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Nutrient removal and microalgal biomass production on urine in a short light-path photobioreactor

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ABSTRACT

Due to the high nitrogen and phosphorus content, source-separated urine can serve as a major nutrient source for microalgae production. The aim of this study was to evaluate the nutrient removal rate and the biomass production rate of *Chlorella sorokiniana* being grown continuously in urine employing a short light-path photobioreactor. The results demonstrated, for the first time, the possibility of continuous microalgae cultivation in human urine. The lowest dilution factor successfully employed was a factor of 2 (50% v/v urine). Microalgae dominated a smaller bacterial population and were responsible for more than 90% of total nitrogen and phosphorus removal. With a light-path of 10 mm, a maximum volumetric biomass productivity as high as 9.3 g L^{-1} d^{-1} was achieved. The co-existing bacterial population removed up to 70% of organic pollutants from the urine at a rate of 1300 mg COD L^{-1} d^{-1}. Enriching the urine with magnesium, adjusting the N:P molar ratio, and shortening the reactor light-path further increased the volumetric biomass productivity to 14.8 g L^{-1} d^{-1}. The corresponding nitrogen and phosphorus removal rates were 1300 and 150 mg L^{-1} d^{-1}, respectively. The subsequently produced biomass contained 43–53% w/w proteins and 16–25% w/w total fatty acids.

1. Introduction

Innovative, decentralised sanitation concepts have been the subject of several studies (Larsen and Gujer, 1997; Otterpohl et al., 1997; Zeeman et al., 2008). Although source separation concepts continue to be immature, various pilot projects have demonstrated their potential to save water, recover energy, and recover nutrients from black water and/or urine (de Graaff et al., 2010; de Graaff et al., 2011). In the Netherlands, several projects for new sanitation concepts have been established i.e. in 32 apartments in Lemmerweg-Oost in Sneek, a housing complex of 250 houses of Noorderhoek in Sneek, and an office building of 160 employees in Wageningen. Urine separation projects were conducted in several countries such as an office building of 60 employees (Sneek, the Netherlands), the

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Table 1 – Composition of synthetic urine and different batches of human urine.

Composition (g L^{-1})	Synthetic urine[a]	Human urine		
		Batch A	Batch B[b]	Batch C[b]
Total nitrogen (TN)	4326	7167	4358	5310
Phosphate phosphorus (PO_4^{3-}–P)	255	466	200	260
Ammonium nitrogen (NH_4^+-N)[c]	4006	844	393	4660
Chemical oxygen demand (COD)	–	8349	2886	5160
N:P atomic ratio	38:1	34:1	48:1	45:1

[a] Calculated values based on synthetic urine composition.
[b] Urine mixture of males and females from urine diverting toilet.
[c] Ammonium nitrogen concentration was measured at the beginning of the experiments.

Saniphos installation (Zutphen, the Netherlands), the building 'Villa Flora' (Venlo, the Netherlands), the head offices of the German technical co-operation GTZ (Eschborn, Germany), Kullön residential area (Vaxholm, Sweden), Universeum-science and Discovery Museum (Gothenburg, Sweden), and the EAWAG research institute (Zurich, Switzerland), etc (Kvarnström et al., 2006; Lienert and Larsen, 2009).

Because urine is rich in the nutrients: nitrogen and phosphorus, it has potential as a liquid fertilizer or as a nutrient source for the production of microalgae biomass which are a potential source of feedstock for the bio-based production of chemicals and biofuels (Wijffels et al., 2010), although, large-scale cultivation is not yet economically feasible. Coupling microalgae production to wastewater treatment is considered a more economic and sustainable option (Yang et al., 2011). In only a few studies has human urine been employed as the growth medium for microalgae cultivation. Although these studies have demonstrated that microalgae and cyanobacteria can grow and remove nutrients from urine, the studies were conducted in batch systems, and the urine or synthetic urine that was employed was extensively diluted from 50 to more than 100 times (Adamsson, 2000; Dao-lun and Zu-cheng 2006; Yang et al., 2008a,b; Chang et al., 2013).

Studies have shown the feasibility to use microalgae to recover nutrients from different types of wastewaters (Cai et al., 2013). However, no knowledge has yet been made available regarding the potential of coupling microalgae cultivation with the treatment of concentrated urine. From a theoretical point of view, high cell density cultivation is required to afford high nutrient removal efficiencies from high strength wastewaters such as urine. This can only be achieved with short light-path reactor systems that effectively supply light to all of the cells encapsulated inside the microalgal culture. In addition, the complex and partially unknown composition of concentrated urine may affect microalgae growth. Finally, with concentrated urine, hydrolysis of urea into ammonium will result in increased free ammonia concentrations which may inhibit microalgal growth (Azov and Goldman, 1982).

The aim of this study was to assess the minimum dilution factor that would allow for stable treatment of urine and concomitant microalgae production. In addition, it was investigated whether the process could be continuously operated at consistently high rates for nutrient removal and biomass production. For this purpose, *Chlorella sorokiniana* was selected as the inoculum as it had previously demonstrated significant potential in urine treatment (unpublished results). A flat panel photobioreactor with short light-paths of 1.0 and 0.5 cm was selected to support the high density microalgae cultivation. Finally, the possibility was explored to further stimulate biomass production by optimising the urine N:P molar ratio and by supplementing magnesium.

2. Materials and methods

2.1. Microorganism and urine media

C. sorokiniana CCAP211/8K was obtained from the Culture Collection of Algae and Protozoa, Oban, UK. Pre-cultures were grown in M8a medium (Kliphuis et al., 2010) in 250-mL shake flasks with 100 mL of liquid culture at 25 °C. The microalgae were initially cultivated in a light intensity of 20–40 μmol photons m^{-2} s^{-1} and a 16/8 h day/night cycle followed by continuous light of 165 μmol photons m^{-2} s^{-1} and 2% CO_2 enriched air.

In Experiment 1, synthetic urine modified from Yang et al. (2008a,b) was employed. It consisted of (per L): 0.11 g $(NH_4)_2SO_4$, 22.49 g NH_4HCO_3, 0.7 g K_2HPO_4, 0.75 g Na_2H-$PO_4.2H_2O$, 0.53 g $CaCl_2.2H_2O$, 1.23 g $MgSO_4.7H_2O$, 1.4 g K_2SO_4, 9.6 g NaCl, 1 g creatine, 0.1 g phenol, 10 mL Fe-EDTA stock solution and 10 mL micronutrient stock solution. Fe-EDTA stock solution contained per L: 11.6 g EDTA ferric sodium salt and 3.72 g $Na_2EDTA.2H_2O$. Micronutrient stock solution consisted of (per 1 L): 1.3 g $MnCl_2.4H_2O$, 0.32 g $ZnSO_4.7H_2O$, 0.18 g $CuSO_4.5H_2O$, and 0.006 g H_3BO_3. The synthetic urine pH was adjusted to 7 prior to utilisation. In synthetic urine, creatine was mistakenly used instead of creatinine which is the second most-abundant organic metabolite in urine (Bouatra et al., 2013). However, this did not affect synthetic urine COD concentration since creatine and creatinine both require equal amount of oxygen for their oxidation (the same COD input) (Kuntke, 2013).

In Experiments 2 and 3, various batches of urine were employed. Urine batch A was applied in Experiment 2; Urine batch B was administered in Experiment 3 from day 1–90; and batch C was employed from day 91 to the completion of Experiment 3. Urine batch A was directly collected using bottles from male employees from the Sub-department of Environmental Technology, Wageningen University, the Netherlands. Batch B was collected with urine diverting toilets from male and female employees of the Sub-department of Environmental Technology. Batch C was extracted from urine diverting toilets collecting urine from offices of a participating company (Landustrie BV, the Netherlands). The urine batches were maintained in the dark at 4 °C during all experiments. The composition of the various urine batches is depicted in Table 1. Non-diluted urine was supplemented with Fe-EDTA stock solution and micronutrient stock solution at a concentration level of each solution at 33.25 mL per liter. As demonstrated in Table 2, the urine with supplemental iron and micronutrients was diluted 50, 20, 10, 5, 3, and 2 times (1