Cultivation of aquatic plants on cow manure digestate

A technical report

Authors: Sander Huurman, Rommie van der Weide and Wim van Dijk

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Summary

Turning animal manure into energy generates a leftover product: digester bio slurry. Generally this bio slurry is separated in a solid and a liquid fraction. The use of the liquid fraction bio slurry (LFBS) in combination with residual heat and flu gas from the CHP unit could proof an interesting way to turning LFBS in to valuable biomass locally and thereby reducing the manure processing costs.
The goal of the study described here is to assess the application opportunity of LFBS from a cattle manure co-digester in combination with aquatic biomass. In this study different aquatic plant types (submerged, floating and emerged) are used to observe there growth characteristics on different concentrations (1:50 an 1:100 dilutions) of LFBS. In addition to the aquatic plants soy plants are subjected to the same growth conditions to find out their response to a hydroponic culture using LFBS as sole fertilizer. The used plants (cattail, hornwort, water hyacinth and soy) generally grew well under the selected conditions. Unfortunately hornwort (submerged plant type) was overgrown by duckweed on the surface of the test setup, this might had led to lower biomass productions. Cattail, hornwort and soy were only tested in outside conditions. Water hyacinth was only tested in greenhouse conditions, first in a small scale setup (12 plastic boxes of 60x40 cm) followed by an upscaling step to a race way pond of 175 m^2. While water hyacinth is notorious for its growth potential (potentially invasive in tropical and sub-tropical regions) in this study cattail showed higher specific biomass growth. Maximum projected dry matter biomass yields of 32.9 and 38.9 t/ha/year respectively. Soy beans were tested as an alternative to aquatic plants. Beans were directly sown in rockwool, which was used as rooting media floating in the bio slurry dilution. Remarkably the soy plants grew well and even developed beans, the projected yield was comparable to field yield. LFBS proofed to be a suitable nutrient source for the tested plants in an aquatic environment. Especially in countries, such as The Netherlands, where bio slurry and water is abundant, the cultivation of aquatic or terrestrial plants in a LFBS dilution could be an attractive alternative for soil bound agriculture.

Keywords: bio slurry, aquatic biomass, digestate, water hyacinth, duck weed, cattail, soy

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Acronyms and abbreviations

CHP unit	Combined heat and power unit
DM	Dry matter
DO	Dissolved oxygen
EC	Electric conductivity
Ha	Hectare
LFBS	Liquid fraction bio slurry (in this study originated from cow manure co-digestion)
N	Nitrogen
P	Phosphorus
RWP	Race way pond
t/ha/yr	tonne/hectare/year

1 Introduction

Manure and digestate generally have a negative economic value in The Netherlands. The relatively high density of livestock introduces a larger volume of manure (or manure products) than safely and responsibly can be used in the Dutch agriculture. Surplus manure is processed and mainly exported.

To reduce manure processing costs alternative, possibly valuable, methods can be introduced in the manure processing chain. Possible additional links in the chain could be the production of various organisms on manure, digestate or digestate fractions. For example:

- Cultivation of mushrooms
- Cultivation of insects and worms
- Production of (aquatic) plants in aquatic environments
- Production of algae

All of the examples are can be setup in systems in addition to the normal agricultural fields. The general goal is to reduce manure or digestate volume, recycle nutrients and possibly introduce some additional profit from the chain.

This research focuses on the production of plants in an aquatic environment using LFBS from a cow manure fed co-digester. The goal of this research is to gather details on plants growth and nutrient use, based on the found results a hectare yield and nutrient use is projected.

Four different plant types are selected to investigate their response to a LFBS nutrient mixture. The experiments performed are not intended to optimize growth of a certain type of biomass on the used concentrations LFBS, plant details are described in chapter 4.

2 Plant selection

Water hyacinth

Water hyacinth is known for its invasive character in waterways, canals and lakes in tropical and sub-tropical regions. The water hyacinth is native to south-America but has spread all over the globe, mainly in tropical regions [1]. Water hyacinth biomass can reach densities up to 300.000 kg wet weight/ha (approx. 30.000 kg DM/ha) [2]. Over the years various research topics like, food, feed, phytoremediation and bioenergy using water hyacinth have been explored. The strong growth potential of the water hyacinth in combination with residual heat from a CHP makes it an potentially interesting biomass type to grow on LFBS in non-tropical regions. The use of water hyacinth is currently prohibited by the European union because of its invasive character in southern Europe. Despite the regulation research on this type of plant still is valuable as a model for regions where the plants is a native, possibly invasive, species. Probably the use of water hyacinth in prohibited areas is possible under strict exemption if its use is proofed to be beneficial and unintended spread is prevented.

Hornwort

Hornwort is a submerged aquatic plant that normally grows in bushes with a stem length that can reach up to 3 m. Hornwort is an oxygenating plant, the produced oxygen is transferred to the aquatic environment[8]. Hornwort (like other submerged aquatic plants) is able to absorb minerals and heavy metals from water. The plant is a suitable organism for phytoremediation [3,4]. Moreover, hornwort doesn’t develop real roots therefor it needs to absorb nutrients directly from its environments [5]. This might be an advantage compared to other submerged aquatic plants that are more root dependent. Hornwort might be utilized for different purposes, the crude protein content of the plants exceeds 18% based on dry weight [6]. Hornwort meal could be used as a dietary component in fish feed [7]. Possibly hornwort can be refined to extract crude proteins and fat, fibres and xanthophylls. Hornwort and other types of submerged aquatic plants can contain up to 1160 ppm xanthophyll in the dry biomass[6]. The major challenge of growing submerged aquatic plants on a LFBS is the turbidity and brown colour of the nutrient mixture.

Soy

The worldwide soy production in 2016 was approximately 336 million tonnes. Products from soy beans, oil and meal, are used all over the world in different ways. The extracted oil is refined and sold as vegetable oil and is used in different purposes. Roughly 97% of the soybean meal is used to feed livestock. Soybean meal also has its purpose in the food sector [9]. Growing soy in hydroponic culture could be particular useful in regions where soil or climate is not suitable for soil bound crop cultivation. Hydroponics could also provide fresh food in space programmes and in region where arable land declines [10]. When successful, a hydroponic culture of soy plants on LFBS in combination with residual heat from a CHP (or equivalent) could prove an interesting way of feed production.

Cattail

The cattail is a wide spread emerged aquatic plant. Research on cattail is mainly focussed on the removal of nutrients and heavy metals from residual (waste) streams [12, 14, 15]. Part of other research is focussed on biomass production as energy carrier [12,16]. Other sources, mainly internet based, mention the use of cattail as food source (rhizomes, young shoots, pollen) and as an industrial resource (paper fibers, insulation material) (specific sources not mentioned). The cattail is subjected to a LFBS nutrient mixture to investigate its growth potential and nutrient uptake.

3 Experimental setup

The goal of the experiments described in this report is to gather basic plant growth data on LFBS; do certain plants grow on LFBS, if yes how much biomass do they produce and how much LFBS can they process. It must be made clear that all generated results probably can be optimized by a certain degree.

The gathered results will be used to project nutrient uptake and biomass yield on hectare level. In addition this information will be used in a business case in which the biomass cost price will be calculated and compared to grass and maize.

3.1 Outside experiments

3.1.1 General description

The outside experiments were used to grow plants on LFBS that would naturally grow outside on the field or in lakes and canals. In this experiment 4 basins with a filling volume of ~1.3 m^3 were used as a pond in which the selected plant types were grown. To facilitate plant rooting and stability open baskets (LxBxD: 795x545x457mm) were filled with rockwool slabs (Grodan Flortop Rosa 100x15x7.5 cm, 7 slabs/basket), a tight filling was ensured. Per basin two filled baskets were attached to square aluminium tubes (25x25 mm o.d.) and adjusted at a specific height to the water level; for cattail the topside of the rockwool was slightly submerged (~2 cm) and the rockwool topside of the soybean baskets was placed approximately 5 cm above the water level. During the experiment the water level varied due to rainfall, evaporation and water refill. No additional growth measures were introduced for the submerged hornwort. See Figure 1 and Figure 2 for a setup overview.

At the start of the experiment (26 may 2016), one day before the basins were populated with plants, LFBS was added to the already water filled (tap water: pH 8.54, EC 367 µS/cm) basins at a concentration of 1%, the LFBS was thoroughly mixed to get a uniform nutrient mixture. To find out if aeration (addition of oxygen and generate mixing) of the nutrient mixture would induce differences in plant growth in 2 of the 4 basins continuous aeration using a ceramic sparger was installed at approximately 3/4 of the basin depth. Basin number 1 and 2 were equipped with aeration. The used air flow was ~400 l/hr. The nutrient mixture was kept at a constant temperature of 20^0C during the experiment using a geothermal heating station.

During the experiment the EC (Hanna Instruments, DiST 5 EC/TDS Tester) and pH (WTW, pH 3310) were manually measured periodically, fresh LFBS was added to the basins in which the EC value was dropped to ~500 µS/cm, the added volume was equal to the volume added at the start of the experiment (1% final concentration) and mixed thoroughly. The water level in the basins were adjusted to the starting level periodically.

The plant growth was monitored during experiment progress. At the end of the growing season, October month, all biomass was harvested and analysed. After harvesting the biomass the left over liquid level was normalized and mixed thoroughly prior to sampling for left over nutrient analyses. Samples we stored at -20^0C.

Figure 1: Overview of the experimental setup, the 4 basins in front are used in the experiment. The baskets were lined up in north-south direction. The green piping is used for heat and/or cold supply to regulate the basin temperature.


Figure 2: Basin detail, baskets mounted on different heights on aluminium tubes. Showed here are basin 1 (back) and 2 (front), the bubbles from the air supply are clearly visible.

3.1.2 Plant details

3.1.2.1 Cattail

Cattail plants used to populate the rockwool filled baskets were gathered form a pond located in the garden of Wageningen UR, PAGV, Lelystad. The gathered plants measured ~1 m in height and were trimmed down to 60 cm above the base of the roots. The roots were trimmed to approximately 5 cm length. Each basket was populated with 6 cattail plants, the plants were placed between rockwool slabs, no additional plant support was installed.

Figure 3: left: freshly harvested cattail plants, both roots and shoot trimmed. Right: planted cattail, the water is clearly brown coloured by the LFBS.

3.1.2.2 Soy

Soy bean seeds were planted directly in the rockwool at approximately 1.5-2 cm depth, the top of the rockwool was closed after planting to prevent direct sunlight interference and birds feeding on the seeds. In total 18 seeds were planted per basket at a 15x15 cm square distance, the northern half of the sown seeds (9 seeds) were inoculated with rhizobium (*Bradyrhizobium japonicum)* bacteria to induce symbiotic nitrogen fixation in the soy plant roots by these bacteria. Successful root inoculation is mostly visible in the formation of root nodules.

3.1.2.3 Hornwort

Hornwort plants were obtained at Moerings waterplanten in Rosendaal, approximately 750 gram of wet weight material was used per basin as starting material. The plant were loosely spread throughout the available surface area in the basin.

3.2 Greenhouse experiments

3.2.1 Water hyacinth small scale

In this water hyacinth experiment 2 different LFBS are compared on biomass growth, in addition half of the boxes were aerated continuously in order to compare a more oxygen rich liquid environment with a non-aerated environment.

In the greenhouse at the Acrres test facility a row of 12 white plastic boxes (lxbxd 60x40x30) was setup in east-west direction on concrete Stelcon slabs, the boxes were spaced ~15 cm from each other. Counting from the west the following conditions were present in the boxes:

Table 1: Water hyacinth experimental setup, boxes inside greenhouse

<table>
<tr><th>Box number</th><th>Test condition (TC) number</th><th>Liquid volume (l)</th><th>Fraction LFBS (%)</th><th>Air supply*</th></tr>
<tr><td>1,2,3</td><td>1</td><td rowspan="4">60</td><td>1</td><td rowspan="2">Yes</td></tr>
<tr><td>4,5,6</td><td>2</td><td>2</td></tr>
<tr><td>7,8,9</td><td>3</td><td>1</td><td rowspan="2">No</td></tr>
<tr><td>10,11,12</td><td>4</td><td>2</td></tr>
</table>

**Air supply: ~100 l/hr continuously*

At the start of the experiment (26 may 2016) the boxes were all filled with 60 litre tap water (tap water: pH 8.34, EC 392 µS/cm), this volume was equal to a liquid height of 23 cm in the middle of the boxes, depth measurement is later used a reference for measuring the liquid level and refilling the boxes. After adding tap water each box is fertilized using 0.6 litre (final LFBS concentration of 1% when dissolved/suspended) freshly separated LFBS (not older than 24 hours) after which the fertilized water is mixed manually until evenly coloured. The mixture is left to stand for 1 day to acclimatize to the greenhouse temperatures. The next day water hyacinths are added to the boxes, in total approximately 0.5 kg fresh weight was added to each box. At the start of the experiment the used LFBS concentration (1%) was kept the same for all boxes to be make sure the water hyacinths are all subjected to similar nutrient conditions. A data logger was used to monitor the temperature in the greenhouse at plant level.

During the experiment the pH and EC value were manually measured periodically, fresh LFBS was added to the experiment conditions in which the EC value was dropped to ~500 µS/cm. After adding LFBS water-LFBS mixture was mixed manually. In addition to pH and EC measurements the water level in the boxes was monitored and adjusted to the start level roughly once a week. Samples of the used LFBS at each addition moment were stored in a -20^{0}C freezer for analyses.

To monitor the biomass growth water hyacinths ware harvested when the boxes were fully filled with plants, approximately 50% of the plants were harvested each time. At the end of the experiment all plants were harvested. From a couple of harvests water hyacinths were dried at 104^{0}C for 24 hours to determine their DM content, these plants were used to determine the composition of the water hyacinths. The harvested, not dried, plants were used to start up the race way pond experiments, additional information available below (water hyacinth race way).

3.2.2 Water hyacinth race way pond

To validate the found biomass productivity in the small scale experiments one of the algae race way ponds (RWP) at the Acrres test facility was used as a reference. The RWP was filled (21 July 2016) with 100 m^3 tap water to which fresh LFBS was added to a concentration of 1%. The existing submerged Flygt mixer is used (motor speed ~40Hz) to mix the water and LFBS, the pond's central heating system using residual heat from the CHP is used to heat up the nutrient mixture (temperature set point: 35^0C). To populate the RWP all harvested water hyacinths, except sample material, from the small scale experiments are transferred.

To generate an estimation of the biomass productivity approximately 60 m^2 plants was harvested when the population reached a fresh weight density of ~10 kg/m^2, after harvest the population was redistributed evenly across the RWP. During the following time period the biomass density was monitored, the time required to reach the harvest density of ~10 kg/m^2 again gave an estimate of the productivity.

4 Results and discussion

The results of the experiments are described per plant type. For each plant type the projected hectare yield is calculated and the volume of "processed" LFBS per plant type is calculated based on the mineral composition of the harvested plant material.

4.1 Cattail

The cattail plants used to populate the baskets didn't show any visible growth for the first 1.5 weeks after planting, after 2 weeks the firs new shoots started to emerge from the rockwool and the growth in height of the original plants started to continue. Approximately 5.5 weeks after planting cattail root- and shoot growth outside the baskets was visible. During the growth season the plants did not flower, this observation is normal for the cattail as they flower on one year old shoots [18]. Except for some small, short-termed, greenfly infestations, there were no additional observed infections by plant diseases during the growth season.

At the end of the growth season all cattail shoots, in- and outside the basket, were harvested. The roots that grew outside the basket were also harvested. Roots inside the rockwool were not harvested. Table 5 shows harvested biomass, wet- and dry weight, DM percentage, plant height and extrapolated biomass yield of roots and shoots combined per hectare.

Table 2: Harvested cattail plant length, dry matter content and fresh and dry biomass at the end of the growth season.

Basin	Max. plant height (cm)**	Harvested wet weight (g)			Total harvested dry weight (g)		DM% (all shoot material combined)		Projected hectare yield (t DM/ha)***
		shoots		roots	shoots	roots	shoots	roots	
		inside basket	outside basket						
1*	180	7512	5602	13507	1440	1628	11.0%	12.1%	38.3
2*	172	4155	4401	12183	820	1260	9.6%	10.3%	26.0
3	169	4703	4288	9303	973	948	10.8%	10.2%	24.0
4	158	3986	3802	8626	760	883	9.8%	10.2%	20.5

**basins with aeration*

***plant height measured on harvested material*

****combined root and shoot yield per hectare linearly extrapolated from basket yield*

The highest biomass growth was developed in basin 1, both the share of roots and shoots were higher compared to the other basins. Basin 1 also produced the longest cattail plants, basin 2 produced similar sized shoot length. The shoots in basin 3 and 4 were clearly shorter than the shoots in basin 1 and 2.

The projected yield is calculated based on a first year cattail harvest. D.R. Dubbe et. al.[19] described a doubling of biomass yield in the second year of an 2 year cattail experiment, the explanation for this increase in yield was the existence of already developed rhizomes (roots) which facilitated a 2 months earlier start of the exponential growth period compared to the first year. The yield and nutrient removal in the used experimental conditions of this report is also expected to be higher in the second and following years of growth.

From each basin root and shoot samples were collected, dried and analysed on crude protein, total nitrogen, phosphor, potassium, magnesium and calcium. No significant difference (ANOVA, $p>0.05$) could be found between all separate analyses parameters when comparing the separate basins with each other. This indicates that the different growth conditions had no effect on the plant composition and mineral uptake based on the analysed parameters. When comparing roots and shoots a significant difference (T-test, $p<0.05$) was found in all analyses parameters, all concentrations except potassium and calcium showed a higher value in the root biomass. These results are comparable with the results that D.R. Dubbe et. al. [19] found in their research. At the beginning of the cattail growth season shoots generally contain higher concentrations minerals and proteins, towards the end of the growth season the roots continue to increase in mineral and protein concentration while the concentrations in the shoots decrease. The roots function as an energy storage for the winter season and as energy source for next year's shoots.

Based on the plant composition a yearly nutrient uptake is calculated assuming an average of the projected biomass yield (Table 3). Based on the average composition of the added LFBS in this experiment (appendix 1) and average phosphorus uptake a yearly LFBS consumption by cattail is calculated, a hectare of cattail culture could "process" 398 tonnes of LFBS/yr, this calculated volume contains surplus nitrogen to sustain the cattail biomass. Compared to the Dutch regulations for phosphate use on grassland the uptake by cattail is 4.4 times higher than the neutral dosage limit of 90 kg phosphate per hectare per year (2017) [20]. In reality not all biomass will be harvested for further processing, depending on the way of cultivation not all root biomass will be harvested. When only harvesting shoot biomass 181 tonnes LFBS can be "processed", assuming the recorded biomass growth for the first year, a second year cattail culture is likely able to process more LFBS.

Table 3: Cattail composition, calculated protein production and nutrient uptake

	Average plant composition (g/kg DS)					
	crude protein	phosphor	total nitrogen	potassium	magnesium	calcium
Roots	98.5±7.2	3.1±0.3	14.2±2.6	33.6±3.4	2.3±0.2	5.6±0.4
Shoots	85.1±7.4	2.2±0.2	10.3±1.7	39.5±3.1	1.7±0.1	12.6±0.9

	Protein production t/ha/yr	Nutrient uptake kg/ha/yr*	
		phosphor	nitrogen
Roots	2.68	84.7	387.6
Shoots	2.31	58.9	297.7
	Total	143.6	667.3

**based on the average projected biomass yield of 27.2 t/ha/yr*

In both the cattail and soy experiments the biomass yield was highest in basin 1, the lowest biomass yield for both plants was recorded in basin 4. As the wind in The Netherlands mostly comes from south-east direction this possibly might have affected the growth in basin 3 and 4 which are directed westwards, basin 1 is located north-east of basin 4 and might have had an advantage due to the shelter effect that basin 1 provided. In a larger scaled operation biomass yields are expected to be comparable to the yields in basin 1 except for an area of plants that are located on the edge of the field. Another possible explanation for the difference in biomass is the continuous aeration in basins 1 and 2, possibly the addition of air creates a more suitable environment for cattail growth. Since the projected biomass yield and the plant height of basin 2 and 3 are relatively comparable but different from basin 1 and 4 the effect of wind on the biomass seems more likely as basin 2 and 3 have the same position relative to the main wind direction.

In this study the use of cattail biomass is not further practically explored. Additional studies on a larger scale can be performed to investigate the use of cattail for bioenergy, feed, food or chemicals. The tests in this study mainly served to get a rough idea on the growth potential of cattail on LFBS, in further studies different cattail varieties can be compared and optimized for their growth on LFBS.

In literature cattail is mentioned as a bioenergy source. Cattail can, for example, be used in an anaerobic digester to produce biogas/methane (CH_4). Sources [21,22] claimed a biogas production of 150-447 ml CH_4/g volatile solids depending on conditions and cattail parts used, roughly 90% of the cattail dry matter consists out of volatile solids. The above ground cattail parts generally yielded the highest CH_4 volume. The use of cattail as a source of food or feed could also be valuable, in this context harvested plant parts and harvest time are important factors to investigate. Cattail parts have been shown to be an excellent biobased building material for insulation as well as structural components [24].

4.2 Hornwort

The hornwort plants in basin 1 and 2 (with aeration) disappeared quit quickly, after 2.5 weeks the plants were not visible at the surface any more. During the first week it was observed that small particles, likely originated form the LFBS, deposited on the hornwort plants. The particle deposition occurred in a matter of days. The particle deposition might inhibited necessary sunlight for plant growth, additionally the particles might have got too heavy for the plants to stay afloat.

Hornwort growth in basin 3 and 4 (without aeration) showed more success, fresh green shoots started to appear after 1 week. During the experiment duck weed started to populate the surface of the basins, manually it was tried to control duck weed growth as much as possible to maximize sunlight availability for the hornwort plants. After 7 weeks of hornwort growth it was decided to harvest the plants and end the experiment to avoid additional growth limitation due to possible duck weed interference.

The harvested hornwort biomass seemed more dense/bushy (more needles per plant length) than the input material, this could be induced by the plant itself to compensate for the slightly turbid and brown coloured nutrient mixture or possibly the nutrient rich environment caused the plant to grow more dense. Harvested biomass (DW) was extrapolated to a yield per hectare assuming a yearly hornwort growth period of 25 weeks and assuming that the observed growth speed (including duck weed shadow) was representative for an average hornwort growth speed on a yearly basis when using similar (temperature and nutrient concentration) conditions. Except for dry weight determination no additional analyses were performed on the harvested biomass.

Table 4: Hornwort biomass yield

Basin	Harvested wet weight (g)	Harvested dry weight (g)	DM%	Projected hectare yield (t DM/ha)
3	2800	260	9.3%	11.9
4	2500	210	8.4%	9.0

This experiment showed that hornwort is able to grow on a 1% LFBS nutrient mixture, remarkably enough the growth was even better than expected given the opaque brown nutrient mixture properties. Optimization of the culture conditions might lead to even better yields than already achieved. Larger scale experiment could proof valuable to verify the found plant composition and possible plant use. Alternatively other submerged aquatic plants can be subjected to the same conditions for comparison and possibly new uses.

4.3 Soy

The soy plant seeds were sown later than usually on the field (R.D. Timmer et.al, 2016), Dutch field sowing time is from the end of April till half May, the seeds in the experiment were sown on the 6th of June. As described before the soy seeds were sown in a 15x15 cm square distance in rockwool, the first seedlings started to appear 2 weeks after sowing. After 4 weeks no new seedlings sprouted from the rockwool. During plant growth a large variation in development was observed within a single basket, 4 weeks after sprouting some plants already reached a height of ~20 cm while others only measured ~5 cm. The observed differences in plant height were not caught up during the growth season. The first flowers started to appear ~4 weeks after sprouting, bean pods started to develop ~1 week after the flowers wilted. During the growth season no plant diseases were observed.

The germination rates in basin 1, 2 and 4 were lower than normally observed in the field, 50, 61 and 50% respectively. The germination rate in basin 3 was 71% which was similar to field results (R.Timmer personal communication, October 2016). The relatively low germination rate in basket 1,2 and 4 was not compensated by additional biomass growth per plant, at the end of the growth season the space above the baskets was not fully filled with soy plant biomass, from above empty rockwool spots were visible. Basket 3 and 4 showed poor soy plant growth as showed in Table 5.

The soy plants were harvested when wilted and dried out. The length, number of seed pods, number of seeds and the seed mass were recorded. Additionally the top part of the root system was removed from the rockwool and reviewed for root nodule formation.

Figure 4: soy plant growth stages. Plant age from left to right: 3, 6, 11, 12 and 14 weeks. Most right picture clearly shows the seed pods.

Plant height and seed harvest are described in Table 5. The average plant height in the basins 1 and 2, as well as basin 3 and 4, were not significantly different from each other ($p>0.05$).The average plant height in basins with aeration (basin 1 and 2) was significantly different (higher) ($p<0.05$) from the basins without aeration (basin 3 and 4). Seed weight had a positive correlation coefficient with plant height (0.98) and was significant ($p<0.05$).

Although only the northern half of the sown soy seeds were inoculated with *Rhizobium* root nodules formed on inoculated and not-inoculated soy plant roots. Possibly the *Rhizobium* bacteria were able to spread through the wet rockwool and inoculate the southern half of the sown seeds. Based on the made observations the bean yield and formation of root nodules do not seem to correlate, it must be remarked that only the top soy plant roots are scored for root nodules, deeper, well-hidden roots are not examined.

Table 5: Soy plant length and harvested seed mass at the end of the growth season.

Basin	Plant height (cm)			Average seed pods/plant	Seeds		Projected hectare yield (t DM/ha)
	high	low	average		number	mass (g)	
1	74	31	57.0±13.2	39.6±15.9	650	155.7	4.8
2	59	26	45.8±12.0	29.2±16.5	564	138.8	4.3
3	45	22	29.2±7.4	15.2±12.1	363	81.3	2.5
4	42	17	26±6.7	10.6±9.7	201	55.3	1.7

**hectare yield linearly extrapolated from basket yield*

The variation of the plant height and seed production seems to be related to the aeration installed in basin 1 and 2, possibly a higher oxygen concentration in the nutrient mixture generated a more favourable growth condition for the soy plants (DO not measured during experiments) compared to the non-aerated basins. A similar effect was also observed in the cattail plant length, basin 1 and 2 developed larger plants than basin 3 and 4. Another possible explanation for the differences in plant height and seed yield is the effect of wind, since the wind direction in The Netherlands is generally from south-west direction basin 3 and 4 (both oriented in western direction) could have functioned as a windshield for basin 1 and 2 and thereby providing better growth conditions. The first explanation seems most significant as differences were already detected in an early plant development stage when all plants were protected from wind by the basket side walls. In later development stages the soy plants were wind protected by the cattail plants, therefor growth differences in soy plants by the effect of wind is assumed to be minimal.
An effect of *Rhizobium* root nodules on plant height cannot be concluded as only the top part of the soy plant root system (top 5-6 cm) was screened for root nodules, roots that were embedded deeper in the rockwool were not screened. The baskets containing soy plants were placed north of the cattail baskets, as the cattail grew significantly taller than the soy plants they casted a shadow on the soy plants during midday time, the shadow on all individual soy baskets is assumed similar and thereby not causing a difference in potential soy plant development.
To estimate the soy bean production on hectare level the found results were extrapolated (Table 5). No correction for edge effects was included assuming that a higher bean yield was achieved if the soy plants had received full sunlight at midday. The projected yield in basin 1 and 2 on hectare level is similar to what is found on field experiment in 2016 (R.Timmer personal communication, December 2016).

Although the yield in basin 4 is very promising compared to field yield additional larger scale experiments are required to optimize the conditions and verify the found bean yield. Larger scale experiments need to be compatible to a possible “commercial” scale conversion of LFBS to a valuable biomass.

Water hyacinth

4.3.1 Water hyacinth small scale

In general the water hyacinths grew well on the LFBS nutrient mixture. Except for a period of plant yellowing in test condition (TC) 1 and 2 (see Table 1) no signs for mineral deficiency or plant diseases were noticed. The average logged temperature during the experiment was 23.7±5.3^{0}C, with a temperature maximum and minimum of 42.1 and 15.3^{0}C respectively.

During the experiment plants were harvested regularly, per test condition the DM content was determined three times, average DM was used to calculate the total harvested DM and the hectare yield. The total wet weight biomass yields of the test conditions (each 3 boxes) were all significantly different from each other ($p<0.05$), the highest yield was recorded in TC 4, the lowest in TC 2 (Table 6).

Table 6: Harvested water hyacinth biomass, dry matter content and hectare yield projection.

Test condition	Total harvested wet weight (kg)	Total harvested dry weigth (kg)*	DM%	Projected hectare yield (t DM/ha)**
1	12.6	0.66	5.2%	26.8
2	11.8	0.60	5.0%	24.1
3	13.9	0.75	5.4%	30.5
4	15.8	0.80	5.1%	32.9

*total dry weight harvest calculated with DM% from dried test condition sample

**hectare yield linearly extrapolated from test condition yield

During the experiment the nitrogen levels (ammonia, nitrate, organic N) were not analysed, therefore it is difficult to fully explain the differences in pH between the TC's. The calculated dry weight biomass showed a negative correlation coefficient (-0,96) with the average hydroxide (OH^-) concentration in the TC's, the correlation was significant ($p<0.05$). Since the average OH^- concentration correlates to the calculated dry weight biomass it is plausible that the plants themselves generate the variations in observed pH (see Figure 6). The differences in pH between aerated and non-aerated TC's can be explained by different processes which play a role mainly in aerated TC's. Most likely ammonia stripping (removal of ammonia via air bubbles), ammonification (conversion of organic nitrogen to ammonium) and nitrification (conversion of ammonium to nitrate) are active pH influencing processes. The plant influence on environment pH is caused by excretion of H^+ or OH^- to compensate for ammonia or nitrate uptake respectively [20].

After 1.5 week plant yellowing in TC 1 and 2 was detected, this could indicate a deficiency in nitrogen and/or magnesium. As a result of continuous aeration in combination the relative high pH of TC 1 and 2 a nitrogen deficiency caused by ammonia stripping is the most likely cause of the observed yellowing. 2 weeks after first detection the affected yellow leaves turned green again and new plant sprouts didn't show yellow colouration at all. This observed recovery indicates more available nitrogen, possibly due to better ammonia solubility caused by the small drop in pH or by the nitrification of ammonia to nitrate which is not volatile.

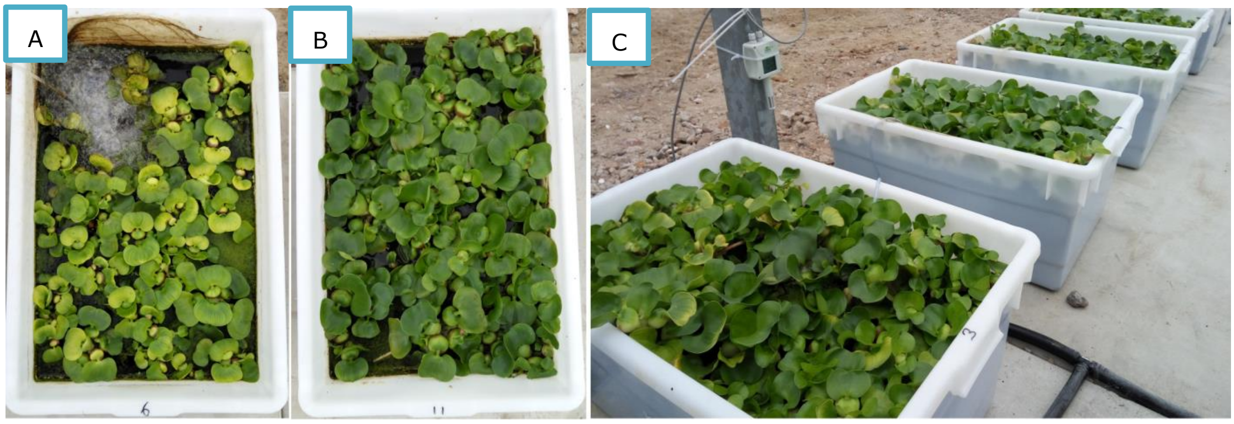

Figure 5: A: TC 2, plant yellowing clearly visible. B: TC 4, same LFBS concentration as TC 2, no yellowing visible. C: Experiment setup, the box on foreground (nr 3) still shows some residual yellowing but the bulk of the biomass looks identical to the non-aerated conditions (not visible in this figure)

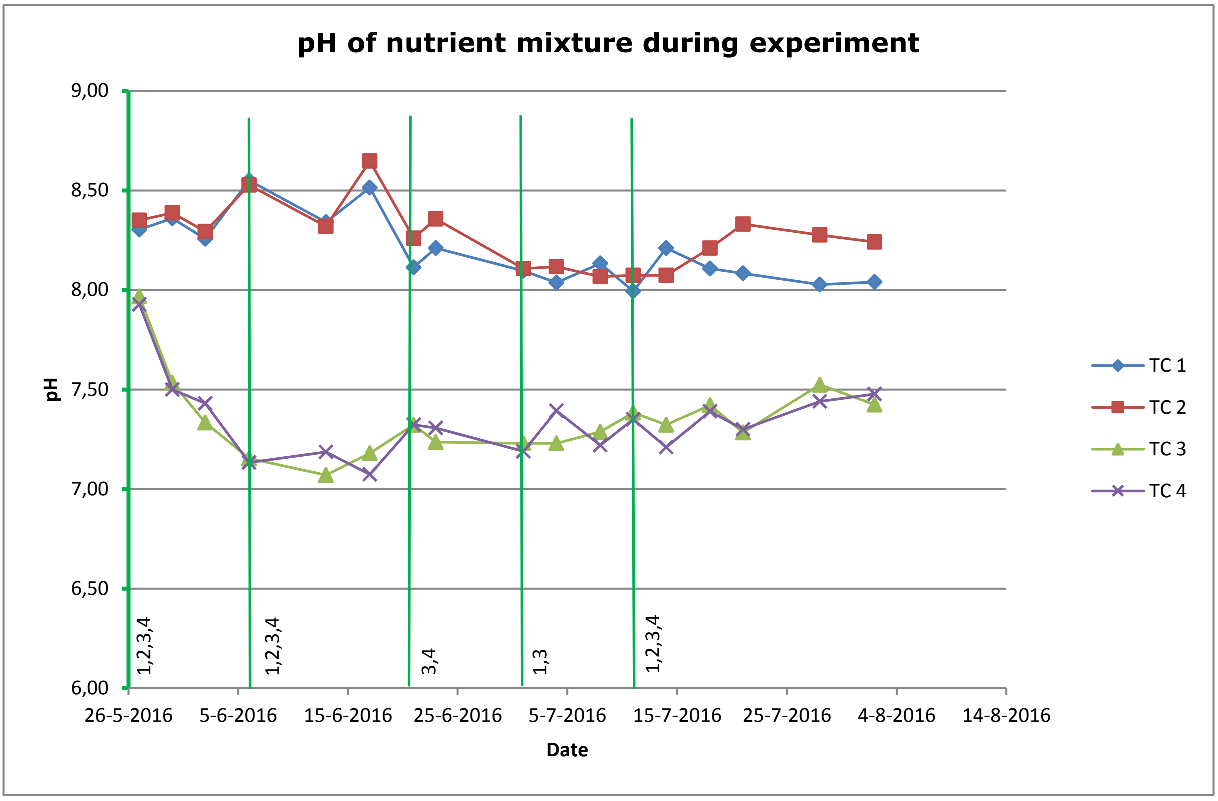

Figure 6: pH of nutrient mixture during the experiment, green lines represent addition of LFBS, at the bottom of the lines the test condition which received LFBS are given

In both TC 1 and 2 and TC 3 and 4 a stabilization in pH arose when the surface area of the boxes was fully covered with water hyacinths and biomass was harvested regularly. At this stage the addition and removal (by uptake, stripping, mineralization, etc) of nutrients created a more or less steady state in the test conditions. Most likely there is an influence on the pH by other nutrients like phosphates and carbonates, these are not discussed in this report.

The average found evapotranspiration was 5.31±0.22, 5.05±0.09, 5.25±0.06 and 5.45±0.16 mm per day for TC 1, 2, 3 and 4 respectively. No control was used to distinguish plant transpiration and water surface evaporation from each other. The measured evaporation differences between the TC's were not significantly different from each other (ANOVA, $p>0.05$), this could indicate that water evaporation from the water surface has the largest influence on the total evaporation.

During the experiments samples were collected and dried for analyses, at the end of the experiment all samples were analysed on crude protein, total nitrogen, phosphor, potassium, magnesium and calcium.

Based on the plant composition a yearly nutrient uptake is calculated assuming an average of the projected biomass yield (Table 6). Based on the average composition of the added LFBS in this experiment (appendix 1) and average phosphorus uptake a yearly LFBS consumption by water hyacinth is calculated. One hectare of water hyacinth culture could "process" 524 tonne of LFBS/year, this calculated volume contains surplus nitrogen to sustain the water hyacinth biomass. Compared to the Dutch regulations for phosphate use on grassland the uptake by water hyacinth is 5.8 times higher than the neutral dosage limit of 90 kg phosphate per hectare per year (2017) [20].

Table 7: Average composition of water hyacinth, calculated protein production and nutrient uptake.

Average plant composition (g/kg DS)					
crude protein	phosphorus	total nitrogen	potassium	magnesium	calcium
164.5±24.3	6.6±2.0	28.5±5.6	69.3±4.5	5.6±1.2	19.0±4.0
Protein production t/ha/yr	Nutrient uptake kg/ha/yr*				
	phosphorus	total nitrogen	potassium	magnesium	calcium
4.70	189.3	812.7	1979.6	160.0	542.8

**based on the average projected biomass yield of 28.6 t/ha/yr in greenhouse conditions*

The cultivation of water hyacinth without the use of a greenhouse could be considered, this reduces initial investment costs but also reduces LFBS processing capacity by the water hyacinths.

4.3.2 Water hyacinth race way pond

At the 20th of September the water hyacinth fresh weight density reached ~10kg/m^2, in total 573 kg fresh biomass was harvested. In 35 days the biomass density in the RWP restored to ~10kg/m^2. Based on the harvested surface area, regrowth time and plant dry weight it was calculated that 24.6 t DM/ha/yr could be harvested. Compared to the experiments in the boxes the yield was lower, 4 t/ha/yr and 8.3 t/h/yr for the average and the best found yield respectively. The day length at the start of the experiment was ~12 hours at the end of the experiment ~10 hours, compared to a day length of ~16 hours at the longest day period of the year significantly less daylight is available for plant growth. It is expected that a year round biomass yield experiment would harvest more biomass than found in this experiment.

In general the propagation of water hyacinth in an energy extensive system (RWP without continuous mixing) proofed to be possible and it is expected that the maximum projected yield found in the box experiments (32.9 t/ha/yr) could be achieved or even exceeded.

4.3.3 Biogas potential of water hyacinth

According Malik [26] the use of water hyacinth as a co-product in a co-digester seems challenging, due to the low specific weight (spongy plant tissue) of the plant material it tends to float in conventional digesters. Another challenge seems to be the low dry matter content of the plants. Air drying the water hyacinth combined using a high impact mill to destroy the spongy plant structure could improve the usability of water hyacinth as a digester co-product. The biogas potential of water hyacinth in anaerobic digestion varies widely, depending on conditions a yield of 52-560 ml CH_4/gr volatile solids is mentioned. Approximately 80-90% of the water hyacinth dry matter are volatile solids. (Various resources, not listed)

It can be concluded that the use of water hyacinth as a co-digestion co-product requires some technical development and additional research to investigate its true potential and value. Possibly the water hyacinth has its use as biobased material, the spongy core could be used an insulation material. Possibly the plants can be ensiled in combination with other agricultural crops as a feed source.

5 Business case

Based on the results found in this research a business case is set up in which the production costs for water hyacinth and cattail is calculated. In addition the economic value for the plants is calculated when used as a source of biogas or livestock feed.
The results of these economic calculations are combined with a micro algae cultivation business case. The combined results can be found in WPR report 736, Production of aquatic biomass on residual streams, Evaluation research 2013-2016.

6 Conclusions

- This study proofs that the tested plants (cattail, water hyacinth, hornwort and soy) can grow on liquid fraction bio slurry when used in a 1 or 2% dilution in water under aerated or non-aerated conditions.
- The highest projected dry matter yield was 38.9, 32.9 and 11.9 t/ha/yr for cattail, water hyacinth and hornwort respectively
- The highest projected soy bean yield, 4.8 t/ha, is comparable or better than field yield.
- Aeration of the liquid fraction bio slurry dilution has a negative effect on the growth (and energy costs) of water hyacinth and hornwort but has a positive effect on de seed production in soy. The effect of aeration is not fully clear for cattail.

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Annex 1 LFBS composition

Average composition of LFBS used in the experiments as nutrients source for cattail, soy and hornwort. Average composition is based on analyses performed by Eurofins, December 2016.

		LFBS used in basins	LFBS used in boxes
Component	Unit		
Dry matter	g/kg	40.7	41.6
Ash	g/kg	14.7	15.0
Organic matter	g/kg	26.0	26.7
Nitrogen (N)	g/kg	3.2	3.1
N-NH3	g/kg	1.6	1.6
N-org	g/kg	1.6	1.5
Phosphor (P)	g/kg	0.3	0.4
Phosphate (P_2O_5)	g/kg	0.8	0.9
Potassium (K)	g/kg	4.0	3.8
Potassium oxide (K_2O)	g/kg	4.8	4.6
Magnesium (Mg)	g/kg	0.5	0.5
Magnesium oxide (MgO)	g/kg	0.8	0.8
Sodium (Na)	g/kg	0.6	0.6
Sodium oxide (Na_2O)	g/kg	0.8	0.8
N/P ratio		9.5	7.8

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