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# Evaluation research activities project “Oesterbroedfarm”

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1. **Innovating the algae culture of shellfish production: a step towards a more sustainable lighting system – Just van der Endt**

After describing the state of the art, Just van der Endt looked at different ways to improve the algae culture systems of the hatchery of Roem van Yerseke by investigating 2 factors that may limit the production of the algae in the Seacap system, being the availability of photons and to a lesser extent of nutrients. A calculation sheet evaluates the impact of different light systems on the production cost of the micro-algae, based on the preliminary results of the different experiments and certain assumptions.

A lot of effort has been invested in the execution of the experiments and the writing of the report, but due to practical constraints which in some cases negatively affected the experimental design, results should be interpreted with caution.

Based on the collection of data by the student on the current algae production, some observations can be formulated :

* Diatoms are more difficult to grow than flagellates, as expected. The average culture time in the bags varies between 1 – 1.5 month for diatoms, while flagellates last on average for 2 months. Since the growth rate and nutritional requirements of diatoms are different than for flagellates, the concept of two independent culture systems for diatoms and flagellates may be a good decision.
* For flagellate species especially, the culture time of one species can vary from 30 days to 75 days before collapsing. This variation indicates that the culture parameters are not constant and sometimes not optimal. This may have several reasons :
	+ Not enough temperature control in the culture room
	+ No systematic replacement of the TL lights, while the lights should ideally be replaced every year.
	+ CO2 regulation (and thus pH) is not optimal, which is not surprising since all bags, whether being harvested or upcoming, are supplied with CO2 through the same line (cf above remark diatoms).

The experimental work on nutrients doesn’t allow statistical processing of the data and the very short duration of the growth experiments also makes it impossible to draw some conclusions. However, the main focus of the report lays on the use of alternative light sources, being LED lights. The introduction of the LED technology is not only important for every household, but also for the horticulture and aquaculture. One of the major costs of producing microalgae is the energy consumption due to the 24hour light regime applied to maximize the biomass production. LED lights offer an interesting alternative to the widely used TL lights, since they are supposed to consume less energy and produce less heat. Besides, the energy is more efficiently used (higher external quantum efficiency EQE) since only specific wavelengths are generated which are useful for the microalgae envisaged. It is therefore crucial to invest in high quality LED’s that emit the promised wave lengths since they have to match the maximum absorption wave length spectrum of the pigments that are present in the algae. For all microalgae, Chlorophyll a (maximum absorption at 465 and 665 nm) is the most important pigment for photosynthesis, but green algae for example also possess Chorophyll b (maximum absorption at 450 and 640 nm) (Fig. 1).

Using blue LEDs might seem economically interesting as these have a higher EQE by emitting more energy rich radiation (Van Giel *et al.*, 2010). But, since photosynthesis is a quantum process that always yields the same output per absorbed photon the energy content of the light is only relevant to a lesser extent (Wang *et al.*, 2007) (cf Fig. 1). Besides, the red radiation is most efficient for plant photosynthesis since at 400nm, 1Watt produces 3.4 µmol while at 700nm, 1 Watt produces 5.8 µmol photons (Hemming S. 2004)





Fig. 1 Absorption of the light by different pigments present in microalgae and the rate of photosynthesis in function of the wavelength (Source : <https://www.simply.science/images/content/biology/cell_biology/photosynthesis/conceptmap/Photosynthetic_pigments.html>)

However, one identified problem is the weak intensity of the wavelengths, reducing its power to penetrate till the center of the algal culture vessel (term called self-shading). Contrary to what is written in the report, light in the red spectrum has a deeper penetration into the algae culture, than the more energetic blue light fraction (Richmond and Cheng-Wu, 2001). This makes the use of a red light source especially interesting for high density cultures. Increasing the penetration depth and therefore the photic zone allows the use of higher volumes, without compromising productivity. Nevertheless, further investigation is necessary.

The design of the light experiments is not ideal since comparison between the different runs of the small-scale experiments can not be compared according to the author since too many factors changed. However, it is clear that the growth of *Isochrysis* and *Sceletonema* under LED white light (W) and LED3:1 (R:B) was comparable and that a minimum of 12µmol/m2/s was necessary to have growth. Growth was slow however (from 70 000 to 1 million cell/ml in 12 days) but a positive control with TL light was not included for easy comparison.

The description of the big scale experiment is difficult to follow, but it would seems that similar growth was obtained for *Isochrysis* with TL lights and LED 6:1 (W:R) when a light intensity of 290-300 µmol/m2.s was respected, corresponding with the standard distance between the algae bags and the TL light and a distance of 80cm between the bags and the LED light. Photoinhibition was suspected when the distance between the bags and LED light was reduced to 4 cm and light intensity increased to 5000 µmol/m2.s. This corresponds with the literature that confirms that light saturation of the photosystems I and II in the chloroplasts is obtained at 300-600 µmol/m2.s and values above that threshold lead to the production of reactive forms of oxygen and consequently to cell damage. It is not clear why the option of LED 3:1 (R:B) was omitted from this large scale experiment.

The calculations of the costs of different scenario’s (Table 3) whereby TL lights are replaced by LED’s are interesting although the following observations can be made:

* No scenario was included where the current set-up of the algae room was re-designed in order to have easy access to the TL lights for yearly replacement and make an increase of the total number of bags from 67 to 100 possible
* The changes in algae production due to the yearly replacement of TL, or the doubling of LED lights or the reduction in length of LED lights to 150cm, are not well justified and based on very preliminary results which are not statistically supported. The proposed setting of having the LED-lights in the center of 4 bags, was never tested.
* The long life span of the LED lights (11.5 years) is in contradiction to the observations during the small-scale experiment, but experience from the horticulture industry may confirm this.

On the other hand, the fact that LED lights produce less heat, is confirmed by previous studies and may offer a serious advantage. Harter et al. (2012) calculated that for TL lights about five times more electrical energy was needed for cooling, compared to LED.

Conclusion

Taking into account that at least similar production levels can be reached with LED 6:1 (W:R) as with TL lights, that less heat is produced and production costs in general seem to be lower, it could be worthwhile for Roem van Yerseke to further investigate the possibilities of switching from TL to LED light.

It would be interesting to include the option of LED 3:1 (R:B) since the results were encouraging in the small-scale experiment and energy is used in more efficient way than with white light. Literature shows that microalgae can be grown with LED emitting a small spectrum although one has to take into account the specificity of the microalgae species.

Finally, besides measuring the biomass produced under different light conditions, quality parameters should be followed up as well during the follow-up experiments such as cell size, cell composition (% lipids, C/N ratio), DW and quantum yield (indications of health).

1. **Internship of Lisa Bakker at hatchery of Roem van Yerseke**

Two practical research questions were tackled during the internship of Lisa Bakker :

* Can the hatching rate of cupped oyster eggs (*Crassostrea gigas*) be enhanced by improving the protocol ?
* Can the microalgae *Chaetoceros calcitrans* be (partially) omitted from the oyster larval diet since this diatom causes clogging of the sieves in the larval tanks ?

To answer the first question, several sperm/egg ratios and different contact times were tested in three different experiments. All treatments were carried out in triplicate which enable statistical analysis of the data.

Based on the results, one can cautiously conclude that a sperm/egg ratio between 10 and 500 is acceptable whereby for lower ratios (up to 100) a contact time of 24hr is fine but for higher ratio a contact time of 12 minutes is more appropriate.

However, from a microbiological point of view, it is advisable not to apply a contact time of 24hr since dead sperm cells offer a rich carbon source for the bacteria to proliferate. Since the student was only looking at the hatching rate and not at the fertilization rate, it is not clear why the hatching rate in general was very low. The best treatments had a hatching rate of below 50%, between 20-30% in general. This can be due to the fact that not enough eggs were fertilized or that fertilized eggs couldn’t develop because of bacterial contamination or because of polyspermy. The substantial differences in hatching rate between the replicates, could be indication of such problems.

As a rule of thumb, a ratio of 10 sperm cells per egg are recommended to fertilize eggs of bivalves. However, close monitoring of the fertilization process is recommended by taking samples every 5 minutes of the egg/sperm mixture and observing the appearance of the first polar body which indicates successful fertilization of the egg. The number of sperm cells surrounding the eggs should also be monitored and should be around 10. In case there are not enough sperm cells, they can be added.

As a rule of thumb, the eggs and sperm are used as soon as possible after release by the broodstock animals (within 15 minutes). After adding the sperm to the eggs, the eggs can be left to rest for 20-30 minutes (with plunging) before being washed thoroughly to remove the excess of sperm and being stocked in the incubation tank. If oysters are not allowed to spawn naturally by temperature shock and the eggs are collected from the gonads by stripping, it may be a good policy to give the eggs time to absorb water before fertilization. The normally pear-shaped eggs when first removed, should round off in contact with seawater within 20 minutes (FAO 2004).

The experiments to find an answer whether *C. calcitrans* is necessary for the larvae culture of the cupped oyster, are also carried out in triplicate, which allows statistical interpretation of the results. It was a good policy to restock all the tanks after 2 weeks, since there were big differences in stocking densities between the bottles. Perhaps this should have happened every week (after determining the survival) to rule out slow larval growth because of lack of feed, since the bottles could not individually fed.

Looking at the raw data, a bigger sample size for measuring the larvae is recommended. A total of 10 larvae per bottle is not representative for the population. A minimum of 50 larvae per bottle should be considered and even a bigger sample size of 100 larvae at week 1, week 2 and end of experiment. This extra work can be compensated by sampling only twice a week for example instead of 3 times a week.

Conclusion

Despite these comments, the outcome of the feeding experiments look reliable enough to be applied in the hatchery. The adaptation of this new feeding scheme will solve the practical issue of sieve clogging during the first week of larvae culture. Alternatively, another diatom could have been tested, like *Sceletonema* (short chained) or *Thalassiosira* as a replacement for *Chaetoceros calcitrans*. These diatoms do not have setae which most probably are responsible for the fast clogging of the sieves.

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