Lettinga Award 2017 **Final Report**

1. Author/applicant (= contact person)

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2. Project title

Dark photosynthesis: *anaerobic biosynthesis of food from wastewater and electricity*

Proposal abstract

Agriculture must be coupled to wastewater treatment for a sound circular economy. In addition, the growing world population increases the pressure on nature areas and enhances water scarcity. By dark photosynthesis, food is produced in a bioreactor in a water and energy efficient way. Hereby no arable land is used which will reduce the pressure to break down our 'last' natural sites. Dark photosynthesis is an idea in which photosynthetic micro-organisms (e.g. algae) are grown in the dark while an electrode provides the electron donor and conditions needed for growth. By providing a nutrient rich wastewaters (e.g. urine) high value biomass can be produced which is in potential food of feed grade. So far, phototrophic micro-organism do grow bioelectrochemically with light. We have hypothesis that we can create conditions which will also allow growth in the dark. With this award we will experimentally validate our idea and will use real (diluted) urine as primary nutrient. This way we expect to open new doors of research on anaerobic biosynthesis technologies which use wastewater as raw feedstock.

For what specific purpose was the Lettinga Award of 10.000 Euro used?

The Lettinga Award was used to do experiments while using a Microbial Electrosynthesis System (MES) and a hydrogen with fed far-red-light supplied bioreactor using several selected phototrophic organism as biocatalyst. The objective was to show anaerobic biosynthesis of high value potentially food grade biomass from wastewater and electricity. The experimental work was primary done by Mathijs van der Zwart, 3 MSc thesis students (Yi Jiun Chu; Math Lambalk; Javier Reynoso Lobo). Technical and experimental design input was completed with David Strik, Prof. Cees Buisman and postdoc Ludovic Jourdin.

The detailed budget use is given in below table while other explanations are given in the text. We tested several species in Microbial Electrosynthesis Systems (MES) we obtained from culture stocks including Prosthecochloris sp; Rhodobacter sphaeroides; Shewanella sp.; Geobacter sp. ; Cupriavidus necator; *Prosthecochloris aestauii* and *Arthrospira platensis* (commonly known as Spirulina). We tested the species either pure or mixed them with each other. Under MES conditions, unfortunately, no growth was observed (see budget use on species). We also tested a mixed undefined culture and did let it grown in a MES with a full

light spectrum. Under these conditions growth did occur; these results we send for a poster presentation at AD conference in Delft (see attached poster-presentation).

Based on all the gained insights we decided to do the follow-up experiments with provision of limited light supply using a specific bandwidth of light (which should stimulate the PS1 system and limit the PS2 system according to our hypothesis) and Spirulina as organism. This organisms is a cyanobacterium which received a GRAS (generally regarded as safe) status by the FDA in 2003 (GRN. No 127), and more recently it received the label 'superfood' due to its nutritional characteristics including a high protein conten. Hereby we used H2 as electron donor and developed a new set-up to provide light (see images in presentation at AD conference in Delft). (see budget use on several parts including stir-plates).

We were successfully in the growth of spirulina supplied with hydrogen and far red light. The reactor was not kept aseptic; a such a bioreactor microbiome was created with significant growth and presence of spirulina species. In addition we showed that this organism is able to grow on fresh diluted real human urine (of one of the students). (see budget use on analyses costs). The results of this work were presented at the AD conference in Delft (see attached presentation) and are currently processed into a scientific research article. We show hereby firstly that a H2-far-red light bioreactor process to grow a spirulina enriched microbial biomass is feasible. This is a relevant step towards the idea of the anaerobically synthesis food from wastewater and electricity.

Table Budget use

Monsterkosten	76106773, Freight + Handling fee	26.62
Monsterkosten	76106773, Prosthecochloris sp.- active culture	151.25
Monsterkosten	76106773, Rhodobacter sphaeroides (van Niel 1944) Imhoff et al. 1984 - Freeze Dried	96.80
Monsterkosten	76106773, Shewanella sp. - Freeze dried	96.80
Monsterkosten	76106773, Geobacter sp. Freeze dried	96.80
Monsterkosten	76106773, Cupriavidus necator Makkar and Casida 1987 - Freeze dried	96.80
Chemicaliën	76103770, HYDROQUINONE 99.5%	15.61
Chemicaliën	76103240, METHYL VIOLOGEN DICHLORIDE HYDRATE, 98%	69.58
Chemicaliën	76103240, Totaal kosten	18.15
Chemicaliën	76108420, 3-(3,4-DICHLOROPHENYL)-1,1-DIMETHYLUREA,	44.24
Laboratoriumbenodigdheden	76103990, BAR STIRRER MAGN. IKAFLON 30 ROUND PTFE	35.95
Laboratoriumbenodigdheden	76104521, Verzend/verpakkingskosten	30.25
Laboratoriumbenodigdheden	76104521, 50 cm zuiver platina draad dia. 0.3 mm	175.87
Laboratoriumbenodigdheden	76108688, SAG 7.82 - Gloeobacter violaceus	215.26
Laboratoriumbenodigdheden	76109994, multilayer gasbags 0.6 L	369.05
Laboratoriumbenodigdheden	76110275, Mag2 mix 15 eco multi position stirrer	924.56
Laboratoriumbenodigdheden	76111021, Mag2 mix 15 eco multi position stirrer	924.56
Laboratoriumbenodigdheden	76114216, titanium draad, grade 2 0.5mm	140.66
Laboratoriumbenodigdheden	76114216, verzending	30.25
Laboratoriumbenodigdheden	86101911, Ref electrode	760.17
Technisch Installatiebeh.	86100256, pompslang oranje-groen	99.70
Technisch Installatiebeh.	86100256, Minimale ordertoeslag	24.20
Technisch Installatiebeh.	86101891, pompslang oranje-groen	49.85
Porti	76111481, suzhou berchan trading	159.23
Drukwerk	VB DSMZ 01707447-1 76108916	565.07
Analyses	ElectroFoodSynthesizer august 2017	328.60
Analyses	ElectroFoodSynthesizer august 2017	328.60
Analyses	ElectroFoodSynthesizer september 2017	37.50
Analyses	ElectroFoodSynthesizer september 2017	37.50
Analyses	Analyses oktober 2017 ETE lab	752.60
Analyses	Analyses oktober 2017 ETE lab	752.60
Analyses	analysekosten ETE-lab december 2017	1,221.40
Analyses	Analysekosten ETE-lab november 2017	762.00
Analyses	Analysekosten ETE-lab januari 2018	832.80
		10,270.88