Nitrification in bulk water and biofilms of algae wastewater stabilization ponds

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Abstract Nitrification is limiting in facultative wastewater stabilization ponds. The reason why nitrification is considered to be limiting is attributed to low growth rate and wash out of the nitrifiers. Therefore to maintain a population, attached growth is required. The aim of this research is to study the relative contribution of bulk water and biofilms with respect to nitrification. In order to achieve this, transparent pond reactors representing water columns in algae WSP have been used. To discriminate between bulk and biofilm activity, 5-day batch activity tests were carried out with bulk water and biofilm sampled. The observed value for \( R_{\text{nitrbulk}} \) was \( 2.7 \times 10^{-1} \) mg-N L\(^{-1}\) d\(^{-1}\) and for \( R_{\text{biofilm}} \) was 1,495 mg-N m\(^{-2}\) d\(^{-1}\). During the 5 days of experiment with the biofilm, ammonia reduction was rapid on the first day. Therefore, a short-term biofilm activity test was performed to confirm this rapid decrease. Results revealed a nitrification rate, \( R_{\text{biofilm}} \), of 2,125 mg-N m\(^{-2}\) d\(^{-1}\) for the first 5 hours of the test, which is higher than the 1,495 mg-N m\(^{-2}\) d\(^{-1}\), observed on the first day of the 7-day biofilm activity test. \( R_{\text{biofilm}} \) and \( R_{\text{nitrbulk}} \) values obtained in the batch activity tests were used as parameters in a mass balance model equation. The model was calibrated by adjusting the fraction of the pond volume and biofilm area that is active (i.e. aerobic). When assuming a depth of 0.08 m active upper layer, the model could describe well the measured effluent values for the pond reactors. The calibrated model was validated by predicting effluent Kjeldahl nitrogen of algae ponds in Palestine and Colombia. The model equation predicted well the effluent concentrations of ponds in Palestine.

Keywords Biofilm; bulk water; modelling; nitrification; wastewater stabilization ponds

Introduction

Nitrogen pollution on the world water bodies is increasing and effects have become visible since the 1960s when increasingly reports are given of eutrophication of water bodies (Gijzen and Mulder, 2001). In response, high environmental standards and stringent regulations are being adopted by developed nations. Many developing countries have followed suit and have set strict standards, which in practice do not function because of prohibitive costs for treatment plants required to satisfy those standards (Veenstra and Alaerts, 1996). Several advanced treatment technological innovations have come up but developing nations cannot afford them, yet urbanization and population are on the rise (Gijzen and Khondker, 1997; Yu et al., 1997; Gijzen et al., 2004). This is compounded further by the millennium development goal seven which advocates for reduction of half the proportion of people without access to safe drinking water by 2015 (WSSCC, 2004).

Increase in accessibility to safe drinking water and sanitation implies generation of more

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wastewater. It is estimated that 80–90% of water consumed is converted to wastewater (Mara et al., 1992).

In trying to address the issues of wastewater treatment, most developing countries have opted for wastewater stabilization ponds (WSP) as the major treatment technology. This is due to its cost-effectiveness in construction and maintenance. In fact Mara and Pearson (1998) recommend the use of WSP in developing regions. They have found them to perform similarly to advanced systems especially in COD removal. However, their major shortcomings include narrow zone for nitrification since the aerobic zone is limited to the upper 0.50 m (Baskaran et al., 1992); long hydraulic retention time and low attached bacterial biomass (McLean et al., 2000; Zimmo et al., 2000); short-circuiting (Shilton and Harrison, 2003; Shilton et al., 2000); high concentration of total suspended solids (TSS) in the effluent (Mara, 2004); and large area for construction (Pearson, 1996).

This study investigates limitation of nitrification due to lack of attachment surface for nitrifiers. In this study, nitrification rates in the bulk water and in the biofilm have been investigated. This is mainly geared towards developing, calibrating and validating a model that can be used to design a pilot scale system. In order to achieve this, transparent pond reactors representing water columns in algae WSP have been used. Bulk water and biofilm have been sampled from these reactors and used in the study. The data generated is used to develop a model under different environmental conditions and the approved model is being used to design a pilot scale algal biofilm pond reactor.

Materials and methods

Four transparent pond reactors with a surface area of 0.043 m², an effective depth of 0.95 metre and a volume of 0.041 m³ were used. The pond reactors A1–A3 were placed in series, while A0 was a single reactor. The reactors simulated the water column in algae wastewater stabilization ponds (Figure 1). Synthetic wastewater (Table 1) of moderate strength (Metcalf and Eddy, 2003) was continuously fed at a flow rate of 0.66 L d⁻¹ into A0 and A1, which translates into a theoretical mean retention time of 2.6 days for each pond reactor. Nitrifiers and denitrifiers were introduced into the system at the start of the experiment by seeding with 100 ml of aerobically and anoxically grown activated sludge. Later on, green algae were also introduced in the reactors and allowed to colonize the system. The set-up was exposed to 12-hour light and dark regimes by illumination with a light intensity of 125–129 μEm⁻² s⁻¹. This provided sufficient light and represented natural conditions. The lamps also provided heat that resulted in a mean ambient temperature of 24°C. The average influent NH₄-N and COD concentration was 40 mg L⁻¹ NH₄-N and 96 mg L⁻¹ COD respectively.

**Figure 1** Experimental set up showing flow patterns of A0, A1, A2 & A3
Bulk water activity tests

1 litre of bulk water was collected from A3 and placed into two 2-litre glass beakers. Similarly, synthetic wastewater devoid of microorganisms was prepared with an ammonia concentration adjusted to 20 mg L\(^{-1}\) – values similar to that of the effluent bulk water of A3 and placed into two beakers to serve as control. All beakers were continuously aerated and exposed to light for 7 hours. Water samples were collected on an hourly basis and the ammonium concentration determined. The experiment was continued for 7 days. Ammonia, pH and oxygen levels were monitored on a daily basis. All the experiments were run in duplicates.

Biofilm activity test (7 days duration)

Algal biofilm was scraped from the walls of A3 at 0.05 m depth. A small round sampler of area 2.7 \(\times\) 10\(^{-3}\) m\(^2\) was found to collect an average of 3.30 g ± 0.47 wet weight of biofilm. Biofilm of 11.28 g (equivalent to biofilm area of 0.0092 m\(^2\)) and 11.30 g (0.0098 m\(^2\)) were sampled for duplicate studies. These were placed in beakers (area 0.0177 m\(^2\)) containing 1 litre of synthetic wastewater (ammonium concentration of 20 mg L\(^{-1}\)) devoid of microorganisms. Control experiments using the same synthetic wastewater devoid of microorganisms and biofilm were run in parallel. All the beakers were continuously exposed to 12-hour light/dark regimes. The experiment was left to run for 7 days without aeration. Ammonia, pH and oxygen were monitored on a daily basis.

Biofilm activity test (7 hours duration)

After the 7 days duration of the experiment above, the biofilms were reused to run a short-term experiment. This experiment lasted for a period of seven hours. The biofilms were weighed and the wet weight had increased from 11.28 g to 12 g (0.00981 m\(^2\)) and 11.30 g to 13.73 g (0.01123 m\(^2\)) respectively. The same experimental procedures as above were repeated. Water samples were collected hourly and ammonia concentration, pH and oxygen determined.

Biofilm plate activity tests (7 hours duration)

To investigate further nitrification rates of biofilm from A3, glass biofilm plates of 0.03 m by 0.08 m were suspended in this pond reactor at 0.05 m depth (Steen, 2000). The plates were retrieved from the pond reactor after two weeks and hung in glass beakers containing 0.5 litres of synthetic wastewater containing 20 mg L\(^{-1}\) NH\(_4\)-N. Ammonium nitrogen, nitrates, pH, dissolved oxygen and temperature were monitored after every two hours.

### Table 1 Composition of synthetic wastewater (modified after Moussa et al., 2003)

<table>
<thead>
<tr>
<th>Macro nutrients</th>
<th>Concentration (mg/L)</th>
<th>Micro nutrients solution</th>
<th>Concentration (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH(_3)OONH(_4)</td>
<td>93.75</td>
<td>EDTA</td>
<td>10</td>
</tr>
<tr>
<td>NH(_4)Cl</td>
<td>87.70</td>
<td>FeCl(_2)6H(_2)O</td>
<td>1.5</td>
</tr>
<tr>
<td>Na(_2)HPO(_4)H(_2)O</td>
<td>26.70</td>
<td>H(_3)BO(_3)</td>
<td>0.15</td>
</tr>
<tr>
<td>MgSO(_4)7H(_2)O</td>
<td>90.00</td>
<td>CuSO(_4)2H(_2)O</td>
<td>0.03</td>
</tr>
<tr>
<td>CaCl(_2)2H(_2)O</td>
<td>4.72</td>
<td>KI</td>
<td>0.18</td>
</tr>
<tr>
<td>KCl</td>
<td>36.00</td>
<td>MnCl(_2)4H(_2)O</td>
<td>0.12</td>
</tr>
<tr>
<td>Micronutrient solution</td>
<td>0.6 (ml/L)</td>
<td>Na(_2)MoO(_4)2H(_2)O</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ZnSO(_4)7H(_2)O</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CoCl(_2)6H(_2)O</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Nitrification rates obtained in activity tests fitted in mass balance equation

$R_{\text{biofilm}}$ and $R_{\text{nitrbulk}}$ values obtained in the batch activity tests were used as parameters in the mass balance model equation below (adapted from Zimmo et al. (2004)).

$$V_{\text{reactor}} \times \frac{d[KjN]}{dt} = Q \times [KjN]_{\text{inf}} - Q \times [KjN]_{\text{eff}} - (R_{\text{overall}} \times V_{\text{reactor}}) + U_{\text{vol}} + S_{\text{sed}} \quad (1)$$

where

$[Kjn]_{\text{inf}} = \text{Influent Kjeldahl Nitrogen (g/m}^3\text{)}$

$[KjN]_{\text{eff}} = \text{Effluent Kjeldahl Nitrogen (g/m}^3\text{)}$

$R_{\text{overall}} = \text{Overall nitrification rate (}(R_{\text{nitrbulk}} \times V_{\text{active}}) + (R_{\text{biofilm}} \times A_{\text{biofilm}})/V_{\text{reactor}}\text{))}$

$R_{\text{nitrbulk}} = \text{Nitrification rate in the bulk (g/m}^3\text{/d)}$

$V_{\text{active}} = \text{Aerobic volume of the bulk water column (m}^3\text{)}$

$R_{\text{biofilm}} = \text{Nitrification rate in the biofilm (g/m}^3\text{/d)}$

$A_{\text{biofilm}} = \text{Aerobic biofilm area (m}^2\text{)}$

$Q = \text{Flow rate (m}^3\text{/d)}$

$U_{\text{vol}} = \text{Ammonia volatilization (g/d)}$

$S_{\text{sed}} = \text{Ammonia removal by sedimentation (g/d)}$

$V_{\text{reactor}} = \text{Total volume of pond reactor}$

Ammonia volatilization was estimated using Equation (2) developed by Zimmo et al. (2004).

$$Y = 3.3 \times 4.90 \quad (2)$$

where,

$Y = \text{Ammonia volatilization rate (mg} - \text{N/L} \text{ m}^2\text{d)}$

$X = \text{Ammonia concentration (mg} - \text{N/L)}$

Sedimentation was monitored using cotton wool wrapped and tied on one end of a wooden rod as described by the white towel test of Malan (Pearson et al., 1987); this was done on a monthly basis. Sediment accumulation was found to be negligible, probably because the synthetic wastewater did not contain any suspended matter.

The overall model (Equation 1) was calibrated by adjusting the fraction of the pond volume and biofilm area of pond reactors A0–A3 that is active (i.e. aerobic). A depth of 0.08 m was applied to calculate the active areas and volumes. The calibrated model was then used to predict effluent Kjeldahl ammonium nitrogen of pilot scale in Palestine (Zimmo, 2003) and that in Colombia (Caicedo, 2005) respectively.

Results and discussion

In general it can be mentioned that the activity tests showed that nitrification occurred in both the bulk water and in the biofilms. However, in the pond reactors, the contribution of the biofilm nitrification was much more important than the bulk activity.
Bulk water, 7 hours experiment

The short-term investigation on the activity of microorganisms in the bulk water did not show a change in ammonia concentration for the entire 7-hour period of incubation. A mean ammonia concentration of $21.1 \pm 0.6 \text{ mg L}^{-1} \text{NH}_4\text{-N}$ and $19.6 \pm 1.1 \text{ mg L}^{-1} \text{NH}_4\text{-N}$ was measured in the duplicate experiment 1 and 2, respectively. This indicates that the activity is low in the bulk despite the fact that $8.8 \text{ mg L}^{-1}$ of oxygen and a pH of 8 were recorded. Dissolved oxygen of less than $0.50 \text{ mg L}^{-1}$ is thought to inhibit nitrification but in this case, oxygen was not limiting. The pH values measured are also within the optimum pH ranges of 7.2–9.0 required for suspended growth (Metcalf and Eddy, 1991). Nitrifiers can only maintain themselves while attached to surfaces that prevent wash out. They rarely live as free suspended bacteria (Hammer and Knight, 1994). This could be the major reason for the absence of nitrogen removal in the bulk water. Zimmo et al. (2000) also note that the lack of surface area for nitrifiers and denitrifiers is a major limitation of nitrogen removal in algae ponds. The control experiment showed a similar trend with a mean ammonia concentration of 20–21 mg L$^{-1}$ NH$_4$-N.

Despite the fact that the bulk water was aerated by bubbling of air, ammonia volatilization did not play an important role. An ammonia volatilization rate of $8.4 \text{ mg-N m}^{-2}\text{d}^{-1}$ was obtained, which is negligible.

Because the 7-hour experiment did not show any change in the ammonia concentration, the experiment was continued for 5 extra days. The results show a slight drop of the ammonia concentration for the first four days. The concentrations dropped from 20.1 to 17.4 mg L$^{-1}$ NH$_4$-N and from 19.6 to 16.8 mg L$^{-1}$ NH$_4$-N in duplicate 1 and 2, respectively. After the fourth day, the ammonia concentration rapidly dropped to 9.5 mg L$^{-1}$ NH$_4$-N, giving an overall ammonia decrease of 10.6 mg L$^{-1}$ NH$_4$-N for the entire period of seven days. The reason for this observation could probably be attributed to growth of nitrifiers. Nitrifiers are known to be slow growers which would require more time to build their population.

As in the bulk water, there was rapid drop of the ammonia concentration in the control after four days. The overall ammonia decrease from the control was 6.5 and 8.7 mg L$^{-1}$ NH$_4$-N in control 1 and 2 respectively. These values are slightly lower than the values from the bulk water. Ammonia loss in the control is exclusively due to volatilization, while in the bulk water, both ammonia oxidation and volatilization may have been important. Taking values of 10.6 mg L$^{-1}$ NH$_4$-N and 8.7 mg L$^{-1}$ NH$_4$-N as decrease in bulk water and control, it can be assumed that 1.9 mg L$^{-1}$ NH$_4$-N was lost due to nitrification. This gives a bulk water nitrification rate ($R_{\text{nitr,bulk}}$) of $2.7 \times 10^{-1} \text{ mg-N L}^{-1}\text{d}^{-1}$. The result indicates very low nitrification rates in the bulk water. This is in agreement with McLean et al. (2000) who made a similar observation for algae lagoons. They observed that lagoons with a high density of suspended algae had higher nitrification rates. The algae provided an attachment surface for nitrifier growth. Nitrifiers are known to prefer attached growth. From these results, it can also be concluded that long term bubbling of air strips the water of ammonia.

Biofilm activity test, 7 days experiment

The ammonium concentration in the biofilm experiment rapidly dropped from 18.0 to 3.8 mg L$^{-1}$ NH$_4$-N within one day (Figure 2). The concentrations then gradually dropped and after the fifth day, very low concentrations ($<1 \text{ mg L}^{-1} \text{ NH}_4\text{-N}$). While the ammonia concentrations were dropping, nitrate levels were building up (Figure 2). This was clear evidence that nitrification was occurring in the beakers. Biofilm nitrification rate ($R_{\text{nitr,biol}}$) of 1,495 mg-N m$^{-2}\text{d}^{-1}$ was obtained, which is comparable to 1,652 mg-N m$^{-2}\text{d}^{-1}$ obtained for the glass biofilm plates (Table 2). This shows that the biofilm glass plates
can also be used to determine the nitrification rates of the pond reactor. In comparison, the control had an average ammonia concentration of 23.0 ± 2.8 mg L\(^{-1}\) NH\(_4\)-N after the seven days of incubation (Figure 2). This shows that ammonia volatilization was minimal. Ammonia volatilization rate of 5.9 mg-N m\(^{-2}\) d\(^{-1}\) was obtained for both treatments (Table 2). It can then be concluded that ammonia loss in the biofilm experiment was mostly due to nitrification. However, the amount of nitrate measured was lower than the amount of ammonia reduced. It was found that nitrites did not accumulate. It is then believed that nitrification and denitrification occurred simultaneously, with denitrification taking place in the deeper anoxic micro environments of the biofilm (Kuenen and Robertson, 1994). This could have resulted in the low concentration of nitrates measured. The decrease of ammonia within the first day as shown in Figure 2 was further investigated and the results are discussed below.

**Biofilm activity test, 7 hour experiments**

A short-term biofilm activity test was performed to confirm this rapid decrease. Both duplicates 1 and 2 containing biofilm samples show a gradual drop in ammonia concentration with time (Figure 3). A regression line was fitted and ammonia reduction rates of 0.85 and 0.83 mg L\(^{-1}\) h\(^{-1}\) (R\(^2\) = 0.93 and 0.86) were obtained for duplicate 1 and 2 respectively. The average values of these reduction rates revealed a biofilm nitrification rate (R\(_{\text{biofilm}}\)) of 2.125 mg-N m\(^{-2}\) d\(^{-1}\) for the first 5 hours of the test, which is higher than the 1.495 mg-N m\(^{-2}\) d\(^{-1}\) observed on the first day of the seven-day biofilm activity test (Table 2). The results for the short-term experiment are more reliable since the experiment was closely monitored. Both values for R\(_{\text{biofilm}}\) are comparable to 720–2,640 mg-N m\(^{-2}\) d\(^{-1}\) (Leu et al., 1998) but higher than 480–720 mg-N m\(^{-2}\) d\(^{-1}\) (Craggs et al., 2000) and 720–960 mg-N m\(^{-2}\) d\(^{-1}\) (McLean et al., 2000). The control experiment showed a constant ammonia concentration during the first five days of the experimental

![Figure 2: Ammonia and nitrate in biofilm experiment after 7 days](https://iwaponline.com/wst/article-pdf/55/11/93/439287/93.pdf)

**Table 2 Nitrification and volatilization rates of bulk water, biofilm and glass plates of A3**

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Nitrification rate (mg N m(^{-2}) d(^{-1}))</th>
<th>Volatilization rate, Treatment (mg N m(^{-2}) d(^{-1}))</th>
<th>Volatilization rate, control (mg N m(^{-2}) d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>*R bulk (mg-N L(^{-1}) d(^{-1})) 2.7 (\times) 10(^{-1})</td>
<td>2.7 (\times) 10(^{-1})</td>
<td>8.4</td>
<td>6.1</td>
</tr>
<tr>
<td>R biofilm (7 days)</td>
<td>1,485</td>
<td>5.9</td>
<td>5.9</td>
</tr>
<tr>
<td>R biofilm (7 hrs)</td>
<td>2,125</td>
<td>1.02</td>
<td>8.7</td>
</tr>
<tr>
<td>R biofilm (glass plates)</td>
<td>1,652</td>
<td>5.7</td>
<td></td>
</tr>
</tbody>
</table>

*Note that the units for R bulk are expressed in mg-N L\(^{-1}\) d\(^{-1}\)
This again confirms that volatilization was minimal. These results are in agreement to those obtained by Zimmo et al. (2004), which indicated that ammonia volatilization is negligible in wastewater stabilization ponds under these conditions.

**Nitrification rates fitted in mass balance equation**

The mean nitrification rates of the seven hour activity tests of $1,377 \pm 242 \text{mg-N m}^{-2} \text{d}^{-1}$ were used as the $R_{\text{biofilm}}$ in the model equation (1) proposed in this study. When assuming a depth of $0.08 \text{m}$ active upper layer of the algal pond reactors, the model could describe well the measured effluent values for the pond reactors A0, A1 and A2 (Table 3). The calibrated model was validated by predicting effluent Kjeldahl nitrogen of algae ponds in Palestine (Zimmo, 2003) and in Colombia (Caicedo, 2005). Results in Figure 4 indicate that the model equation predicted the effluent concentrations of the pilot systems

**Table 3** Calculated and predicted effluent Kjeldahl nitrogen in the algal pond reactors of this study and those of Caicedo (2005)

<table>
<thead>
<tr>
<th>Pond reactor</th>
<th>Measured values Kj-N (mg/L)</th>
<th>Values calculated by calibrated model Kj-N (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A0</td>
<td>36.6</td>
<td>34.4</td>
</tr>
<tr>
<td>A1</td>
<td>36.7</td>
<td>36.5</td>
</tr>
<tr>
<td>A2</td>
<td>30.9</td>
<td>32.2</td>
</tr>
<tr>
<td>A3</td>
<td>27</td>
<td>19.1</td>
</tr>
<tr>
<td>Caicedo (2005)</td>
<td>36</td>
<td>23</td>
</tr>
</tbody>
</table>

*Figure 3* Ammonia concentration in the 7-hour biofilm experiment

*Figure 4* Predicted (dotted line) and measured (block line) effluent Kjeldahl nitrogen for algal ponds of Zimmo (2003) during warm period of $21^\circ\text{C}$
in Palestine well. The predicted value in Colombia was higher than the measured effluents. Currently, the model is further developed by (among other things) including the effect of environmental conditions on the model parameters. Activity tests are carried out under different environmental conditions and the approved model is being used to design a pilot scale algal biofilm pond reactor.

**Conclusions**

This study has demonstrated the importance of attached growth in the process of improving nitrification in wastewater stabilization ponds. The model equation proposed in this study could be calibrated by the laboratory experiments and predicted well the Kjeldahl effluent nitrogen from a pilot scale system of Zimmo (2003). The model can be further developed and used in the design of algal based WSPs.

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