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GROWTH OF THE GREEN MICROALGA CHLORELLA VULGARIS OUTSIDE A CULTIVATION MEDIUM

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ABSTRACT

Microalgae can be used for various applications, such as food, cosmetics or biofuel production. Usually they are cultivated in bioreactors, which makes harvesting and processing strenuous. Growing them adhering on a surface, i.e. as a biofilm, can reduce these problems. On the other hand, introducing substrates into bioreactors may cause additional problems regarding the light intensity on the microalgae. The next logical step would therefore be to let the microalgae grow on substrates outside bioreactors which are regularly wetted, but not constantly submerged in the medium, to improve light intensity and make harvesting easier. Here we report on experiments with the green microalgae *Chlorella vulgaris* grown on different textile substrates at the air. A polyester (PES) nonwoven as well as a Tencel (cellulose) plush knitted fabric showed the best water retention properties and were thus used for the final tests. *C. vulgaris* started to grow on these textiles in suspension, before the substrates were transferred to cultivation in the air with regular wetting and defined light. The algae biofilms, measured by areal density, were constantly increasing during 4 weeks of cultivation, showing more algae on the Tencel fabric and similar oxygen production rates of the algae on both substrates. The experiments confirm the possibility to let algae grow outside cultivation medium.

Keywords: microalgae, *C. vulgaris*, knitted fabric, textile substrate

1 INTRODUCTION

Green microalgae are useful for different applications, such as food, pharmaceutics, oxygen production, biopolymers or biofuel [1-3]. Typically, commercial cultivation of these microalgae occurs in open ponds or closed photobioreactors [4,5]. In these systems, however, production costs are unnecessarily high since the applied light is partly blocked in a suspension of green microalgae, making many products economically less attractive. Diluting the suspension to allow more light from outside to enter, on the other hand, makes algae harvesting more complicated. One potential solution to solve this problem is growing the microalgae on substrates [6]. This is possible for the edible microalgae *Chlorella vulgaris* and others, as different research groups have tested for various rigid substrates [7-9]. Besides this solution, textile fabrics have been tested as possible substrates, e.g. woven fabrics for the adhesion and growth of *C. vulgaris* [10], *Chlamydomonas reinhardtii* on electrospun nanofiber mats and other nonwovens [11], or *C. vulgaris* on different knitted and woven fabrics [12,13].While microalgae growth on flexible substrates enables easier harvesting after a defined growth period, the problem of reduced light intensity in the photobioreactor can even be increased by the introduction of an additional substrate. This is why two recent projects aimed at investigating the potential growth of green microalgae outside a photobioreactor on different textile substrates, i.e. at the air, with regular wetting of the substrate, but without fully immersing the substrate in water or medium. For the tests, the green microalgae *C. vulgaris* and *Scenedesmus longispina* were used which both grew well on textile fabrics inside a photobioreactor in previous experiments. *C. vulgaris* is a unicellular freshwater microalga with anti-cancer and immune-regulating properties, protection against age-related diseases and a high protein content of > 55% of its dry mass, usually grown under photoautotrophic conditions [14-17]. *Scenedesmus qudricauda* var. *longispina*, often abbreviated *S. longispina*, is a less often investigated colony-forming green microalga, which can be grown unicellular or in different colony forms with usually 2-4 cells, depending on the cultivation medium [18-20].

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Table I - Textile fabrics used in the experiments

Material	Production	Thickness (mm)	Areal weight (g/m²)
100% jute	Woven, plain weave	1.2	265
100% Tencel (cellulose)	Knitted, plush	4.5	1052
100% linen	Woven, plain weave	0.76	148
100% cotton	Woven, twill weave 3/1	1.08	220
65% cotton, 35% linen	Woven, plain weave	0.65	200
100% polyester	Nonwoven	1.45	170
100% polyester	Woven, roughened	2.04	300
99% cotton, 1% elastan	Woven, plain weave	1.2	378

2 MATERIALS AND METHODS

2.1 MICROALGAE CULTIVATION

Microalgae cultures of *S. longispina* (from Culture Collection of Algae at Göttingen University, SAG, Germany) and *C. vulgaris* (from Interaquaristi.de Shop, Biedenkopf-Breidenstein, Germany) were cultivated in tris-acetate-phosphate medium (TAP medium) which was autoclaved at 121 °C before use. Long term cultivation was performed in culture flasks illuminated by warm-white LED lights (CRI930 linear flex band, purchased from ISOLED, Schwoich/Austria) with an average intensity of 14–15 W/m² during a photoperiod of 10 h/d.

Besides this long-term cultivation, both microalgae were also grown on different textile fabrics, in the air as well as in medium, the latter serving as a reference.

2.2 TEXTILE SUBSTRATES

The following textiles substrates were used in the experiments (Table I).

2.3 NEW CULTIVATION BOX

For cultivation of microalgae in the air, a box was constructed and 3D printed which allowed fixing textile fabrics vertically, with closed translucent walls to enable enhancing humidity inside and different possibilities to wet the textile fabrics by drip irrigation or spraying. This setup is depicted in Fig. 1.

Figure 1 Cultivation box.

As an easier alternative, a simple commercially available plastic box was used for cultivation in which the textile fabrics were placed horizontally on a mesh without direct contact to the medium at the bottom of the box. Generally, the exact design of the box is not very important, as long as irrigation and sufficient light intensity are ensured. In both cases, warm-white LED strips are used for illumination.

Tests were performed from September to November 2020 and from February to March 2022 in Bielefeld, Germany.

2.4 CHARACTERISATION

The textile fabrics were firstly investigated in terms of water retention by immersing them in water, placing them horizontally on a mesh and measuring the mass of water retained in the fabric at defined times.

Algae concentrations on the textile fabrics were measured by cutting 3 samples of area 1 cm² per textile fabric, putting each sample into a test tube, filling it up with 2 ml deionized water and separating the algae from the fabrics with a UP200HT ultrasonic processor (Hielscher Ultrasonics Gmbh, Teltow). Absorption measurements in the supernatant were performed by a Biochrom WPA Biowave DNA Life Science spectrophotometer (Biochrom, Cambridge, United Kingdom).

Oxygen production of the microalgae was measured by a Clark electrode (Oxygraph+, Hansatech Instruments Ltd., Norfolk, United Kingdom) on three samples of area 1 cm² per textile fabric.

All graphs are prepared with OriginPro 2021.

3 RESULTS AND DISCUSSION

Firstly, the textile fabrics are investigated with respect to their water retention capabilities. Fig. 2 depicts the results of these experiments performed for about two days.

The Tencel shows optimum water retention properties and was thus used in all subsequent tests. In addition, the polyester nonwoven was chosen for some subsequent tests as an example for a fabric from man-made fibres.

Next, tests with *S. longispina* on the Tencel fabric were performed. In preliminary tests of the cultivation box (Fig. 1), the wetting method was optimized so that wetting was regular over the whole mounted textile fabric.

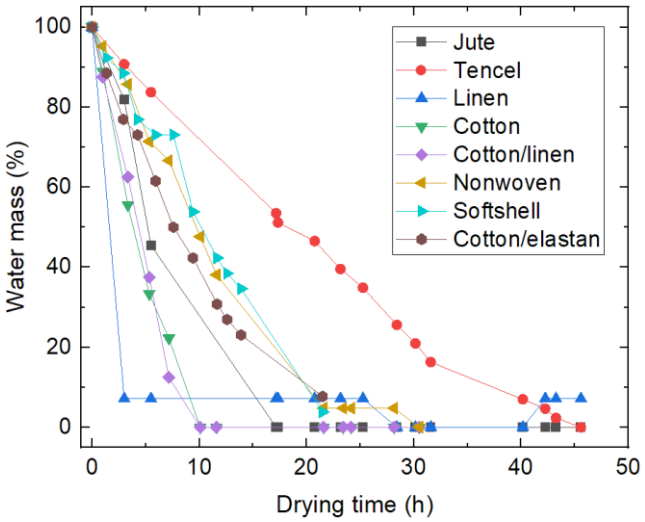

Figure 2 Water retention tests on different textile fabrics.

In the first tests, the microalgae were cultivated on the Tencel fabric inside the medium for 7 days and afterwards cultivated in air for further 7 days, with spray-watering all 3 h for 7 s at a pump intensity of 61% in test T1 and all 5 h for 5 s at 49% for test T2, resulting in overall pumped water of 1.5 L (T1) and 0.7 L (T2), respectively. In both tests, the microalgae were slowly washed off the textile fabric, as depicted in Figure 3.

Figure 3 Tencel fabrics after tests T1 and T2, respectively.

In the next test series, cultivation in medium was extended to 14 days and cultivation in air to 18 days, while the other parameters were approximately equal to the previous tests. These tests showed a much thicker algae biofilm after the cultivation in medium, but the microalgae were still detached from the textile fabric during cultivation in air. This can be explained by the strong impact of the sprayed fluid on the surface of the textile fabric.

For the next test series, irrigation was thus changed towards drip irrigation. Fig. 4 shows a textile fabric pre-cultivated in medium for 14 days, before and after further cultivation in air with drip irrigation for 18 days. After this first proof of principle, cultivation under drop irrigation in air was further optimized. The algae culture used for these tests was changed to *C. vulgaris* due to its good availability and widespread use in various applications.

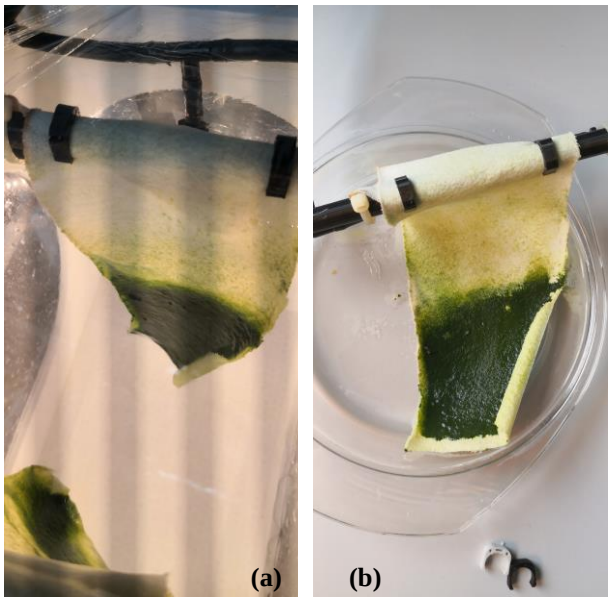

Figure 4 Tencel fabric (a) at the beginning and (b) at the end of cultivation in air for 18 days.

Due to previous experience with these microalgae [13], the medium was changed to diluted phytoplankton fertilizer according to Guillard f/2 (Planktino, applied in a concentration of 1 mL/L). Besides Tencel, a polyester nonwoven (cf. Table 1) was used as a substrate for cultivation. Instead of the custom-made cultivation box (Fig. 1), a commercially available box was used in which the fabrics were placed on a metal mesh. According to the previous results, drip irrigation was used for watering, applying different intervals (3 min all 24 h in the first week, 2 min all 24 h in the second week, 1 min all 24 h in the third week, and 1 min all 48 h in the fourth week). Warm-white LED light was applied for 8 h/day. Pre-cultivation in medium was performed for 12 days, cultivation in air for 28 days. The areal density of microalgae on both textiles under examination, measured by cutting 3 samples per textile, is given in Fig. 5. On the one hand, the microalgae grow well on both substrates during cultivation in air. The slope of the growth on Tencel is higher than for the microalgae growth on the PES nonwoven. On the other hand, the initial areal density of the microalgae on the PES nonwoven is significantly lower than the value measured on the Tencel fabric. This results in a higher relative increase of the growth on the PES nonwoven by a factor of (22 ± 7) during cultivation in air, as compared to a factor of (5.6 ± 1.1) during the same time for the microalgae density on the Tencel fabric. Both values are lower than those measured in suspension [12,13], which may be attributed to the increased space in three dimensions. Finally, Fig. 6 shows the oxygen production of *C. vulgaris* grown on Tencel and PES nonwoven substrates, respectively. In both cases, the oxygen production is slightly increased during cultivation in air, indicating that the microalgae have a normal metabolism and are not negatively influenced by these culture conditions.

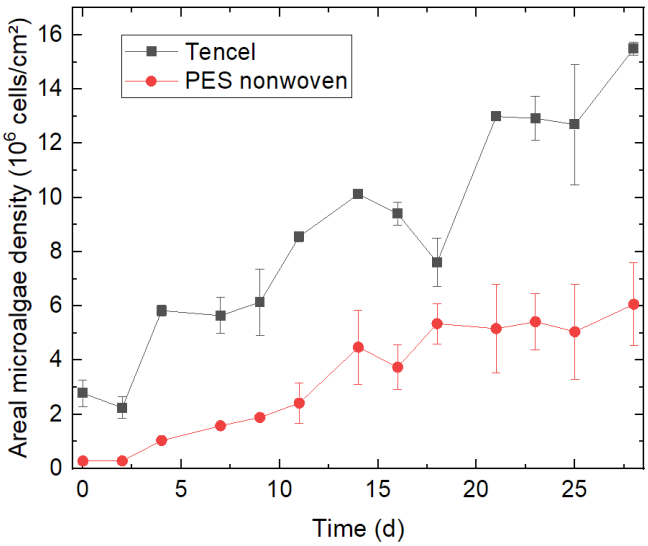

Figure 5 Areal density of *C. vulgaris* grown on different substrates in air.

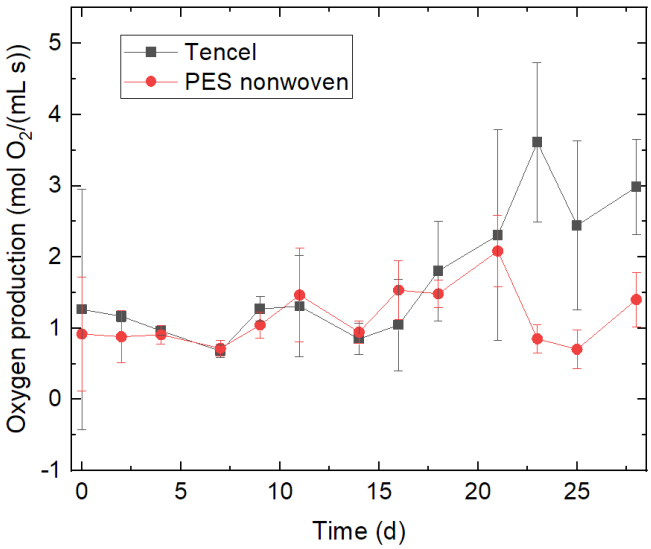

Figure 6 Oxygen production of *C. vulgaris* grown on different substrates in air.

4 CONCLUSIONS

To improve illumination and facilitate harvesting, green microalgae were grown on textile substrates in air. The experiments showed that drip irrigation is advantageous as compared to spray irrigation. During a 4 week cultivation period in air, after 12 days of pre-cultivation in medium, the irrigation intervals could even be elongated, indicating that the algae could adapt to reduced humidity.

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