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Influence of culture medium recycling on the performance of *Arthrospira platensis* cultures

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ABSTRACT

To reduce the water footprint of microalgae biomass production, it is essential to recycle the culture medium. The influence of medium recycling on the performance of the cyanobacterium *Arthrospira platensis*, the most widely cultivated microalgae, was investigated. *Arthrospira* was harvested with a 20 μm mesh size microstrainer, which is the benchmark harvesting technology for *Arthrospira* production. Repeated recycling of the culture medium resulted in a decline in growth rate and the maximum quantum yield of photosynthesis (F_v/F_m) when compared to a control culture in fresh medium. This decline was accompanied by accumulation of organic matter in the culture medium (up to 104 mg C L^{-1}). This organic matter consists of 70% of sugars, mostly rhamnose-rich polysaccharides with uronic acids. Accumulation of polysaccharides resulted in a decrease in the filtration rate through the microstrainer used for harvesting. Part of the biomass escaped harvesting and was returned to the culture with the recycled medium. This resulted in a change in the *Arthrospira* population and reduction in the harvesting efficiency, but this change in population had no effect on the growth rate. The growth rate of *Arthrospira* in the recycled culture medium was primarily influenced by organic matter that accumulated in the medium.

1. Introduction

Microalgae have received a lot of interest in recent years as a new source of biomass for production of biofuels or other bioproducts [1]. Like other crops, production of microalgae requires water [2,3]. The biomass concentration of microalgae in open cultivation systems is typically only about 0.5 g dry biomass L^{-1}. Consequently, 2000 L water is required to produce 1 kg of dry biomass. This is comparable to rice or soybeans, two terrestrial crops with a notoriously high water demand. This water footprint should be taken into account when considering microalgae as a new source of biomass [4]. In contrast to terrestrial crops, microalgae do not actively evaporate water through evapotranspiration. Microalgae require water mainly as a medium to remain in suspension, which implies that a large proportion of medium used for production of microalgae can thus theoretically be re-used, provided that essential nutrients have been repleted. Water losses are therefore limited to evaporative losses from the water surface, biomass-bound water and water used in biomass processing. Recycling of the culture medium after harvesting can reduce the water demand by 84% [5]. This results in a water demand of only 320 L kg^{-1} dry biomass, which is low compared to most terrestrial crops.

Today, global production of microalgae is still modest (about 10,000 ton $year^{-1}$) and only a handful of species are produced on a large scale. The bulk of the total global production can be ascribed to a single species, the cyanobacterium *Arthrospira platensis*, commercially known as 'Spirulina' [6]. Production of *Arthrospira* is relatively straightforward compared to other microalgae because contamination of the cultures can easily be avoided by maintaining a high alkalinity. Under nutrient-replete conditions, *Arthrospira* has a high protein content and is attractive as animal feed. When nutrient-stressed, it can accumulate up to 70% of carbohydrates in the form of glycogen, which makes it an attractive feedstock for production of biofuels [7]. *Arthrospira* also contains several high-value biochemical compounds such as the polyunsaturated fatty acid gamma-linolenic acid or the natural blue pigment phycocyanin [8,9]. Because the culture medium contains high concentrations of carbonate/bicarbonate (>8 g L^{-1}), most of which is not consumed but serves only to maintain a high alkalinity in order to avoid contamination of the culture, recycling of the medium in *Arthrospira* cultivation is not only important to reduce water consumption but also to reduce the need for chemicals.

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Despite the fact that recycling of the culture medium can lead to massive savings in water and chemical consumption, there have been relatively few studies that have investigated the feasibility of culture medium recycling. As far as we know, no studies have investigated medium recycling in *Arthrospira* cultures. Nevertheless, it is known that culture medium recycling causes problems in industrial production of *Arthrospira* [10]. In other species of microalgae, some studies found that recycling of the culture medium had little effect on the performance of microalgal cultures (e.g., *Chlorella vulgaris*, Hadj-Romdhane et al. [11]; *Scenedesmus* sp., Kim et al. [12]; *Chlorella zofingiensis*, Zhu et al. [13]) while others observed a significant inhibition of growth after repeated medium recycling (e.g. *Nannochloropsis* sp., Rodolfi et al. [14]).

Medium recycling may influence the performance of the culture in two essentially different ways. First, dissolved substances that are excreted by the microalgae will accumulate in the medium. Microalgae can excrete large amounts of dissolved organic matter in the culture medium [15]. This organic matter may include growth-inhibiting substances, such as specific free fatty acids (e.g. [16]). A large part of this organic matter consists of polysaccharides and these may influence the rheological properties of the culture medium [17,18]. Not only dissolved substances but also particulate cell debris can accumulate in the medium and this may also influence the performance of the culture, as has been shown for *Nannochloropsis* [14]. Secondly, because harvesting is never 100% efficient, part of the microalgal population will inevitable escape harvesting and will be returned to the culture when the medium is recycled. This selective force may result in evolution of the population and this may influence the performance of the culture [19]. One of such changes will most likely be a decrease in the harvesting efficiency of the biomass, but other properties may change as well, including a reduction in growth rate.

The goal of this study was to investigate the influence of repeated recycling of the culture medium on the performance of an *Arthrospira* culture. Therefore, we compared the performance of an *Arthrospira* culture in which the culture medium was repeatedly recycled with the performance of a control culture that received fresh medium. It was also investigated to what extent changes in culture performance were due to changes in the *Arthrospira* population resulting from incomplete harvesting or due to changes in the medium. Changes in growth rate, biomass composition as well as harvesting efficiency were monitored.

2. Materials and methods

2.1. Arthrospira strain and culture conditions

The *A. platensis* strain 21.99 (SAG, Germany) was used in all experiments (further referred to as *Arthrospira*). *Arthrospira* was cultured in Zarrouk medium modified by Cogne et al. [20]. Cultures were maintained in 1 L bottles. The cultures were aerated with 0.2 μm filtered air (5 L min^{-1}) and stirred using a magnetic stirrer. The culture bottles were irradiated from one side with daylight fluorescent tubes, resulting in a light intensity of 100 μmol photons m^{-2} s^{-1} at the surface of the flasks. The photoperiod was set at 16:8 (light:dark) and room temperature was held constant at 20 ± 2 °C.

2.2. Culture medium recycling experiment

To evaluate the influence of medium recycling on the performance of *Arthrospira* cultures, three replicate sequential batch cultures in which the medium was replaced weekly with fresh culture medium (fresh medium treatment, FM) were compared with sequential batch cultures in which the medium was replaced with recycled culture medium (recycled medium treatment, RM). In the RM treatments, the biomass was harvested using a microstrainer, as this is the standard harvesting method in commercial production of *Arthrospira* [21]. A nylon mesh with a pore size of 20 μm was used. Preliminary tests had shown that this mesh size had a harvesting efficiency (Eq. (1), see below) of 88%. Nylon meshes with a wider mesh size had a substantially lower harvesting efficiency (30 μm: 83%, 41 μm: 76%, 64 μm: 64%, and 180 μm: 5%). In the FM treatment, 80% of the culture was removed and replaced with fresh culture medium. In the RM treatment, 90% of the culture medium was removed and replaced with recycled culture medium. Because harvesting was only 88% efficient in the RM treatment, initial biomass concentrations were comparable in both treatments despite the fact that the amount of medium that was replaced was different. The cultures were restarted every 10 days and this cycle was repeated 4 times. To avoid nutrient limitation, the main macronutrients N and P were repleted in the RM treatment based on the amount of biomass that was harvested and the content of nutrients in the biomass (10% N and 0.8% P [22]). Previous experiments had shown that repletion of iron and trace elements was not necessary.

2.3. Biomass analyses

Biomass concentration was monitored by measuring the absorbance at 750 nm [23]. Spectrophotometric measurements were calibrated by gravimetrical analysis, after filtration of a known volume on a preweighed GF/C filter. The exponential growth rate was calculated from changes in biomass concentration over the one-week period following medium replacement. The maximum quantum yield of photosystem II or F_v/F_m is a highly sensitive indicator of stress in photosynthetic organisms [24]. We measured F_v/F_m using an AquaPen PAM fluorometer (Photon Systems Instruments, Czech Republic) Samples were dark-adapted for 30 min prior to fluorescence measurements. F_m is defined as the maximum fluorescence, measured after supplying a high intensity light pulse. F_0 is defined as the minimum fluorescence, measured with minimal irradiance. F_v is then referred to as the variable fluorescence (F_m–F_0) [25].

At the end of the experiment, total sugars as well as the pigments phycocyanin, chlorophyll a and carotenoids were measured in the biomass. Proteins were determined by the Bradford assay [26] and sugar content using the phenol–sulphuric acid method according to Dubois et al. (1956) with glucose as standard [27]. Carotenoids and total chlorophyll in the biomass were measured according to Lichtenthaler and Buschman [28] and phycocyanin according to Yoshikawa and Belay [29].

2.4. Culture medium analyses

In the culture medium, the concentration of dissolved organic carbon (DOC) and sugars was analysed. The culture medium was first filtered over a Whatman GF/C glass fibre filter. DOC was measured using a Shimadzu TOC analyser after acidification to pH 2 and sparging to remove (bi)carbonate. Total sugars in the medium were measured according to the Dubois' phenol sulphuric acid method [27], using glucose as the standard. To evaluate the monosaccharide composition of the sugars excreted in the recycled culture medium after 4 recycles, the organic matter was concentrated using a 3000 Da ultrafiltration membrane and dialyzed to remove salts. The concentrate was then freeze-dried before the polysaccharides were hydrolysed with trifluoroacetic acid. For HPAEC–PAD, monosaccharides were isocratically separated on a CarboPacTM PA1 column (250 mm) with 18 mM NaOH for 30 min followed by a linear gradient from 0 to 1 M sodium acetate in 200 mM NaOH for 20 min (Dionex ICS3000 system). The total protein content of the freeze-dried concentrate was measured by the Lowry method [30].

2.5. Monitoring of harvesting efficiency

Because straining using the nylon mesh did not retain all *Arthrospira* filaments during harvesting, changes in the efficiency of harvesting