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Phycoremediation of Olive Mill Wastewater (OMW) Using Cyanobacteria for Sustainable Biofertilizer and Biofuel Production

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Agenda

- **Introduction**
- **Objective**
- **Materials**
- **Methodology**
- **Results**
- **Concluding remarks**

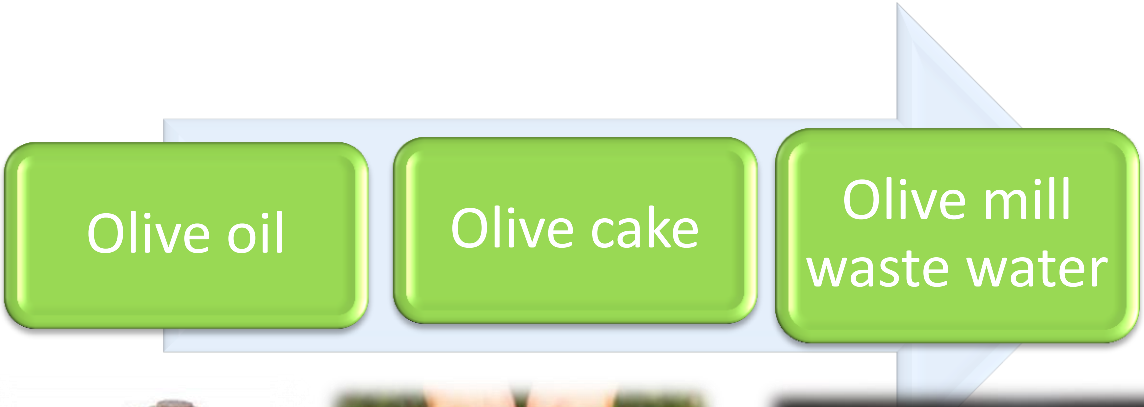
Introduction

- Demand for olive oil is rapidly expanding worldwide due to its healthy image.
- More than 97% of the world production of olive oil is concentrated in the Mediterranean basin.
- Olive oil mill wastewaters (OMW) generated in huge quantities over a short period every year (November - April)

- **In Mediterranean countries, Several hundred thousands cubic meters of untreated OMW is discharged into rivers, open areas and cesspools and represent significant environmental problems**

Olive Oil Production

- **Olive oil production unfortunately, leads to the generation of significant amounts of both solid and liquid wastes.**



Olive mill wastewater "OMW"

- **Olive mill wastewater (OMW), known as alpechin, disposal may cause adverse effects on soils, surface- and ground waters due to organic compounds content (organic acids, lipids, alcohols and polyphenols) which are considered as phytotoxic.**

- **One ton of olives approximately produces 0.8 ton of OMW which are characterized as acidic (pH 4-5), with an average COD content of 80 g/L, high concentrations of suspended solids (7–15 g/L) and phenolic compounds (2–10 g/L).**

Olive mill wastewater “OMW”

Advantages	Disadvantages
• OMW direct disposal in soil can be used as a soil conditioner/ fertilizers amendment and proposed as one of the most suitable methods to restore soil fertility . • OMW has the potential to increase organic matter contents of soils (contains more than 94% organic matter) and other nutrients.	• The continuous disposal of OMW in soil without any treatment might causes higher levels of soil salinity • Unfavorable impact on soil microbial population/ctivity, plants, aquatic ecosystems and even in air media because of its high content s of fatty acid, phenolic compounds, mineral salts, COD and BOD.
• The disposal of this waste in rivers increases the organic matter and K, Fe, Zn and Mn contents.	• The disposal of this waste in rivers decreases the dissolved oxygen.
• This wastewater contains valuable resources of fatty acids, sugars, phosphates, organic matter and a wide range of nutrients that could be recycled	• A limiting factor to the biodegradation of OMW is its high content of phenolic antimicrobial and phytotoxic compounds

What the problem?

OMW is difficult to treat by common biological processes due to its high COD, low pH and the presence of organic compounds (phenols, polyphenols and polyalcohols) with low biodegradability and high toxicity, which may explain the lack of a well-established technology for their treatment.

- Phycoremediation of OMW into valuable products via microalgae cultivation and formulate liquid bio-organic fertilizer.

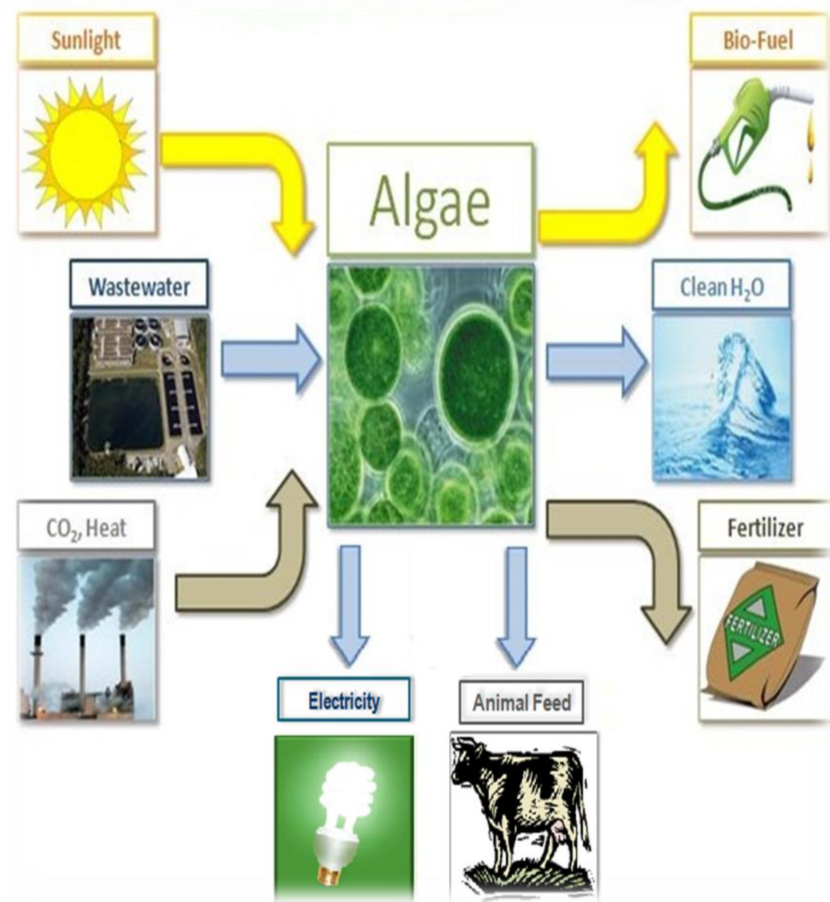
- Highlight the optimum bioreactors design for biomass production and its utilization in biofuel industry.

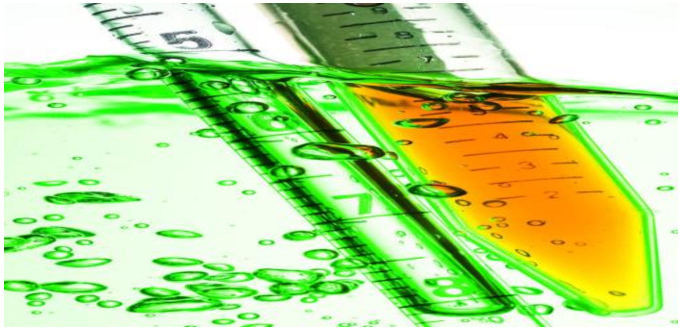
Phycoremediation

Phycoremediation is the process of employing algae for the removal or biotransformation of pollutants, including nutrients and xenobiotics from wastewater and CO_2 from waste air with concomitant biomass propagation.

Why Algae?

- Cost- effective, ecofriendly and a safe process.
- High nutrient value therefore, can be suitable as a live feed for aquaculture
- Removal waste CO_2 due to photosynthetic fixation (carbon credits).
- Useful bio-chemicals (nutraceutical, pharmaceutical, bio-fertilizer and bio-fuel) from the algal Biomass.





Materials

1)Blue Green Algae

2) Olive Mill Wastewater (OMW)

Methanol (99.8% purity) as a reacting alcohol and concentrated sulfuric acid (H_2SO_4 conc.) as a biodiesel transesterification catalyst are supplied from El-Nasr Pharmaceutical Chemicals Company (ADWIC), Egypt.

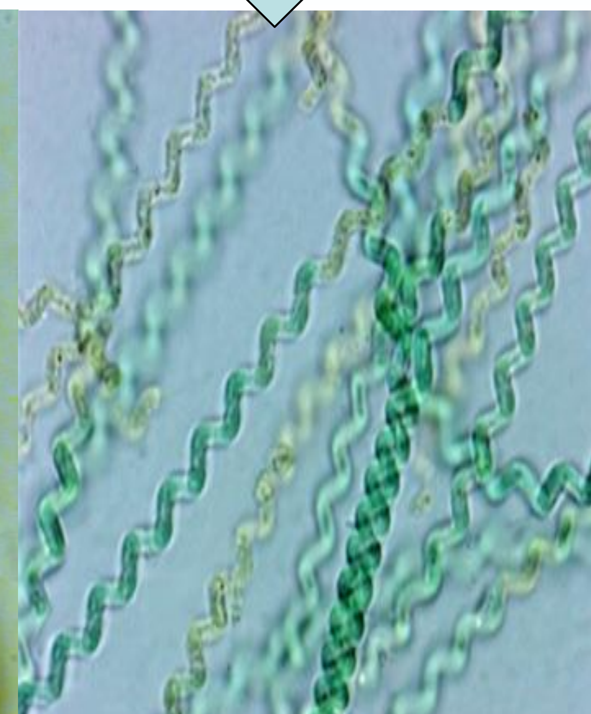
Blue Green Algae (Cyanobacteria)

Three cyanobacteria strains were kindly supplied from Department of Microbiology; Soils, Water and Environment Research Institute (SWERI); Agricultural Research Center (ARC*)*, Giza, Egypt.

Nostoc muscorum

Anabaena oryzae

Spirulina platensis



1)Blue Green Algae (Cyanobacteria)

- N_2-fixing *Nostoc muscorum* and *Anabaena oryzae* were maintained separately on BG_{11} medium (Rippka *et al.*, 1979).
- The non N_2-fixing *Spirulina platensis* was grown on Zarrouk medium (Zarrouk, 1966).
- Cultures were incubated in growth chamber under continuous shaking (150 rpm) and illumination (2000 lux) at 27± 2°C for 30 days to be used as inoculum for lab experiments.

Table 1: Characterization of cyanobacteria cultures

Parameter	*Nostoc muscorum*	*Anabaena oryzae*	*Spirulina platensis*
pH	8.2	6.70	10.45
Optical density at 560 (nm)	1. 2	0.84	2.85
Total chlorophyll (mg l^{-1})	5.39	5.31	13.50
Dry weight (g l^{-1})	0.85	0.55	1.84

2) Olive mill wastewater “OMW”

- **Olive mill wastewater (OMW) was obtained from FIFA farm (km48 of Cairo-Alexandria, Egypt).**

- **The raw OMW samples were generated by the three-phase olive-oil extraction process of 2015 seasons.**

- **Samples were left in tanks to settle for 10 days under laboratory conditions. The light supernatant after the sedimentation was filtered by passing through a sieve (mesh size about 200 µm) and was kept to be used as substrate for cyanobacteria cultivation.**

Table 2: Chemical analysis of OMW during 2014 and 2015 seasons

Season	pH	EC ds.m^{-1}	Organic Matter (%)	Organic carbon (%)	COD (%)	BOD (%)	Total phenols (g/l)	N %	P %	K %	Na %	C/N ratio
Before sedimentation												
2014	5.20	16.70	21.20	12.60	11.75	4.15	9.46	1.62	0.68	1.14	3.11	84.92
2015	4.82	17.33	20.42	11.60	10.25	2.85	7.70	1.18	0.78	1.46	2.89	87.94
Average	5.01	17.02	20.81	12.10	11.0	3.50	8.58	1.40	0.73	1.30	3.00	86.43
After sedimentation												
2014	5.40	15.71	14.11	8.18	10.01	3.51	8.56	1.20	0.70	0.47	3.00	6.81
2015	6.75	15.33	12.75	7.40	6.67	2.65	6.82	1.10	0. 40	0.36	2.88	6.73
Average	6.07	15.75	13.43	7.79	8.34	3.08	7.26	1.15	0.55	0.42	2.94	6.77

PHYCOREMEDIATION PROCESS

(4) Algal treated OMW

(3) Supernatant

(2) Sedimentation stage

(1) Raw OMW

Phycoremediation Process

- **Cyanobacteria culture suspensions at log phase, either individually or in a mixture of 1:1:1 v/v, were inoculated at the rate of 20% into 500 ml conical flasks containing 200 ml of different sterilized dilutions of OMW/tap-water (25, 50 and 100% v/v).**

- **In case of *Spirulina platensis*, all dilutions were supplemented with 5 g/l $NaHCO_3$.**

- **The non-inoculated OMW dilutions were used as control.**

- **The cultures were incubated at 27±2°C under continuous shaking (150 rpm) and illumination (2000 Lux) for two weeks.**

Up-scaling production of Cyano-OMW biofertilizer

- **About 50-liter plastic tanks were used to prepare Cyano-OMW biofertilizer by diluting 20L of non-sterilized OMW with 20L tap water (1:1 v/v).**
- **Tanks were under continuous aeration by air pumps for two days before cyanobacterial inoculation.**
- **10 L of cyanobacterial mixed culture at log phase (1:1:1 v/v) were added to 40 L of OMW-tap water diluted (1:1).**
- **Incubated for two weeks under laboratory conditions and continuous aeration to be applied for one season in the field.**

Biodiesel production methodology

System set up

- The cultivated cyanobacteria in OMW will be utilized as feedstock for biofuel synthesis based on their chemical composition.
- It is proposed to convert the algal lipids into fatty acid methyl esters (FAMEs) or biodiesel using direct transesterification process.
- The acid catalyst is firstly dissolved in the reacting alcohol and then fed to the biomass where in-situ transesterification reaction takes place.
- At the end of process, the residual cake is removed and distinct layers of biofuel and glycerol are generated.
- Refining of products is achieved before storage.

Results

- Results indicated that cultivating three cyanobacteria strains, either individually or in combination, on different dilutions of OMW indicated affected significantly on the chemical characteristics of OMW after being exposed to algal growth development for 2 weeks as shown in Table 3.

- Figure 1. show the pollutant removal percentages as indidicated by COD and phenolic compounds as affected by cyanobacterial biomass development after cultivation on OMW for two weeks

Table3: OMW specifications after microalgae growing for two weeks

Treatment	OMW %	pH	E.C ds m^{-1}	D.M g l^{-1}	COD g l^{-1}	O.C g l^{-1}	O.M g l^{-1}	Total phenols g l^{-1}
Un-inoculated	25	4.00	4.20	17.21	7.00	2.63	4.53	2.75
	50	4.90	6.00	23.14	10.00	3.75	6.47	4.38
	100	5.60	10.50	25.22	17.16	6.43	11.09	7.38
Mean value		4.83	6.90	21.86	11.4	4.27	7.36	4.84
Anabaena oryzae	25	4.30	1.60	18.72	2.18	0.82	1.41	1.20
	50	5.3	2.40	25.82	5.80	2.18	3.75	1.35
	100	4.50	5.00	26.11	9.20	3.45	5.95	3.75
Mean value		4.70	3.00	23.55	5.73	2.15	3.70	1.99
Nostoc muscorum	25	4.20	2.30	19.23	3.00	1.13	1.94	1.21
	50	5.20	3.00	26.05	4.65	1.75	3.01	1.75
	100	4.50	4.65	25.67	7.94	2.98	5.13	3.06
Mean value		4.63	3.32	23.65	5.20	1.95	3.36	1.95
Spirulina platensis	25	8.00	2.50	20.87	2.04	0.86	1.49	0.41
	50	8.00	3.25	27.27	3.66	1.37	2.37	1.90
	100	8.50	4.50	26.02	4.20	1.58	2.72	3.30
Mean value		8.17	3.42	24.72	3.30	1.27	2.19	1.87
Mixed Cyanobacteria	25	8.30	1.65	20.28	1.40	0.53	0.91	0.40
	50	7.50	1.45	28.32	2.20	0.82	1.42	0.27
	100	8.00	4.90	26.81	2.60	0.97	1.68	3.20
Mean value		7.93	2.67	25.14	2.07	0.80	1.34	1.29
OMW% Average	25	5.76	2.45	19.26	3.12	1.19	2.06	1.19
	50	6.18	3.22	26.12	5.26	1.97	3.40	1.89
	100	6.22	5.91	25.97	8.22	3.08	5.31	4.14

E.C.= Electric Conductivity; D.M= Dry Matter; COD= Chemical Oxygen Demand; O.C.= Organic Carbon; O.M.= Organic Matter

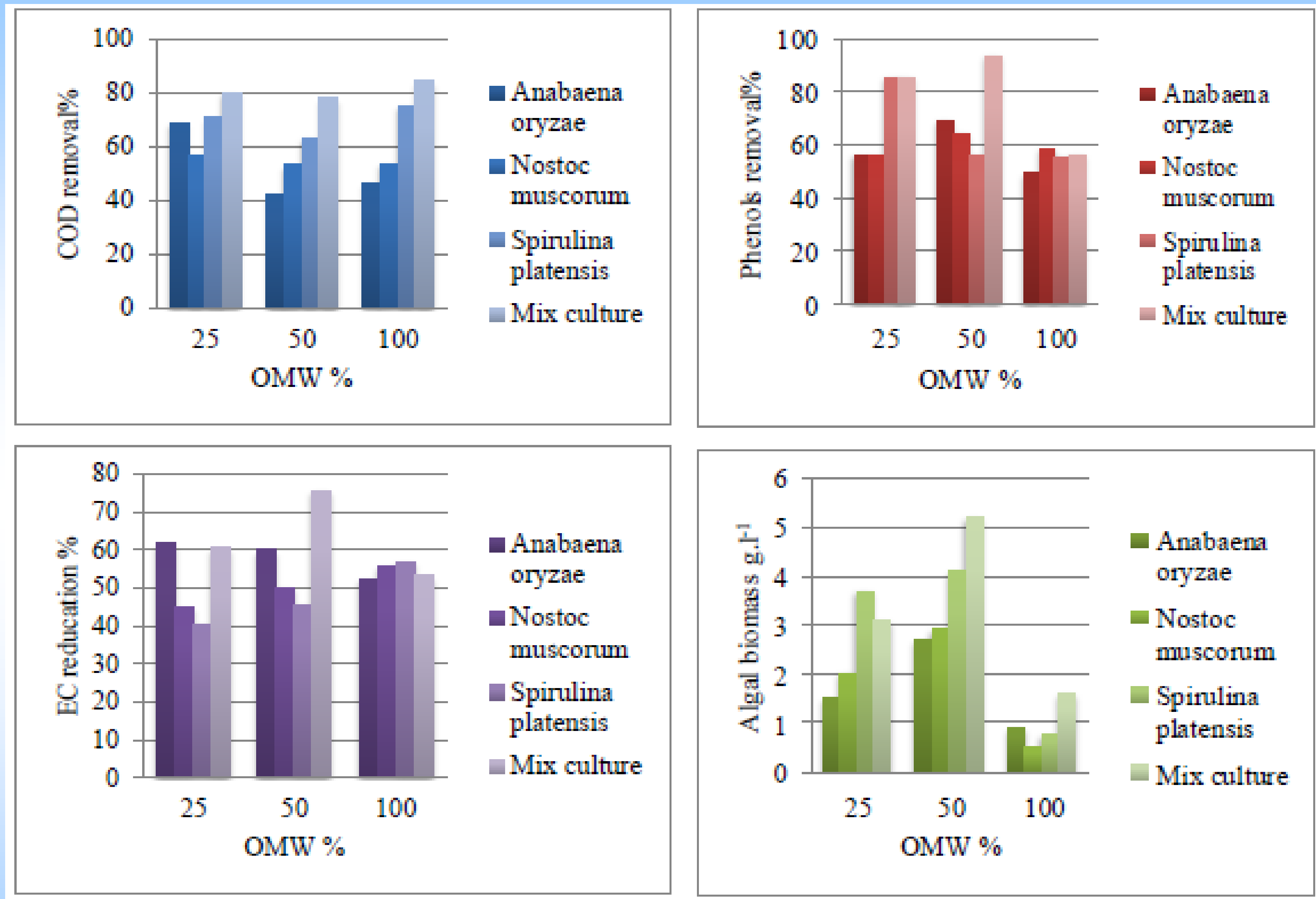


Figure 1. Phycoremediation Results

- **OMW of 50% concentration inoculated with cyanobacterial mixture was selected to be used as biofertilizer (Cyano-OMW) on large scale.**
- **In this concern, a strategy of multiple successive processes (sedimentation, filtration, aeration and bioremediation) was applied for Cyano-OMW large scale production.**
- **The physiochemical and microbiological properties of the bio-formulated Cyano-OMW are listed in Table 4. It proves that the product is neutral (pH 7.5 - 8.5) with moderate EC (2.5 – 3.75), very rich in macro and micro nutrients as well as free from pathogens.**

Table 4. Specifications of bio-formulated Cyano-OMW

Property	Cyano-OMW
Color	Brownish-green
Odor	-
pH	7.50 - 8.50
EC ($dScm^{-1}$)	2.50 – 3.75
Total solids (gl^{-1})	25.00 – 30.00
Chemical Oxygen Demand, COD (gl^{-1})	15.00 – 20.00
Organic matter (%)	2.50 – 3.00
Organic carbon (%)	0.88 – 1.20
Total nitrogen (%)	2.60 – 3.50
Total phosphorus (%)	1.70 – 2.00
Total potassium (%)	2.00 – 3.00
Phenols (%)	0.25– 0.30
Soluble nutrients (mg l^{-1})	
N	1550.0 – 2000.0
P	1000.0 – 1500.0
K	1500.0 – 2000.0
Ca	1500.0 – 1700.0
Fe	50.0 – 60.0
Mn	5.0 – 6.0
Zn	2.2 – 2.5
B	10.0 – 11.5
Microbiological constituents of biofertilizer	
Total bacteria $X10^7$ CFU/ml	30.0 – 40.0
Coliform group	*ND
Faecal coliform	*ND
E.coli	*ND
Sallmonela and Shigella	*ND

*ND: Not Detected

Bioreactor design for phycoremediation of OMW

- **Open raceway ponds or vertical tank reactors are available models as bioreactors for simultaneous propagation of algal biomass and nutrients removal from OMW.**
- **The most primitive algae farmers used large open raceways of liquid medium to grow their algae as shown in Figure 2.**
- **The advantages to raceways are that they are inexpensive, can support a large population of algae, and can use effluent water and CO_2 emissions from local industrial plants.**
- **However, open raceways are vulnerable to contamination and it is difficult to find an efficient way to harvest the algae from these large raceways due to the vast area and the constant water flow.**
- **Cyanobacteria can be grown in vertical plastic sheets or photobioreactors (PBRs). These systems are flexible and its design can be optimized according to the features of the cultivated algal species.**
- **PBRs allow growth of many algal strains and provide a protected environment against contamination. PBRs benefits include lower CO_2 losses, lower water use and higher lipids productivity as can be predicted from Figure 3.**

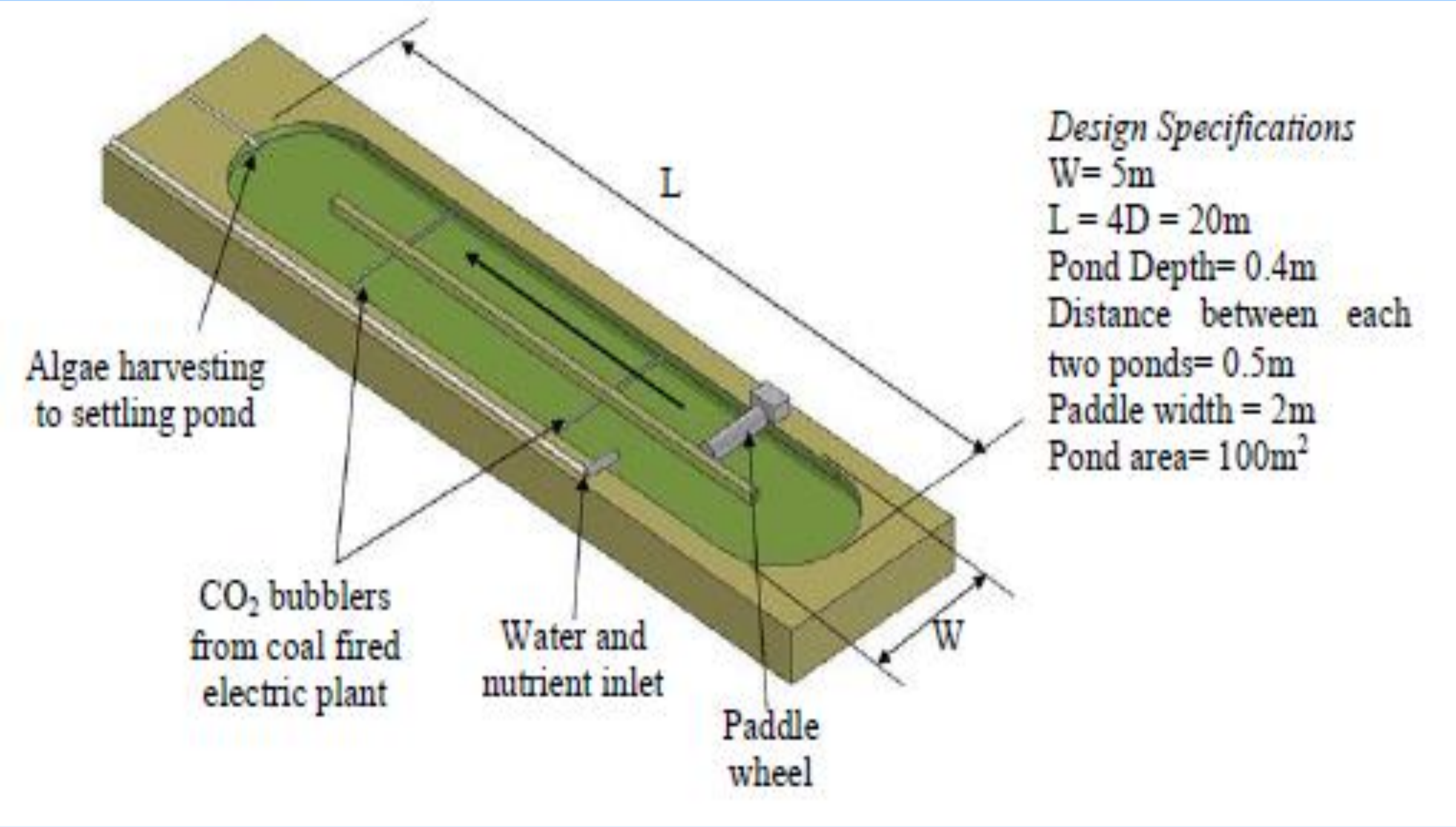


Figure 2. Scaled model of a140m^2 algae open pond bioreactor

Figure 3. Model of PBR investigated in this study

Utilization of cultivated algae in biofuel industry

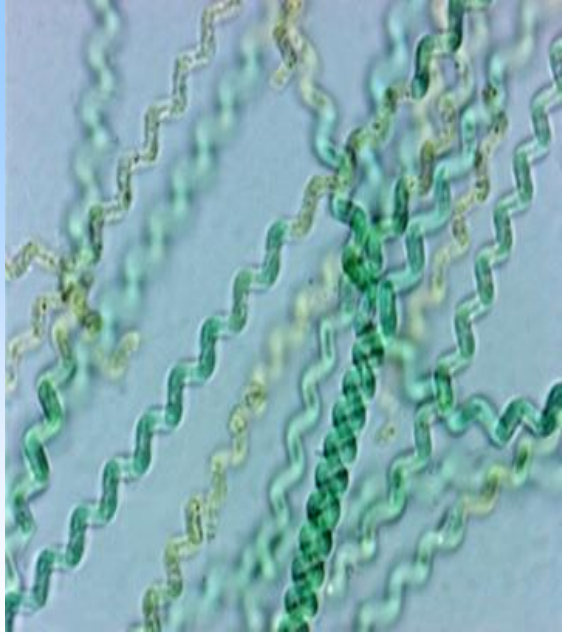
- **Once phycoremediation process has completed, the three microalgae strains are collected and dried followed by chemical analysis; to decide which strain can be considered a promising feedstock for biofuel (biodiesel or bioethanol) production. The biochemical analysis of algal biomasses is demonstrated in Table 5.**

- **As well known, the richest the lipids content algae the most suitable for biodiesel synthesis , so *Spirulina platensis* biomass is suggested for biodiesel production, while the other cyanobacteria are preferred as bioethanol feedstock‘s due to their high carbohydrates content.**

Table 5. Biochemical analysis of algal species investigated in phycoremediation process

Compound	*Spirulina platensis*	*Nostoc muscorum*	*Anabaena oryzae*
	Dry basis %wt		
Lipids	11.05	9.6	10.3
Proteins	62.2	43.2	51.6
Carbohydrates	12.8	38.1	32.2
Minerals	6.95	5.5	4
Fibers	4	2.7	1.1
Nucleic acid	2.5	-	-
Moisture	0.5	0.4	0.8

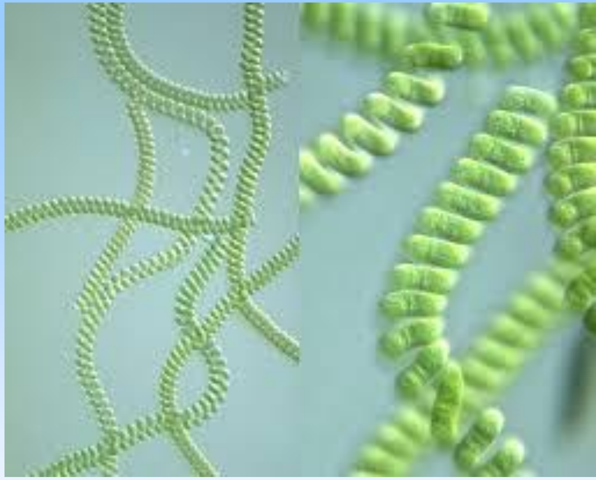
In-Situ Transesterification Process



- *Spirulina platensis* **was investigated for biodiesel production using the in-situ transesterification technology.**

- **It involves simultaneous addition of alcohol and acid catalyst to biomass; as alcohol extracts the lipids and then catalyzed by the acid into fatty acid alkyl esters (FAAEs).**

- **This methodology is suggested to be economical way in algal lipids transformation into methyl esters (biodiesel) without pre-extracted oilgae.**



- Fatty acid profile of the produced biodiesel produced was detected by GC-MS that confirming the predominant fatty acid groups in the *Spirulina platensis* microalgae are Mystic, C14:0 (22.67% by mol) and Palmitic, C16:0 (49.58% by mol) as shown in Table 6.

- Therefore, *Spirulina platensis* is a promising feedstock for biofuel synthesis.

- The biochemical analysis was demonstrated for *Spirulina platensis* cake and proved presence of valuable amount of protein (51.5%wt), hence it is suitable to be animal fodder or poultry diet feed.

- Considerable amount of nitrogen (500 mg/100g), phosphor (900 mg/100g) and potassium (1475 mg/100g) are involved in *Spirulina platensis* . These elements are the main nutrients for plant growth, so it is vital as promising plant nutrition element to be used as N-P-K fertilizer “bio-fertilizer”.

Table 6: Analysis of *Spirulina platensis* processing products

FAME Profile		*Analysis of Algal Cake*		
Fatty acid	*Spirulina* Biodiesel	Component	Value	Unit
Mystic methyl ester (C14:0)	22.67	Carbohydrates	12.6	%wt
Palmitic methyl ester (C16:0)	49.58	Protein	51.5	%wt
Palmitoleic methyl ester (C16:1)	2.75	Moisture	1.5	%wt
Stearic methyl ester (C18:0)	5.56	Ash	7.5	%wt
Oleic methyl ester (C18:1)	2.24	Ca	400	mg/100g algae
Linoleic methyl ester (C18:2)	5.03	P	900	mg/100g algae
Linoleuic methyl ester (C18:3)	7.41	Fe	70	mg/100g algae
Ecosanoic methyl ester (C20:0)	1.06	N_2	500	mg/100g algae
Eicosenoic methyl ester (C20:1)	3.69	K	1475	mg/100g algae
Saturated	78.87			
Monounsaturated	8.68			
Polyunsaturated	12.44			

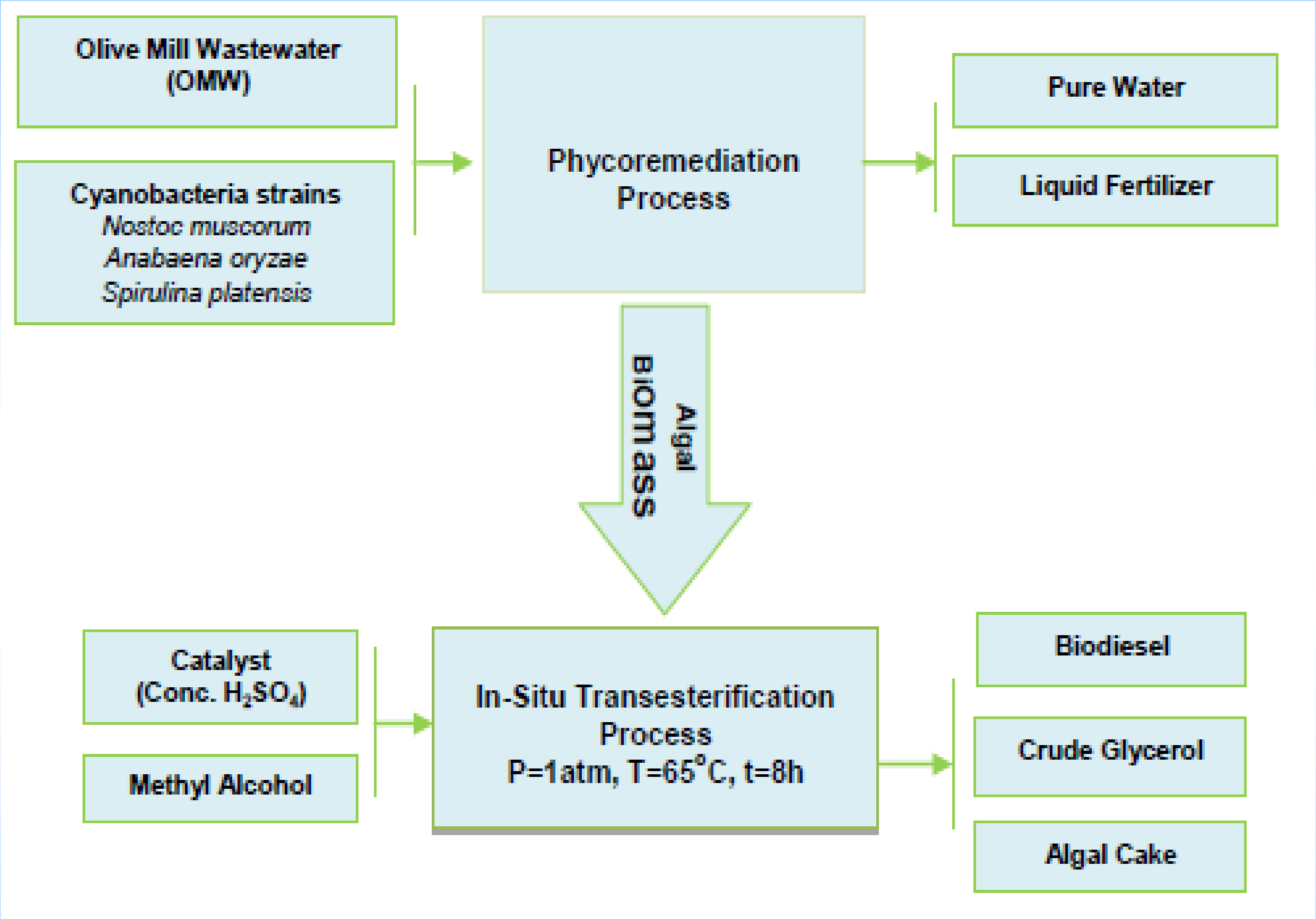


Figure 4: Phycoremediation process followed by biodiesel production from Spirulina

Conclusion

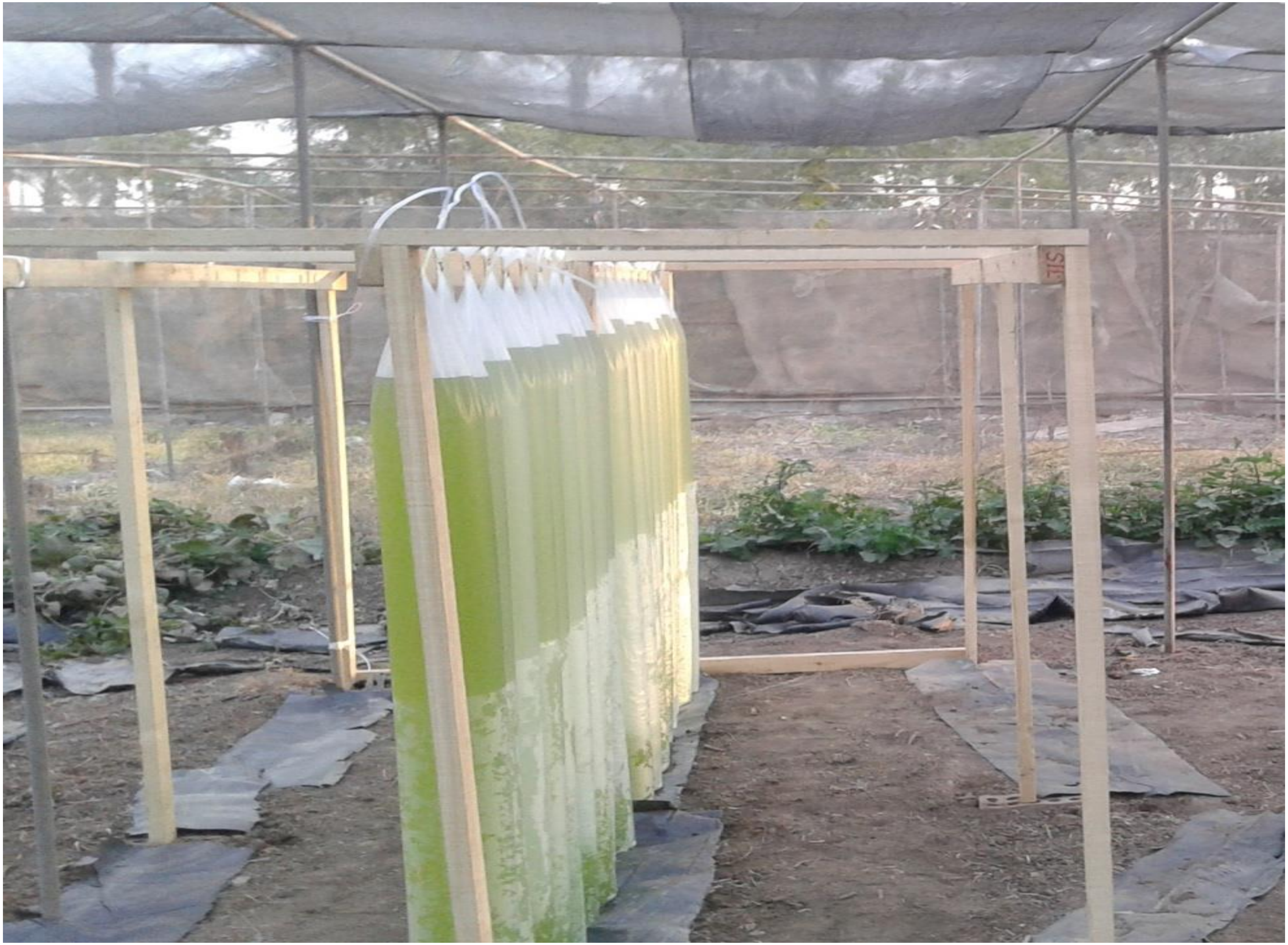
- Phycoremediation is a successful technology for nutrients removal from OMW especially phenolic compounds using 50% OMW and mixed Culture of ***Spirulina platensis, Nostoc muscorum*** and ***Anabaena oryzae***

- Optimum bioreactor design for OMW phycoremediation is an ongoing exercise however; open ponds or PBRs are feasible.

- Biomass production from OMW-Cyanobacteria suspension can be achieved using flocculation followed by centrifugation to obtain 300g dry algae/liter.

- Microalgae cultivated via phycoremediation are sustainable feedstock for biofuel industry as C16:0 is the predominant fatty acid in FAME profile.

- Algal waste can be utilized as solid biofertilizer, animal fodder or poultry feed.



Soils, Water and Environment Research Institute

Objectives:

1. Conducting applied research to serve the following topics: Survey of land resources - Maximize the output of soil, water and fertilizer unit -Balanced fertilization for plants - Management of soils and water - -Recycling of agricultural residues - Bio-fertilizers and Biological agents production - Improvement of soil productivity - Monitoring of soil and water pollutants.
2. Conducting extension services and training programs of the above topics.
3. Quality control of local and imported fertilizers and the inspection of the market.
4. Providing the services to farmers, organizations and the private sectors throughout the special unit of (soil, water and environment unit) in the field of soils, water, production of biofertilizers (Aukadin, Nemaless, Yeast Solutions and Mixed Solutions) ammonia gas fertilization, agricultural residues recycling and production of land cover, land use and suitability maps.
5. On farm irrigation development in the old land sharing with agricultural extension sector and ministry of irrigation and water resources.

Staff of Agricultural Microbiology Research Department

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