#### Use of algae and fungi in WWT

Danielle Bansfield 17.3.2020

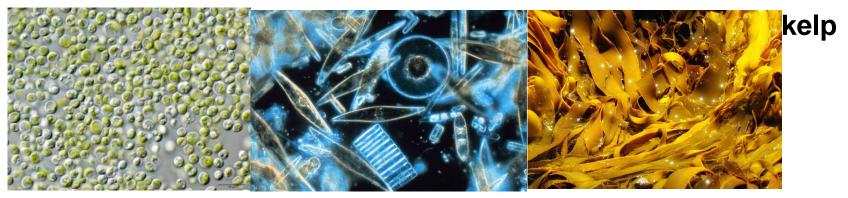
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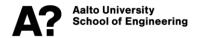


#### Algae

#### Are a diverse collection of photosynthetic eukaryotes They differ in form, and function

e.g.





Most are aquatic and produce own energy (autotrophic) via photsynthesis

Some like dinoflagellates consume organics (heterotrophs)

Some terrestrial and aerial

Some undergo asexual and some sexual reproduction



#### Importance in aquatic food chain

#### **Aquatic Food Chain**

Large fish

Very important

Form base of aquatic food chain

Produce own energy

Providing food for heterotrophs

Small fish Phytoplankton Zooplankton



### **Phycoremediation**

Removal or biotransformation of nutrients/pollutants

Algae is used for:

- 1. Nutrient removal (namely N, P)
- 2. Transformation, conversion and degradation of xenobiotics (hydrocarbons, dyes, phenols)
- 3. Treatment of pH and metallic removal
- 4. Sequestration of CO2
- 5. Detection of xenobiotics by algae based biosensors



### Why not just use bacteria?

Better candidate than bacteria, yeast and plants because of their:

- 1. Fast growth
- 2. Higher nutritional value than bacteria
- 3. Production of valuable biochemicals (bio oils, vitamins)
- 4. Don't compete for arable land
- 5. Less affected by seasonal changes



# Wastewater treatment and Nutrient removal

They are ideal for WWT because they are aquatic and phototrophic (produce energy via sunlight)

Especially microalgae which grow quickly (double every 8hrs)

Good phycoremediators (removal/biotransformation of nutrients or toxins)



Algal base WWT was first developed by Professor William J. Oswald, University of California, Berkley in 50's

He developed the high rate pond (HRP) system still in use today to produce feed, supplements, biochemicals and biomass for fuel production

Later immprovements -> Advanced integrated wastewater pond system (AIWPS)



#### AIWPS

Sewage goes through various series of ponds with various stages of "clean up"

- 1st: anaerobic bacteria break down solids
- 2nd: shallower ponds so sunlight penetrates maximizing algal growth to outcompete bacteria
- 3rd: settling pond from which algal biomass is harvested
- 4th: maturation or settling to remove fecal coliform bacteria



-removes organics up to 1000mg/L of BOD5

-used around the world, especially suited to developing countries because:

- 1. Cheap to construct and maintain
- 2. Easy to maintain and operate (no need for engineering skills and lots of money)
- 3. Customizable

The technology has been refined by Oswald's students

In 60's they started large scale Algal WWTP in Califonia

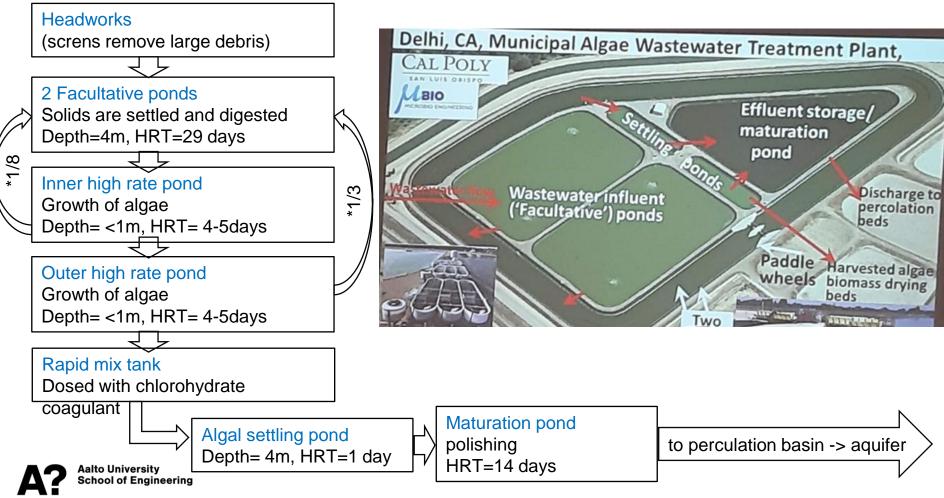


## Case study: Delhi, CA Municipal Algae WWTP

Location: Delhi, California, Population: 10 000 Latitude: 37.4N







\*recirculation of aerated sewage to curb odor

#### Case study: Aqualia, All-gas

Location: Chiclana-de la Frontera, Southern Spain.

Population: ~54 000. Latitude: 36.4N







- 10 yrs from lab -> pilot -> large scale
- Treats the domestic WW of the city
- Algal biomass digested -> methane for use in cars
- Clean water discharged -> local river and agricultural use
- Also feutured in the Netflix documentary "Brave Blue World"









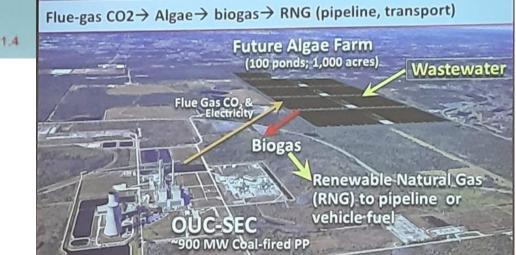
#### Pros

#### **Municipal Wastewater Treatment Technologies**



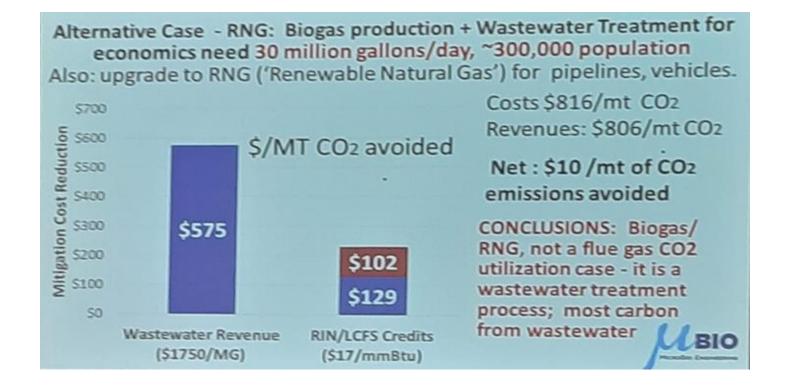
Technology	Number of Facilities	Total Flow <u>MGD</u>	Energy Intensity <u>MWh/MG</u>
Activated Sludge	6,800	25,000	1.3 - 2.5
Biofilm Systems	2,500	6,000	0.8 -1.8
Algae	5,000	2,000	0.4 - 1.4

Low energy use And others mentioned in slide 10



Can provide energy by digestion of algal biomass -> biogas While mitigating CO2 emissions

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#### Cons

- The algal biomass is rarely harvested for used
- Many now fail new treatment standards
- Need relatively large land area
- Low performance in winter
- Pond systems limited to small population centers in warm climates



## Fungi

Yeasts, rusts, smuts, mildews, molds, and mushrooms

\*slime molds and water molds are not fungi!

Eukaryotic i.e. have membrane bound organelles and defined nuclei Differ from plants:

- lack of chlorophyll
- different components in their cell walls and membranes
- Different mode of growth and nutrition



## Fungi

Grow from tips of filaments (hyphae) -> mycelia network -> thallus (mushroom cap)

Secrete enzymes which breakdown organic matter externally then absorbed

Found everywhere

Yeasts do not form mycelium but bud into individual cells

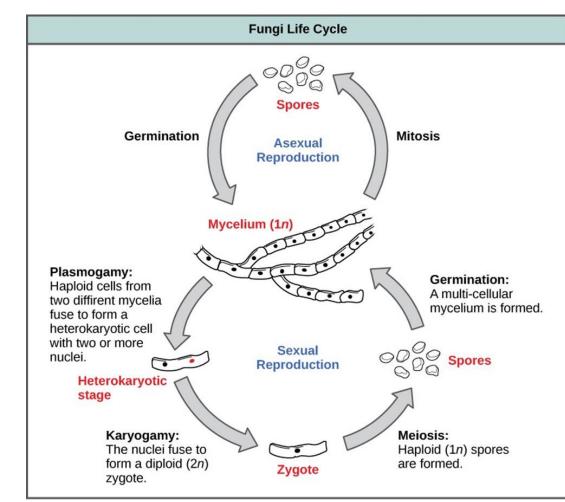


# Fungi

Reproduction

- Asexual: cell division or fragmentation of the thallus, budding in yeasts
- 2) And sexual: cells of 2 hyphae fuse





#### Importance

Important for decomposition of organic matter

And therefore the realease of C, O, N and P back into the soil and atmosphere

Produce numerous industrailly important biochemicals e.g.

Laccase and cellulose (paper industry)

Medicines (antibiotics, statin)

Fatty acids

Vitamins

lipids

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## Fungi in WWT

Well are well suited to WWT because:

The are excellent at aborbing and degrading matter

Degradation is non-specific and broad

Ease of separation from water

Resistant

Used to treat difficult to remove toxins from industrial WW such as dyes (tecxtile industry), heavy metals (mining, printing) and phenolics



### **Biosorption**

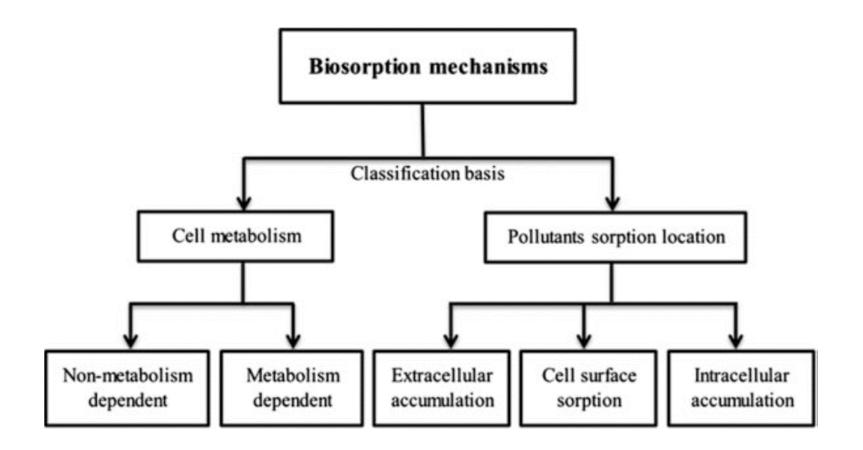
Various functional groups on cell walls such as carboxyl, hydroxyl, amino, sulfonate, and phosphonate, which adsorb compounds

Employ a variety of absorption mechanisms which are not fully understood

Two types:

- 1. Non-metabolism dependent (passive uptake)—ion exchange, precipitation, complexation, physical adsorption
- 2. Metabolism dependent (active uptake)—energy-driven







### Degradation

Fungi produce non-specific degradative enzymes

e.g. laccase, lignase, cellulase

Meaning molecules of similar structure to its substrate can be degraded

e.g. organic pollutants: phenols, dyes (textile and paper making industry), pharmaceuticals, endocrine disruptors and many other xenobiotics



Filamentous fungi have especially great potential for sludge treatment such as:

- Organic solids reduction
- Bioflocculation
- Pathogens removal
- Dewaterability
- Detoxification
- \*As living organisms their efficacy is affected by:
- Temperature, pH, nutrient content and salinity of the WW



#### Fungi/algae associations in WWT

Fungi is usually combined with algae for:

 Improvement of the biomass (increased lipids -> better biofuel production, increased nutrients -> better fertilizer and feed, added biochemicals)

fungi/alage pellets can be used to remove nutrients (namely N,P,) of up to 80 and 70% respectively

This is species dependent of course

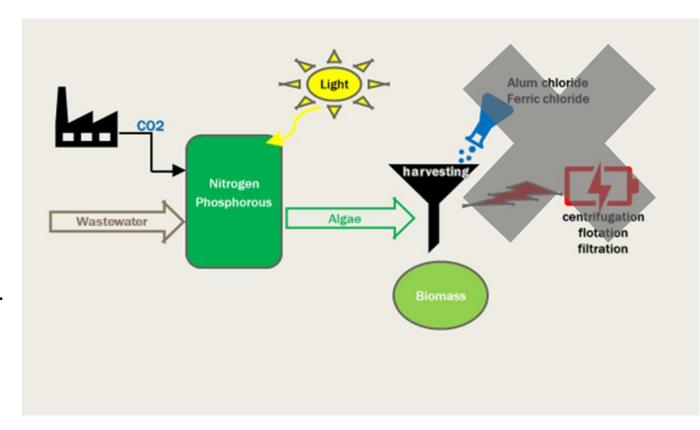


#### Easier/cheaper/more biofriendly harvesting of algal biomass

Traditional algal harvesting is chemical and energy intensive

Fungal-assisted bioflocculation

-> cost effective, environemtaly friendly algal harvesting method.





# Our project at SYKE

(LEVARBIO-project/Leväsieppari project by my colleague Jonna Piiparinen

Investigating growth and removal of nutrients by

Euglena gracilis (algae) in biogas RW

E. gracilis grew in a 50% dilution

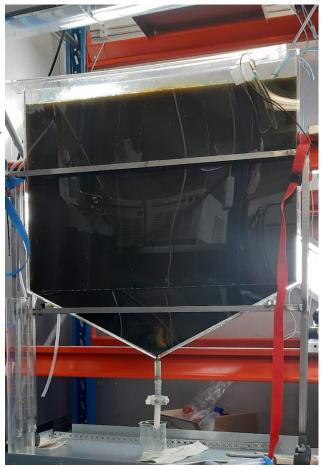
Biomass yield: 1.0 g L-1

Nutrient removal: 13% N, 72% P

Protein content: 24% of the biomass.

Lipid content: low (22-24 mg g-1)





# My project

Idea : to add fungi to Jonna's algal WWT system to improve organic removal and possibly ease harvesting

Chose 8 filamentous species and 2 yeast based on lit.

Found the RW was too nutrient limiting for fungal growth

Refocused my project to investigate other industrial waste streams (food/beverage production and digestate)



In the meantime I did harvesting studies using free *Pleurotus ostreatus* filaments in suspension combined with *E. gracilis* 

Method:

ideal conditions

rotation at 90RPM (fungal self-flocculation induction method) At RT (23C)

Measured concentration of microalgal cells (cells/ml) in suspension over time

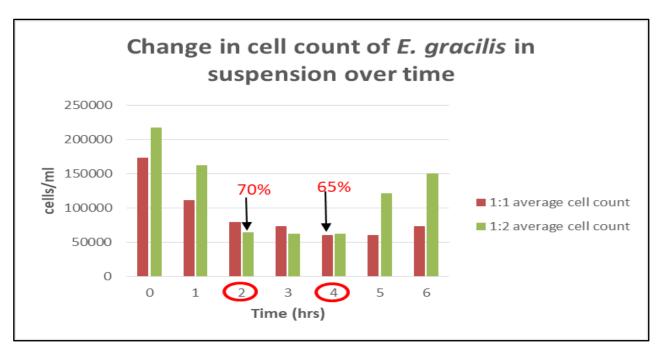
What did I find?...



# ...that PO was able to harvest Euglena by up to 70% in 2hrs in a ratio of 1 part PO to 2 parts EG (ideal conditions)

Dosage affected harvesting

Larger proportion of fungi to algae -> higher removal of algal cells



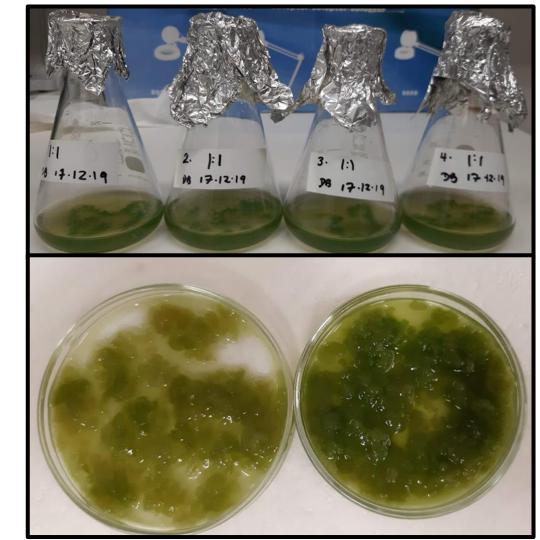


\*ratio = fungi: algae

Aggregates large enough to be collected with common household strainer

Also tested their settling ability using batch settling test

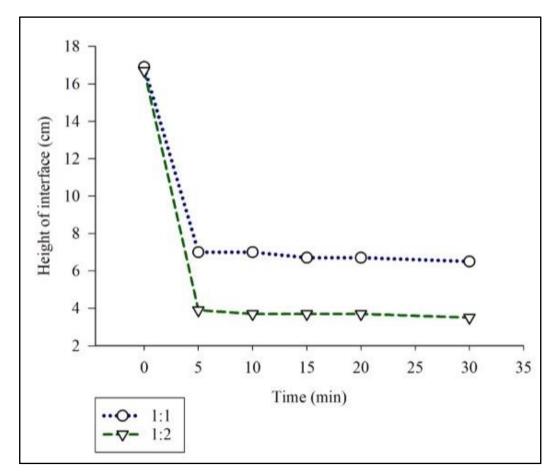




Also tested their settling ability

Dosage of fungi affected settling ability

Larger proportion of algae to fungi -> faster and more compact settling of flocs

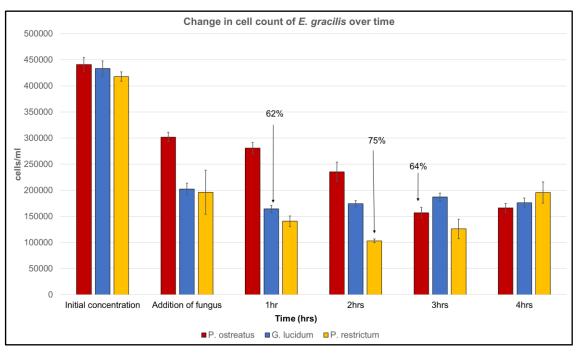




\*ratio = fungi: algae

#### Tested harvesting of *E. gracilis* with other species

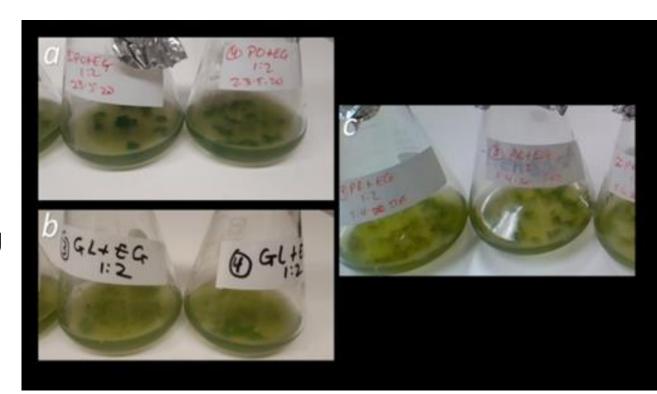
- Used same set up conditions
- Two other species were also able to harvest the algae rapidly (1 - 3 hrs)
- by 62 75 %
- *P. restrictum* gave the highest harvesting results



\*ratio = fungi: algae



These aggregates were also large enough to be collected by screening



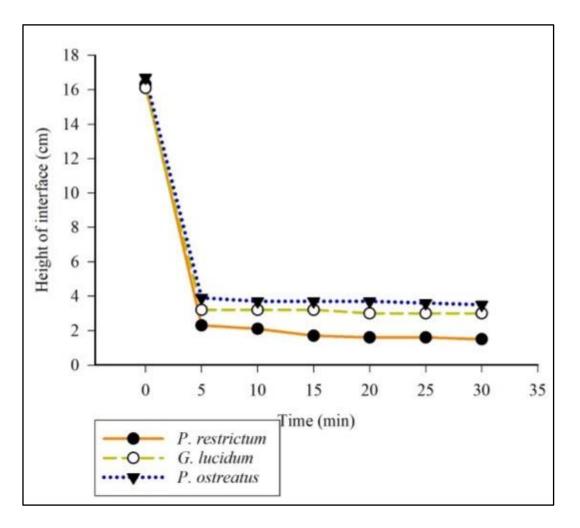


Also conducted batch settling tests on these flocs

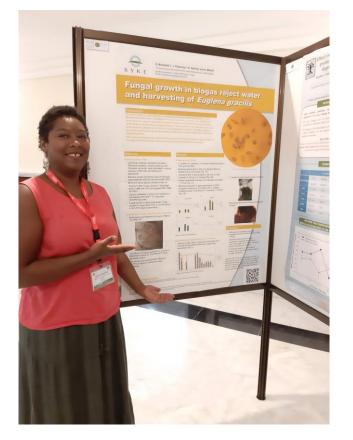
*P. restrictum* also settled fastest and most compactly

Proving to be the best species

These results prove that fungal filaments canbe used to harvest microalgae in



#### Thanks for your attention



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#### References

#### Pics

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