

## Smart solutions to optimize biodiesel production in green microalgae



**To produce biodiesel from algae is a challenge, because algae stop growing while producing oil, raw material for biodiesel. This results in the end of the algae culture. Consequently, time is lost starting up a new algae culture after harvesting. New techniques are now being developed to make oil production more efficient.**

In search for sustainable alternatives for crude oil algae may play an important role. Microalgae may contain as much as 50 percent oil. Depriving algae from essential nutrients, while maintaining optimal light conditions, pushes them to synthesize oil. The stressed algae stop growing and start producing oil. After a few days the whole batch can be harvested. 'Growing algae could potentially be more interesting to produce commercial oils', says Anne Klok, Ph.D. researcher. 'Such a continuous system is easier to operate and requires less down time of the production process, compared to the classical batch system.'

## Welcome

In a bio-based economy, agricultural crops are not only used for production of food and feed but also for chemicals, materials and biofuels. Microalgae are the most promising feed stocks for a sustainable supply of commodities for both food and non-food products. To realize this promise, the scale of production needs to increase with a concomitant decrease in production costs.

AlgaePARC aims to bridge the gap between fundamental laboratory research and applied outdoor research. A good interaction between basic research and applications is essential to speed up commercialization of algae production processes. With our pilot facilities we are able to quickly test breakthroughs obtained in the lab under realistic production conditions, and bottlenecks observed outdoors can be studied systematically under standardized laboratory conditions. Our entire research program is therefore connected. To show this, all our algal research activities are now put under the umbrella of AlgaePARC.

In this edition we would like to show three examples of research projects covering the whole production chain. Anne Klok presents her work on lipid metabolism. Lipids are key components from algae. They can be applied in fuels, food, feed and chemicals. Sina Salim works on algae harvesting, one of the economical bottlenecks in the algae production chain and Ellen Slegers works on scenarios for optimizing large scale production.

We think it is important to have good connections to the industry. We believe microalgae will become an important sustainable feedstock for production of commodities by innovative research. Through collaboration with our industrial partners these innovations can be realized in a bio-based economy

Rene Wijffels  
Bioprocess Engineering,  
AlgaePARC  
&  
Maria Barbosa  
Food & Biobased Research,  
AlgaePARC



## Energy waste

During several experiments, Klok tried to design an algae culturing system where growth and oil production were combined. At optimum light conditions she cultured algae receiving a diet limited in nutrients. Nutrient supply was enough for growth, but not enough to use all light energy for normal cell production. She discovered that oil accumulation in algae was the result of energy imbalance. 'Limited nutrients resulted in reduced growth, while light energy became too much for just growth', she explains. 'We expected that excess light energy was used to produce oil.' But total oil content was disappointing: just 17 percent, not good enough to beat the traditional batch system. The low oil content indicated that not all light energy was used for oil production. Klok found out that algae adapt to dietary conditions quickly and dissipate about 90 percent of excess energy as heat and/or fluorescence. It seemed that algae prefer to waste energy, instead of storing it in oil. Interestingly though, this energy waste was not caused by a blockage of oil synthesis, because nutrient restricted algae did increase their oil production.

## Modified algae

Based on these results, the scientist has several options to increase oil production in algae. 'By inhibiting the process of energy wasting we may increase oil production', Klok says. 'Our experiments have shown that some algae species naturally waste less energy, but algae can also be genetically modified to be more efficient.'

## Floc-forming algae cause a spectacular reduction in harvesting costs

**Ph.D. researcher Sina Salim has discovered extraordinary flocculation potentials of an algae species that is substantially less expensive to harvest after cultivation. The algae, *Ettlia texensis*, naturally forms flocs. This facilitates harvesting and reduces energy costs with more than 85 percent.**

Such modified algae could be designed to reduce energy dissipation and fluorescence or to reduce energy flows to carbohydrate metabolism, resulting in more oil production. In addition to modifying algae to waste less energy, oil production could also be improved by optimizing the culturing process. For example, by using a 2-step process in 2 different compartments: a fast and more efficient growth in one culture is followed by nutrient restriction and quick oil production in another 'Our first experiments indicate that this system might really work well,' says Klok

## Short News

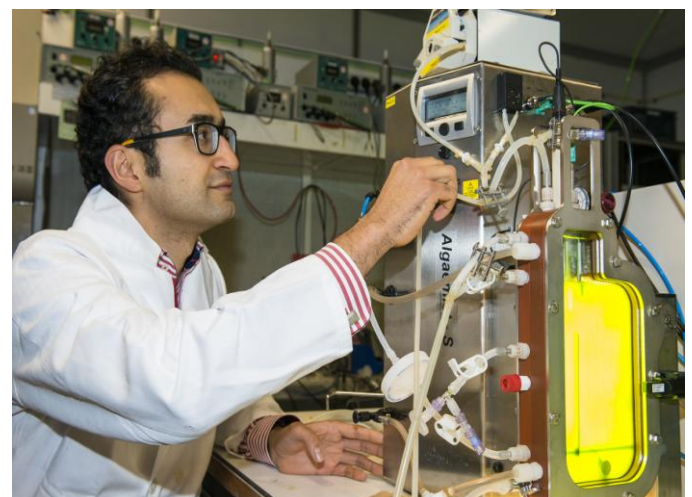
**Completion of three PhD theses** on the effect of flashing light on photo bioreactors productivity, biorefinery and the effect of oxygen accumulation on algae productivity.

## Start of new large projects

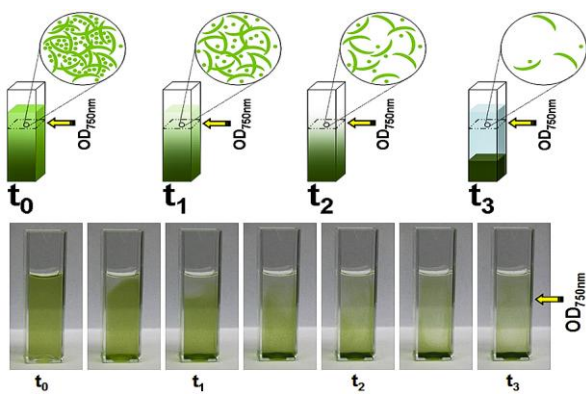
We are coordinating two new large FP7 EU projects on biopolymers (SPLASH) and biofuels (FUEL4ME) from microalgae. We were successful in setting up another large program on biorefinery (AlgaePARC biorefinery) and metabolic engineering.

## Education

Another highlight is the start of an international course on Microalgae Process Design in June: *From cells to photo bioreactors*. Within 1 week it was fully booked. We certainly will organize new editions coming years.



Cost-effective culturing microalgae for oil production still faces some major challenges. One of those is the high energy costs of harvesting. The main reason is the low algae concentration in this type of culturing system. These systems only contain about 0.5 – 2 grams of dry matter per liter. Harvesting algae cells requires separation a of lot of water from algae. Usually, centrifuges are used to do the job. But as a result, lots of energy goes into removing water. Between thirty and fifty percent of the total energy to produce biodiesel from cultured algae in a raceway pond is consumed by harvesting. Expensive and inefficient.



**Fig. 1 Floc-forming algae at different time points**

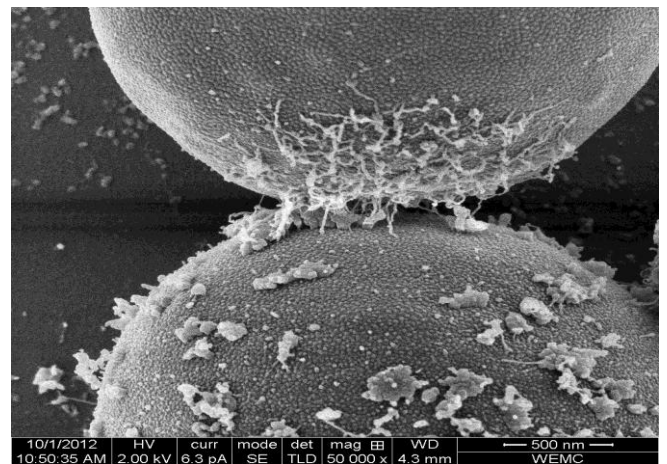
### Ideal algae

One way to harvest the microalgae more efficiently is to increase concentrations before centrifugation. Algae forming flocs together could be a solution, since flocs sink to the bottom more easily than individual cells. As a result, a more concentrated fluid layer will form at the bottom (fig. 1). Harvesting algae from this more concentrated bottom fluid requires relatively little centrifugation energy.

Most algae avoid sticking together due to a negative charge at the cell surface. This results in algae cells repelling each other. This has several good reasons. Algae flocs have a reduced surface to volume ratio, resulting in less light exposure and less nutrients that are absorbed. Some algae species do form flocs or bio-films under certain conditions though. Salim has clear ideas how a model algae should behave: 'The ideal algae grows well in suspension, but eventually forms flocs', he says. 'In addition it also should contain enough oil and other useful components to be commercially interesting.'

### Archived algae

In search of the ideal algae, Salim traveled to Austin (Texas) in the United States, where he could look into features of more than 40.000 archived algae species. He selected 16 floc-forming algae to test out in the lab. From those 16, he selected four that were suitable: they grew well in suspension, formed flocs and contained about 25 to 30 percent oil. The fast growing *Ettlia texensis* proved to be the favorite species. It formed the biggest stable flocs, containing up to hundreds of individual cells, had a relatively high growth rate and contained more than 25 percent fat, based on dry weight. Large flocs were formed because during multiplication the daughter cells stuck to the mother cell. Possibly, polymeric substances either proteins, carbohydrates or a combination function as bonding agent to glue cells together (fig. 2).



**Fig. 2. Electron-microscopic image of bonding algae cells**

### Break through

The scientist started to look for the most efficient way to harvest his favourite floc-forming species. Between harvest and centrifugation he included a sedimentation step to try to increase algae concentrations. During one full day, algae flocs were allowed to settle and concentrate on the bottom. With sedimentation, Salim was able to reduce the total volume for centrifugation to a mere 2 percent of the original volume. Energy costs for centrifugation dropped from around 14 MJ per kg dry algae to 2 MJ. A reduction of 85 percent. Salim: 'Finding species like *Ettlia texensis* is a break through to reduce harvest costs.'



## The perfect design

**The flat panel photo bio-reactor shows the best productivity under most circumstances compared to other algae culturing systems. Ph.D. researcher Ellen Slegers modeled the productivity of several algae culturing systems. She found that in the majority of cases the flat panel reactor was superior compared to other designs, including tube reactors.**

Finding the best method to culture algae is a complex undertaking. High growth rates are obviously important and these depend on a combination of the type of culturing system (i.e. reactor model), algae species, but also on geographical location. But not only productivity is important. Also processing costs, logistical issues like water and nutrient supply as well as the sustainability of the whole process are important factors. For example, algae culture in the Sahara may seem a good idea. High temperatures and large amounts of available light year round result in relatively high productivity. But transport in this area is costly and energy demanding. To come up with the best balance between productivity, costs and sustainability Slegers developed complex mathematical models. 'We modeled the effects of location, reactor design and algae culture and their influence on each other', she explains. 'Choices made are always a matter of balancing pros and cons for any given situation and which end product you are after.'

### Reactor design

From all factors influencing cost-effective algae culturing, the reactor is a basic element. A variety of algae culturing systems in all shapes and sizes is available nowadays, from traditional race-way ponds to sophisticated tubular 3-D designs. Productivity and performance of these different systems vary, as do building and operating costs. Reactor design is the decisive factor allowing optimal growth under certain conditions for a certain algae species. Therefore, Slegers paid special attention to reactor type, design and features and calculated different design scenarios for different locations and algae species. 'For optimal productivity under various conditions, understanding details of reactor design is of paramount importance',



she says. 'For example, in a 3-D tubular reactor tube diameter, horizontal and vertical distance between tubes, height and materials used all influence reactor performance.' Optimal dimensions also vary with geographical location, she found out. What works in Africa does not necessarily work in The Netherlands.

### Excellent performers

Due to novel designs, reactor productivity has substantially increased over the years. For example, depending on species and location, the 3-D tube reactor produces about 1.4 – 6.5 times as much algae biomass per hectare as compared to traditional raceway ponds. Based on her models Slegers showed that flat panel photo bioreactors generally were excellent performers as compared to other reactor types. 'According to our calculations, culturing the algae *Phaeodactylum* in a flat panel reactor in The Netherlands results in twice as much algae biomass per hectare as compared to a 3D tubular reactor', Slegers says. 'But in the Sahara flat panel and 3D reactors performed similar.' This can probably be explained by the lower angle of the sun in the Netherlands as compared to the Sahara. In The Netherlands the sunrays fall on the vertical plates in a more perpendicular angle, resulting in more light and consequently higher algae growth rates. 'Calculations were performed based on current knowledge', Slegers says. 'The next phase is to validate the models.' At AlgaePARC different scenarios and reactor designs can be compared. With research groups down south models will also be validated for sunnier locations.

### Contact

Rene Wijffels, [Rene.wijffels@wur.nl](mailto:Rene.wijffels@wur.nl)

Maria Barbosa, [Maria.barbosa@wur.nl](mailto:Maria.barbosa@wur.nl)