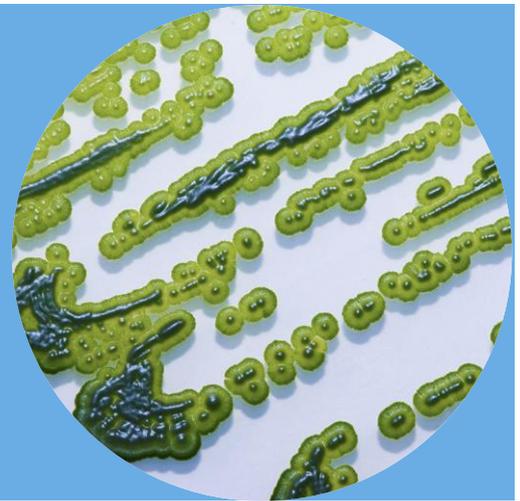


AlgaePARC

Newsletter | Winter 2013-2014



WAGENINGEN UR
For quality of life



Short News

Research at AlgaePARC



Ph.D. researcher Giulia Benvenuti checks one of the last samples of the season.

All reactors have been running non-stop and fully automatically between May and September. The productivity of four different systems could be compared: the raceway pond, horizontal tubular reactor, the 3D stacked tubular reactor and the flat panel reactor. Overall, due to its bigger reactor volume per surface area, the 3D tubular reactor showed a twice as high productivity per square meter compared to the horizontal tubes. But differences between the systems were season-dependent. In fall, productivity of particularly the 3D reactor was substantially lower than in summer, resulting in smaller differences with the horizontal reactor. This was due to a lower angle of the sun resulting in more shade on the lower tubes of the 3D reactor. 'Based on our results, we will compare the different systems with respect to different harvesting strategies for each month during next season', says Maria Barbosa, director of AlgaePARC. 'In other words: how much algae biomass can you harvest in different months in different reactor types for the highest productivity?'

Welcome

It is winter and the last algae reactors have been closed down for the season. This year we started up new projects together with our partners, aimed at developing sustainable and economical production methods for algae-based raw materials and end products. Crucial to success is a strong interaction between fundamental research in the lab and applied research at pilot scale.

Testing reactor performance in AlgaePARC gave new insight of reactor productivity and design, in relation to season. In our laboratory we further developed methods to increase lipid production. By manipulating growth conditions, lipid accumulation has increased to roughly 50 percent of dry biomass. A fat production of about 35 ton per hectare per year will become technically possible. To improve lipid productivity, we aim for continuous production, selection of better lipid-producing strains and genetic improvement of existing strains.

Three new EU projects were started, intended at improving complete production chains: from algae harvesting to extraction of raw materials from algae biomass and product development. Production locations at different latitudes and climates are compared for efficiency, productivity and sustainability. Within this framework large bio-refinery projects have been initiated to improve algae harvest, cell disruption and mild extraction technologies, while keeping energy input as low as possible, while functionality of raw components, remain intact.

We also started projects to involve consumers in our research, by initiating an algae production design challenge and an algae test kit for school kids. Further, together with Arkefly we offer consumers CO₂ compensation flights by investing in our research.

Rene Wijffels

&

Maria Barbosa



Women in Science grant for Maria Barbosa

On October 5th, AlgaePARC director Maria Barbosa received a grant *For Women in Science* of € 25.000 from L'Oréal-UNESCO. This gives talented women the opportunity to focus on writing scientific papers at the Netherlands Institute for Advanced Study (NIAS) in Wassenaar for several months.



Maria Barbosa receives her Women in science award in Amsterdam during the World Science Festival on October 5th.

Barbosa was awarded the grant for her research to implement sustainable microalgae biotechnology in our society. With her award, Barbosa will work at NIAS to translate fundamental research into practical applications aimed at maximizing microalgae production. Her ultimate goal is to develop sustainable microalgae products as raw materials for food, feed, cosmetics, chemicals and fuel. 'Microalgae biotechnology is still in its infancy, but has a huge potential to change into commercial and industrial activities', says Barbosa. 'It offers a lot of fundamental scientific and technological challenges.' Barbosa's activities have resulted in a leading role for Wageningen UR and AlgaePARC in microalgae research.

Start Algae PARC Biorefinery project

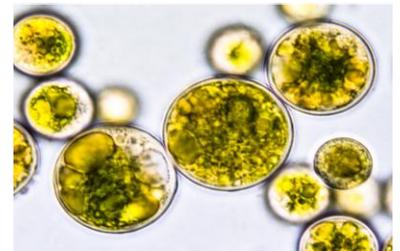
With the kick-off meeting of held on Friday November 13 the official start of the project AlgaePARC Biorefinery is a fact. Eight companies and three knowledge institutions will take part in the project. Suitable extraction and separation methods for the withdrawal of lipids, proteins and carbohydrates from complex mixtures is becoming the bottleneck for efficient production of these compounds for use as raw materials in different industries, for example for the production of fuel, pharma, food and chemicals. AlgaePARC Biorefinery aims at developing sustainable technologies and knowledge to harvest microalgae sustainably and separate algae biomass into different components. The separation includes cell disruption and mild extraction to maintain structure and functionality of different fractions. These biorefinery concepts will be developed at lab scale and implemented at pilot scale. In addition, the project will

obtain basic knowledge on cell wall composition and induced self-disruption of the algae cell.

Start of EU project Miracles

On November 20-22 2013 the kick-off meeting of the EU project MIRACLES was held. This four year R&D project aims at developing technologies to reduce costs and improve microalgae cultivation, harvest, raw material extraction and product development. The project consortium includes 11 prominent research organizations and 15 industries with complementary expertise. 'At this moment, several, mainly economic, obstacles hinder large-scale application of microalgae-based products', says Hans Reith, coordinator of MIRACLES on behalf of Wageningen University. 'Algae biomass is not available in large quantities, and production costs are too high. In addition, there is a need for development of more algae-based end products.' MIRACLES aims to resolve these hurdles by a technology-based fundamental improvement of the production chain, from algae cultivation to final product. To improve the economics of microalgae production, technology will be developed to use atmospheric CO₂. An innovative reactor will be designed with better mass transfer and less energy costs, while new methods to increase production of target compounds in the algae cell, such as valuable oils, will also be developed.

Oil accumulation, visible as droplets, in algae cells.

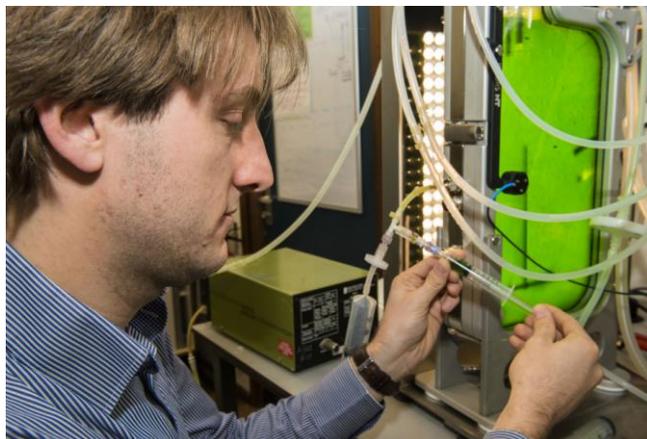


Furthermore, novel membrane-based technologies to harvest algae will be applied, reducing costs and increasing sustainability by recycling water and nutrients. To improve product extraction from algae biomass, innovative bio-refinery techniques will be developed, including mild extraction and purification methods to maintain the product's functionality. We will explore production of algae in regions where agriculture is not possible today such as in Norway and in the Antofagasta desert in Chili. Finally, applications of microalgae-based raw materials will be developed for food, aquaculture and several non-food applications such as resins, bio-plastics and cosmetics.

Science

Optimizing edible oil production in microalgae

Scientist Guido Breuer increased the efficiency and production of algae oil production substantially by selecting the right algae species and optimizing growth conditions.



The coming decades, the world population will grow towards nine billion people. Consequently, there will be increasing demands for high quality foods, including edible oils from plant-based resources. With just conventional agricultural methods and agricultural soils becoming increasingly scarce, it will not be possible to meet these higher demand in a sustainable way. Anticipating on the need to increase future food production, industries are exploring additional and sustainable sources for these edible 'bulk oils' to apply in food products.

High yield

For several reasons, microalgae are very promising alternatives for the production of important food components. A large variety of species produce carbohydrates, proteins and oils of different compositions. Culturing microalgae is a sustainable agricultural industry. Compared to traditional agricultural crops, these microscopic organisms need very little fresh water when cultivated in closed photobioreactors. In addition, they can be cultured in areas unsuitable for crop growing. Hence, there is no competition for traditional farmland. One of the main advantages of algae over traditional crops is their high yield per hectare. Using results obtained in the laboratory, Breuer calculated that microalgae can annually produce up to thirty-five tons of oil per hectare. About ten times more than traditional oil-producing plant species. For this reason, Breuer started

his research project to optimize edible oil production in cultured microalgae.

Photosynthetic efficiency

Under standard growth conditions, with no nutrient limitations, the algae cell prefers to synthesize functional biomass, such as protein. However, when nitrogen becomes limited, the cell is unable to synthesize these functional structures and starts synthesizing carbohydrates or oil (fig. 1). This way, it continues to utilize light that is absorbed by the cell's pigments and photo-oxidative stress is prevented. Furthermore, carbohydrates, and especially oil, might be used by the cells as an energy-dense reserve, that can be metabolized into functional biomass when growth conditions improve.

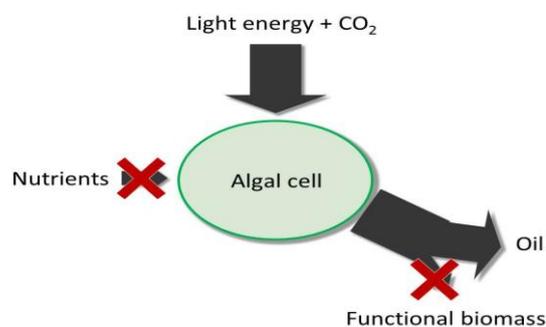


Fig. 1. Nutrient limitation results in less functional biomass and more oil production.

So, a nutrient (nitrogen)-restricted diet results in oil production, but at the same time the rate of photosynthesis decreases. The amount of oil that can be synthesized and the efficiency of the cell's photosynthesis is dependent on the species (fig. 2). To find the most promising algae species with respect to oil production and photosynthesis under nitrogen limited conditions, Breuer did an extensive literature study. Based on these results, he pre-selected nine potentially suitable oil producing algae to test further in the laboratory. All species selected produced oil when put on a diet limited in nitrogen. But there were big species differences in the amount of oil produced and these species showed different photosynthetic efficiencies when nitrogen was limited. Based on his test results, Breuer concluded that the species *Scenedesmus obliquus* performed best. 'This algae species is particularly robust and produces a lot of oil', Breuer explains. 'In addition, it keeps its photosynthetic

efficiency at a high level for a relatively long time after nitrogen has been used up’.

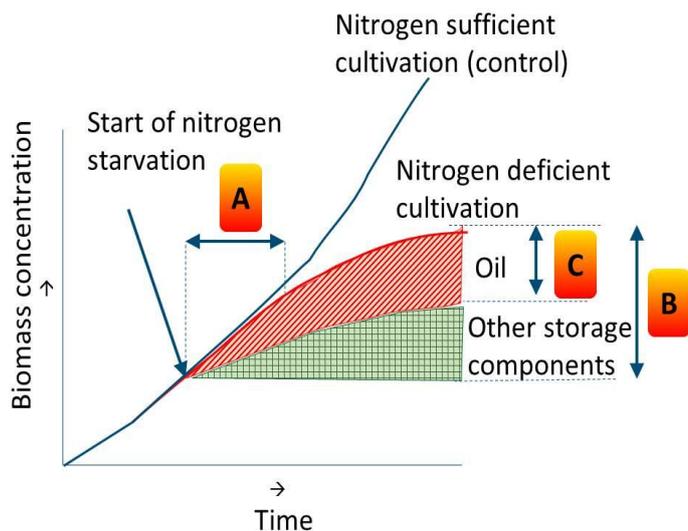


Fig. 2. The impact of nitrogen starvation on microalgae. After nitrogen is depleted, photosynthesis continues for a certain period. During this period, the algae produce oil and carbohydrates. Differences between species are A) the duration of the period in which photosynthesis continues after nitrogen is depleted, B) the total amount of biomass that can be produced after nitrogen is depleted, and C) the amount of oil that can be produced.

Wastewater cleaning using microalgae biofilms

Ph.D. researcher Nadine Boelee looked into different scenarios to use microalgae biofilms to clean wastewater. Algal biofilms are especially efficient when algae grow together with bacteria. Boelee concluded that these symbiotic biofilms are very promising for year round wastewater cleaning in countries at lower latitudes, where temperature and irradiation are sufficiently high.

Conventional wastewater plants use microbial processes, sometimes in combination with suspended microalgae and/or chemicals to remove nitrogen and phosphorous from wastewater. In The Netherlands, the maximum concentrations of these nutrients in discharge water are by law 10 mg/ml for nitrogen and 1 mg/l for phosphorous. For discharge in 'sensitive' waters, guidelines are even more stringent: 2.2 mg/l and 0.15 mg/l for nitrogen and phosphorous, respectively. In 2015 EU regulations require

Upside-down

After selecting the most efficient species, the next step to maximize oil production was by optimizing growth conditions. Breuer tested the oil-producing performance by varying amount of light, temperature, and pH. Best performance was achieved at a temperature of 27.5 degrees Celsius and neutral pH. Also, the algae were able to utilize light most efficiently at low light intensities. Nevertheless, part of the algae's metabolism was still directed towards carbohydrate production.

To maximize the algae's oil production even further, the algae's metabolism has to be manipulated, aiming at increased oil synthesis. Therefore, in the near future the microalgal response to nitrogen starvation will be studied in more detail to better understand cellular and metabolic processes in the algae cell. 'When nitrogen is depleted, lots of physiological changes happen in the algal cell', Breuer explains. 'The cell starts making oil, it's photosynthetic efficiency drops, and chloroplasts change. Metabolism is completely turned upside-down.' These basic processes have to be better understood to direct an even bigger part of the algae's metabolism towards oil production while optimizing photosynthetic efficiency.



strict purity guidelines for all water bodies. To reach these targets, post treatment of wastewater effluent can be needed. Microalgae could be a sustainable alternative to existing (post) treatment methods since they use nitrogen and phosphorous for growth. Microalgae could be a sustainable alternative to existing (post) treatment methods since they use nitrogen and phosphorous for growth.

More sustainable

Microalgae may grow in suspension, but also in biofilms. A biofilm looks as a slimy, green layer and consists of large numbers of microalgae entrapped in a gel-like matrix (fig. 1). They may grow on almost any surface.



Fig. 1 Algae biofilm growing on an artificial matrix.

Removing nutrients from wastewater by using algae biofilms is potentially more sustainable than traditional methods. Less energy and chemicals are needed, while microalgae utilize CO₂ as a carbon source and produce valuable biomass. Microalgal biofilms have several advantages over suspended algae cultures. 'Biofilms grow on a surface separate from waste water, and can easily be harvested by simply scraping off the algae layer', Boelee explains. 'Biomass density of a biofilm is hundreds of times higher than that of algae in suspension. Consequently, harvesting requires no energy-consuming centrifugation step.' (fig. 2).

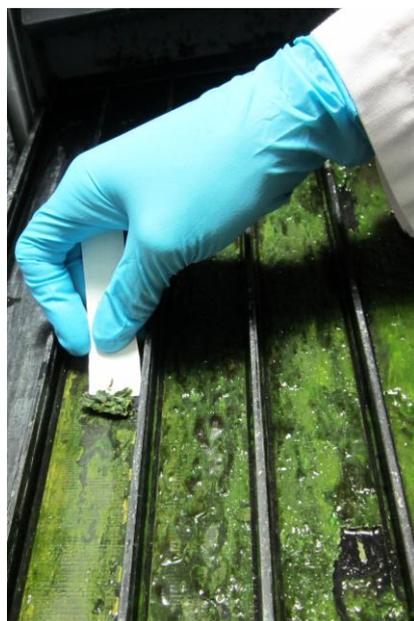


Fig. 2. Harvesting algae bio-films can easily be done by simply scraping off the algae layer.

Lab scale reactor

Boelee investigated the possibility to use microalgae biofilms to reduce nutrients in wastewater to reach 2015 emission targets. In collaboration with Wetsus, Leeuwarden, she tested their performance using both lab scale and pilot scale phototrophic biofilm reactors of different designs. First she built a lab scale reactor aimed at investigating the capacity of microalgal biofilms as a post treatment step for the effluent of a wastewater plant. The maximum uptake of nitrogen and phosphorous by the algae was 1.0 and 0.13 g/m²/day respectively, while target values of the new EU regulations were still reached. She estimated that a full scale post-treatment reactor of this design would need about 10 ha per 100.000 people, producing 2000 kg of dry biomass per day.

Pilot scale reactor: lower productivity

An up-scaled pilot version of the laboratory reactor was built to test its capabilities 'in real life' at the municipal wastewater plant in Leeuwarden (fig. 3) from June to October. The algae biofilm was growing on a vertically placed carrier material, where a continuous thin layer of wastewater was flowing over.



Fig. 3. Pilot scale algae biofilm reactor at the municipal waste water plant in Leeuwarden.

This vertical system had substantially lower productivity than the original lab version and consequently also lower nitrogen and phosphorous removal rates: 0.13 g/m²/day and 0.023 g/m²/day, respectively. This amount varied due to light availability and temperature fluctuations during the day. Target values for 2015 were not reached. 'Unfortunately, this type of reactor is unsuitable for the Dutch climatic conditions, possibly because temperatures and irradiance are limiting', Boelee concludes. 'As a result, nutrient removal is too

low, while the surface area needed is too large.' The reactor can be made more energy-efficient, by placing the carrier material horizontally, instead of vertically, resulting in reduced pumping. In addition, automatic sampling, pH control and limiting heat loss are important tools to increase reactor efficiency.

Large capacity

Further experiments with a symbiotic microalgal biofilm, where algae and bacteria grow in the same biofilm, showed promising applications for this design. Boelee studied this biofilm in the lab (fig. 4), with ammonium, phosphate and acetate mimicking a degradable organic pollutant. Microalgae remove nitrogen and phosphorous, use carbon dioxide (CO₂), while producing oxygen. The bacteria use oxygen (O₂) for aerobic degradation of organic pollutants, while producing CO₂. Once the system was running, there was no need to supply extra O₂ or CO₂. The symbiotic biofilm showed a large capacity to clean waste water with relatively high nutrient concentrations, removing 3.2 g nitrogen and 0.41 g phosphorous/m²/day. 'If this symbiotic biofilm is used as a single method to remove all nitrogen and phosphorous from wastewater, nutrient concentrations should be adapted to available light intensity, otherwise a second cleaning step is needed', Boelee concludes. 'In southern locations the symbiotic biofilm is a very promising method for year-round wastewater cleaning.'

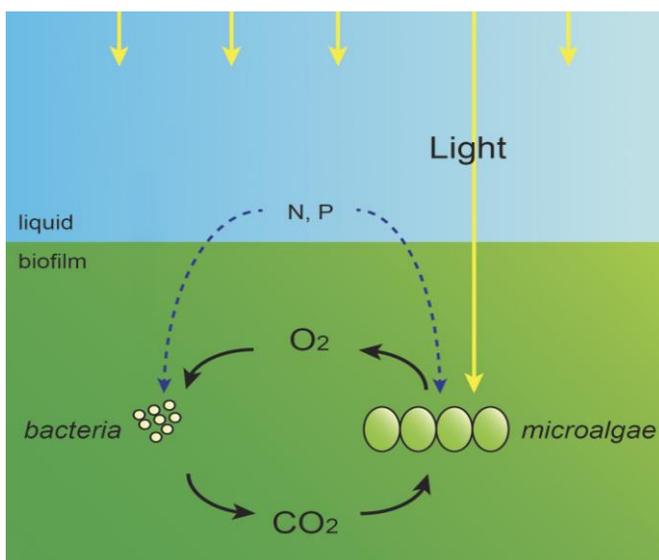


Fig. 4. Schematic overview of the symbiotic microbial biofilm reactor. Micro algae utilize light and CO₂, while producing biomass and O₂ that is utilized by bacteria.

Agenda

Young Algaeneers Symposium

The second edition of The Young Algaeneers Symposium will take place in Narbonne and Montpellier, France, from April 3-5 2014. The conference organizing committee will take care of transport from Montpellier to Narbonne the 3rd of April. For more information see: <http://yas2014.sciencesconf.org/>

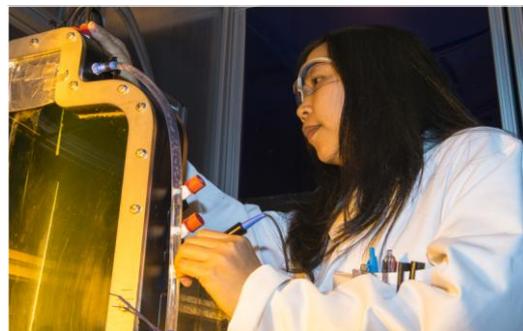
Ph.D. defense

On March 21 2014 Ellen Slegers will defend her thesis entitled 'Scenario Studies for Algae Production.' Slegers analyzed the effects of location, reactor design and the type of algae culture, as well as their influence on each other on algae growth and productivity.



Ph.D. defense

Kanjana Tuantet will defend her thesis 'Production of Microalgae in Human Urine' on May 20 2014. Tuantet developed a running and productive system where nutrients from urine can be recovered from concentrated urine.



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