ALGAE CULTIVATION USING DIGESTATE AS NUTRIENT SOURCE: OPPORTUNITIES AND CHALLENGES

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ABSTRACT: In recent years, algae production has attracted increasing scientific interest, mostly because of potential high biomass yields for production of lipids, protein and chemicals. For large-scale production of algae, large amounts of nutrients are needed. Replacing commonly used artificial fertilisers with recycled or alternative sources of nutrients is highly important, both for lowering algae production costs and for displacement of phosphate rock mining. This study evaluated the possibility of producing algae biomass in an economically viable way using digestate as a source of nutrients and by combining the cultivation of algae with a codigester and CHP unit for usage of flue gass and residual heath. A short literature review is made and results from a pilot plant and a small scale expeiment in the Netherlands are given. Several algae species can grow on diluted digestate. Combining algae cultivation with codigestion profits if algae are sold as feed additive. Renewed regulations ask a risk analyses and additional analyses to allow the algae produced to be sold as feed.
Keywords: algae, anaerobic digestion, CO_2 capture, pilot plant, proteins, wastewater treatment

1 INTRODUCTION

A co-digestion process for manure and co-products is usually integrated with a CHP unit to produce electricity by burning the biogas. In areas with a high density of livestock, such as the Netherlands, the amount of manure or digestate that can be distributed on farm land is limited due to regulations implemented to prevent eutrophication and related problems. As a result, disposal of manure and digestate represents an important cost factor for many livestock farmers. Use of digestate in algae cultivation is an interesting option, as algae production yields can theoretically be higher than that of 'normal' crops, reducing the land area needed for recycling the nutrients [16].

In recent years, algae production has received growing scientific attention, mostly because of potential high biomass yields for production of lipids, protein and chemicals [1, 16]. For large-scale production of algae, large amounts of nutrients – mainly phosphorus and nitrogen – are needed. Replacing the commonly used artificial fertilisers with recycled or alternative sources of nutrients is highly important [6].

A digester is often combined with a CHP unit to produce electricity. The exhaust gases and excess heat can be used to enhance algae production. After digestion, a useful destination for the digestate has to been found. This creates important costs in producing biogas and electricity with co-digestion. In particular, aqueous fractions of digestate with relatively low amounts of nutrients has to be transported and sometimes also sanitised before discharge to the environment.

The objective of this study is to evaluate the possibility of producing algae biomass in an economically viable way using digestate as a source of nutrients and in combination with a digester.

2 MATERIAL AND METHODS

2.1 Data collection

The background to the study was research results on algae cultivation using digestate presented in the literature and obtained in recent pilot-scale studies in the Netherlands. In determining whether and how digestate can be used for cultivating algae, information is needed and partly collected on:

- The concentration of nutrients (e.g. N, P, trace elements) related to the nutritional requirements of various species of algae
- The influence of digestate treatment on nutrient availability (free or bound in organic material)
- The presence of toxic levels of certain components – for algal growth and for processed algal biomass (e.g. Cu) or beneficial compounds (C sources for mixotrophic algae [30])
- The influence of suspended particles on light transmission – and consequently growth rate – in algal growth medium
- Possible contamination with pathogenic microorganisms

2.2 Pilot plants

Many publications address algae cultivation using digestate, manure or wastewater as the nutrient source [2, 3, 7, 14, 18, 24, 29, 30], but only a few report the results of pilot-scale studies [8, 19]. In the Netherlands, pilot plants for algae production, fed with digested manure and digested brewery waste, have been started. The aim is to improve the economics of local biorefinery projects and increase local recycling of nutrients, organic residues and water.

At the open public-private pilot site of ACRRES (Application centre of Renewable Resources) (1, 2), an innovative small-scale biorefinery concept is being constructed and tested, with the aim of local maximisation of biomass valorisation for food and non-food purposes. The concept is directed at transporting high value products (end-products and semi-manufactured products), resulting in minimised transport of water and maximised local re-use of nutrients. This type of small-scale biorefinery can be a step in the development and implementation of a biobased economy. The valorisation of residues and associated processes play a key role in enhancing the economic efficiency and sustainability of production of green gas, electricity and basic components for feed, food or fuels based on (co)fermentation and bioraffinage (Fig. 1).

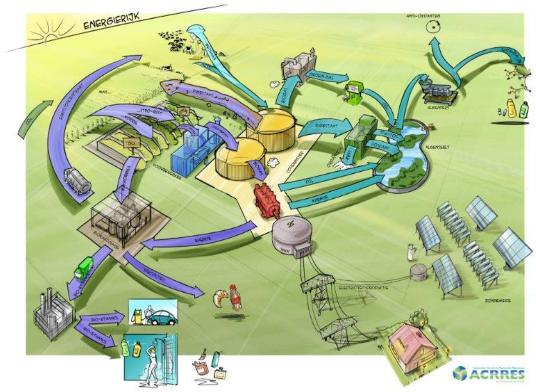

Figure 1: Schematic presentation of the ACRRES pilot site.

In order to research and test new production methods, nutrient recycling and soil quality issues, the pilot project currently comprises:

- A pilot digester, CHP (123 kwE) and associated clamp silos and digestate storage
- A bio-ethanol plant with a capacity of 150 000 L per year (3)
- An installation for hydrolysis of recalcitrant biomass for fermentation processes and other follow-up processes
- Open production systems for algae situated in a greenhouse or in open field ponds of both 250 m^2 and 100-200 m^3 depending on culture depth, and open LED-lighted bioreactors ranging in size from 1 to 60 m^3
- An installation to upgrade biogas to green gas (4),
- Arable land and grassland available to produce biomass and cattle (dairy farm) to produce manure.

In addition, the possibility of decentralised processing of (aqueous) residues that occur locally within farms, landscape and water management organisations and processing industries is being explored. The ACRRES concept being tested is not a fixed solution, but keeps evolving with new developments in technology and environmental research. At the moment, the addition of a refinery process for manure or digestate is being considered. In a broader perspective, ACRRES is open to new and innovative technologies that can help to maximise local utilisation of available resources.

At the ACRRES pilot site, the algae culturing and harvesting technology of the company Algae Food & Fuel (AF&F) (5) is being tested. The ponds are used to grow algae fed by minerals from digestate or artificial fertilisers and CO_2 from the flue gases of the nearby digester and the CHP engine. Growth rate of algae is further increased by using excess heat from the CHP engine to support algae productivity. The algae are harvested 3-5 times a week almost daily year round. Temperature, absorption and minerals in the water are monitored. Dry weight and species composition are determined. At present, the algae biomass produced is mostly used for high value feed applications (feed additive). Together with our partners [31], we plan to add a pilot plant for testing algae refinery methods to allow separation of the biomass into valuable components such as proteins and PUFAs.

The algae production site of ACRRES is one of the pilots in the interreg project Energetic algae (Enalgae) (6). An economic model in Excel is being developed in the WP 2: Action 7 Markets and economics. This model version 3.0 is based on the open algae ponds built at ACRRES, Lelystad [21]. Data from this pilot will be presented in this paper, both on measured productivity as well as economic calculations.

In cooperation with commercial partners, data are also collected at smaller LED-lighted pilot bioreactors (1 m^3) cultivating algae and removing nutrients from the digested effluent of a brewery [25].

At least two commercial agricultural companies in the Netherlands also cultivate algae in combination with a digester. Experiences and data from these farms are being collected but are not presented in this paper. Kelstein (7) combines an organic dairy farm with a digester and cultivation of algae sold mainly as dried feed additives. This firm operates LED lighted open bioreactors and open algae ponds of 5000 m^2 or 2500 m^3, in which artificial fertilisers are used. In one of these ponds, organic algae production is being trialled, using extraction of thick fraction digestate for phosphate and micronutrients and using ammonia containing dry air damped from the thin fraction of the digestate. The farmer Kroes has started to cultivate algae in closed photo bioreactors on the roof of his farm to feed his calves with algae water. For the cultivation of algae and duckweed, source-separated urine and the thin fraction of digested manure are being used. The ideas of Ecoferm (8) are practised on this farm.

2.3 Small-scale experiments

The possibility for algae species to grow on treated digestate was studied in more detail in small-scale experiments. In earlier experiments [21], it became clear that feeding with CO_2 in the culture is needed for relevant results concerning dose-response relations of algae growth with digestate.

The small-scale growth experiments were run in 25-L cultures in the climate-controlled trial greenhouse of PPO in Lelystad, at a temperature of 15-25 °C through exposure to growth lamps. To stimulate growth, the cultures were fed with CO_2 (breeders' mix) based on a timer (during daylight time 10 minutes per hour of CO_2). In addition, algae were circulated by placing two aquarium pumps in these mini ponds [22].

The cultures were inoculated with a 2-L seeder mix of species proven to grow on digestate dilutions in earlier experiments [21]. This ACRRES mix consists of > 90% from *Chlorella* sp., *Scenedesmus* sp. and *Phaedactylum* sp. After inoculation, the growth of the cultures was followed by determining absorption at 440 nm and 695 nm using a Hach Lange DR3900. Additional cell counts were performed using a Bürker Türk counting chamber (Labor Optik), with a light microscope under 400x magnification (Leica DM). In some experiments the dry weight of the algal cultures was also determined. Treatments comprised replacement of the artificial growth medium with increasing amounts of thin fraction digestate (0, 25, 50, 75 or 100%). The experiments were performed in triplicate and the results analysed using Genstat 16th edition SPI.

3 RESULTS AND DISCUSSION

3.1 Microalgae species selection

Around 40 000 algae species are believed to exist, of which only a few thousands are kept in collections and a few hundred have been investigated for chemical content [1]. Even less research has been devoted to investigating the growth possibilities of microalgae on digestate (Table 1).

Table I: Overview of microalgae species used and able to grow in experiments with digestate

Genus	Species	Source
Chlorella	sp	[3,7,10, 30]
Chlorella	*sorokiniana*	[14]
Chlorella	*vulgaris*	[11,29]
Dunaliella	*tertiolecta*	[11]
Euglena	*gracilis*	[11]
Microspora	*willeana*	[17, 33]
Neochloris	*oleoabundans*	[12]
Oedogonium	sp	[17, 33]
Phormidium	*bohneri*	[7]
Rhizoclonium	*hieroglyphicum*	[17, 33]
Scenedesmus	sp	[2, 3, 24]
Scenedesmus	*accuminatus*	[18]
Scenedesmus	*dimorphous*	[29]
Scenedesmus	*obliquus*	[7]
Synechocystis	sp	[3]
Ulothrix	*aequalis* Kütz	[33]
Ulothrix	*ozonata*	[17, 33]

The microalgae that are able to grow on diluted digestate include both freshwater species and marine species.

The mineral content of algae differs between species and production sites. Some detailed data have been collected by ECN for the freshwater species *Chlorella* and two samples of the marine species *Synechococcus* (Table II). The mineral content of the dried algae provides an indication of the minerals needed by the algae as nutrition.

Table II: Mineral content of dried algae in g/kg dry weight (9)

	Chlorella	*Synechococcus*	
Mineral	#2333	#2327	#2328
Nitrogen (N)	113.0	115.6	105.0
Phosphorus (P)	12.6	11.2	11.9
Potassium (K)	5.4	11.3	8.7
Sulphur (S)	6.9	5.0	7.7
Iron (Fe)	5.9		
Magnesium (Mg)	3.7	4.2	3.5
Calcium (Ca)	1.0	1.6	2.2
Sodium (Na)	0.7	2.3	3.4
Manganese (Mn)	0.3	0.4	0.5
Chlorine (Cl)	0.1	0.2	1.2

3.2 Sources of digestate

Different algae species have different preferences for growth medium. The amounts of nutrients in the artificial fertiliser medium used by ACRRES for algae production in open ponds are shown in Table III. The medium was developed by experimenting with different available cheap fertilisers used in horticulture. Because many of the salts are in compounds with potassium, the medium has a rather high potassium concentration. In the ponds, CHP gas is injected for the provision of CO_2. Because of the sulphur and small traces of metals in the CHP gas, hardly any sulphur is added to the artificial fertiliser medium.

When digestate is used instead to fertilise algae production, it has to be diluted if it originates from co-digestion with dairy, swine or poultry manure. Table III shows the mineral content of growth medium made from digestate diluted 200-fold. Dilution is also necessary because of the absorption of light by the organic substances present in digestate. At 200-fold dilution, the optical density (OD695) is already 0.28 without any algae growing. Because some of the algae species used are mixotrophic, the lower light intensity is probably compensated for by use of the organic C sources in the digestate.

The N/P ratio of the diluted digestate is comparable to that of the artificial fertiliser mixture and is fairly close to the Redfield ratio of 7, which is good for growing algae. However, the concentrations of chlorine and some minerals are much higher in the digestate. The high chlorine concentration is probably the reason why the marine species *Dunaliella tertielecta* can growth on higher amount of digestate than *Chlorella vulgaris* [11].

Table III: Mineral content (mL/L) in growth medium based on artificial fertilisers and on 200-fold diluted digestate (#1 = thin fraction after screw press filtration of digestate from the ACRRES dairy farm co-digester, #2 = thin fraction digestate from the Heeten piggery manure co-digester, #3 = digestate from a chicken manure co-digester)

	Fertiliser	*Digestate*		
Mineral	ACRRES	#1	#2	#3
Nitrogen (N)	25	25	25	37
Phosphorus (P)	4	4	2	6
Potassium (K)	77	25	8.7	25
Sulphur (S)	0.5	2	7.7	?
Iron (Fe)	0.1	?	?	?
Magnesium (Mg)	1	1	3	4
Calcium (Ca)				
Sodium (Na)		10	3	?
Manganese (Mn)	0.1	?	?	?
Chlorine (Cl)	4	120	327	?
Zinc (Zn)	0.01	0.07	0.33	0.26
Copper (Cu)	<0.01	0.02	0.15	0.10

? not determined

Copper is a mineral which is also used to control algae when they are not wanted. A concentration of more than 0.1 mg/L of zinc and copper can reduce the growth of some algae [4,32]. Because the concentrations of these minerals vary between different digestates, this issue needs special attention. It may require an adjustment in species selection towards Copper and zinc tolerant species. The other option is to adjust the method used for pre-treatment of the digestate before usage in algae production including several methods to achieve the thin fraction, ammonia stripping, air flotation, membrane filtration and reversed osmosis can also be used [9]. Pre-treatment of digestate will also influence the availability of the nitrogen and phosphorus (bound or free).

The high concentrations of minerals in animal manure-based digestate distinguish it from digestate originating from breweries. Digestate from brewery process water can be used for algae production without dilution [25]. Because part of the nutrients are organic bound, resting time before using this digestate or probably other pretreatments f.e. disolved air flotation increase the growth possibilities of the algae.

3.3 Growth of algae on digestate and in combination with a digester

In small-scale experiments, growth of the algae species mix with different percentage replacement levels of the fertiliser by diluted digestate was determined (Fig. 2).

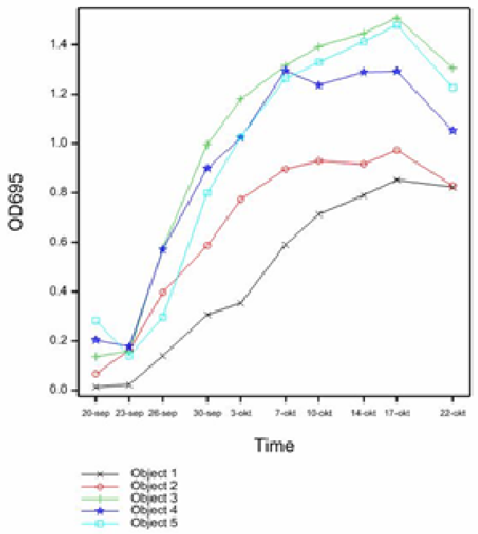

Figure 2. Growth of algae species, determined as increase in optical density (OD695) over time, with different combinations of artificial fertiliser (f) and digestate #3 content in table III (d). 1: 100% f; 2: 75% f and 25% d; 3 50% f and 50% d; 4 25% f and 75% d; 5 100% d) [22].

The optical density increased with increasing replacement of part of the artificial fertiliser by digestate. However, with a higher percentage of digestate, the algae needed some days to acclimatise to the new medium. After 14 days of growth, dry weight of algae was significantly higher when at least 50% of the nutrients came from digestate (Table IV). Nitrogen was as nitrate in the artificial fertilizer medium and as ammonium in the digestate. This can be the reason of even better growth on diluted digestate, but also the availability of C sources for the mixotrophic algae in the digestate [30].

Table IV. Dry algae mass after two weeks of growth with different dilutions of fertiliser and digestate, content of fertilizer and digestate #3 in table III [22]

Object	Dry weight algae (g/L)	
1 100 % fertiliser (f)	0.253	a
2 25% f/ 75% d	0.476	b
3 50% f/ 50% d	0.864	c
4 75% f/ 25% d	0.678	bc
5 100% digestate #3 (d)	0.651	bc
LSD	0.215	
p-value.	<0.01	

Algae can produce more proteins per hectare soil than normal arable crops and also use more nitrogen per unit surface area [16]. In open ponds with a light use efficiency of 1.5%, algae theoretically produce as much as 25 ton dry weight per hectare in Dutch light conditions in water of 25 °C [13, 21]. With a measured protein content in the harvested algae of around 50%, this would mean a protein yield of 12.5 ton per hectare. The protein yield of arable crops is in the range 1-2 ton per hectare [27, 28].

Besides recycling part of the nutrients by combining algae cultivation with co-digestion [26], additional expected benefits are the possibility to use the residual heat and the flue gases of the CHP unit for algae cultivation.

In 2013, it was found that using the residual low value heat (1.8 GJ per pond per day) in a floor heating system under the algae ponds increased the temperature by around 5 °C in the outdoor open pond and 10 °C in the indoor pond. Because a set-point of 25 °C was used, floor heating was not active in the summer months in the pond in the greenhouse. With a simple yield model based on Dutch light and temperature conditions [21], the effects of the additional heat on algae cultivation in the Netherlands were estimated (Fig. 3). The effects of using residual heat on yield are highest in spring, when the low culture temperature is limiting algae growth while light conditions rapidly improve (Fig. 3).

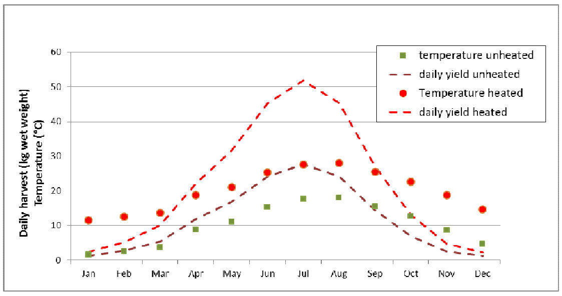

Figure 3. Estimation of the effect of increasing medium temperature by 10 °C, using the residual heat of the CHP unit, on algae growth in open ponds at ACRRES, Lelystad, the Netherlands.

The expected yield in open ponds under Dutch light conditions with 1.5% light use efficiency and 25 °C is 25 ton dry weight algae per hectare. Corrected for the lower temperatures in autumn, winter and spring and with the extra heat provided by the CHP unit, the expected yield is 16.9 ton/ha (comparable with light use efficiency of 1%). However, at the start of the data collection we harvested less biomass (Fig. 4). The main reason for this was that the injection of flue gas had to be tested first for safe use in the greenhouse [21]. In Figure 4, the measured yield in the ponds (data points) is expressed as a percentage of the light use efficiency achieved based on the measured amount of incoming light. A light use efficiency of 1, 0.5 and 0.25% corresponds to 16.8, 8.4 and 4.2 ton dry algae per ha, respectively. The black line represents injection of flue gas. Thus, the harvest in weeks 26-30 was achieved without the addition of flue gas. However, the ponds were aerated with air. Weeks 31-48, flue gas was added manually to the system for a period of 2 hours per day. After this test period, continuous addition of flue gas was started, using pH to control the rate. However, technical problems soon developed, so this pH-based flue gas injection only functioned in week 49, and in weeks 50-52 no flue gas was injected.

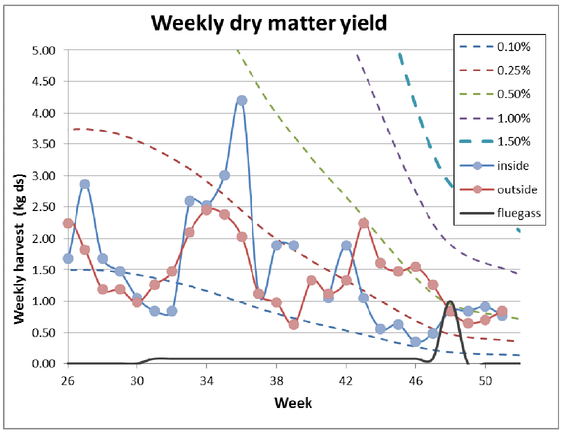

Figure 4. Productivity of the algae ponds in Lelystad in 2012 (kg dry matter yield of biomass per week) compared with the theoretical productivity based on percentage conversion of solar energy into biomass (dashed lines). Black line shows flue gas addition to the process.

In weeks 26-30 (without flue gas injection), about 0.1% of the energy from sunlight was converted into biomass and harvested (Fig. 4). In the long period with flue gas injection for 2 hours per day (weeks 31-48), a conversion of roughly 0.25% relative to the average light intensity was achieved. However, there was also an increase in the conversion efficiency in this period. This was not the result of a larger amount of biomass harvested, but of a decrease in the light intensity. Part of the increased efficiency is most attributable to inertia in the system, as yield follows daylight intensity with a lag phase. In addition, algae growing at a lower light intensity may be better able to convert the energy available into biomass.

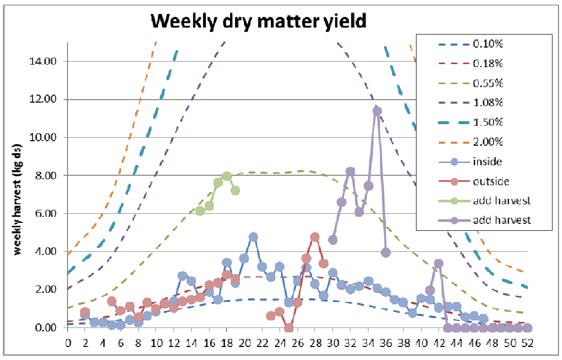

Figure 5. Productivity of the algae ponds in Lelystad in 2013 (kg dry matter yield of biomass per week) compared with the theoretical productivity based on percentage conversion of solar energy into biomass (dashed lines). During week 14-18 and 29-36 additional harvesting installed.

During data collection in 2013 (Fig. 5), it became clear that the harvesting system also reduced the productivity of algae growing in the ACRRES pilot process. In 2013, during weeks 14 to 18 and 29 to 36 productivity was increased to more than 9 ton/ha by installing another harvesting system in the outdoor algae pond. In 2014, a refined harvesting system using flocculants in combination with dissolved air was installed and will be tested later this year. Preliminary test results indicate a further increase in yield, combined with less energy usage for harvesting.

3.4 Economics of algae growth in combination with a co-digester

An economic model in Excel is being developed in the Interreg project Energetic Algae (EnAlgae) WP 2: Action 7 Markets and economics. This model is based on the open algae ponds built at ACRRES, Lelystad, and will be further improved with the experiences of Enalgae partners and other experts [21]. This model is freely available to anyone wishing to discuss and improve the economics of algae cultivation.

Calculations with version 3 of this model have been made to determine the economics of algae production in an open pond in the Netherlands [21]. Based on the expectation that it will be reasonable to achieve productivity of dry algae yield of 17 ton per hectare and year, the cost price of one kg of dry algae mass can be calculated. Based on the assumptions used [21] and for a scale of 1 hectare of algae, the calculated cost price is around 6 Euro per kg. This is in line with calculations by Wijffels (10).

The main contributors to the cost price in our calculations are the cost of capital (interest, depreciation, maintenance and insurance) and labour and power costs. The cost price is almost doubled when algae cultivation is not coupled to a digester for the use of flue gas and residual heat. At a selling price for the dry algae biomass of 35 Euro per kg dry matter (as a valuable feed additive [not just for its energy content), the calculated return on investment for the ACRRES site is 20-25%.

3.5 Use of commercially produced algae and legislation

Many studies on the possibilities of utilising microalgae biomass in human and animal nutrition have been published [see reviews by 1, 15, 20, 23]. Algae often contain many valuable proteins, which can be safely digested by several species of farm animals as part of their diet [1]. Algae also contain valuable vitamins and pigments. They are the main producers of the omega-3 and -6 fatty acids in the food chain [20, 23]. Claims that they can be a useful additive to improve the health of animals and humans have been supported, at least partly, by research [1, 15, 20]. Positive effects reported to result from algae consumption include: a low of mortality rate in young turkeys, lower microbial infection in chicks, increased milk production in cows, lower feed-based cholesterol concentration in the blood of cows, higher feed conversion efficiency in pigs, decreased intensity of pain in humans suffering from fibromyalgia syndrome, lower levels of toxins in human breast milk, and improvement of IQ and serological blood parameters in humans. However, these effects are species-specific and dependent on growing conditions and they also differ between studies, so more research is needed [1]. Applications of algal biomass for generating value-added animal products is also seen as a great potential [15].

EU feed legislation changed recently. In the past, algae biomass was not permitted to be sold as a feed additive if manure or digestate had been used as the nutrient source. Since 2013, the GMP+ rules have changed (11). Now, algae biomass can be used as a feed additive if a risk analysis has been conducted on the production method and the products which will be sold have been analysed to ensure that they contain acceptable levels of certain compounds and pathogens.

For use in human food, each algae species and production method has to been registered as a novel food. The species of micro-algae that are currently used as food or food ingredient are restricted. Mainly *Spirulina (Arthrospira)*, *Chlorella*, *Dunaliella* are used. In some specific regions other species can be found, like *Nostoc* and *Aphanizomenon* [20].

4 CONCLUSIONS

Cultivating algae in open ponds is more profitable when carried out in combination with a digester. This is also more favourable from an environmental perspective, because nutrients and organic by-products can be recycled and flue gas and residual heat can be used.

Several species of algae can be cultivated using the diluted digestate from co-digestion of manure and other organic wastes as the main nutrient source.

The economics of algae cultivation currently require a high selling price for the algae, for example as a valuable feed or food additive. However, such high value markets require prior risk analysis to confirm that it is safe to produce algae with treated digestate and additional analyses to sell it as a GMP+ feed.

Algae are claimed to have a number of proven and unproven benefits as a feed or food additive in terms of health improvements and value added to meat and eggs. The proteins and oils in algae are also valuable in the diet of animals and humans. Algae can be cultivated in circumstances where crops cannot be grown, can achieve much higher productivity than crops and can recycle phosphorus from water. Thus, increasing algae production deserves attention in a world with limited resources and a growing population.

5 NOTES

(1) Short company film about ACRRES at http://www.youtube.com/watch?v=vSYgjIqWA8I
(2) Short film closing the loop with algae at ACRRES at http://www.omroepflevoland.nl/Nieuws/95170/nieuwe-algenkas-officieel-geopend (in Dutch)
(3) More information about the company producing bioethanol in combination with a digester at http://byosis.com/producten/byosense.html (in Dutch)
(4) More information of the pilot to test the upgrade of biogass to green gas on http://www.dirkse-milieutechniek.com/dmt/do/webPages/202356/Biogasupgrading_small_size.html
(5) More information about the company producing LED lamps and cultivation systems for algae at http://www.algaefoodfuel.com/english/home/
(6) More information at www.enalgae.eu
(7) More information about the dairy farm producing algae for feed at http://www.kelstein.nl/ (in Dutch)
(8) More information about the ecoferm concept practized by a commercial calf meat producer http://www.innovatienetwerk.org/en/concepten/view/109
(9) Data from database accessed 19062014: https://www.ecn.nl/phyllis2/Browse/Standard/ECN-Phyllis#microalgae
(10) Recent note at http://www.wageningenur.nl/nl/nieuws/Duurzame-algenteelt-krijgt-vaste-voet-aan-de-grond.htm
(11) Relevant regulations GMP+: https://www.gmpplus.org/pagina/2241/gmp-fsa-certification.aspx, https://www.gmpplus.org/bestand/4224/gmp-b6---en-20130301.pdf.ashx, , https://www.gmpplus.org/pagina/9/standards.aspx

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