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High-density cultivation of microalgae continuously fed with unfiltered water from a recirculating aquaculture system

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ABSTRACT

Water from recirculating aquaculture systems (RAS) has been shown to be a suitable growth medium for microalgae and their cultivation can, therefore, be used to reduce RAS emissions. However, while efficient wastewater treatment is possible, the nutrient content of RAS water limits attainable microalgae biomass densities to 1–2 g l^{-1} at best, which requires frequent harvesting of microalgae. We have taken advantage of the constant evaporation of water from an open thin-layer photobioreactor (200 l volume, 18 m^2 illuminated surface, artificial supply of CO_2) to continuously add water from RAS to a microalgae culture and thereby provide nutrients for continued growth while evaporating all water. To test for a possible inhibitory effect of RAS water on microalgae growth, components of mineral medium were omitted stepwise in subsequent cultivations and replaced by RAS water as the only source of nutrients. This approach showed that microalgae can be grown successfully for up to three weeks in RAS water without additional nutrients and that high (20 g l^{-1}) biomass densities can be attained. While growth in wastewater did not reach productivities measured in mineral medium, analysis of growth data suggested that this reduction was not due to an inhibitory effect of the RAS water but due to an insufficient supply rate of nutrients, even though RAS water contained up to 158 mg l^{-1} NO_3-N. It is, therefore, concluded that this method can be used to fully treat the wastewater discharge of a RAS. Furthermore, because both water evaporation from and microalgae growth in the photobioreactor correlated positively with each other due to their shared dependency on solar radiation, supply of nutrients continuously adjusts to changes in demand. It is estimated that the area of a photobioreactor required to treat all emissions of a RAS requires approximately 6.5 times the area of the latter.

1. Introduction

Aquaculture is a growing industry. While fishery production by capture has stabilized, aquaculture has experienced steady growth for the last two decades and is becoming the major source for human fish consumption [1]. This development places a heavy burden on the environment as it causes an increase in both feed demand [2] and wastewater discharge [3]. Solutions must be brought forward to make aquaculture more sustainable.

In recirculating aquaculture systems (RAS), the process water is recycled within the system through mechanical and biological filters, which reduces the demand of fresh water to < 10% of that of conventional aquacultures [4]. Thus, RAS technology not only decreases wastewater discharge, it also increases the concentrations of nitrogen and phosphorous in the circulating water to levels that can support the production of plants [5]. This allows co-cultivation technologies such as aquaponics, where nutrients in the wastewater originally introduced with fish feed serve as fertilizer for the production of vegetables [6].

Here, we investigated the use of RAS water to cultivate microalgae, a promising renewable resource that is commercially used in a number of applications [7]. Microalgae cultivation lends itself to a coupling with aquaculture [8] because microalgae are the basis of the natural food chain in aquatic ecosystems and, thus, are an important food source to rear larvae of many fish species [9]. Furthermore, microalgal protein and lipids are candidates for the partial substitution of fish meal and fish oil in feed production [10]. However, the production of such low-cost commodities is hindered by high production costs [11].

In order to make the production of microalgae more economical, a number of solutions have been proposed, among them the recycling of waste products from other processes such as flue gas or wastewater to supply carbon and other nutrients respectively to the microalgal culture [12,13]. Besides lowering production costs, such a strategy has the added benefit that the cultivation process becomes more sustainable [14].

Wastewater from aquaculture, and in particular water from RAS, lends itself to the cultivation of microalgae for several reasons: First, nutrient composition in aquaculture wastewater matches the demand of microalgae and batch growth experiments support this [15–17]. Second, RAS water has a low content of organic substances, which minimises the growth of contaminating bacteria in a microalgal culture. Third, unlike in many other types of waste water, where ammonium is the main nitrogen compound, RAS water contains nitrate, which is more stable and less toxic for microalgae [18].

If microalgae are to be used beyond a mere treatment of the wastewater itself, it is important to attain high biomass densities to facilitate downstream processing. The concentration of the limiting nutrient

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in the wastewater sets the upper limit for microalgal growth, which then can be estimated by applying the Redfield ratio. Assuming that nitrogen is the limiting nutrient and occurs at a concentration of approximately 100 mg l^{-1} in a RAS, microalgal biomass may reach a maximum concentration of 1–2 g dw l^{-1}, after which nitrogen levels are depleted. This corresponds to published values [19–21].

Microalgal biomass densities of 30 g dw l^{-1} or higher are possible with phototrophic cultivation, if conditions are optimized and a non-limiting supply of nutrients is ensured [22,23]. Because nutrient concentrations in wastewater are typically low [12], its subsequent addition requires the removal of an equal amount of culture volume and, thus, prohibits attaining a dense culture of microalgae.

Here, we propose to overcome this limitation by feeding water from a RAS to an open thin-layer photobioreactor [24]. Due to its open design and high surface-area-to-volume ratio, up to 50% of the culture volume (5–6 l m^{-2}) evaporate on a single day [24] and must be balanced by supplying additional water. By using RAS water to offset evaporation, nutrients are provided constantly and the cultivation becomes a fed-batch instead of a batch cultivation. In addition, because both evaporation and photosynthesis are correlated due to their dependency on incident sunlight, the delivery of nutrients scales with its demand.

To test whether the continuous supply of RAS water allows a productivity that is equivalent to cultivation in an artificial medium and allows to yield biomass densities beyond 1–2 g dw l^{-1}, experimental cultivations of *Chlorella vulgaris* and *Tetradesmus obliquus* (syn. *Acutodesmus obliquus*, *Scenedesmus obliquus*) were conducted in an open thin-layer photobioreactor (200 l culture volume). This was done in a series of cultivations where a mineral medium was replaced stepwise by unfiltered water taken directly from a RAS.

2. Materials and methods

2.1. Cultivation of microalgae

All cultivations were carried out in an open thin-layer photobioreactor [24, constructed by BCS Engineering s.a., Brno, Czech Republic], situated in a foliar greenhouse on the Grüental campus of the Zürich University of Applied Sciences in Wädenswil, Switzerland (47°13′2.09“ N, 8°40′53.58” E). The reactor consisted of an inclined culture surface made of glass sheets in a steel frame on which an algal suspension was circulated (Fig. 1). At the lower end of the surface, the suspension was collected in a tank and then pumped up again with a centrifugal pump. The culture surface had an inclination of 1.7%, a length of 18 m, and a width of 1 m. On the surface, the layer of algal suspension had thickness of 6–8 mm and a velocity of 0.5 m s^{-1}. The thickness of the suspension layer was regulated by coupling an ultrasonic sensor to the pump via a proportional integral (PI) controller.

Chlorella vulgaris (strain CASSIE/CCAP 211–52) was used for all cultivations in 2014 (Table 1). Early in 2015, an undescribed *Chlorella*-specific infection prevented its subsequent use and a natural isolate of *Tetradesmus obliquus* was used instead. If not indicated otherwise, a mineral fertilizer [25] was added regularly, so that nutrients never became limiting. The fertilizer consisted of the macronutrients $(NH_2)_2CO$ (3.05 mM), KH_2PO_4 (0.29 mM), $MgSO_4$ (0.14 mM), EDTA FeNa (0.02 mM), $CaCl_2$ (0.13 mM), and the micronutrients H_3BO_3 (2.78 μM), $CuSO_4$ (0.77 μM), $MnCl_2$ (2.77 μM), $CoSO_4$ (0.37 μM), $ZnSO_4$ (1.55 μM), $(NH_4)_6Mo_7O_{24}$ (23.2 nM), $(NH_4)VO_3$ (20.0 nM). Molar concentrations given allow growth of 1 g l^{-1} microalgae biomass (dry weight) and the fertilizer was dosed accordingly.

During the day, food-grade CO_2 was injected into the suction pipe of the suspension circulating pump. Dissolved CO_2 was measured just before the point of injection by means of a Severinghaus electrode (InPro®5000i, Mettler Toledo, Greifensee, Switzerland), which then regulated the supply of CO_2 via a PI controller. CO_2 partial pressure in the algal suspension was kept at 10 mbar. At night, no CO_2 was supplied. Switching between day and night modes was based on local sunrise and sunset times.

The pH of the algal suspension was recorded every minute with a pH electrode (InPro®3253i, Mettler Toledo). The electrode was calibrated before every cultivation. The same sensor was used to measure temperature of the microalgal suspension.

Growth was monitored by daily dry weight measurements (HB43-S-Halogen Moisture Analyzer, Mettler Toledo) of the algal suspension.

Photosynthetically active photon flux density (PPFD; μmol m^{-2} s^{-1}) was measured with two sensors (SKL2620, Skye Instruments Ltd., Powys, UK) placed above and below the glass platform. The number of photons absorbed was calculated as the difference between the measurements of both sensors. PPFD was converted to photosynthetically active radiation (PAR, W m^{-2}) by multiplication with $2.02 \cdot 10^{-5}$ μmol l^{-1} J [23]. Data on global radiation (W m^{-2}) outside of the greenhouse in which the photobioreactor was located were obtained from the Federal Office of Meteorology and Climatology (station Wädenswil, 10-min averages). These data were used to estimate the loss of light inside the greenhouse. It was assumed that 45% of the energy of global radiation are within the PAR spectrum [26].

The volume of the circulating algal suspension was continuously monitored by means of a pressure sensor in the suspension tank. Total volume was 200 l and water loss by evaporation was balanced whenever the volume fell below 195 l. A water meter at the inlet pipe measured the amount of added water and was used to estimate evaporation (readings were taken every minute). Depending on the experimental conditions, water was either partially desalted tap water (Ministil Clean HT, BWT, Austria) or unfiltered water from a RAS (see below).

Concentrations of nitrogen compounds (ammonium, nitrite, nitrate) in water samples were measured with photometric test kits (Hach-Lange, Rheineck, Switzerland) or ion-chromatography (930 Compact IC Flex, Metrohm, Zofingen, Switzerland).

2.2. Recirculating aquaculture system

RAS water was supplied from two different systems in 2014 and 2015. The RAS used in 2014 consisted of three interconnected circular fiber glass fish tanks with a total volume of 5 m^3. Water from the tanks was drained through a central bottom-outlet to a 60-μm drum filter (Hydrotech HDF 501, Veolia, Saint-Maurice, France) into a moving-bed filter, enriched with pure oxygen, and returned to the tanks.

Tanks were stocked with 100-g Pike Perch (*Sander lucioperca*) to a stocking density of 20 kg m^{-3} and were fed with commercial fish feed (Aller Metabolica, Emsland Aller Aqua GmbH, Golssen, Germany). Daily feed amounted to 1.5% of the fish body weight (1.53 kg d^{-1}).

The RAS used in 2015 hat a total volume of 4 m^3 and consisted of one rounded square fish tank with a volume of 2.9 m^3. Water from the tank was drained through a central bottom outlet to a 60-μm drum filter (L500, Lavair AG Klimatechnik, Aach, Germany) into a moving bed biofilter enriched with pure oxygen and returned to the tank. Faeces and fish feed leftovers removed with the drum filter were collected in a radial flow settler and the supernatant was returned back. Settled sludge (7–10 l) was manually removed from the system three times per week.

The tank was stocked with 500-g Tilapia (*Oreochromis niloticus*) to a stocking density of 8.5 kg m^{-3}. Fish were fed 2% of their body weight per day with commercial fish feed (Tilapia Vegi 4.5 mm, Hokovit, Hofmann Nutrition AG, Bützberg, Switzerland).

2.3. Experimental cultivations of microalgae

To be able to assess whether the use of RAS water for microalgae cultivation has an influence on the growth performance, consecutive cultivations were carried out, where artificial medium was replaced stepwise with unfiltered water from the RAS (Table 1). This approach