FACULTY OF BIOSCIENCE ENGINEERING

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VALORISATION OF AGRICULTURAL WASTEWATER STREAMS BY PRODUCING DUCKWEED AS AN ALTERNATIVE PROTEIN SOURCE

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Introduction

- Protein supply for animal feed applications in the European union (EU) relies mainly on the import of soybean meal.
- In 2013, 69% of the protein-rich feed materials (excluding fish meal) were imported into the EU-28. The low self-sufficiency exposes the EU to possible trade distortions.
- As a result, there is an increasing demand for alternative protein sources produced in the EU.
- Duckweed is potential alternative protein source in animal feed.
- There's an abundance of nutrient rich wastewater in agriculture which has led to eutrophication in Flanders. This nutrient content can potentially be reduced by duckweed.

Potential of duckweed

- The duckweed family (Lemnaceae) have an excellent productivity.
- In optimal conditions a production of 180 ton dry weight (DW) ha^{-1} y^{-1} can be reached.
- In field conditions, a production between 10 and 55 ton DW ha^{-1} y^{-1} can be reached.
- Its high protein content (15 – 45%) and excellent amino acid composition make it a very suitable protein source for both food and feed.
- Easy to harvest.

Laboratory scale tests

1) Sampling natural occurring duckweed and selection for salt tolerance

Doubling time of 9 duckweed clones sampled from 2 naturally occurring species in Flanders and grown on high and low salinity media

		Low Electric Conductivity 0.6 mS/cm		High Electric Conductivity 7.4 mS/cm	
Species	Clone	Doubling time (days)	std.	Doubling time (days)	std.
L. minuta	Destel2	6.2	0.3	12.9	1.0
L. minuta	Lovendegem	7.1	0.4	14.9	2.3
L. minor	Gent1	8.7	0.9	24.8	3.7
L. minor	Tielt-Wi	9.1	0.4	NA	NA
L. minor	Kalken	9.4	0.9	16.5	2.6
L. minuta	Gent2	9.7	0.3	16.7	1.2
L. minuta	Drongen	10.4	1.6	32.8	18.2
L. minor	Kluizen	13.7	1.2	NA	NA
L. Minuta	Destel1	15.2	2.2	NA	NA

NA — *Negative growth was observed, thus doubling time is not applicable*

Doubling time — *The time for species following an exponential growth to double their fresh weight*

2) Testing potential clones on different agricultural effluents

Doubling time of 3 duckweed clones grown for 4 weeks on 4 different agricultural effluents under glasshouse conditions

Medium\Clone	Destel2 *L. minuta*	Pittem *L. minor*	Uddel *L. minor*
Aquaculture	11.5	10.5	10.3
Pig urine (1/30)[1]	28.0	14.0	18.8
Influent[2]	NA	17.6	13.5
Effluent[2]	9.1	8.5	9.3
Spirulina (1/20)[3]	NA	NA	10.7

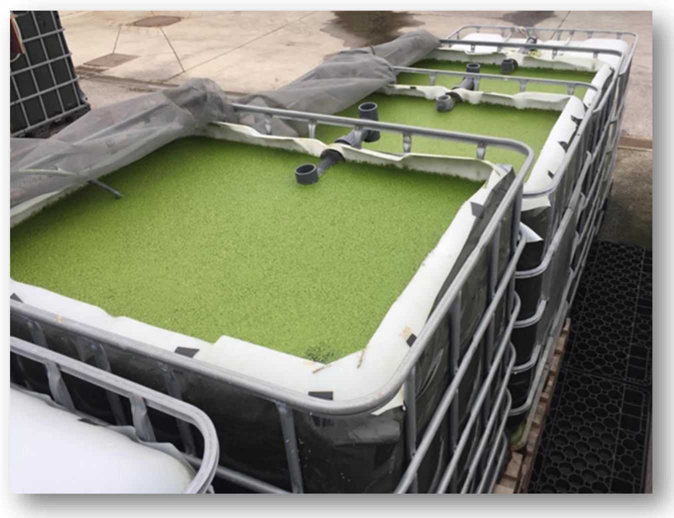
[1] diluted 30 times to decrease salt stress
[2] influent and effluent of a constructed wetland
[3] effluent from the production of Spirulina, diluted 20 times and corrected for its pH.

Different agricultural effluents were tested as a growing medium for the selected duckweed clones. Destel2 was selected because of its salt tolerance. Additionally, two clones naturally occurring in constructed wetlands in Pittem and Uddel were tested. It was assumed these were naturally adapted to higher salinities.

Duckweed grown on the effluent of a constructed wetland for manure processing showed the best results, closely followed by duckweed grown on aquaculture effluent. *L. minor* Pittem grown on constructed wetland effluent had the best performance (**from 5 g up to 49 g FW in 4 weeks**).

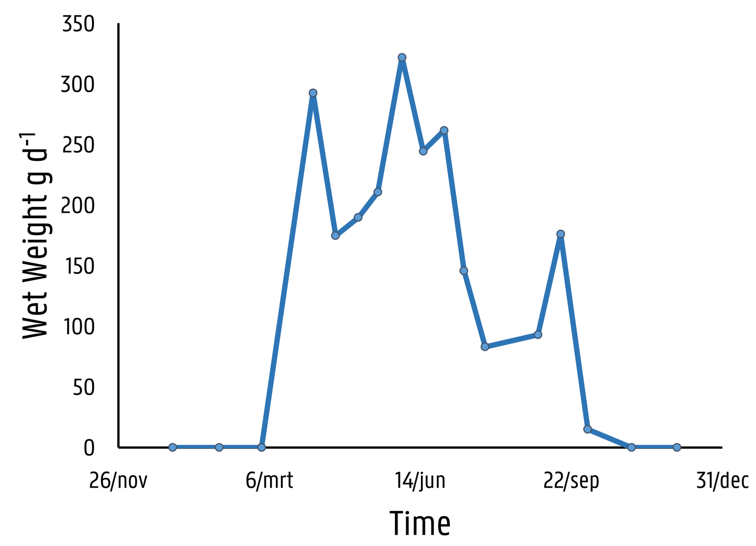
In a next phase, these results were scaled up. The effluent of aquaculture was selected because of its smaller variability throughout the season and its good performance. As the clone Uddel grew the best on this effluent, we selected this clone for further upscaling.

Scaling up on a cascade of agricultural effluent



A cascade consisting of 3 cubicontainers (1.2 × 1.2 × 1.2m) filled with aquacultural effluent from the production of Zander (*Sander lucioperca*) was constructed. Over a period of 12 months, a production of 2.2 kg DW of duckweed was produced. This corresponds with **5.1 ton DW ha^{-1} y^{-1}**.

The protein content of the duckweed was analyzed on three points during the year. A protein content of 28% of the dry weight was measured, which corresponds with a full scale production of **1.4 ton protein ha^{-1} y^{-1}**.



Daily growth rate of Lemna minor on a cascade containing aquacultural effluent observed during the year 2017

During this experiment, duckweed growth was maximal in July and reached 17 g DW m^{-2} day^{-1} or a doubling time of **7.6 days**. Growth decreased in spring and autumn, and eventually stopped from November until April. During the growing season, an average doubling time of 11.5 days was reached. Additionally, an average daily nitrogen and phosphorus uptake of 5.5 g N day^{-1} and 1.4 g P day^{-1} was measured, respectively.

Validation of results on pilot scale

The final step is to grow duckweed on a pilot scale. For this purpose, a lagoon (14 × 9.7 × 1.4 m) was constructed and duckweed growth was initiated. Starting from September 2017, growth was established on nutrient rich water. The following growing season, the goal will be to produce a critical mass of duckweed so further analyses for feeding potential can be carried out.

Future perspectives

1. Identifying critical parameters and investigate their influence. For example, the effect of a high salinity and its interaction with nutrient availability is important for the use of agricultural wastewaters streams as these streams contain generally a salt content above optimum for duckweed growth.
2. Calculating a full mass balance on a pilot scale during one growing season. In this way, the phytoremediation capacity of duckweed for N, P, S, Na, K, Cl, Ca, Mg, Zn, Fe, and Cu will be estimated under Belgian climate conditions. Additionally, water quality parameters as DO, BOD, COD, pH, and EC will be monitored.
3. Estimating the variation of starch, protein and amino acid composition of duckweed grown on different agricultural waste waters.

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