Nutrients removal from anaerobic effluent with *Chlorella vulgaris*.

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Abstract

The removal of nutrients (nitrogen and phosphorus) from an anaerobically treated effluent by *C. vulgaris* was studied under different conditions. Three initial concentration of ammonium (68.4, 79.6 and 94.6 mg NH_4-N·L^{-1}) and microalgal biomass (94, 229 and 344 mg·L^{-1}) were evaluated. High removal for NH_4-N (88.0 ± 6.9%) and PO_4-P (54.3 ± 12.2 %) were obtained. The nutrients removal efficiency in the tests depended on the initial NH_4-N concentration, which can inhibit algal growth at high concentration and the growth of *C. vulgaris* in the tests depended on the initial algal biomass. The highest biomass (781.4 ± 90.1 mg·L^{-1}) and the highest specific growth rate (0.60 ± 0.02 d^{-1}) were obtained when the initial biomass was 344 mg·L^{-1} and the initial ammonium concentration was 94.6 mg·L^{-1}. For lower initial biomass the high concentration of ammonium seems to inhibit the process. According with results, *C. vulgaris* is a good alternative for nitrogen and phosphorus removal from wastewater.

Keywords

Nutrients; microalgae, wastewater treatment

INTRODUCTION

Large amount of wastewater is generated daily and must be treated before being discharged into the rivers. In developing countries with tropical climate like Colombia and Brazil, anaerobic treatment is used widely due to the advantages over those aerobics i.e. consume less energy, produce less sludge and biogas is generated. However, the discharge of the anaerobic effluent into water bodies may cause eutrophication of aquatic ecosystems due to high concentrations of nutrients. Therefore, the post-treatment of effluent is necessary (Cai, Park, et al., 2013).

Microalgae have been used in wastewater treatment since 50´s decade and recently has received more attention due to the microalgal biomass may be converted into added value products as additives for animal food, biomolecules, biofuel or biogas (Borowitzka and Moheimani, 2013). Treatment with microalgae for nutrients removal have been used for a wide variety of wastewater: municipal wastewater (Barrera Bernal, Vázquez, et al., 2007; Zamalloa, Boon, et al., 2013), agro-industrial wastes (Posadas, Bochon, et al., 2014; Hernández, Riaño, et al., 2013), domestic wastewater (Kumar, Dasgupta, et al., 2011) and industrial wastewater (Chong, Wong, et al., 2000). In addition, microalgae have been used to treat effluents at different stages in wastewater treatment plants (Ji, Abou-Shanab, et al., 2013; Ruiz-Martinez, Martin Garcia, et al., 2012). Most of anaerobically treated effluent have lower carbon levels and the nitrogen is mainly in the form of ammonium which can be used by microalgae to grow. However, a strong control on pH must be performed to avoid the volatilization of ammonia by stripping, the pH of the medium must be below 8 units (Escudero, Blanco, et al., 2014).

C. vulgaris has been used in wastewater treatment to assimilate nitrogen and phosphorus from it. He et al. (2013) studied the impact of *C. vulgaris* with and without co-existing bacteria on nutrients removal from municipal wastewater. They found NH_4 removal was due to *C. vulgaris* and the DOC removal was by bacteria. Liang et at. (2013) used *C. vulgaris* to remove nutrients from synthetic-like municipal wastewater. NH_4 and total phosphorus removal reached were 29% and 55%.

In this study, an anaerobically treated effluent was used to cultivate *C. vulgaris* in batch operation. The aim was to assess the influence of initial concentration of ammonium and the initial microalgal biomass concentration on nutrient removal and the biomass growth.

MATERIAL AND METHODS

Experimental set-up

The *C. vulgaris* was supplied by Biomass Laboratory at Universidad Industrial de Santander. The stock culture was grown at room temperature in 1 L Schott bottles with Bold´s Basal medium (BBM) (Bischoff and Bold, 1963). The bottles were sealed with stoppers and side openings for gas exchange. These flasks were aerated by using an air compressor and a diffuser in each bottle. Artificial light was provided at 4700 Lux using fluorescent lamps and light/dark cycle 12 hr/12hr was used.

To evaluate the influence of initial microalgal biomass (DW_o) and initial ammonium concentration (NH_{4o}) on microalgal growth and nutrients removal from anaerobically treated effluent, a 3^2 factorial design was used. DW_o were 94, 229 y 344 $mg \cdot L^{-1}$ and NH_{4o} were 68.4, 79.6 and 94.6 $mg \cdot L^{-1}$. Three replicates and a control without algae were used. Table 1 shows the operating conditions used.

Table 1. Operating conditions

DW_o ($mg \cdot L^{-1}$) **NH_{4o} ($mg \cdot L^{-1}$)**	**94**	**229**	**344**
68.4	B1N1	B2N1	B3N1
79.6	B1N2	B2N2	B3N2
94.6	B1N3	B2N3	B3N3

In each series of experiments, 500 ml suspension of precultured cells and 500 ml anaerobic effluent obtained from wastewater treatment plant at Universidad Pontificia Bolivariana, Colombia, were mixed in 1 L Schott bottles. The experiments were conducted with the same system used in the cultivation process. The pH in the bottles was kept between 7.5 and 8.0 units to avoid the volatilization of ammonia by stripping.

Analytical methods

Microalgal biomass, NH_4-N and PO_4-P concentrations, were monitored on the samples taken each two days. The pH was measured using a multiparameter HACH HQ40D. Algal biomass was determined by measuring optical density (OD_{550}) at 550 nm; prior, calibration curves were prepared between OD_{550} and dry weight (DW). Samples were centrifuged at 3600 rpm by 10 min to separate microalgae and the supernatant was used for NH_4-N and the PO_4-P measurements. NH_4-N concentrations were carried out using the Grow Master for Nutrient Analysis (Hanna Instruments) and the PO_4-P concentrations were determined by the Vanadomolybdophosphoric Acid Colorimetric Method (APHA, AWWA, et al., 2012).

Kinetic modelling

The initial substrate utilization rate (Ri, mg·L^{-1}·d^{-1}) was obtained using Eq. (1), where S_o (mg·L^{-1}) is the initial substrate concentration (NH_4-N or PO_4-P) as initial time, t_o (d) and S_t (mg·L^{-1}) is the corresponding substrate concentration at t_t (d). The specific rate of substrate removal, R_{xi}, was calculated by dividing R_i to the initial microalgal biomass concentration, DW_o (Eq. 2).

$$R_i = \frac{S_o - S_t}{t_o - t_t} \tag{1}$$

$$R_{xi} = \frac{R_i}{DW_o} \tag{2}$$

Statististical analysis

Three-way repeated-measures ANOVAs were used to study the main effects of factor levels NH_{4o} (68.4 mg·L^{-1}, 79.6 mg·L^{-1}, and 94.6 mg·L^{-1}) and DW_o (94 mg·L^{-1}, 229 mg·L^{-1}, and 344 mg·L^{-1}) and their interactions. The Mauchy's Test was used to test for sphericity. Post-hoc t-tests with Bonferroni adjustment was used for multiple average comparisons and to detect any differences between pairs of variables, at a significance level of $p<0.05$.

RESULTS AND DISCUSSION

Influence on microalgae growth

The mean values of DW with time at different NH_{4o} and DW_o are depicted in Figure 1. The NH_{4o} ($F_{2,10}$= 14.289, $p < 0.00117$) and DW_o ($F_{2,10}$= 248.077, $p < 3.010e\text{-}09$) have a significant effect on biomass concentration at the time, in addition to the interaction between NH_{4o} and DW_o was significant ($F_{4,20}$= 23.945, $p = 2.194e\text{-}07$). Mauchly's Test of Sphericity indicated that the assumption of sphericity had not been violated for DW_o ($p = 0.5758$) and NH_{4o} ($p = 0.9523$).

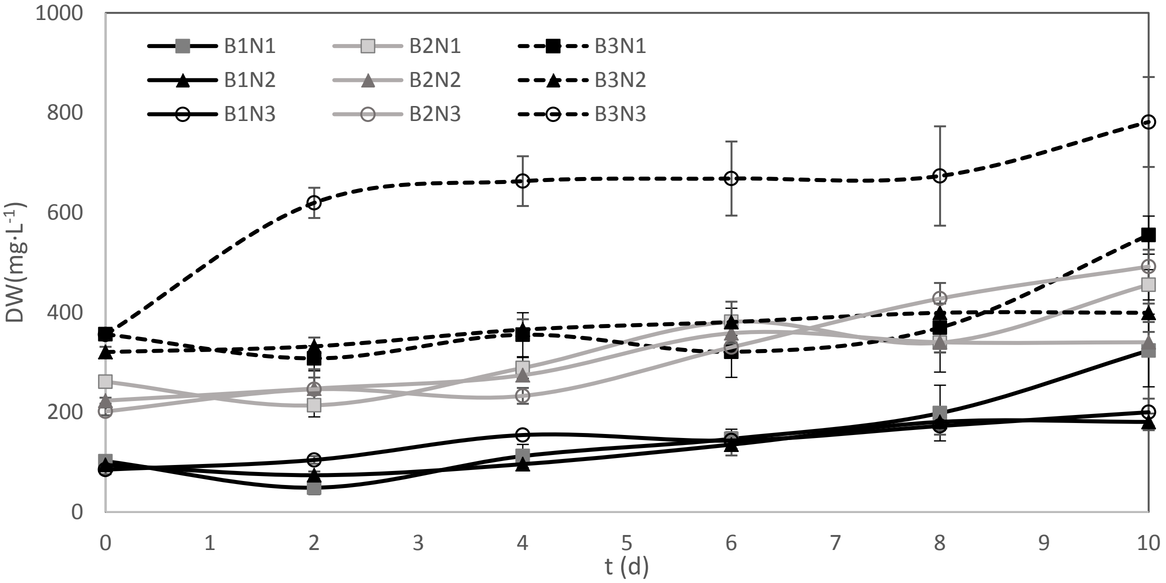

Figure 1. Variation of DW for NH_{4o} and DW_o. Each point represents mean value from five replicates determinations with standard deviation.

The results show that B3N3 had the highest biomass (781.4 ± 90.1 $mg\cdot L^{-1}$) after 10 days of incubation and the highest specific growth rate (0.60 ± 0.02 d^{-1}) whilst B1N2 and B1N3 have the lowest biomass at the end of the incubation time. These findings are according with post-hoc t-tests with Bonferroni adjustment for multiple comparisons which found significant difference between in NH_{4o} = 94.6 $mg\cdot L^{-1}$ and the other values used (68.4 $mg\cdot L^{-1}$ and 79.6 $mg\cdot L^{-1}$) ($p < 0.05$, Bonferroni test), but no significant difference was found between 68.4 $mg\cdot L^{-1}$ and 79.6 $mg\cdot L^{-1}$ ($p > 0.05$, Bonferroni test). When the DW_o was changed significant difference between in DW_o = 344 $mg\cdot L^{-1}$ and the other values used (94 $mg\cdot L^{-1}$ and 229 $mg\cdot L^{-1}$) ($p < 0.05$, Bonferroni test) but there was not significant difference between 94 $mg\cdot L^{-1}$ and 229 $mg\cdot L^{-1}$ ($p > 0.05$). The growth in B1N2 and B1N3 may be inhibited by the high concentration of ammonium as it has been reported by other authors (Escudero, Blanco, et al., 2014; Aslan and Kapdan, 2006).

Influence on nutrients

The mean values of NH_4-N with time at different NH_{4o} and DW_o are depicted in Figure 2. As seen in Figure 2, NH_4-N concentrations decreased rapidly at the beginning of the experiment (firsts four days) with ammonium removal rate approximately 11 $mg\cdot L^{-1}\cdot d^{-1}$, while the last five days those were around 3 $mg\cdot L^{-1}\cdot d^{-1}$. Aslan and Kapdan (2006) reported maximum NH_4-N removal rates of 10.5 $mg\cdot L^{-1}\cdot d^{-1}$ for *C. vulgaris* while Jimenez et al. (2004) obtained 56.06 for *Nannochloris sp.* suggesting that removal rate depends of microalgae used.

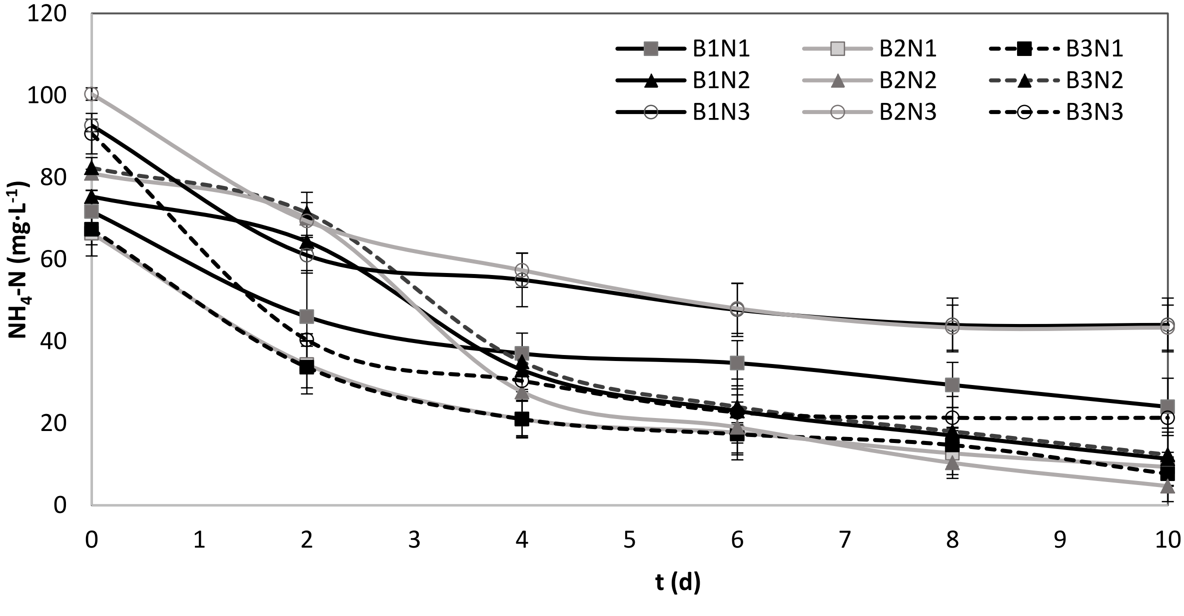

Figure 2. Variation of NH_4-N concentration for NH_{4o} and DW_o. (a) NH_{4o} = 68.4 $mg\cdot L^{-1}$, (b) NH_{4o} = 79.6 $mg\cdot L^{-1}$ and (c) NH_{4o} = 94.6 $mg\cdot L^{-1}$. Each point represents mean value from three replicates determinations with standard deviation.

The statistical analysis showed a significant main effect for NH_{4o} ($F_{2,10}$= 10.675, p = 0.0033), and DW_o ($F_{2,10}$= 16.280, p = 0.00071) on NH_4-N concentration at the time, in addition to the interaction between NH_{4o} and DW_o ($F_{4,20}$= 70.426, p = 1.694e-11). Mauchly's test of sphericity indicated that the assumption had not been violated for DW_o ($p = 0.242$). Post-hoc t-tests with Bonferroni adjustment for multiple comparisons showed that systems with $NH_{4o} = 68.4\ mg\cdot L^{-1}$ have significant difference with those with $94.6\ mg\cdot L^{-1}$ but no significant difference was found with $79.6\ mg\cdot L^{-1}$. When the initial algal biomass was changed there was significant difference ($p<0.05$, Bonferroni test) between $344\ mg\cdot L^{-1}$ and the other values used ($94\ mg\cdot L^{-1}$ and $229\ mg\cdot L^{-1}$), but no significant difference was found between $94\ mg\cdot L^{-1}$ and $229\ mg\cdot L^{-1}$ ($p > 0.05$, Bonferroni test).

The NH_4-N removal efficiency was higher, 80% for $NH_{4o} = 68.4$ and $79.6\ mg\cdot L^{-1}$ and it was around 60% for $94.6\ mg\cdot L^{-1}$. Aslan y Kapdan (2006) indicated that effluent water quality decreases with increasing nutrient concentration,they found removal efficiency around 50% for NH_{4o} between 41.8 and $92.8\ mg\cdot L^{-1}$ but when NH_{4o} was higher than $129\ mg\cdot L^{-1}$ the removal efficiency decreased to less than 24%. Miao et al (2016) reported NH_4 removal rates were approximately 32% - 50% and the highest was 98.69% by 25% synthetic domestic wastewater.

When DW_o was $344\ mg\cdot L^{-1}$ the NH_4-N average removal efficiency was 83.4 while for $94\ mg\cdot L^{-1}$ and $229\ mg\cdot L^{-1}$ the NH_4-N average removal were 78.9 and 67.9%, respectively. The NH_4-N removal rate of anaerobic effluent depended on the initial microalgal concentration and the $NH_{4o.}$ For lower DW_o the high concentration of ammonium seems to inhibit the process but with the higest DW_o the maximum growth rate and removal efficiency were reached.

In Figure 3, PO_4-P concentrations decreased slightly and the PO_4-P removal rate was around $0.6\ mg\cdot L^{-1}\cdot d^{-1}$ for $NH_{4o} = 68.4\ mg\cdot L^{-1}$ and approximatively $1.3\ mg\cdot L^{-1}\cdot d^{-1}$ when NH_{4o} were 79.6 and $94.6\ mg\cdot L^{-1}$. The PO_4-P removal rate have been reported to be $2.0\ mg\cdot L^{-1}\cdot d^{-1}$ for *C. vulgaris* (Aslan and Kapdan, 2006) and 10.15 for *Nannochloris sp.* (Jiménez-Pérez, Sánchez-Castillo, et al., 2004).

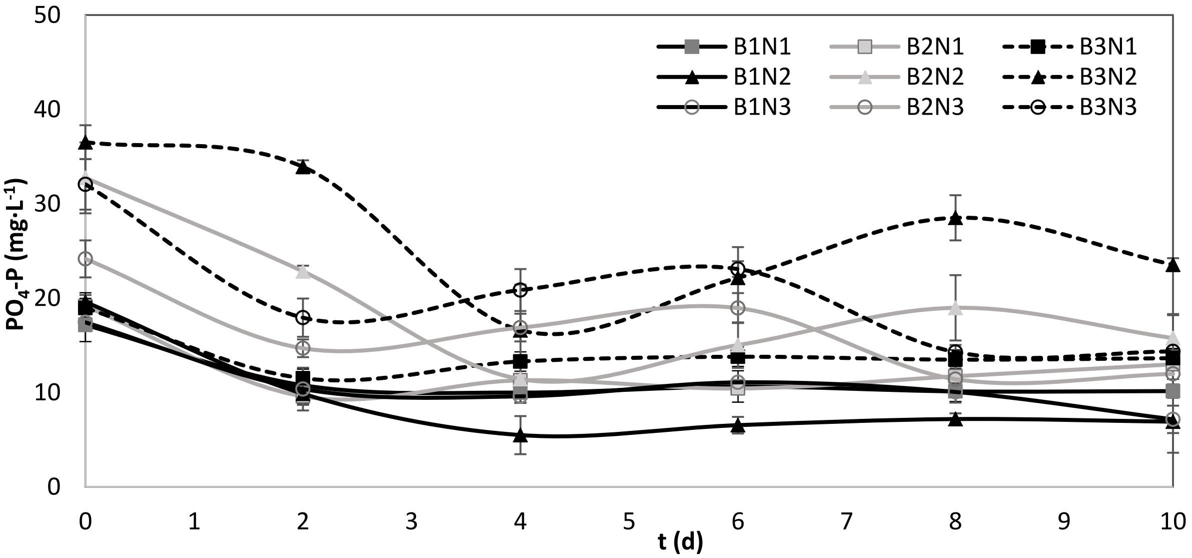

Figure 3. Variation of PO_4-P concentration for NH_{4o} and DW_o. (a) $NH_{4o} = 68.4\ mg\cdot L^{-1}$, (b) $NH_{4o} = 79.6\ mg\cdot L^{-1}$ and (c) $NH_{4o} = 94.6\ mg\cdot L^{-1}$. Each point represents mean value from three replicates determinations with standard deviation.

The NH_{4o} ($F_{2,10}= 6.11$, $p = 0.0184$) and DW_o ($F_{2,10}= 390.06$, $p = 3.247e-10$) have a significant effect on PO_4-P concentration at the time, in addition to the interaction between NH_{4o} and DW_o ($F_{4,20}=$

18.39, p = 1.764e-06). The PO_4-P removal efficiency was approximatively 30% for NH_{4o} = 68.4 mg·L^{-1} and it was around 50% for 79.6 mg·L^{-1} and 94.6 mg·L^{-1}. These removal rates are lower than other studies. He et al (2013) reported that total phosphorus was consumed in 10 days with removal rates of 98%. However, the pH was around 9 during tests and the phosphorus removal can be due to precipitation of phosphates as struvite (De-Bashan and Bashan, 2004).

When the initial algal biomass was changed Bonferroni's adjustment indicated there was highly significant difference ($p<0.05$) between all DW_o levels. The PO_4-P average removal efficiency were 54.7, 44.4 and 39.1% for 94, 229 and 344 mg·L^{-1}, respectively. Other authors have been reported removal rates higher than those obtained in this study, for example, Ji et al (2013) reported that total phosphorus was completely removed within 4 days by C. vulgaris. However, the initial concentrations were lower than 2 mg·L^{-1}. Aslan and Kapdan (2006) obtained 78% removal efficiency for initial PO_4-P = 7.7 mg·L^{-1} but when the initial concentration was increased the removal rates were less than 30%.

Nitrogen and phosphorus specific removal rates

Figure 4 show the specific rate removal for NH_4-N and PO_4^{-3}. The NH_{4o} ($F_{2,10}$= 6.55, p =0.0058) and DW_o ($F_{2,10}$= 94.38, p = 1.6e-11) have a significant effect on specific removal rate for NH_4-N. According with results, C. *vulgaris* obtained better specific removal rates for nitrogen and phosphorus in the anaerobically treated effluent when DW_o was 94 mg·L^{-1} and the growth of *C. vulgaris* in the tests depended on the initial algal biomass. Specific removal rate obtained in this study were higher than those reported for other authors. Ji et al (2013) reported R_{xi} values of 16.8 mgN·g $cell^{-1}$·d^{-1} and 3.1 mgP·g $cell^{-1}$·d^{-1} but the using tertiary municipal wastewater which have low concentrations of nutrients (TN = 8.7 ± 0.5 and TP = 1.7 ± 0.5).

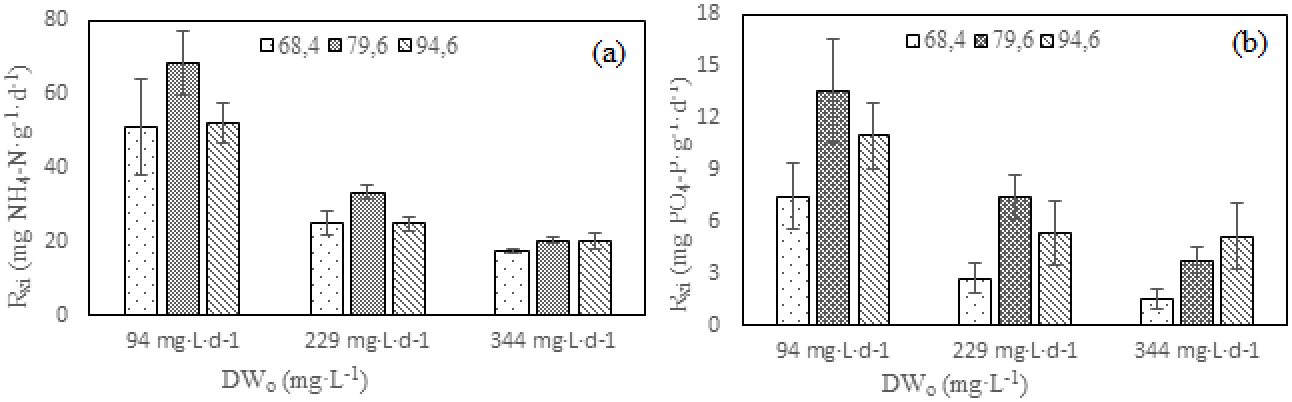

Figure 4. Specific rate removal for NH_4-N (a) and PO_4^{-3} (b)

CONCLUSIONS

The initial concentration of ammonium and the initial microalgal biomass concentration have an effect on nutrient removal and the biomass growth were investigated. The results show that B3N3 had the highest biomass (781.4 ± 90.1 mg·L^{-1}) and the highest specific growth rate (0.60 ± 0.02 d^{-1}). The growth in B1N2 and B1N3 may be inhibited by the high concentration of ammonium.

The NH_4-N removal efficiency was higher when the initial concentration of ammonium was lower and the PO_4-P removal efficiency was approximatively 30% for NH_{4o} = 68.4 mg·L^{-1} and it was around

50% for 79.6 mg·L^{-1} and 94.6 mg·L^{-1}. The better specific removal rates for nitrogen and phosphorus in the anaerobically treated effluent occur when DW_o was lower.

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