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Screening and Optimization of Case Specific Sustainable Mixotrophic Microalgae Medium

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Abstract

The use of microalgae in wastewater treatment has been shown to be a compelling avenue towards sustainability. Dense algal cultures grown mixotrophically on carbon and nitrogen rich wastewater effluents have provoked increased interest following the wavering economic viability of photoautotrophic algae biomass productions. Recent work in our research group at the Technical University of Denmark addressed a new microtiter screening method evaluated on algal species capable of heterotrophic assimilation of carbon and nitrogen abundant in industrial wastes and their subsequent growth rate analysis. The aim was to test the method, find the right combination of industrial waste waters, bioremediate the waste waters and build up high added value product. Residuals of the biomass could potentially be utilized for bioenergy purposes.

Aims

- Developing a microtiter screening method to identify viable mixotrophic algae for the treatment of industrial waste.
- Develop a microtiter screening protocol for testing algal growth viability on various waste media mixtures.
- Analyze batch and chemostat cultivations with reference and mixed medium to validate results from the microtiter plates, and estimate the continuous growth potential.
- Explore the potential of various mixtrophic algae in large scale reactors receiving various industrial waste water effluents.

Microtiter Strain Selection

Mictrotiter Strain Selection Method & Results (See Table-1)

Table 1: Several algal species were tested for their viability growing heterotrophically on various prepared media.

Genus	Species	Type	Media	Temp °C	pH	Results
Galdiera	***sulphuraria***	red-algae	FW-F/2	42	2	No Growth in tested conditions. Deeper green color than chlorella sp.
Arthrospira	***sp.***	cyanobacteria	Z8	26	6 to 11	Minimal growth, difficult to cultivate
Chlorella	***vulgaris***	green-algae	MWC+Se, PP, Bold	15	7.5	No growth in tested conditions.
Phaeodactylum	***tricornutum***	diatom	L1	15	7	Fast dense growth in all growth conditions. Brown Color. Easy Cultivation and morphology and contamination visibility.
Pyrocystis	***noctiluca***	diatom	TL30	20	7	No growth in tested conditions and in 12:12h light:dark cycle in L1 and F/2 media.
Chlorella	***prototheocoides***	green-algae	EG, EG:JM, Bristol-proteose, Proto-ENV	28	6 to 7	Fast dense growth for single condition it was tested in. Optimal pH for various waste substrates Can Achieve up to 64% lipids.

Scale-up Batch and Chemostat Fermentations

Batch Method & Results (see Table-2).

Table 2: Chlorella *prototheocoides* grown under mixotrophic and heterotrophic metabolisms on a combination of waste media. Waste mixtures may contain wet oxidized cow manure (WO), crude glycerol (Gly.), or potato water from potato factory (Pot).

Condition	Metabolism	C-source (g/L)	WO (%)	Gly. (%)	Pot. (%)	Growth Rate (1/h)	Color
Reference-Glucose	Mixotrophic	20	0	0	0	0.1322	Dark Green
Reference-Glycerol	Heterotrophic	20	0	0	0	0.1084	Light Green
Balanced Low-Conc.	Mixotrophic	0	0.5	0.35	2	0.0861	Dark Green
N-Limited Low	Mixotrophic	0	1	0.9	2.5	0.1007	Yellow
N-Limited-High	Heterotrophic	0	3	2.7	7.5	0.0579	Med. Green

Chemostat Method & Results

Continuous operation was initiated after each batch cultivation exponential phase but could only be achieved for *Chlorella prototheocoides* grown mixotrophically on glucose reference media (see Figure-1).

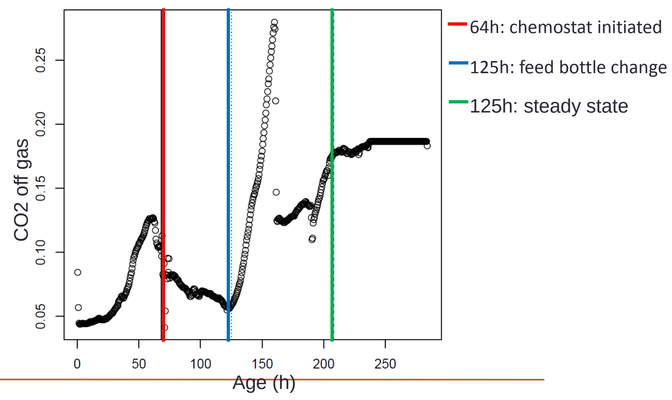


Figure 1: Plot of chemostat off gas readings of *Chlorella prototheocoides* grown under mixotrophic conditions on glucose reference.

Conclusions

- A microtiter screening method identified Chlorella *prototheocoides* as a viable algal strain for experimentation.
- A microtiter method can be used to estimate the growth of specific mixotrophic algal strains grown on industrial wastewater mixtures.
- *Chlorella prototheocoides* can be grown under mixotrophic conditions in batch mode on a combination of waste media , while achieving growth rates that may be competitive with glucose reference media.
- Steady state cultivations of Chlorella *prototheocoides* are possible under reference glucose media but may risk contamination during purely heterotrophic metabolisms and both mixotrophic and heterotrophic metabolisms grown on waste mixtures.
- Fouling of media containing wastewater mixtures must be addressed for continued development of this project.

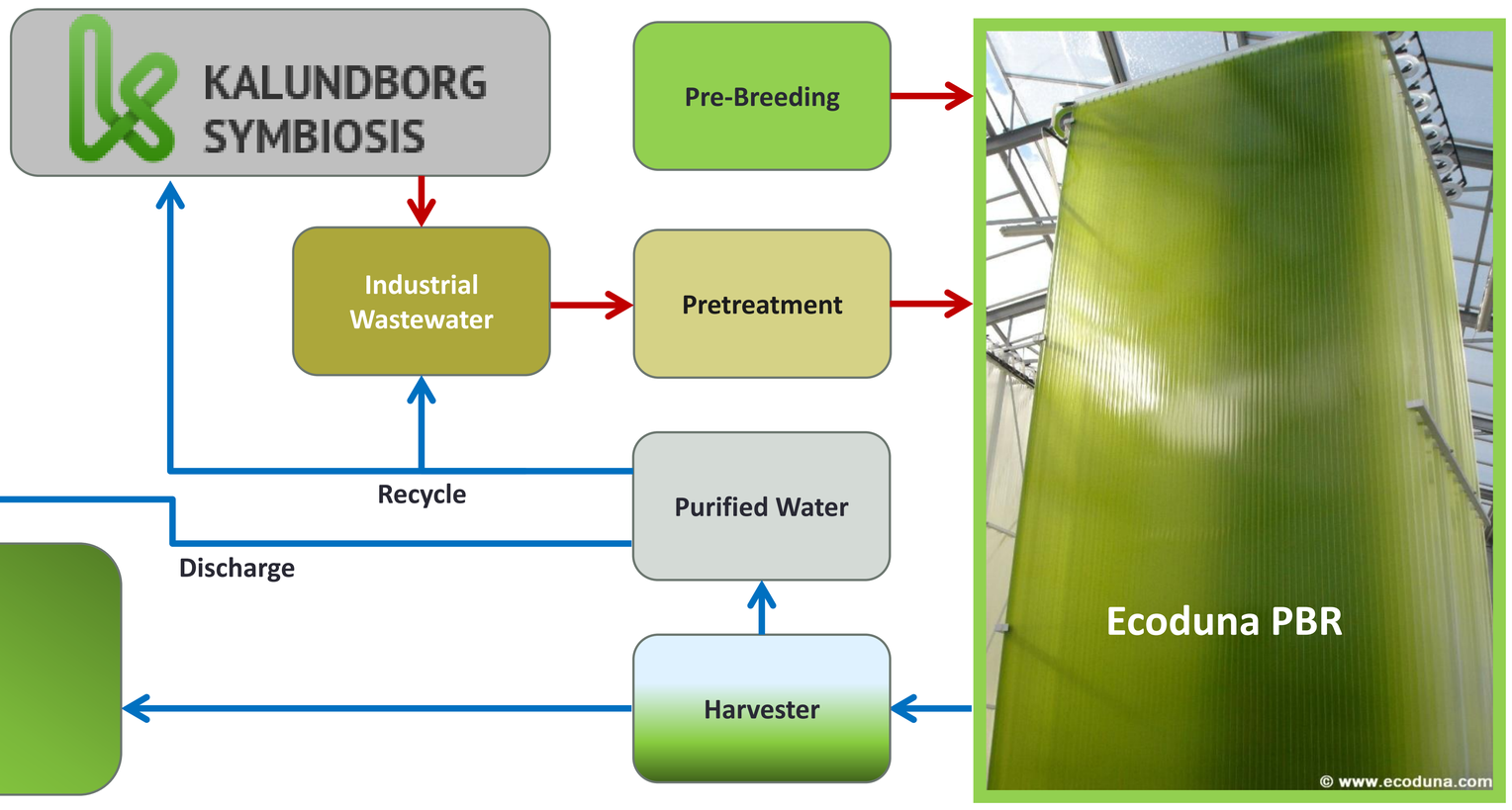
E4Water Project

Selection of wastewater streams for treatment with algae
Waste streams from external and internal partners in Kalundborg will be analyzed for their treatability by algae.

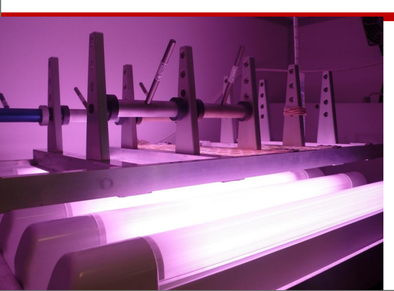
Selection of microalgal strains
1-2 microalgal species, and 1-2 microalgal consortiums will be identified for practicality based on wastewater criteria.

Scale-up experiments
Selected microalgae will be grown in single stage continuous bioreactor configuration at a test facility.

Solid-liquid separation
Simultaneous liquid phase separation and algal biomass quality will be evaluated.



Photos: *Martin Borch* Funded by:



E4WATER Economically and Ecologically Efficient Water Management in the European Chemical Industry

SEVENTH FRAMEWORK PROGRAMME

Cluster Biofuels Denmark

KALUNDBORG SYMBIOSIS