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# Characterization of nutrient removal and microalgal biomass production on an industrial waste-stream by application of the deceleration-stat technique

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ABSTRACT

Industrial wastewaters can serve as a nutrient and water source for microalgal production. In this study the effluent of an internal circulation (IC) reactor anaerobically treating the wastes of a biotechnology production facility were chosen as the cultivation medium for *Chlorella sorokiniana* in batch and continuous cultures. The aim was to evaluate the rates of nutrient removal and biomass production possible at various dilution rates. The results demonstrate that the industrial wastewater served as a highly effective microalgae culture medium and that dilution rate strongly influenced algae productivity in a short light-path photobioreactor. Batch culture on undiluted wastewater showed biomass productivity of 1.33 g $L^{-1}day^{-1}$, while removing over 99% of the ammonia and phosphate from the wastewater. Deceleration-stat (D-stat) experiments performed at high and low intensities of 2100 and 200 (μmol photon $m^2s^{-1}$) established the optimal dilution rates to reach volumetric productivity of 5.87 and 1.67 g $L^{-1}day^{-1}$ respectively. The corresponding removal rates of nitrogen were 238 and 93 mg $L^{-1}day^{-1}$ and 40 and 19 mg $L^{-1}day^{-1}$ for phosphorous. The yield on photons at low light intensity was as high as had been observed in any previous report indicating that the waste stream allowed the algae to grow at its full potential.

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## 1. Introduction

Microalgae are considered promising for production of biofuels, bulk chemicals (Wijffels and Barbosa, 2010) and certain higher value products (Borowitzka, 2013), although cost of production currently limits biofuel feasibility. Recent economic analysis has indicated that utilization of algae for high-volume, low-value products become more feasible when coupled with nutrient removal (Lundquist et al., 2010). Moreover, use of wastewater resources would significantly reduce the water footprint and nutrient requirement of algae biomass production (Yang et al., 2010). Whether the primary purpose is nutrient removal or biomass production, economic feasibility demands optimized production (Morweiser et al., 2010).

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In several studies recovery of nutrients from municipal, industrial or agricultural waste-streams, by algae have been investigated. Each waste stream varies in nutrient content, nutrient bioavailability, content of potential inhibitors to algae, and amount available (Cai et al., 2013a, 2013b). Streams also vary in their content of pollutants that would preclude the use of the algae biomass for higher value products, especially in food or feed applications (Markou et al., 2014). Although algae have some plasticity, there are limits to N:P ratio beyond which performance suffers (Mayers et al., 2014). Nutrient content in wastewaters shouldn't drastically exceed the needs of the algae, for example the municipal anaerobic digestate in Cai et al. (2013b) was so high in nutrients and coloration that the authors chose to dilute it nearly 20-fold in order to achieve optimal productivity and nutrient removal. Such dilution either increases water footprint or requires a second source of low-nutrient wastewater.

The limitation of productivity of algae cultures by "self-shading" has long been understood (Nielsen et al., 1962). As a result, "culture productivity depends on the intensity of the light source, as well as on the exponential decrease of radiation intensity (modulated by cell density) across the optical path" (Richmond et al., 2003). Dilution rate (inverse hydraulic retention time) determines cell density and therefore is a critical factor to control productivity in continuous photobioreactors. Furthermore, microalgae may photo-acclimate to higher light intensities by reducing pigmentation to prevent photo-oxidative stress (Geider et al., 1996). Due to the combined effects of self-shading and pigmentation adjustment, the optimal dilution rate should be empirically optimized for any combination of species, light intensity and path length to maximize production. For example, in the otherwise identical conditions, dilution rates of 0.05 and 0.24 $h^{-1}$ resulted in respective biomass concentrations of 6 and 2 g $L^{-1}$ and productivities of 7.2 and 12 g $L^{-1}$ $D^{-1}$ (Cuaresma et al., 2009), while higher dilution rates quickly led to declining productivity and eventually washout.

The deceleration-stat (D-stat) method has previously been established as an effective way to optimize biomass productivity as a function of dilution rate. As a result of the time consuming nature of a set of experiments optimizing dilution rate in continuous algal cultures, researchers began using methods in which the dilution rate was steadily changed with time, termed acceleration stats (A-stats) (Barbosa et al., 2005, 2003) or deceleration stats (D-stats) (Hoekema et al., 2006). With algae, as with other organisms (Müller et al., 1995), such experiments can yield informative transient or pseudo steady state data. If the dilution rate, $D$ ($day^{-1}$) differs from the growth rate $\mu$ ($day^{-1}$) by a small amount ($\leq$5%), the culture will adapt to the changing conditions. Hoekema et al., 2013 demonstrated that the approach could save up to 94% of the time associated with a series of chemostat experiments and still accurately predict optimal productivity of a photobioreactor system. However until now, the method has been used only to optimize biomass productivity or composition, not nutrient uptake from wastewaters.

For this work, wastewater was collected from a newly installed reactor at a large biotechnology and pharmaceutical production facility. The reactor is an internal circulation reactor containing granular sludge (IC reactor) and was installed in order to produce biogas while treating some of the facility's COD (McHugh et al., 2003). The aim of this study was firstly to evaluate the appropriateness of IC reactor effluents as a source of nutrients and water in a series of batch experiments in photobioreactors. After observing that the wastewater was suitable for algae cultivation, a deceleration-stat was employed to estimate the optimal productivity and nutrient removal by the photobioreactor system.

## 2. Materials and methods

### 2.1. Microorganism, medium and wastewater

Chlorella sorokiniana CCAP 211/8 K was stored on agar plates containing modified M8a medium (Cuaresma et al., 2009). A colony from the plate was used for the inoculum for all experiments as it was maintained in 100 mL flask under continuous illumination of approximately 75 μmol $m^{-2}s^{-1}$ in a room controlled at 20 °C. The cultures were acclimated to the wastewater for several repeated batches before beginning experiments.

Wastewaters from the anaerobic treatment reactor were sampled at Novozymes plant in Kalundborg, January 2014 and filtered at the Liqtech facility in Ballerup DK using a 0.04 micron ceramic cross flow filter. Batches of the wastewater were frozen until used. Samples were sent to Eurofins for full wastewater analysis (Table 1).

Two types of photobioreactors (PBR's) were used (Table 2). Two flat-panel reactors (Algaemist reactor, Wageningen University) with short light-path (14 mm) and high illuminated-surface to volume ratio of 0.71 $cm^{-1}$ (see Fig. 1a) is described further with technical drawings are available in Breuer et al. (2013). Also, a pond simulating reactor (The environmental photobioreactor (ePBR) by Phenometrics inc.) with a long light-path (250 mm) and low illuminated-surface to volume ratio of 0.05 $cm^{-1}$ (see Fig. 1b) is described in detail in Lucker et al. (2014); Tamburic et al. (2014).

**Table 1 – Measured composition of wastewater and calculated concentration of mineral medium.**

| Element | IC reactor effluent (mg $L^{-1}$) | M8a medium (mg $L^{-1}$) |
|---|---|---|
| N | 190 | 443 |
| P | 11–12 | 214 |
| K | n.m. | 211 |
| S | ≥1.2[a] | 51.9 |
| Na | 1500 | 151.8 |
| Mg | 17 | 38.9 |
| Cl | 1200 | 11.3 |
| Fe | 0.23 | 16.8 |
| Mn | 0.24 | 3.85 |
| Ca | 120 | 3.61 |
| Zn | 0.23 | 0.65 |
| Cu | 0.0034 | 0.44 |
| B | 0.18 | 0.011 |
| VFA | 400 | 0 |
| COD | 590 | n. m. |

n.m. = not measured.
[a] only sulphates and sulfides measured.

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