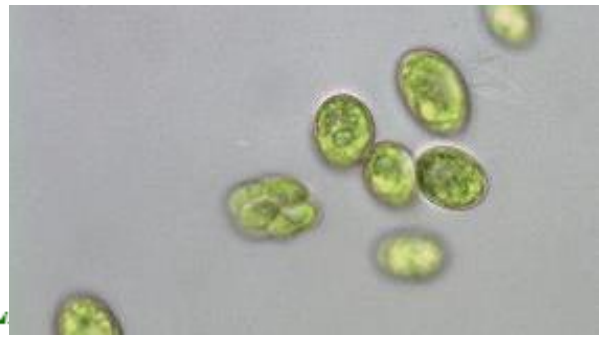
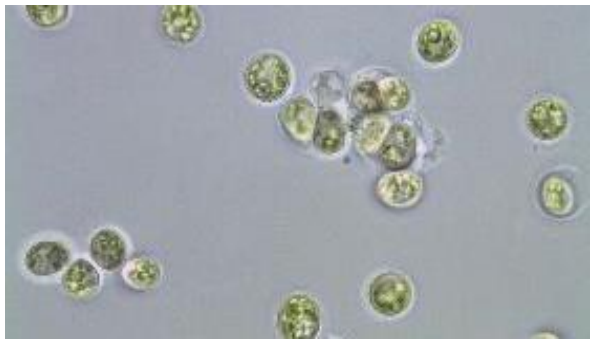
GRUPPO RICICLA
DIPARTIMENTO DI SCIENZE AGRARIE E AMBIENTALI
PRODUZIONE, TERRITORIO, AGROENERGIA

Integrated Algae bio refinery: nutrient and carbon recycling from waste

SU Min, PhD Student
Supervisor: Prof. Fabrizio Adani
GRUPPO RICICLA
DISAA - Università degli Studi di Milano

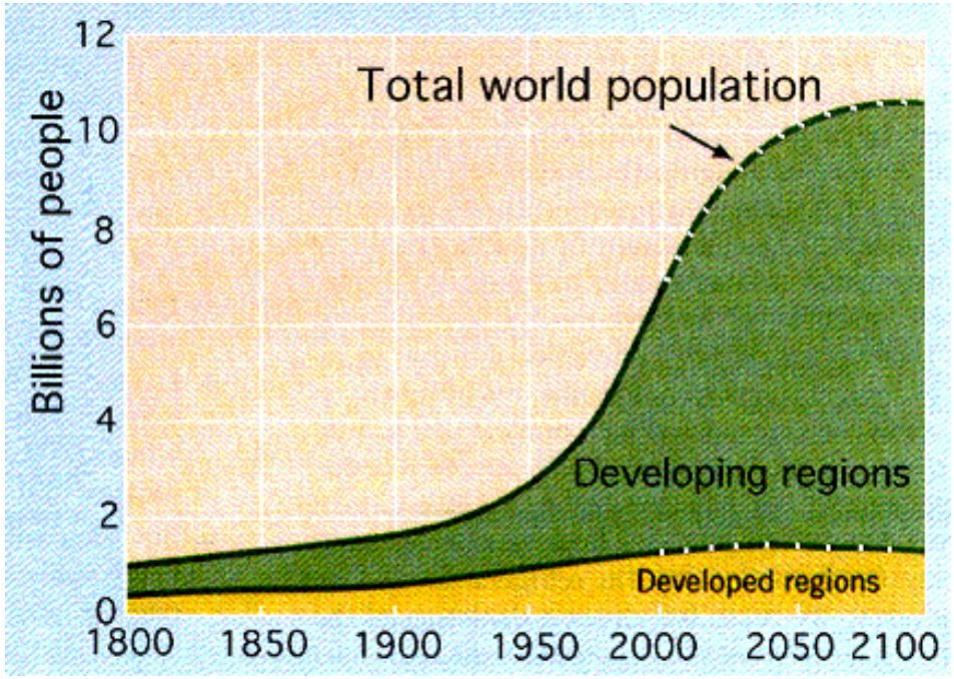




SABANA
Sustainable Algae Biorefinery for Agriculture aNd Aquaculture

Presentation Outline

1. Background
2. Project Concept Review
3. Research activities
4. Conclusion of current stage & Outlook of the project

1. Background



World Population

FAO: Agricultural consumption: 60% higher (in 40 years)

80% wastewater without treatment

Energy consumption(↑48%) in 2030

2.2 billion wasted/year

1. Background – Problem Posing

"We consume our planet's resources at a faster rate than their regeneration!"

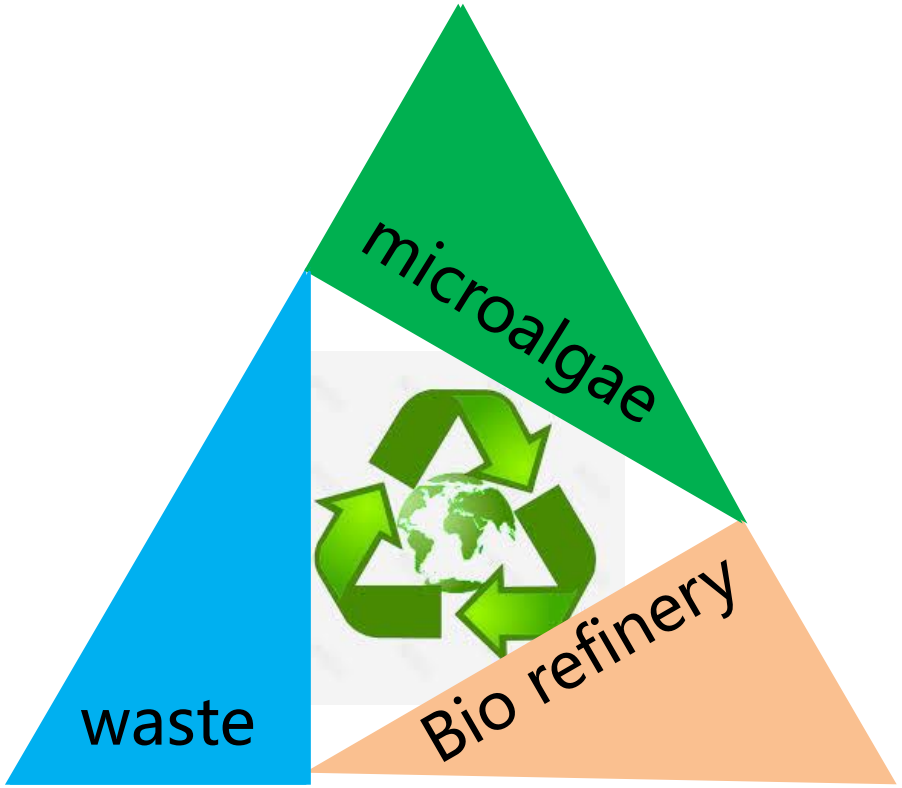
Sustainable Processing

↘

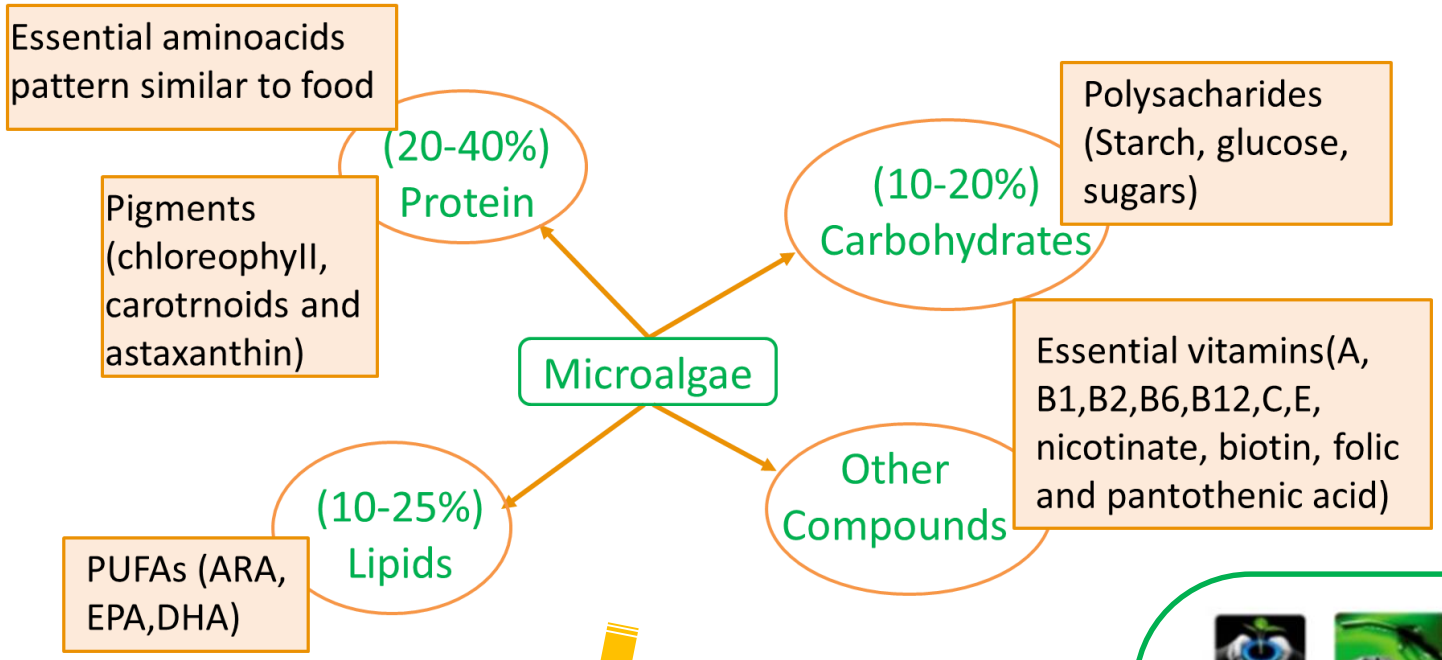
bio refinery

1) WHY BIOREFINERY?

- Sustainable Strategies

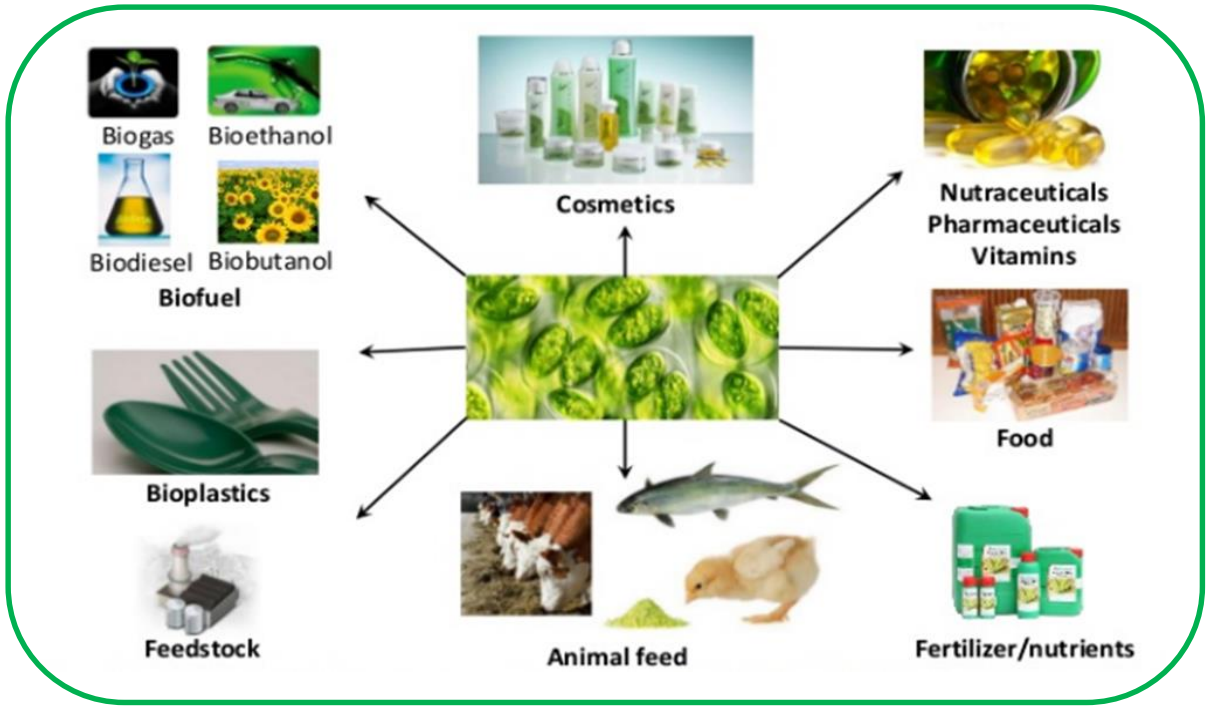


1. Background – Problem Posing

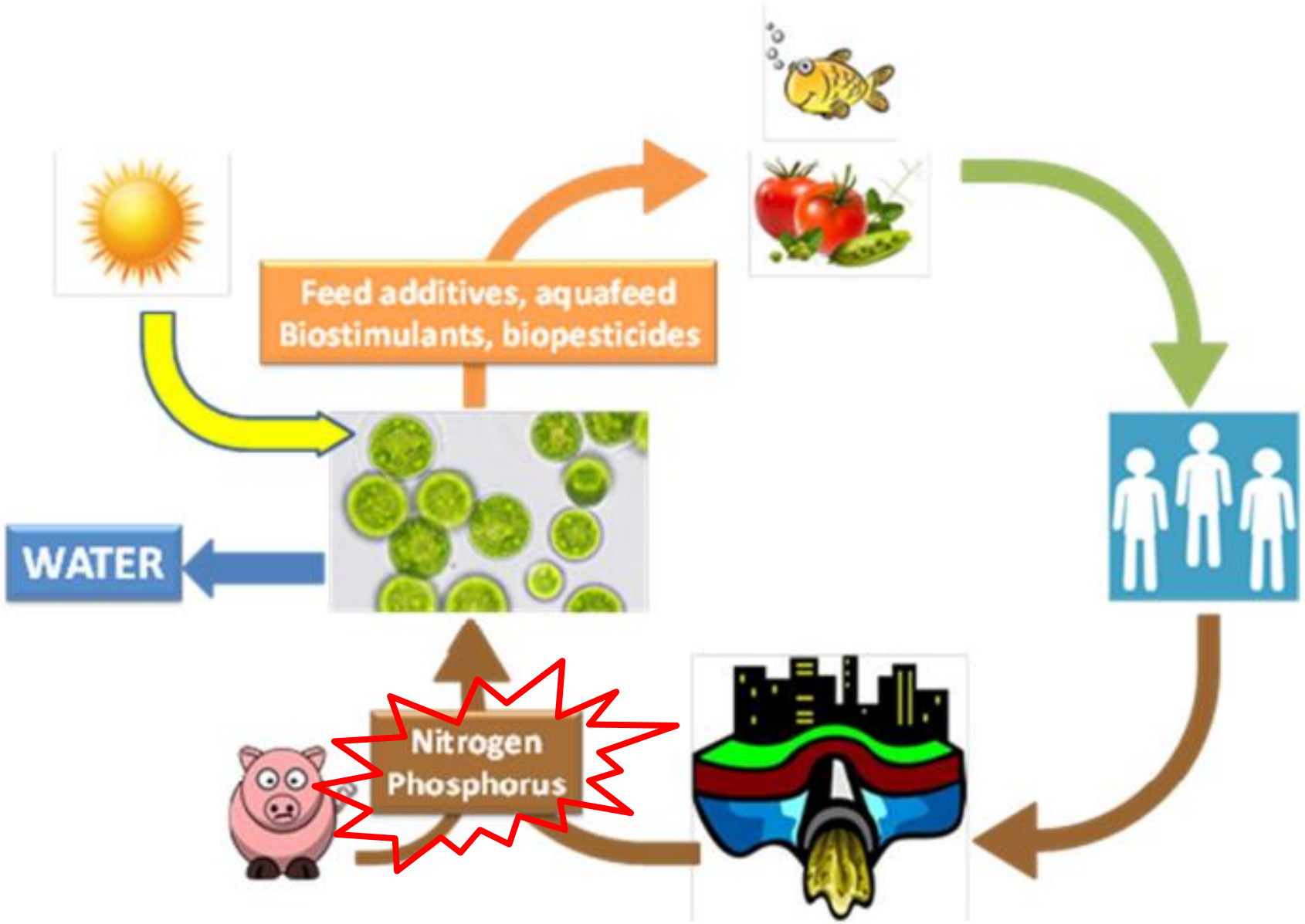


2) WHY MICROALGAE?

a variety of applications in different areas



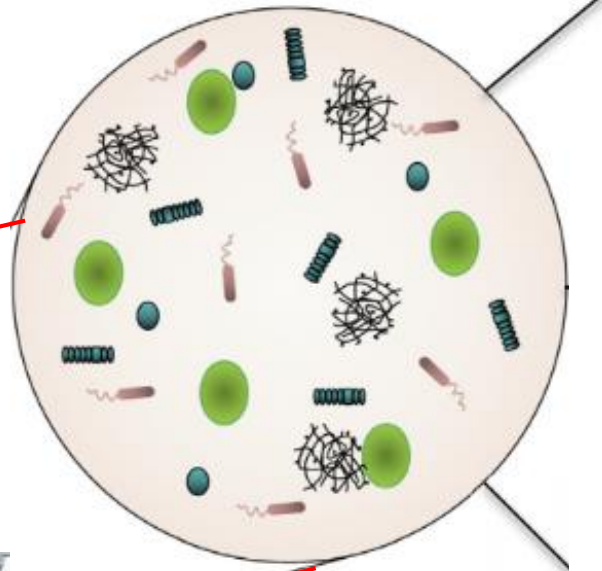
1. Background – Problem Posing

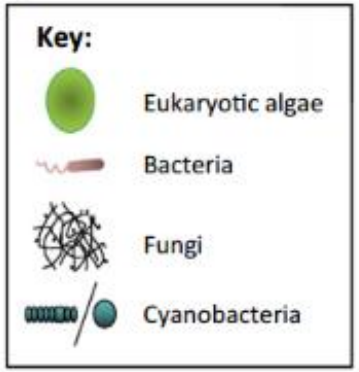


2. Concept review & Research activities

digestate

wastewater





Growing Stage

Metagenomics to identify microalgae strain

Microalgae species Isolation

characterize the promising strains

2. Concept review & Research activities

1. To grow microalgae in different wastes from factories/fields in Lombardy Region, Milan, Italy.
2. To analyze the metagenomics of the culture.
3. To identify and isolate promising microalgae species.
4. To produce biostimulants & evaluate biostimulant activity.

3. Microalgae cultivation Experiment

3.1 Raw Materials

3.2 Experiment Design

3.3 Results and Discussion

3.4 Conclusion

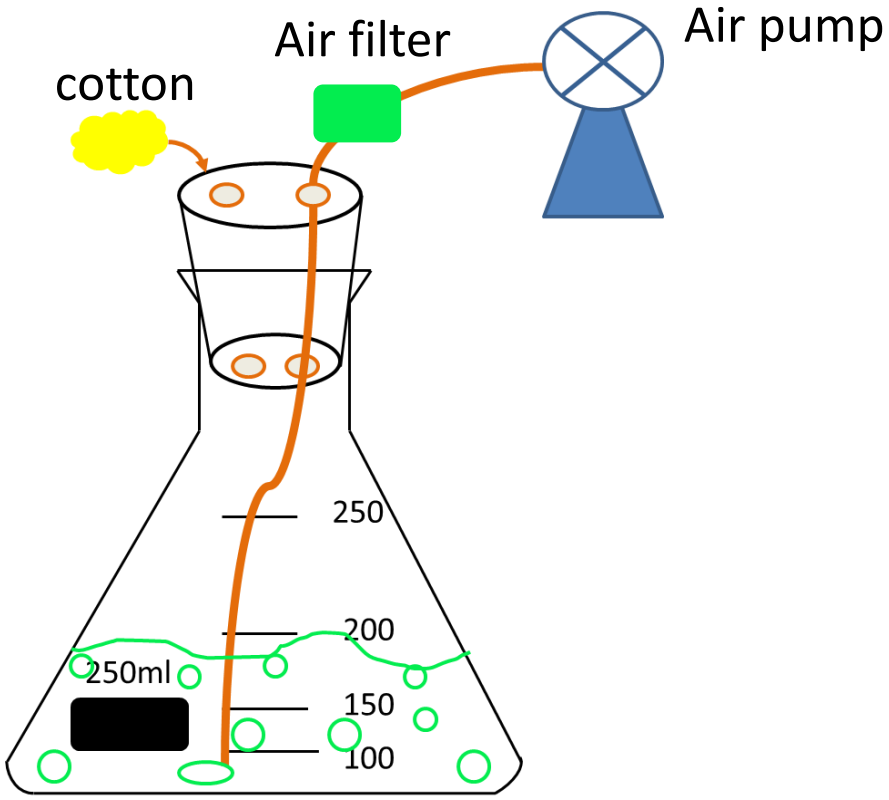
3.1 Materials

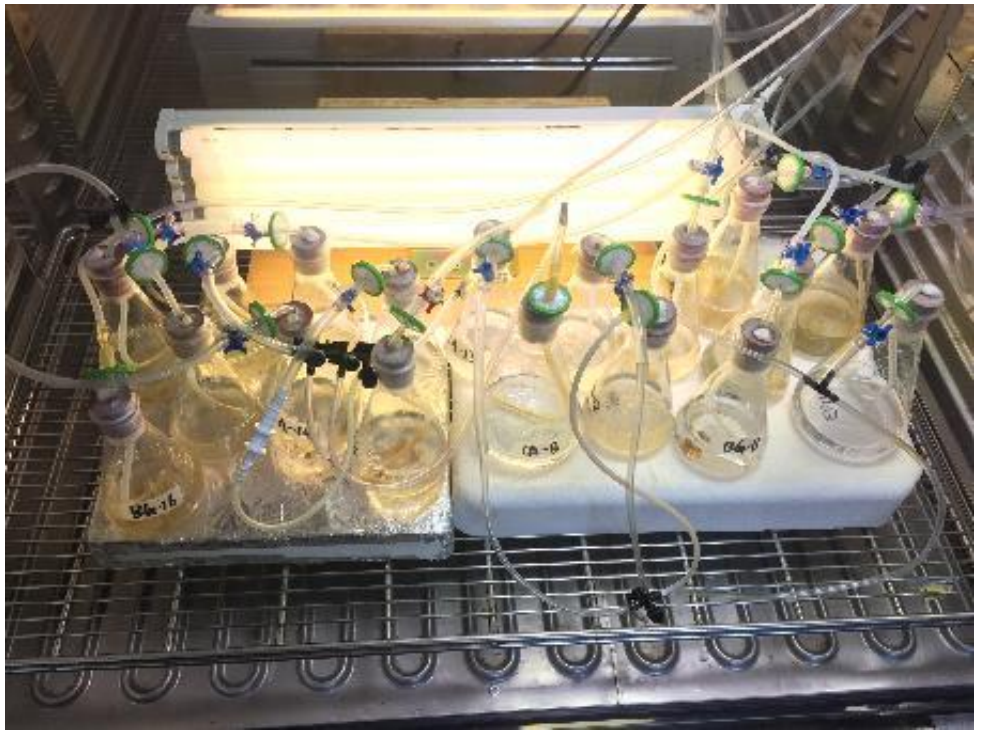
Table 3.1 Raw materials in this research

Number	Sample Name	Stage	Origin	Plant Name	Location
S1	Cow slurry	Input	Cattle stables	Romani	Mantua
S2	Liquid digestate	Output	Biogas plant	BLU Energy	Suzzara
S3	Solid digestate	Output	Biogas plant	BLU Energy	Suzzara
S4	Cow slurry	Input	Cattle stables	Zilocchi	Pegognaga
S5	Liquid digestate	Output	Biogas plant	Agrienergia	Pegognaga
S6	Solid digestate	Output	Biogas plant	Agrienergia	Pegognaga
S7	Cow slurry	Input	Cattle stables	Cervignano	Lodi
S8	Digestate	Output	Biogas plant	Ghiande	Pegognaga
S9	Wastewater	Output	Urban WW plants	cap holding	peschiera borromeo
S10	digestate	Output	AD plant	Lucra	Lodi
S11	OFMSW (liquid)	Input	AD plant	Lucra	Lodi
S12	OFMSW	Input	AD plant	Lucra	Lodi
S13	Sludge	Input	AD plant	Acqua&Sole	Vellezzo Bellini (PV)
S14	Digestate	Output	AD plant	Acqua&Sole	Vellezzo Bellini (PV)
S15	Digestate	Output	Cattle stables	Cervignano	Lodi
S16	Cattle manure	Input	Cattle stables	Cervignano	Lodi

*OFMSW: Organic Fraction of Municipal Solid Waste

3.2 Experiment Design





- Algae cultivation Experiment conditions
  - Continuous filtered air
  - Continuous illumination (25uEm^{-2}s^{-1})
  - 26° incubator

3.2 Experiment Design

Sample Number	Raw Materials	Experiment 1 (3 stages)		
		Stage 1	Stage 2	Stage 3
		original diluted	nitrate (20mg/l)	BG-11
S1	Cow Slurry			★
S2	Liquid Digestate of 1			
S3	Solid Digestate of 1			★
▲ **S4**	Cow Slurry	▲		
S5	Liquid Digestate of 4			★
S6	Solid Digestate of 4			★
S7	Cow Slurry			
S8	Digestate			★
▲ **S9**	Wastewater	▲		
S10	Digestate of 12		★	
S11	Liquid fraction of 12		★	
S12	OFMSW			
▲**S13**	Ingestate Sludge	▲		
S14	Digestate of 13			
S15	Input of 8			
▲**S16**	Cattle Manure	▲		

***Colored cells means microalgae grew**

Results:

In experiment 1, 11 samples grew microalgae.

3.2 Experiment Design

sample	Raw Materials	Experiment 1			**Experiment 2**
		original diluted	nitrate (20mg/l)	BG-11	**BG-11**
S1	Cow Slurry				
S2	Liquid Digestate of 1				
S3	Solid Digestate of 1				
▲ **S4**	Cow Slurry	▲			▲
S5	Liquid Digestate of 4				
S6	Solid Digestate of 4				
S7	Cow Slurry				
S8	Digestate				
▲ **S9**	Wastewater	▲			▲
S10	Digestate of 12				
S11	Liquid fraction of 12				
S12	OFMSW				
▲ **S13**	Ingestate Sludge	▲			▲
S14	Digestate of 13				
S15	Input of 8				
▲ **S16**	Cattle Manure	▲			▲

WHY?

The cultivation period

3.2 Experiment Design

Methodology for growing microalgae from waste need to be set up!

3.2.1 Cultivation period

3.2.2 Nutrient solution selection

3.2.3 Key Parameter (Culture Optical Density) Unify

3.2.2. Nutrient solution selection

Table 3.1 Nutrient Receipts for culturing microalgae

Solution Name	suitable for	Receipt characterater	pH	reference
Allen's Blue-Green Algae (BG-11)	widely used for freshwater green algae and cyanobacteria	nitrate and phosphate levels are exceptionally high	7.4	Allen 1968, Allen and Stanier 1968, Rippka et al.1979
Bold's Basal (BBM)	widely used for many kinds of freshwater algae	unsuitable for algae with vitamin requirements	6.6	Bold 1949, Bischoff and Bold 1963
CA Medium, modified	widely used for culturing oligotrophic desmids	contains both KNO_3 and NH_4NO_3 as nitrogen sources	7.2	Ichimura and Watanabe 1974, Watanabe et al. 2000

- 3 nutrient solutions among total 27 were chosen (R. Anderson, 2017)

3.2.3. Key parameter Unify

Table 3.2 Optical Density value of different dilution rates (OD)

Sample number	Dilution Rate								
	Original	10.0%	5.0%	2.0%	1.0%	0.8%	0.4%	0.2%	0.1%
S1	OR	OR	OR	2.24	1.17	0.40	0.19	0.09	0.05
S2	OR	OR	1.54	0.64	0.33	0.07	0.03	0.01	0.01
S3	Solid	OR	OR	2.03	1.15	0.32	0.12	0.07	0.03
S4	OR	1.9	0.96	0.39	0.2	0.05	0.02	0.01	0.01
S5	OR	OR	OR	2.41	1.32	0.43	0.22	0.11	0.06
S6	Solid	OR	OR	2	1.09	0.29	0.08	0.06	0.03
S7	OR	OR	OR	1.19	0.64	0.28	0.13	0.11	0.05
S8	OR	OR	OR	2	1.04	0.54	0.25	0.12	0.06
S9	OR	2.14	1.13	0.4	0.2	0.10	0.05	0.04	0.01
S10	OR	OR	1.39	0.5	0.29	0.08	0.10	0.02	0.01
S11	OR	OR	1.72	0.59	0.38	0.09	0.04	0.03	0.01
S12	OR	OR	OR	1.97	0.93	0.46	0.22	0.11	0.08
S13	OR	OR	OR	OR	1.47	0.88	0.42	0.22	0.11
S14	OR	OR	OR	OR	1.38	0.73	0.35	0.17	0.09
S15	OR	OR	OR	1.53	0.88	0.37	0.18	0.08	0.06
S16	OR	OR	OR	OR	1.68	0.67	0.32	0.17	0.08

OR: Over Ranged

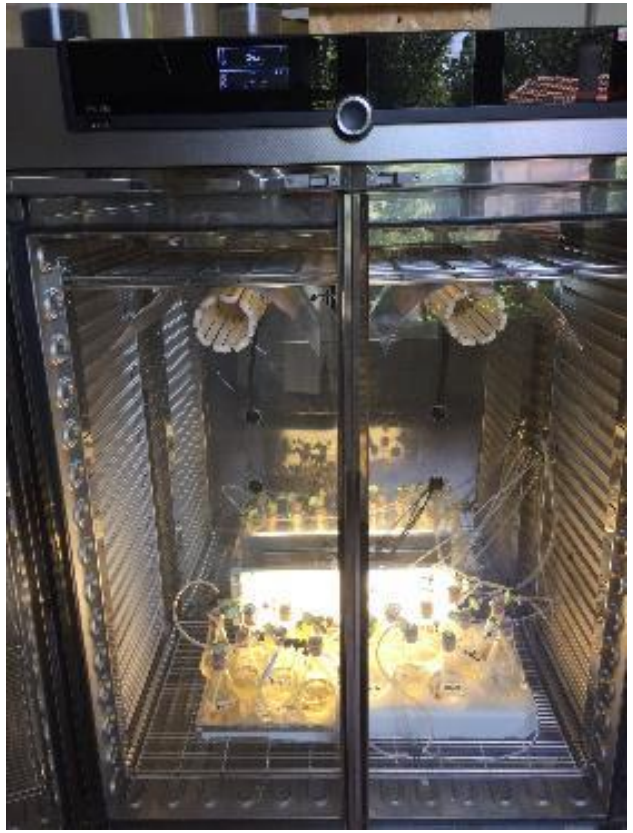
1) 0.1%-0.8% of dilution rate is suggested (OD ranges from 0.01 to 0.88)

2) In principle, the lower OD of microalgae cultivation, the better

4. Experiment Design

Sample numbers for lab incubator:

5 samples * 3 solutions + 3 blanks =18 flasks



- In total for one cycle, 16 samples need 6 months

3.2 Experiment Design

sample	details	Experiment 3	Experiment 4
		Unified OD=0.1	Unified OD=0.3
		BG-11	BG-11
S1	Cow Slurry		
S2	Liquid Digestate of 1		
S3	Solid Digestate of 1		
S4	Cow Slurry		
S5	Liquid Digestate of 4		
S6	Solid Digestate of 4		
S7	Cow Slurry		
S8	Digestate		
S9	Wastewater		
S10	Digestate of 12		
S11	Liquid fraction of 12		
S12	OFMSW		
S13	Ingestate Sludge		
S14	Digestate of 13		
S15	Input of 8		
S16	Cattle Manure		

Results：

In experiment 4 (OD 0.3), 11 samples grew microalgae.

3.2 Experiment Design

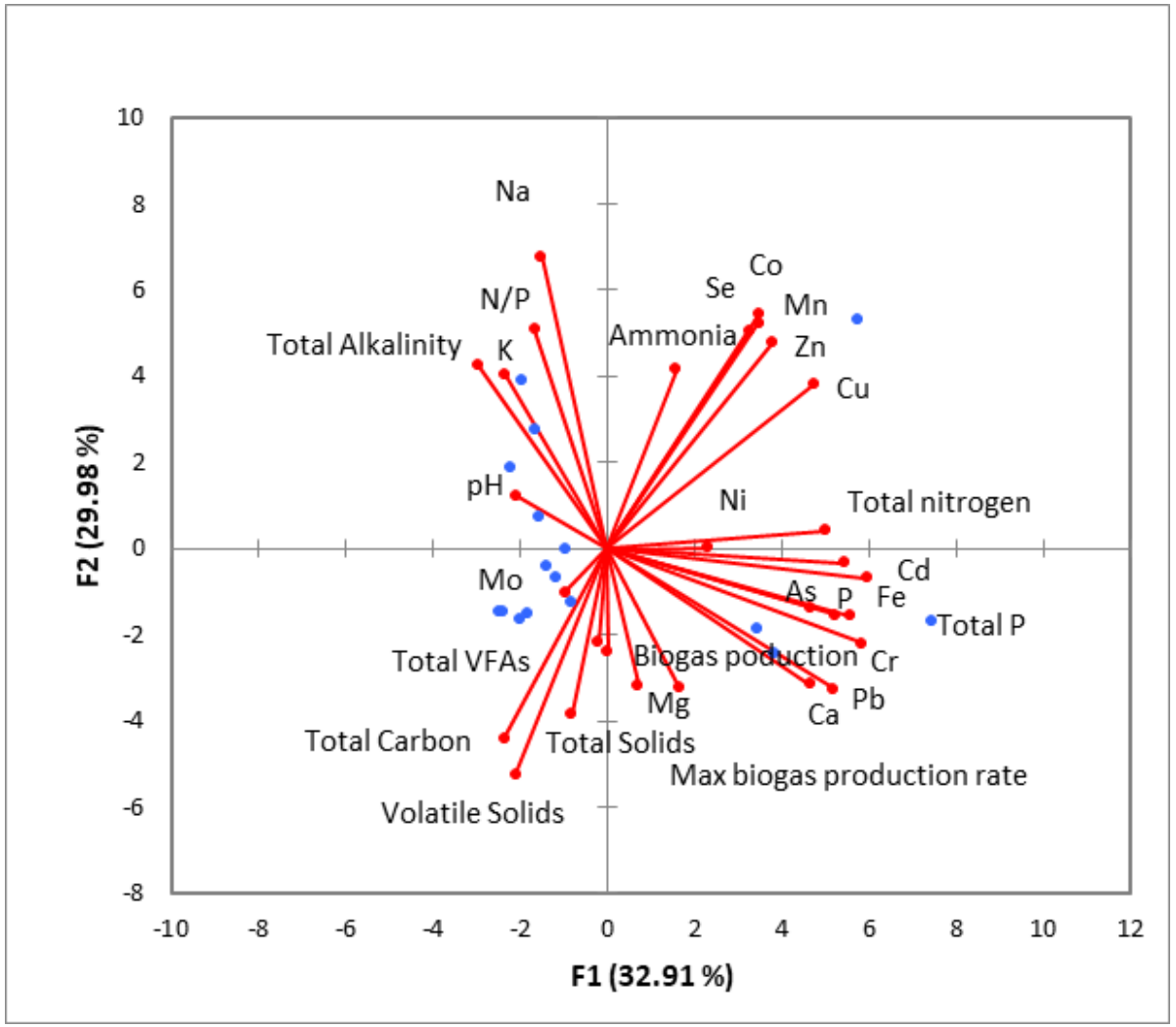
sample	details	Experiment 1 (3 stages)			Experiment 2	Experiment 3	Experiment 4
		(g TS/200ml)			(g TS/200ml)	OD=0.1 (g TS/200ml)	OD=0.3 (g TS/200ml)
		original diluted	nitrate 20mg/l	BG-11	only BG-11	BG-11; BBM; CA	BG-11; BBM; CA
S1	Cow Slurry	0.25	0.25	0.25	0.25	0.03	0.1
S2	Liquid Digestate of 1	0.05	0.05	0.05	0.05	0.05	0.15
S3	Solid Digestate of 1	0.19	0.19	0.19	0.19	0.14	0.37
S4	Cow Slurry	0.05	0.05	0.05	0.05	0.05	0.15
S5	Liquid Digestate of 4	0.13	0.13	0.13	0.13	0.02	0.05
S6	Solid Digestate of 4	0.21	0.21	0.21	0.21	0.17	0.45
S7	Cow Slurry	0.21	0.21	0.21	0.21	0.03	0.12
S8	Digestate	0.18	0.18	0.18	0.18	0.02	0.06
S9	Wastewater	0.10	0.10	0.10	0.10	0.03	0.09
S10	Digestate of 12	0.06	0.06	0.06	0.06	0.03	0.11
S11	Liquid fraction of 12	0.05	0.05	0.05	0.05	0.03	0.10
S12	OFMSW	0.28	0.28	0.28	0.28	0.03	0.09
S13	Ingestate Sludge	0.38	0.38	0.38	0.38	0.02	0.07
S14	Digestate of 13	0.31	0.31	0.31	0.31	0.02	0.07
S15	Input of 8	0.11	0.11	0.11	0.11	0.02	0.05
S16	Cattle Manure	0.45	0.45	0.45	0.45	0.04	0.12

3.3 Results and Discussion

1) Methodology had set up for growing microalgae from waste:

- ✓ BG-11 nutrient solution
- ✓ Optical density at 0.3
- ✓ At least 6 months for 16 samples cultivatuon

3.3 Results and Discussion



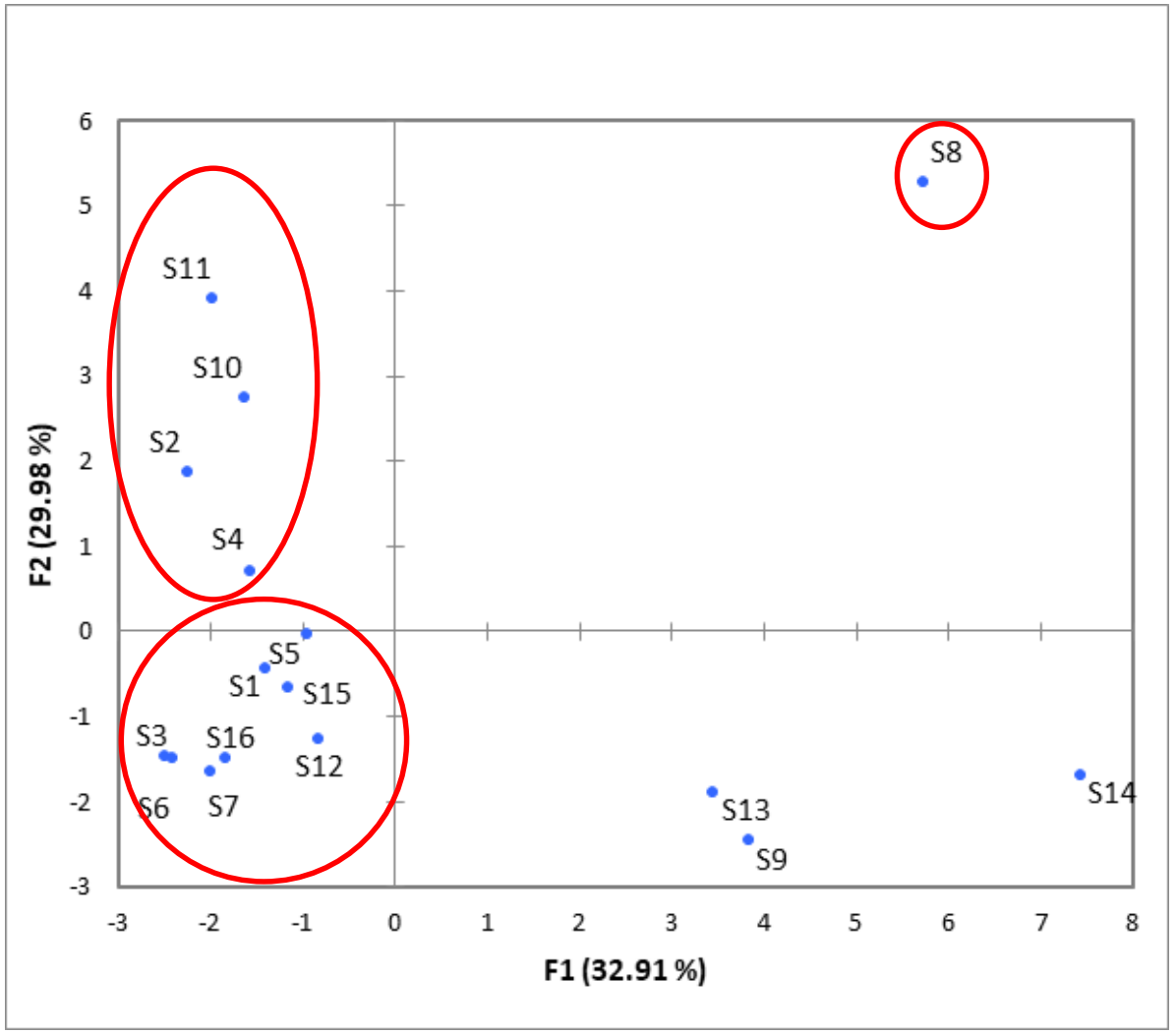
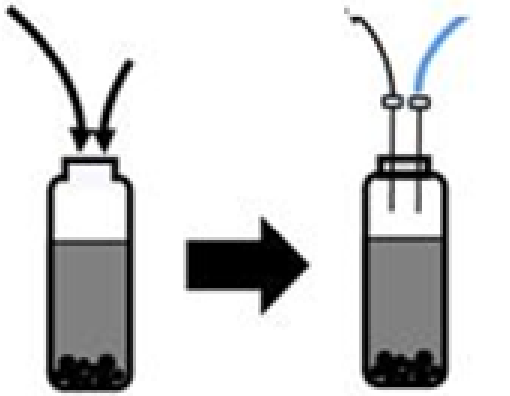


Figure 3.3. Principle Component Analysis of all characterization of raw materials

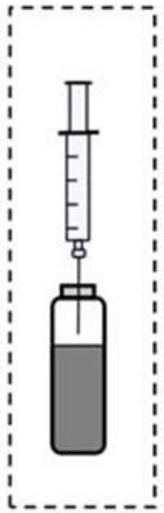
3.3 Results and Discussion

Reactivated inoculated sludge 60ml
Dried samples 0.6g

N_2



Biogas production



Experiment Conditions:

- 90 days in 37°
- Batch mode
- 3 models for analyzing the biogas production potential

1. Modified Gomperz Model

$$P_{(t)} = P_0 \exp\left\{-\exp\left[\frac{R_{max}e}{P_0}(\lambda - t) + 1\right]\right\}$$

2. First-order Model

$$\ln\left(\frac{Cs_0}{Cs}\right) = kt$$

3. Two-phases Model

$$Y = Y_{max}[1 - Pe^{-k_1 t} - (1-P)\, e^{-k_2 t}]$$

Concept review & Research activities

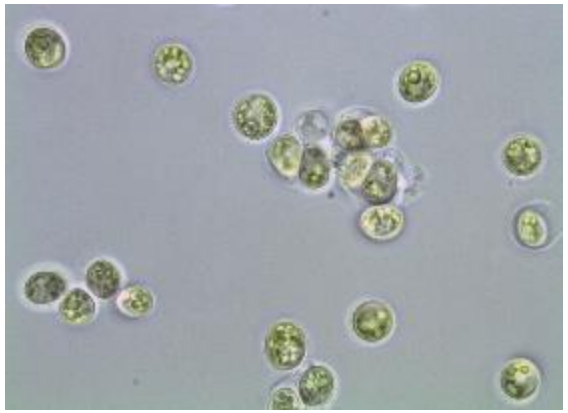
1. To grow microalgae in different wastes from factories/fields in Lombardy Region, Milan, Italy.
2. To analyze the metagenomics of the culture.

✓ DNA was successfully isolated using the “Dneasy PowerSoil Kit” for further metagenomics analysis.

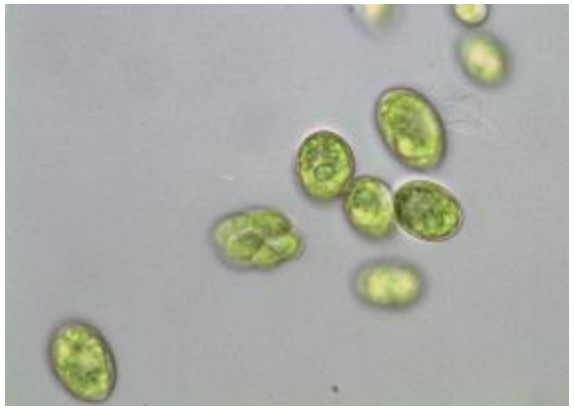
3. To identify and isolate promising microalgae species.
4. To produce biostimulants & evaluate biostimulant activity.

4. Experiment Design

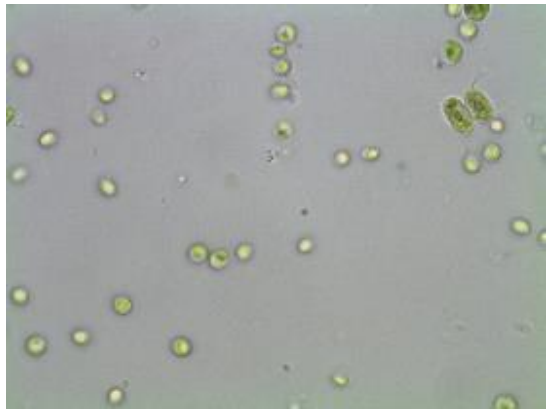
- First evaluation by flasks color change & microscope observation



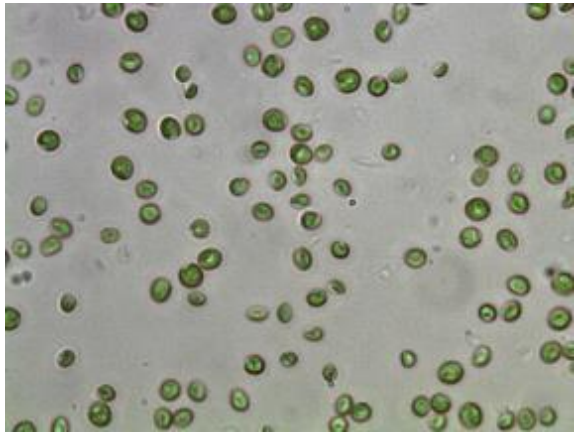
BG-S4(100*)



BG-S8(100*)

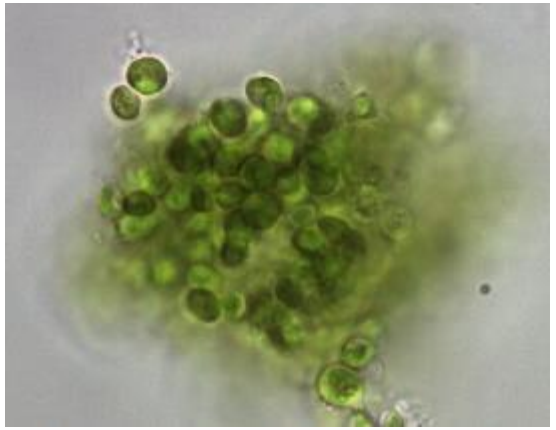


BG-S9(100*)



BG-S10(100*)

BG-S11(100*)



BBM-S9(100*)

4. Conclusion and Outlook

1. Microalgae cultivation methodology (from waste) was investigated.
   At least 2 months & BG-11 & OD 0.3

2. Chemical and biological characterization were analyzed to distinguish the similarity and differences from each waste.

3. After metagenomics, all the characterization analysis results will present clearer according to different microalgae species.

Thank you for attention !

GRUPPO RICICLA

web site: http://users.unimi.it/ricicla/

When you make almost as much as oxygen as trees but nobody shows love to you