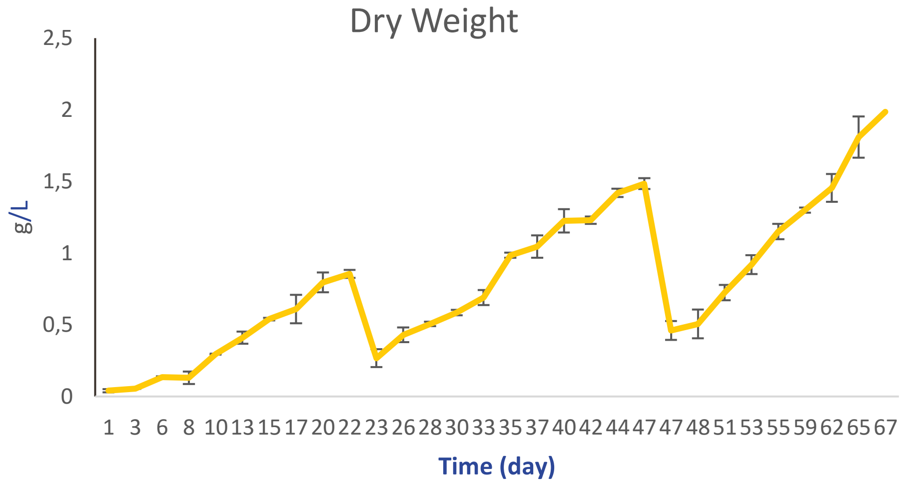




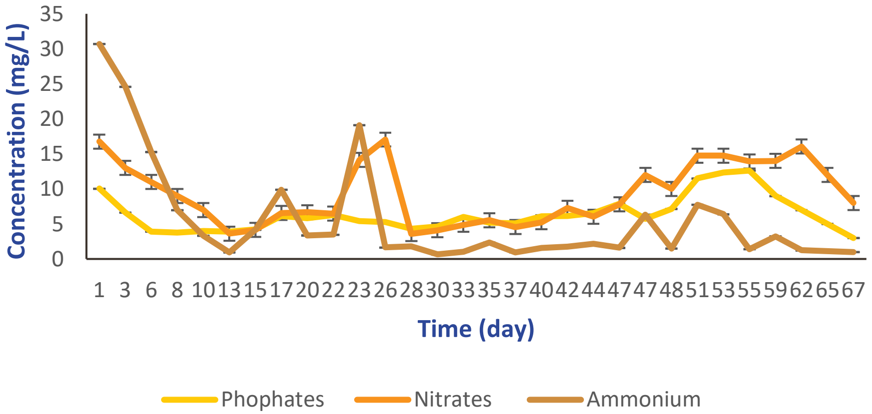
10 days of the *Scenedesmus obluquus* growth at Lab scale of 1 L of cultivation
Initial inoculum 10%

High scale remediation and culture growth





ents remediation



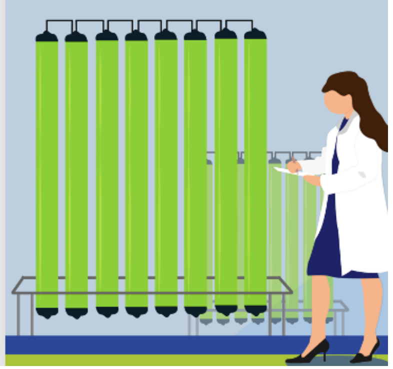
- Acclimation and efficient remediation of waste nutrients
- Using 30-40 mg/L of waste ammonium concentration (100L of non-dulited digestate was used)
- Production of 5 kg of dry weight of Scenedesmus biomass
- Data collection for modelling

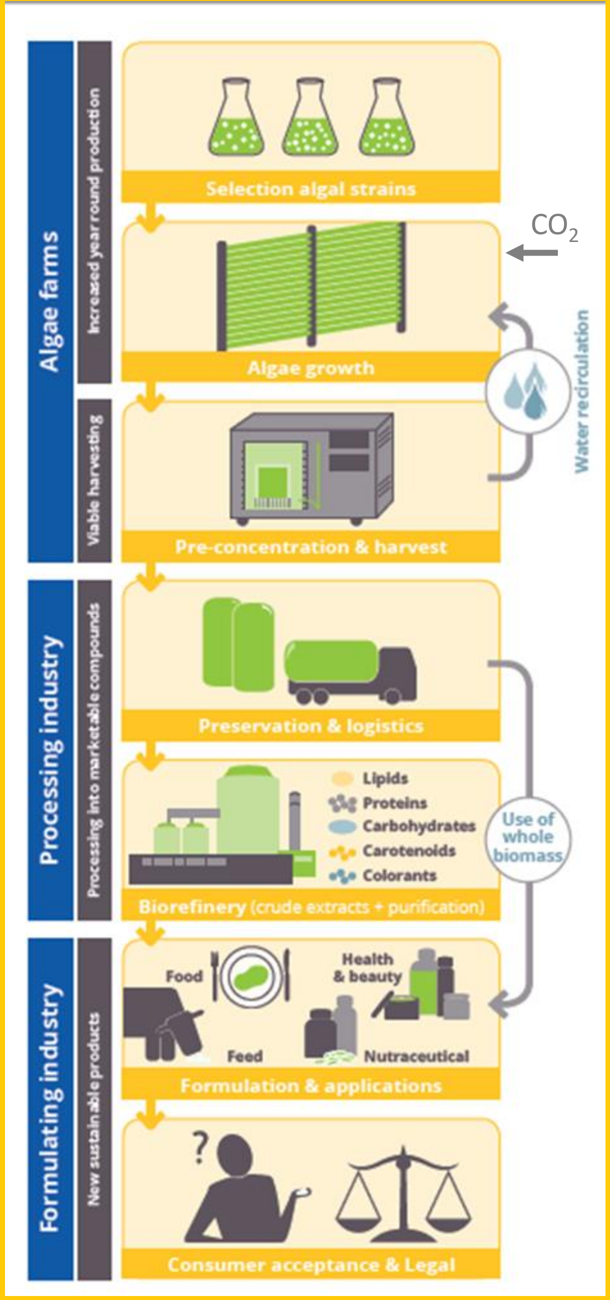
Biomass production

- Several batches of concentrated biomass was produced January-May 2023
- High viscosity of biomass
- High concentration of Exopolysacharides (EPS)

Best practice and takehome message

- **Measurements of N, P and C ; Turbidity, pH of digestate**
- **Pretreatment using low or/and medium/high-cost methods is essential,**
- **Right selection of algal species**
- **Testing at the lab scale and acclimation of the culture**
- **Gradual scale up and close monitoring of the growth parameters and nutrients uptake**
- **Longer duration and frequent supply of the waste nutrients give better results of biomass production**





Acknowledgements

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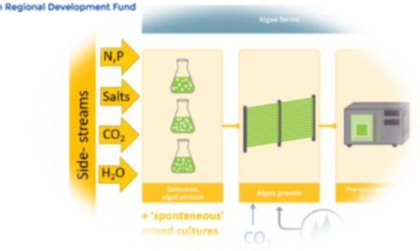

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Website: www.nweurope.eu/idea

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