Nitrification Rates of Biofilms in Algae Wastewater Stabilization Ponds Under Light and Dark conditions

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Abstract

The objective of this study was to investigate nitrification rates in algal biofilms of waste stabilization ponds (WSP) under different conditions of light, oxygen and pH. The purpose was not to accurately predict nitrification rates but to understand how nitrification occurs under different conditions in WSP. Biofilms were grown on wooden plates of 6 cm by 8 cm in a PVC tray fed with synthetic wastewater with NH_4-N and COD concentrations of 40mgl^{-1} and 100mgl^{-1} respectively under light intensity of 85-95 µEm^{-2}s^{-1}. Batch activity tests were carried out using the plates under light conditions (to simulate day time), dim light (to simulate reduced light as in deeper locations in WSP) and under dark conditions (to simulate night time). Also, oxygen as well as pH was varied i.e. at some experiments kept constant and at others left to vary as in WSP. Biofilm nitrification rates under light and dark conditions when pH and oxygen were left to vary did not differ significantly at 95% confidence. Similarly, experiments under light and dark conditions with pH and oxygen kept constant (pH 7.7 and oxygen 8 mg l^{-1}) also did not differ significantly at 95% confidence. When pH was kept constant (7.7) but oxygen left to vary, a significant difference (95% confidence) was found for the nitrification rates under light (692±16 mg-Nm^{-2}d^{-1}) and dark conditions (90±49 mg-Nm^{-2}d^{-1}). Nitrification rates of 592±103 mg-Nm^{-2}d^{-1}, 130±64 mg-Nm^{-2}d^{-1} and 72±95 mg-Nm^{-2}d^{-1} were obtained under light, dim light and dark conditions respectively when the pH was kept constant at 7.7. Only the rates under light and dark conditions were significantly different.

Nitrification rates under light conditions were higher than those under dark conditions, provided that the oxygen concentration under dark conditions decreased at least below 3 mg l^{-1}. When oxygen was supplied by aeration under dark conditions, nitrification rates were not significantly different from those under light conditions. It can therefore be concluded that both aeration and biofilm-photosynthesis provide the nitrifiers in the biofilm with oxygen. This study shows the importance of biofilm and bulk water oxygen production in improving nitrification rates.

Key words: Biofilm, nitrification, light, dim light, dark, WSP, oxygen, pH

Introduction

Waste stabilization ponds are used worldwide as an effective and low cost technology for effluent treatment. The major problem with ponds is the large land requirement for construction and their poor efficiency in reducing nitrogen. They are usually designed as

a sequence of systems (anaerobic reactor, primary, secondary facultative ponds and maturation ponds for nutrient removal). It is suggested that biofilm systems can be combined with maturation ponds hence reducing the required land area (Johnson and Mara, 2005) and improving nitrogen removal. The need to develop compact systems effective in nitrogen removal arises and thus the use of biofilms is increasingly receiving attention.

Introduction of biofilms into WSP has been tried (McLean *et al*, 2000) and has been found to be effective in increasing nitrification rates but still the behavior and performance of biofilms under various conditions in WSP (pH, temperature, light, darkness, oxygen) need to be studied further. Zimmo *et al*, (2002b) has done various experiments on nitrification but mostly on bulk water. Information on nitrification rates of algal biofilms of WSP under different conditions is still insufficient. This study investigates biofilm nitrification rates under light and dark conditions as well as under different pH and oxygen concentrations.

The working hypothesis in this study is that oxygen production in the algal biofilm layer is increasing the oxygen availability to the nitrifiers in the biofilm. Therefore a biofilm placed in a batch with low bulk oxygen concentrations, but in the light will result in higher nitrification rates than under low oxygen bulk concentrations and in the dark.

Methodology

Growth of biofilm

A PVC tray of dimensions 5.5 x 3.7 x 9.0 cm (length, width and depth) was used in this study. The experimental set-up is a continuous flow system similar to that of Gunatilike *et al*. (2006) except that instead of acrylic glass biofilm plates, thin pieces of wood were used. Wood is chosen in this case because it provides a rough surface which is thought to improve the attachment of biofilm. The tray had 60 pieces of wood of dimensions 6 by 8 cm. The system was continuously fed with synthetic wastewater of ammonia and COD concentration of 40 mg N l^{-1} and 100 mg l^{-1} respectively (Babu *et al.,* 2007). The influent flow rate was 0.96 l hr^{-1} and the synthetic wastewater was recycled at a rate of 2.5 l hr^{-1} just before the effluent point to ensure a uniform distribution of ammonia over the reactor. Enriched activated sludge from Hoek van Holland treatment plant was used as inoculum's to establish nitrifier and denitrifier populations in the tray. A volume of 100mls of algae from the column experiments of Babu *et al.* (2007) was introduced into the system. The set-up was exposed to 12 hour light regime of light intensity of 85-95 $\mu Em^{-2}s^{-1}$. This is lower than 133-176 $\mu Em^{-2}s^{-1}$ measured on a sunny day in Uganda. The plates were left to develop biofilms for a period of more than 3 weeks and then transferred to batch reactors for determining the nitrification rates. The same composition synthetic wastewater as used for the continuous flow system was used for the batch system.

Batch reactors for nitrification activity

Condition 1

Light and dark conditions; oxygen and pH left to vary

Biofilm plates were taken out of the tray, gently rinsed with distilled water and hung in two-liter glass beakers containing 1.1 l of fresh synthetic wastewater (not seeded with nitrifiers) of ammonia concentration of 20 mg l^{-1} (Babu *et al*, 2007). Each beaker had only one biofilm plate and tests were performed in duplicates. The beakers were exposed to a light intensity of 85-95 $\mu Em^{-2}s^{-1}$ for a period of 8 hrs. Oxygen and pH were left to vary as in WSP. The temperature was not controlled but was almost constant (22^oC) Samples were taken after every two hours, filtered and analyzed for the concentration of ammonia. Other parameters that were monitored include nitrate, dissolved oxygen, pH and temperature. All parameters were determined according to Standard Methods (APHA, 1995).

This experiment was repeated as described above but in this case, the beakers with biofilm were kept under dark conditions. Similarly, oxygen and pH were left to vary as in WSP. A control experiment with only synthetic wastewater without biofilm plates was also set up and exposed to light as above.

Condition 2

Light and dark conditions; oxygen and pH kept constant

The experiments described above were repeated but this time keeping oxygen and pH constant. The oxygen concentration was kept at 8 mg l^{-1} by continuous bubbling of air while the pH was kept at 7.7 by addition of 150 mg l^{-1} of sodium hydrogen carbonate to the synthetic wastewater.

Condition 3

Light and dark conditions; pH kept constant but oxygen left to vary

In this experiment, the pH was kept constant as described above but the oxygen concentrations were left to vary with time.

Condition 4

Light, dim light and dark conditions; pH kept constant, oxygen fixed per condition

The experiments above were repeated but this time under the conditions of bright light, dim light and darkness. The oxygen level under bright light was kept between 8-9 mg l^{-1} by continuous bubbling with air. The oxygen level under dim light was kept between 3-5 mg l^{-1} by periodic bubbling with air, while that under dark conditions was kept between 1-1.2 mg l^{-1} by periodic bubbling with air and nitrogen gas. The pH was kept constant as described above. Two runs were made for this experiment.

Analysis of results

The results were analyzed using regression analysis in excel. Ammonia concentrations in mg l^{-1} were plotted against time (hours) and the slopes obtained. Regression analysis using the F-test (95% confidence interval) was used to check if the slopes were statistically different. The slopes for light and dark conditions for each experiment were tested.

Results

The figures 1 (a) and 2 show the trends of ammonia reduction with time in batch experiments. The trends were similar in all experiments. Table 1 shows the nitrification rates for all runs.

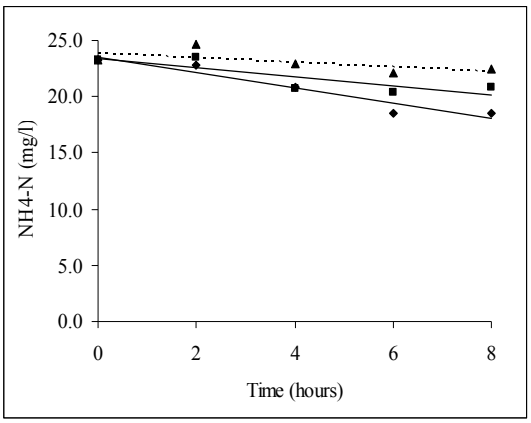

Figure 1 a Trend of ammonium in condition 1. ▲, ■ and ♦ symbols on the graphs represent Control, Dark and Light conditions

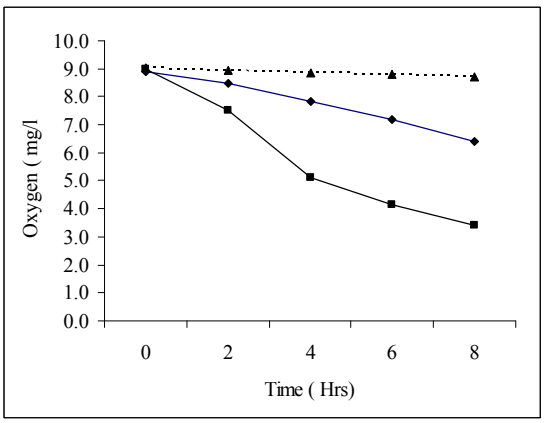

Figure 1b Oxygen variation in condition 1. ▲, ■ and ♦ symbols on the graphs represent Control, Dark and Light conditions

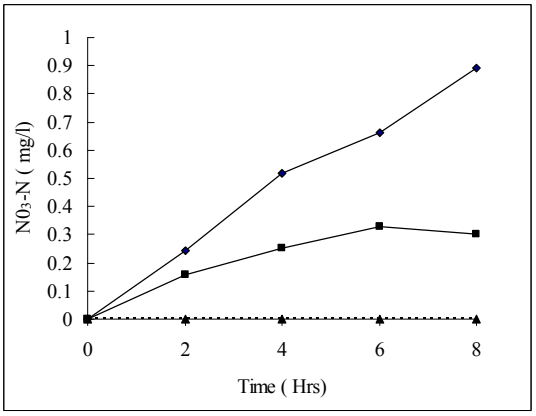

Figure 1c Nitrate accumulation in condition 1. ▲, ■ and ♦ symbols on the graphs represent Control, Dark and Light conditions

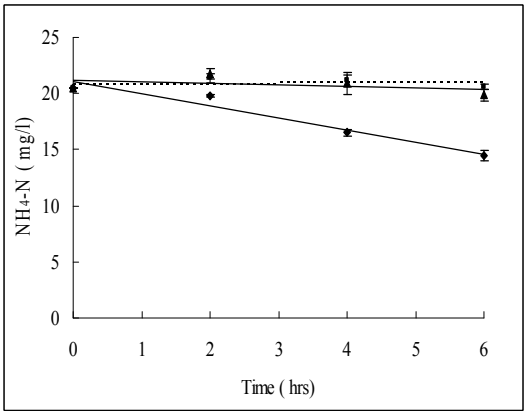

Figure 2 Trend of ammonium in condition 3. ▲, ■ and ♦ symbols on the graphs represent Dim light, Dark and Light conditions

Table 1, Summary of Biofilm nitrification rates (mg-$Nm^{-2}d^{-1}$) for all the 4 different conditions

Condition	**Nitrification rates (mg-$Nm^{-2}d^{-1}$)**			**Significance**
	Light	**Dark**	**Dim light**	
1	855±62	501±34	-	No
2	705±14	562±36	-	No
3	692±16	90±49	-	Yes
4	592±103	72±95	130±64	Yes

1- Light and dark conditions with pH, temperature and oxygen left to vary
2- Light and dark conditions with pH, temperature and oxygen kept constant

3- Light and dark conditions with pH and temperature kept constant and oxygen left to vary
4- Light, dim light and dark conditions

Discussion

Condition 1, Light and dark conditions, oxygen, pH and temperature left to vary

The ammonium concentration dropped from 21 mg l^{-1} to 17 mg l^{-1} for the biofilms exposed to light conditions while under dark conditions, the concentration dropped from 21 to 19 mg l^{-1} (Figure 1a). The ammonia concentration in the control experiment dropped slightly throughout the experimental period.

The nitrification rates calculated were 855 ±62 mg-$Nm^{-2}d^{-1}$ and 501±34 mg-$Nm^{-2}d^{-1}$ for light and dark conditions respectively. These results are in agreement with those obtained by Leu *et al.* (1998), Craggs *et al* (2000), McLean *et al,* (2000) and Lydmark *et al,* (2007). Statistical test shows no significant difference in the nitrification rate under dark and light conditions. This is in disagreement with Verdegem *et al,* (2005) who found higher nitrification rates under light than dark conditions. Although light is important for algal photosynthesis, the effect of oxygen especially under dark conditions in this case, could have masked that of light. The bulk oxygen concentration in both light and dark conditions decreased from 8.9 to 6.4 and 9.0 to 3.4 mgl^{-1} oxygen respectively (Figure 1b). The DO in the dark decreased but remained above 3 mg l^{-1} even after 8 hours. Maybe the DO in the biofilm under these conditions in the dark treatment did not significantly affect the nitrification rate.

The effect of oxygen can be demonstrated from calculations as described by Szwerinski *et a*l, (1986), who found that 4.57 g of oxygen is required to oxidize 1g-N. Using this information, theoretical oxygen consumption is calculated for experiment 1. The volume of synthetic wastewater used in this experiment was 1.1 l therefore ammonium reduction under light and dark conditions were 4.4 mg NH_4-N and 2.2 mg NH_4-N (0.0034g-N and 0.0017g-N) respectively. If we assume that all the ammonium consumed was due to the nitrification process, the calculation gives 15.5 mg O_2 (14.1 mg l^{-1}) and 7.8 mg O_2 (7.1 mg l^{-1}) as the oxygen required for complete oxidation of ammonium under light and dark conditions.

In reality, the DO decreased with only 2.5 mg l^{-1} (Figure 1b) under light conditions compared to the required 14.1 mg l^{-1} from the calculation. It is likely that the extra oxygen for the oxidation of the ammonium was produced by the photosynthesizing algal biofilm. This is in agreement with our working hypothesis which suggests significant oxygen production in the algal biofilm. For the dark experiment, the DO decreased with 5.6 mg l^{-1} of oxygen during the experimental period; this is closer to 7.1 mg l^{-1} estimated from the calculations. This shows that the initial oxygen concentration in the dark experiment was sufficient to support nitrification even after 8 hours.

From the above explanations, one of the mechanisms suggested to cause decrease in ammonia concentration in both the light and dark experiments is ammonia oxidation. Other mechanisms suggested are algal assimilation and volatilization. Evidence to show that nitrification occurred is accumulation of nitrates during the experimental period (Figure 1c). Although there was accumulation, the nitrate concentrations never exceeded

1 mg l^{-1}. This was possibly due to algal up take although Kuenen and Robertson, (1994) have also found denitrification to occur in the deeper anoxic layers of the biofilms. Other authors like Verdegem *et al,* (2005) suggest that algae prefer ammonium to nitrates as N-source. It is only when ammonium concentration is less than 0.03mgl^{-1} Total Ammonia Nitrogen (TAN i.e. $NH_4 + NH_3$) that nitrite and nitrate uptake becomes important. In all cases, the volatilization rates were calculated according to Zimmo *et al.,* (2004) and found to be constant at 5.0 mg-$Nm^{-2}d^{-1}$. This loss is negligible as compared to the higher nitrification rates.

Condition 2; Light & dark conditions, oxygen and pH kept constant

The trend of ammonia removal is similar to that in experiment 1, in which oxygen and pH were uncontrolled. The ammonia concentration under light and dark decreased from 19.2 to 10.9 mg l^{-1} (8.3 mg l^{-1}) and 12.4 mg l^{-1} (6.8 mg l^{-1}), respectively. Nitrification rates were 705±14 mg-$Nm^{-2}d^{-1}$ and 562±36 mg-$Nm^{-2}d^{-1}$ under light and dark conditions, respectively. There is no significant difference (at 95% confidence interval) in nitrification rates and this could be explained by DO concentrations. The bulk DO concentrations under light and dark conditions were from 9.5-6.7 and 8.2-7.7 mgl^{-1} of oxygen after 8 hours. The oxygen concentrations were kept high by bubbling with air and this seemed to favor nitrification. Goncalves and Oliviera, (1996) made similar observations. It appears that the effect of light on oxygen production by photosynthesis is cancelled by bubbling with air. As long as there is sufficient oxygen in the bulk water, the light intensity does not seem to affect the nitrification process.

Condition 3; Light & dark conditions, pH kept constant but oxygen left to vary

The trend of ammonia reduction in this experiment is shown in figure 2. The nitrification rates were 692±16 mg-$Nm^{-2}d^{-1}$ and 90±49 mg-$Nm^{-2}d^{-1}$ under light and dark conditions respectively. This difference is statistically significant. The biomasses of the biofilm in the two experiments were similar (16.7 g VSS m^{-2}) and therefore cannot explain the differences in nitrification rates under light and dark conditions. Similar to the previous experiments, the explanation for the differences in the nitrification rates may be caused by differences in oxygen levels. From results of this experiment, it is observed that within 8 hours, the oxygen decreased to 1.3 mg l^{-1} and 6.3 mg l^{-1} under dark and light conditions respectively. Also, the specific growth rates for nitrifiers under dark and light conditions were 0.008 and 0.0122 hr^{-1} respectively. Both drop in oxygen and lower nitrifier growth rate in dark experiment could explain lower nitrification rate in the dark experiment.

Condition 4; Light, dim light & dark conditions

As with the previous experiments, the ammonia concentration decreased with time under light condition while under dim light and dark conditions the decrease was minimal. The average nitrification rates for the two runs of experiment 4 were 592±103, 130±64 and 72± 95 mg-$Nm^{-2}d^{-1}$ for light, dim light and dark conditions respectively. The mean biomass for the biofilms in the light, dim light and dark experiments were 18.7 ± 1.1 g $VSSm^{-2}$ 18.8± 5.8 g $VSSm^{-2}$ and 15.0± 0.2 g $VSSm^{-2}$ (n=2) respectively. The nitrification rates for dark and light conditions were significantly different, which could be explained by the low DO under dark conditions (1-1.2 mg l^{-1}). The DO under dim light

was relatively high (3-5 mg l^{-1}) and based on the interpretation of the results for condition 1, one would not expect a difference between 'light' and 'dim'. Also the biomass in the light and dim light did not differ; however, the nitrification rates were significantly different. The reasons for this are still not clear. Further research on the relation between bulk water DO and biofilm DO is therefore suggested.

Conclusions

- The results in this study suggest that the effect of light intensity is indirect, via the oxygen availability for the nitrifiers in the biofilm. It seems that as long as there is sufficient oxygen in the bulk liquid, light does not play an important role in the process of nitrification.
- When DO values in the bulk liquid are lower than about 3 mg l^{-1}, then the provision of light for photosynthesis may enhance nitrification rates in the algal biofilm.

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