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# Analysis of nitrogenous and algal oxygen demand in effluent from a system of aerated lagoons followed by polishing pond

Hassan Khorsandi, Rahimeh Alizadeh, Horiyeh Tosinejad and Hadi Porghaffar

# ABSTRACT

In this descriptive-analytical study, nitrogenous and algal oxygen demand were assessed for effluent from a system of facultative partially mixed lagoons followed by the polishing pond using 120 grab samples over 1 year. Filtered and non-filtered samples of polishing pond effluent were tested in the presence and absence of a nitrification inhibitor. Effective factors, including 5-day biochemical and chemical oxygen demand (BOD and COD), total suspended solids (TSS), dissolved oxygen, chlorophyll A, and temperature, were measured using standard methods for water and wastewater tests. The results were analyzed using repeated measures analysis of variance with SPSS version 16. Findings show that the annual mean of the total 5-day BOD in the effluent from the polishing pond consisted of 44.92% as the algal carbonaceous biochemical oxygen demand (CBOD), 43.61% as the nitrogenous biochemical oxygen demand (NBOD), and 11.47% as the soluble CBOD. According to this study, the annual mean ratios of algal COD and 5-day algal CBOD to TSS were 0.8 and 0.37, respectively. As the results demonstrate, undertaking quality evaluation of the final effluent from the lagoons without considering nitrogenous and algal oxygen demand would undermine effluent quality assessment and interpretation of the performance of the wastewater treatment plant.

**Key words** | aerated lagoon, algae, biochemical oxygen demand, nitrification, polishing pond, wastewater

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# INTRODUCTION

The total biochemical oxygen demand (TBOD) of wastewater is the sum of carbonaceous biochemical oxygen demand (CBOD) and nitrogenous biochemical oxygen demand (NBOD) (Gerardi 2002; Vesilind et al. 2009; Bitton 2011). Unlike carbonaceous oxygen demand, which is proportional to the concentration of biodegradable carbonaceous organic compounds, the nitrogenous oxygen demand exerted during the 5-day test is proportional to the number of initial nitrifying organisms present in the sample, as well as to the availability of ammonia (Rich 1999). Typically, due to the slow growth of nitrifiers, it is assumed that the nitrogenous oxygen demand (NOD) does not affect the 5-day biochemical oxygen demand  $(BOD_5)$ experiment (Tchobanoglous et al. 2003; Bitton 2011). Although this assumption is valid on untreated wastewater, it is not correct for treated wastewater, especially for effluent from the system of aerated lagoons followed by polishing pond, because the 5-day BOD in effluent from aerated lagoons often contains NBOD (Mara & Pearson 1998; Mara 2004).

Therefore, the US Environmental Protection Agency authorized the use of a nitrification inhibitor in the BOD<sub>5</sub> test and using the CBOD<sub>5</sub> term, instead of using the BOD<sub>5</sub>, for quality assessment of a secondary treatment effluent especially in aerated lagoons and stabilization ponds (Barth 1981; Dague 1981; Mara & Pearson 1998). According to EPA recommendations, the average 30-day CBOD<sub>5</sub> for secondary treatment effluent should not exceed 25 mg/L (Tchobanoglous *et al.* 2003). However, due to high variations in lagoon and pond effluent NBOD (5–50 mg/L), measuring BOD<sub>5</sub> irrespective of NBOD influence would cause misinterpretation of the results (Mara & Pearson 1998; Mara 2004). Hall & Foxen (1983) found that nitrification was the cause of 60% of violations in the maximum allowable BOD<sub>5</sub> in the aerated lagoon effluent. The interference of nitrification on BOD<sub>5</sub> has been studied and confirmed by various researchers; for example, Dague (1981), Barth (1981) and Chapman *et al.* (1991).

Conversely, a significant portion of  $CBOD_5$  measured in the lagoon and pond effluent is due to the growth and propagation of algae, while the influent streams do not contain any algae (Mara & Pearson 1998; Ramaraj *et al.* 2010).

Algae are photosynthetic organisms containing chlorophyll that can be found in water, air, soil and on plants. Algae growth is directly affected by the concentration of nitrogen and phosphorus in water resources (Griffiths 2009; Ramaraj *et al.* 2010). Wastewater contains excessive amounts of nitrogen and phosphorus (Tchobanoglous *et al.* 2003; Khorsandi *et al.* 2011a, 2011b), therefore, providing suitable conditions for algae growth and proliferation due to the long retention time in the lagoons (Yang *et al.* 2008).

A substantial proportion of  $BOD_5$  and chemical oxygen demand (COD) in aerated lagoon effluent is due to interference from the presence of algae, since algae make up more than 80% of total suspended solids (TSS) in effluent from aerated lagoons (Mara 2004; Gronlund *et al.* 2004).

Rich (1999) investigated the interference caused by algae on TSS, COD and BOD<sub>5</sub> parameters in effluent from aerated lagoon systems and concluded that the standard limit of 25 mg/L for CBOD<sub>5</sub> and 30 mg/L for TSS is rarely reached due to the growth of algae.

The effects of algal and nitrogenous oxygen demand on the effluent quality of polishing ponds have not been studied fully and simultaneously; furthermore, their roles are often overlooked in the interpretation of effluent quality in wastewater treatment plants (WWTPs). Therefore, the aim of this study is, for the first time in Iran, to investigate the analysis of nitrogenous and algal oxygen demand in effluent from the system of aerated lagoons followed by polishing pond.

#### **METHODS**

This descriptive-analytical study was implemented in the Miandoab WWTP, Iran, from July 2009 to June 2010. Miandoab WWTP consists of three facultative partially mixed lagoons in series followed by a maturation pond, which acts as a polishing pond. Due to the incompleteness of the wastewater collection network, the process is working with 57% of its final capacity, causing an increase in total hydraulic retention time from 14.57 to 25.3 days and in algae growth and nitrification occurrence in the lagoons and polishing pond. The characteristics and the flowsheet for the studied lagoons system are illustrated in Table 1 and Figure 1, respectively. During the study, 120 grab samples were collected from the polishing pond effluent with time intervals of 7 to 10 days at 08.00, 12.00 and 18.00.

All tests were performed according to Standard Methods for the Examination of Water and Wastewater (APHA 2005). Dissolved oxygen (DO) and pH measurements were carried out using a YSI 55 DO meter (YSI Company Inc., USA) and a Schott pH meter model CG-824 (Schott UK Ltd, UK), respectively. COD experiments were analyzed via the closed reflux-colorimetric method using a Spectonic 20 D spectrophotometer (Thermo Fisher Scientific Inc., USA). BOD measurements were determined using an OxiTop respirometer (WTW GmbH, Germany). After extracting chlorophyll A with acetone, it was measured using the 10200 H spectrophotometric method. A few drops of effluent including algae were microscopically observed by Leica DM500 microscope (HACH, Germany). Allylthiourea (HACH, Germany) was used as a nitrification inhibitor, and the samples were filtered through M&N 89/90 filters (Macherev-Nagel GmbH & Co. KG, Germany) with 0.5-micrometer pore size.

To determine algal COD, COD tests were conducted on filtered and non-filtered samples.

For studying the interference of nitrogenous and algal BOD on the quality assessment of effluent, BOD<sub>5</sub> tests were performed under the following conditions, and results were compared by repeated measures analysis of variance (RMANOVA) using SPSS version 16 software:

- 1. Filtered sample, in the absence of nitrification inhibitor.
- 2. Filtered sample, in the presence of nitrification inhibitor.
- 3. Non-filtered sample, in the absence of nitrification inhibitor.
- 4. Non-filtered sample, in the presence of nitrification inhibitor.

Table 1
 Characteristics of facultative partially mixed lagoons and polishing pond in the Miandoab WWTP, Iran

Lagoon/pond	Length m	Width m	Depth m	HRT day	OLR Kg TBOD/ha.d
F.L <sub>1-1</sub>	150	100	3.2	7.7	1,280
F.L <sub>1-2</sub>	150	100	3.2	7.7	1,280
F.L <sub>2</sub>	300	100	3.2	7.8	NA
F.L <sub>3</sub>	300	100	3.2	7.8	NA
Polishing pond	165	100	1.5	2	560

F.L = facultative partially mixed lagoons, TBOD = NBOD + CBOD.



Figure 1 | Flowsheet of facultative partially mixed lagoons followed by polishing pond in the Miandoab WWTP, Iran.

Comparison of the BOD<sub>5</sub> for non-filtered samples in the absence of nitrification inhibitor (nfBOD<sub>5</sub>-nATU) and in the presence of nitrification inhibitor (nfBOD<sub>5</sub>-ATU) illustrates the impact of partial nitrification on BOD<sub>5</sub> in the presence of algae. Comparison of the BOD<sub>5</sub> for filtered samples in the absence of nitrification inhibitor (fBOD<sub>5</sub>-nATU) and in the presence of nitrification inhibitor (fBOD<sub>5</sub>-ATU) and in the presence of nitrification inhibitor (fBOD<sub>5</sub>-ATU) and in the presence of nitrification inhibitor (fBOD<sub>5</sub>-ATU) shows the effect of partial nitrification on BOD<sub>5</sub> in the absence of algae (Stander & Theodore 2008).

Comparison of the BOD<sub>5</sub> for non-filtered samples in the presence of inhibitor (nfBOD<sub>5</sub>-ATU) with the BOD<sub>5</sub> for filtered samples containing inhibitor (fBOD<sub>5</sub>-ATU) indicates the impacts of algae on BOD<sub>5</sub> in the absence of nitrifiers. Because of the low prevalence of non-algal suspended solids in effluent from the polishing pond, the TSS in the effluent samples was considered as algal solids.

The difference between the BOD<sub>5</sub> for non-filtered samples without inhibitor (nfBOD<sub>5</sub>-nATU) with the BOD<sub>5</sub> for filtered samples containing inhibitor (fBOD<sub>5</sub>-ATU) indicates the influence of both algae and nitrification on the BOD<sub>5</sub> results.

## **RESULTS AND DISCUSSION**

The results of monthly average of effluent quality parameters from the Miandoab polishing pond are given in Table 2. In addition to DO, pH, temperature and TSS, it also presents other parameters including  $BOD_5$  and COD, which were analyzed under various conditions. Alkaline pH and high DO in the effluent confirm the significant impact of algae on maturation ponds. These conditions for DO and pH also provide an appropriate environment for nitrifier organisms. Both algae and nitrification could affect effluent quality.

The monthly mean variations of  $BOD_5$  in different modes are shown in Figure 2 for determining NBOD, algal BOD and the simultaneous effects of them on  $BOD_5$ . The quality parameters for polishing pond effluent shown in Table 2 and Figure 2 indicate that the annual mean TBOD<sub>5</sub> of polishing pond effluent, considered as the nfBOD<sub>5</sub>-nATU term, was 61 mg/L; this consisted of 27.4 mg/L (44.92%) for algal CBOD<sub>5</sub>, 26.6 mg/L (43.61%) for TNBOD<sub>5</sub> and 7 mg/L (11.47%) for sCBOD<sub>5</sub>.

Considering the long retention time in lagoons and ponds, the sCBOD<sub>5</sub> and non-algal solids in effluent from the system of aerated lagoons followed by polishing pond is less than 10 mg/L (Mara & Pearson 1998). In fact, a vast portion of the effluent's TSS and CBOD<sub>5</sub> is caused by algae growth (Gronlund *et al.* 2004). Conversely, due to the optimum growth conditions for nitrifiers, the TNBOD<sub>5</sub> mainly comprises TBOD<sub>5</sub> in the lagoon and pond effluent. Thereby, usage of TBOD<sub>5</sub> to assess the effluent quality of secondary wastewater treatment processes, particularly stabilization ponds and lagoons, is regarded as an uncertain parameter. Instead, the CBOD<sub>5</sub> for the filtered samples are used according to EPA guidelines (Rich 1999).

In EU countries, the fCBOD<sub>5</sub> and fCOD of lagoon effluents for discharge to surface waters are set at  $\leq$ 25 mg/L and  $\leq$ 125 mg/L, respectively; while TSS in non-filtered effluent from lagoons can reach up to 150 mg/L (Mara & Pearson 1998; Gronlund *et al.* 2004).

Despite the improving efficiency of biological activity in summer, according to Figure 2, the average  $TBOD_5$  in polishing pond effluent decreased in warm weather due to optimum growth conditions for algae and nitrifying bacteria. Neglecting these findings may be responsible for falsely attributing the design and operation of wastewater treatment plant as defective.

RMANOVA showed that the differences between the annual means BOD<sub>5</sub> in the four groups discussed in material and methods were statistically significant (P < 0.001), and differences in the monthly means within these groups were also significant (P < 0.001).

Month	N	DO mg/L	рН	Temp. °C	TSS mg/L	fCOD mg/L	nfCOD mg/L	fBOD <sub>5</sub> -NATU <sup>a</sup>	fBOD <sub>5</sub> -ATU <sup>b</sup>	nfBOD <sub>5</sub> -ATU <sup>c</sup>	nfBOD5-nATU <sup>d</sup>
July 2009	18	$1.2\pm0.9$	$7.8\pm0.1$	$25.2\pm1.3$	$84.9\pm22.6$	$64\pm7.4$	$120.8\pm24.2$	$18.8\pm3.1$	$11.9 \pm 1.9$	$58\pm7.1$	$104.2\pm29.2$
August 2009	6	$4.1\pm3.4$	$8\pm0.1$	$24.4\pm0.9$	$51.1\pm22.6$	$63.2\pm5.7$	$98 \pm 17.5$	$9\pm2.7$	$8.8\pm2.6$	$33\pm 9.3$	$60.7\pm21.4$
September 2009	9	$3.2\pm1.5$	$8\pm0.1$	$24.1\pm0.9$	$45.6\pm2.9$	$71.9\pm4.6$	$86.5\pm3.1$	$9.3\pm2$	$8.3 \pm 2$	$19.4\pm2.1$	$26.3\pm8.8$
October 2009	6	$6.9\pm1.7$	$8.2\pm0.1$	$19\pm1.3$	$57.1\pm10.1$	$55.5\pm9.7$	$89.4\pm6.9$	$7.5\pm3.8$	$5.7\pm1.7$	$21.5\pm7.8$	$34 \pm 12.1$
November 2009	12	$7.7\pm1.4$	$8.2\pm0.1$	$16.3\pm1.1$	$60.6 \pm 12.1$	$66.5\pm3$	$100.7\pm10.2$	$5.5\pm0.9$	$4.9\pm0.9$	$27.8\pm4.8$	$41.3\pm5.3$
December 2009	12	$12.1\pm2$	$8.3\pm0.1$	$10.4\pm1.4$	$65.8\pm8.7$	$66 \pm 9.7$	$110.4 \pm 12.4$	$5.8\pm2.8$	$4.7\pm1.9$	$22.2\pm3.7$	$31.7\pm7.2$
January 2010	12	$12.9\pm1.2$	$8.4\pm0.1$	$9.9 \pm 1.3$	$87.8\pm4.5$	$46\pm 6.2$	$132.3\pm7.9$	$4.4\pm0.5$	$3.6\pm 0.8$	$22.7\pm1$	$57.7 \pm 5$
February 2010	9	$11.7\pm1.4$	$8.3\pm0.1$	$8.8\pm 0.8$	$86\pm3.9$	$36.8\pm4.8$	$128.7\pm4.8$	$5.2\pm1.1$	$4.5\pm0.9$	$27.4\pm2.2$	$55.1 \pm 11.5$
March 2010	9	$10.6\pm0.9$	$8.3\pm0.1$	$9.5\pm0.7$	$91.2\pm2.9$	$43.9\pm5.6$	$143.7\pm6.2$	$6.7\pm0.7$	$5.8\pm0.8$	$33.3\pm4.5$	$50.2\pm19$
April 2010	9	$8.6\pm2.6$	$8.3\pm0.2$	$13.6\pm2.9$	$106.3\pm13.8$	$50.7\pm5.6$	$162.8 \pm 18.2$	$6.3\pm1.7$	$5.7\pm1.7$	$36.9\pm7.1$	$55.8\pm5.9$
May 2010	9	$2.8\pm1.6$	$7.9\pm0.2$	$19.2\pm2.5$	$87.6 \pm 14.4$	$48.7\pm6.1$	$154.4 \pm 12.1$	$8.4\pm1.2$	$8.4\pm2.1$	$46.2\pm8.4$	$90.7\pm9.6$
June 2010	9	$5.1\pm3.9$	$7.9\pm0.1$	$23.8\pm2.7$	$72.6\pm10.5$	$46.7\pm8.2$	$114.5\pm27.1$	$12.8\pm4.1$	$9.1\pm1.3$	$49.1\pm 6.6$	$94.7\pm3$
Total	120	$7 \pm 4.6$	$8.1 \pm 0.3$	$17.2 \pm 6.6$	$75.9 \pm 21.4$	$55.9 \pm 12.5$	$120.7\pm27$	$9 \pm 5.4$	$7 \pm 3.2$	$34.4 \pm 14.4$	$61 \pm 30.5$

Table 2 | The quality parameters of effluent from the system of facultative partially mixed lagoons followed by polishing pond in the Miandoab WWTP, Iran

<sup>a</sup>The filtered sample  $BOD_5$  in the absence of nitrification inhibitor.

 $^{\text{b}}$ The filtered sample BOD<sub>5</sub> in the presence of nitrification inhibitor.

 $^{\rm c}{\rm The}$  non-filtered sample  ${\rm BOD}_5$  in the presence of nitrification inhibitor.

 $^{\rm d} The \ non-filtered \ sample \ BOD_5$  in the absence of nitrification inhibitor.



Figure 2 | The monthly average BOD<sub>5</sub> variations of effluent from a system of facultative partially mixed lagoons followed by a polishing pond in the Miandoab WWTP, Iran. (a) Nitrification effect on BOD<sub>5</sub>; (b) algae effect on BOD<sub>5</sub>; (c) nitrification and algae effects on BOD<sub>5</sub>; (d) comparison of algae and nitrification on BOD<sub>5</sub>.

RMANOVA made a clear distinction between the annual mean COD of filtered samples (fCOD) and non-filtered samples (nfCOD), and overall significant difference of the monthly mean within these groups (P < 0.001).

Taking into consideration Table 2, the annual mean fCOD in polishing pond effluent was 55.9 mg/L, but algal COD with an annual average of 64.8 mg/L increased the total annual average COD to 120.7 mg/L, making up 53.7% of the total COD.

Therefore, nitrogenous and algal oxygen demand result in an increase in the effluent  $TBOD_5$  and COD. These results are consistent with the findings of Rich (1999) about aerated lagoon systems consisting of four cells in series in order to undertake partial polishing of effluent (1999). However, Rich (1999) did not use statistical analysis for the results.

Figure 3 presents the result obtained from the monthly mean variations of algal COD, algal CBOD<sub>5</sub> and TSS of the effluent. Moreover, the ratio of the monthly mean of algal CBOD<sub>5</sub> and algal COD to TSS are shown in this diagram.

A non-linear regression having *R* squared of 0.77 between the algal COD and the TSS demonstrates that algae have a significant impact on effluent quality, particularly on COD. Although linear correlation was further confirmed between



Figure 3 | The monthly average algal COD, algal CBOD<sub>5</sub> and TSS, and correlation between the algal COD and algal CBOD<sub>5</sub> with TSS. (a) Monthly variations of algal COD/TSS and algal CBOD<sub>5</sub>/TSS; (b) monthly variations of algal COD, algal CBOD<sub>5</sub> and TSS; (c) correlation between algal COD and TSS; (d) relationship between algal CBOD<sub>5</sub> and TSS.

them with a coefficient of 0.87 using one-way ANOVA (P < 0.001), it was less preferable due to it being less consistent with expected algal COD and TSS near the origin of zero.

According to Figure 3, the annual average ratios of (algal COD)/TSS and (algal CBOD<sub>5</sub>)/TSS were 0.80 and 0.37, respectively, which are consistent with Sperling (2007) results. To demonstrate the role of algae in quality parameter assessment of stabilization pond effluent, Sperling (2007) showed that the ratios of (algal COD)/TSS and (algal CBOD<sub>5</sub>)/TSS in the effluent from stabilization ponds were 1–1.5 and 0.3–0.4, respectively.

Figure 3(d), having a high degree of scatter, indicates that algal CBOD5 has low correlation with TSS due to the dependence of algal CBOD on TSS concentration and also on the biodegradability of carbonaceous organic compounds present in different types of algae.

The algal green color and microscopic observations confirmed the presence of algae in the effluent. In addition, the mean of chlorophyll A during the experiment was  $345 \mu g/L$ . Based on Mara's (2004) findings, as all wastewater stabilization pond algae are composed of ~1.5 per cent chlorophyll A by weight, and because it is easily measured, expression of algal biomass in terms of chlorophyll A is more meaningful. Accordingly, 23 mg/L algal biomass, as shown in this study, would generate the theoretical oxygen demand (Yang *et al.* 2008; Dalrymple *et al.* 2013) of 28.5 mg/L. This computational finding is consistent with the experimental algal CBOD<sub>5</sub>. It should be noted that the nature of algal BOD<sub>5</sub>, COD and TSS is different from that of raw sewage because the oxygen produced by the algae in receiving water is higher than the oxygen consumed by other aquatic organisms (Mara 2004). However, in local standard procedures, there is no assessment methodology for nitrified effluent containing algae. Therefore, some managers consider algal growth in lagoons and ponds as undesirable (Meneses *et al.* 2005). As a result, they try to improve and upgrade the treatment plant. However, algae are useful in the uptake of nitrogen and phosphorus from wastewater and are also beneficial to agriculture (Naddafi *et al.* 2005; Machibya & Mwanuzi 2006; Mansouri *et al.* 2017), because they will increase organic content over time, and finally increase the water retention capacity of soil (Papadopoulos *et al.* 2004; Ensink *et al.* 2007; Sharafi *et al.* 2012).

#### CONCLUSION

The aim of this study is to investigate the analysis of nitrogenous and algal oxygen demand in the effluent from system of aerated lagoons followed by polishing pond. Findings demonstrated that the annual mean of total 5-day biochemical oxygen demand in the effluent from a polishing pond consisted of 44.92% as algal CBOD, 43.61% as NBOD and 11.47% as soluble CBOD. The annual average ratios of (algal COD)/TSS and (algal CBOD<sub>5</sub>)/TSS were 0.80 and

0.37, respectively. Based on this study, in addition to monitoring fCBOD<sub>5</sub>, fCOD, and TSS, other parameters such as the algal CBOD<sub>5</sub>, TNBOD<sub>5</sub>, and chlorophyll should be determined for assessment of the treatment plant's performance and correct interpretation of the effluent quality from a stabilization pond or lagoon.

# ACKNOWLEDGEMENTS

The authors would like to thank the Municipal Water and Wastewater Company of West Azerbaijan province, Iran, especially Mr Shamchi (CEO) and Mr Heydarlou (Deputy Director of Sewage) for their cooperation in conducting this research. We also gratefully acknowledge Mr J. Khorsandi for editing the text.

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First received 22 December 2013; accepted in revised form 8 April 2014. Available online 22 April 2014