

Dinitrogen oxide production by a mixed culture of nitrifying bacteria during ammonia shock loading and aeration failure

JE Burgess^a, BB Colliver, RM Stuetz and T Stephenson

School of Water Sciences, Cranfield University, Cranfield MK43 0AL, UK

A number of experiments was conducted in order to establish if N₂O in the exhaust gas from an aerobic consortium of nitrifiers could be used as an indicator for monitoring the nitrification process. Laboratory-scale experiments with an activated sludge system showed a strong correlation between ammonia shock loads and both the concentration of N₂O and the rate of increase of N₂O in the exhaust gas for shock loads less than 1.60 mg ammoniacal nitrogen (NH₃-N) per g total suspended solids (TSS). For greater ammonia shock loads, correlation was found between build-up of nitrite in the aeration tank and the concentration of N₂O in the exhaust gas from the tank. When subjecting the system to aeration failure, a similar pattern was seen, with a correlation between nitrite build-up in the aeration tank and increases in the concentration of N₂O in the exhaust gas. The results from this work suggest that the changes in N₂O concentration in the exhaust gas from a nitrifying process may be a useful parameter for monitoring such processes.
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Introduction

Legislation demands that wastewater is treated to remove the majority of organic matter, colour and solids in order to meet certain water quality objectives before it can be discharged [6]. Biological removal of ammonia — nitrification — is traditionally defined as the aerobic oxidation of NH₃⁺ to NO₃⁻ via nitrite (NO₂⁻). This is mainly carried out by two groups of autotrophic bacteria: ammonia oxidisers (NH₃→NO₂⁻), exemplified by the genera *Nitrosococcus* and *Nitrosomonas*, and nitrite oxidisers (NO₂⁻→NO₃⁻), such as *Nitrobacter* and *Nitrospira* spp. [10,11].

Nitrification is a sensitive process. Inhibitory chemicals and high ammonia concentrations can affect nitrification, thus resulting in suppression or cessation of ammonia oxidation [2,16]. In the wastewater treatment industry, nitrification inhibition is often seen at wastewater treatment works that receive a high proportion of industrial wastewater, and this can result in ammonia in the works effluent and hence the works not meeting the required water quality objectives. Analysis of composite or grab samples of the process effluent in a laboratory is traditionally used to monitor the nitrification process during wastewater treatment [3]. This method of monitoring is resource- and time-consuming and does not allow for early warning of process failure. Some wastewater treatment works have recently experimented with installing biological nitrification toxicity monitors in side streams [9]. This does allow for early warning but such monitors are expensive to install and run, time-consuming and often unreliable due to sensor fouling and the need for frequent recalibration [5].

Recently, it has been shown that the bacteria normally associated with the nitrification process are able to produce nitrogen oxide

(NO) and dinitrogen oxide (N₂O) under aerobic conditions [13,20]. These processes have been described as aerobic denitrification. Build-up of NO₂⁻ during nitrification is a sign that the nitrification process is failing, as NO₂⁻ has been shown to be a strong inhibitor of ammonia oxidation through its action on ammonia monooxygenase [23], and its accumulation indicates that nitrite oxidisers are also overloaded. A number of studies have shown that the accumulation of NO₂⁻ is often seen in nitrifying cultures producing N₂O and NO [12,24,25]. Garrido *et al* [7] demonstrated a relationship between N₂O production and denitrification inhibition in an anoxic filter. This suggests that N₂O in the exhaust gas from a nitrification process could be a useful indicator for monitoring the nitrification process on an industrial scale. The appearance of N₂O and NO₂⁻ in the aeration stage of an activated sludge system should precede the appearance of ammonia in the final effluent by approximately one hydraulic retention time (HRT) of the entire aeration and clarification system. The aim of this study was to establish if the presence of N₂O in the exhaust gas from the aeration tank of an activated sludge system did indeed display the potential to be used to predict and avoid operational failure.

Materials and methods

Experimental

A laboratory-scale activated sludge system consisting of a completely mixed aeration tank (7 l) and a settling tank (1 l) was employed (Figure 1). Temperature, pH and dissolved oxygen (DO) concentrations were continuously monitored using an online computer. The temperature was maintained at 25°C by keeping the aeration tank in a heated water bath and DO was maintained at 5.0–5.5 mg l⁻¹ by regulating the air flow. The pH was maintained at 7.2 by means of a feedback from the online computer using 1.0 M NaHCO₃ as the buffer. The influent to the activated sludge system was a synthetic non-nitrified secondary effluent, which was prepared as a solid free basal medium feed developed and described in detail by Hanaki *et al* [8]. The feed was prepared

Correspondence: Richard M Stuetz, School of Water Sciences, Cranfield University, Cranfield MK43 0AL, UK

^aPresent address: Goldfields Biotechnology Laboratory, Department of Biochemistry, Microbiology and Biotechnology, Rhodes University, Grahamstown 6140, South Africa.

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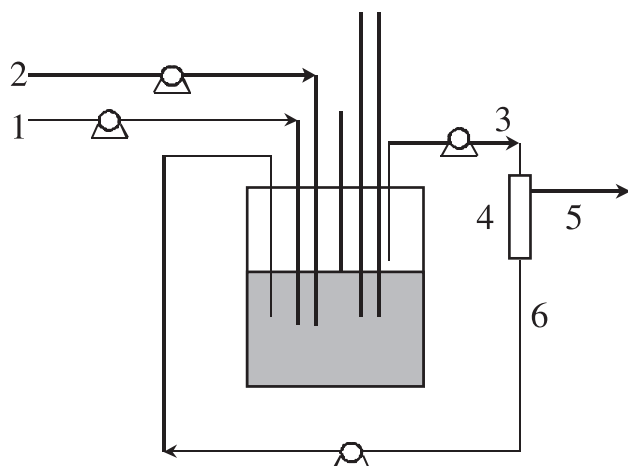


Figure 1 Schematic of laboratory-scale activated sludge pilot plant (not to scale). (1) Feed; (2) buffer; (3) overflow from aeration tank; (4) settling tank; (5) overflow from settling tank; (6) recycling of biomass; (7) thermometer; (8) pH probe.

using AnalaR grade chemicals, including glucose to provide organic matter and 160 mg l^{-1} chemical oxygen demand (COD). Ammonia bicarbonate was the sole source of ammonia and also acted as an alkaline buffer. This gave a steady-state nitrogen loading of $244 (\pm 15)$ and $184 (\pm 12)$ ammonical nitrogen ($\text{NH}_3\text{-N}$) per g total suspended solids (TSS). For the shock loading experiments, the feed ammonia concentration was $701 (\pm 39) \text{ mg l}^{-1}$, the flow rate of the influent was maintained at $2.25 (\pm 0.04) \text{ l d}^{-1}$ (HRT = 3.1 days) and the solids concentration of the mixed liquor was $1.0 (\pm 0.05) \text{ gTSS l}^{-1}$. The sludge retention time (SRT) was maintained at 23 days by adjusting the solids recycle rate from the settling tank. For the aeration failure experiments, the ammonia concentration was $1100 (\pm 52) \text{ mgNH}_3\text{-N l}^{-1}$, the flow rate of the influent was $1.48 (\pm 0.03) \text{ l day}^{-1}$ (HRT=4.7 days) the TSS concentration was $1.27 (\pm 0.05) \text{ g l}^{-1}$ and the SRT was 23 days.

Inoculation and start-up

The nitrifying culture used for inoculation of the aeration tank was kindly provided by Engineered Biological Services (Berkshire, UK). The concentration of nitrifiers in the aeration tank after inoculation was approximately 1.5 gTSS l^{-1} . However, when the system reached steady state, the concentration of nitrifiers had settled at 1.0 and 1.72 g l^{-1} TSS for the shock loading experiments and aeration failure experiments, respectively. During start-up, the initial feed flow rate was approximately 1 l day^{-1} . The effluent from the aeration tank was analysed daily for the presence of ammonia and nitrite. If the concentration of either ammonia or nitrite exceeded 1 mg l^{-1} the feed flow rate was kept constant and the effluent analysed again 24 hours later. If the concentration of either ammonia or nitrite was below 1 mg l^{-1} , the feed flow rate was increased by approximately 0.15 l d^{-1} . This procedure was repeated until steady state was reached. Between each shock loading or aeration failure experiment, the activated sludge system was allowed 3–4 days to regain steady state before another experiment was carried out.

Analytical methods

Total suspended solids of the nitrifying biomass, $\text{NH}_3\text{-N}$, $\text{NO}_2\text{-N}$ and $\text{NO}_3\text{-N}$ were analysed according to standard methods [3]. An

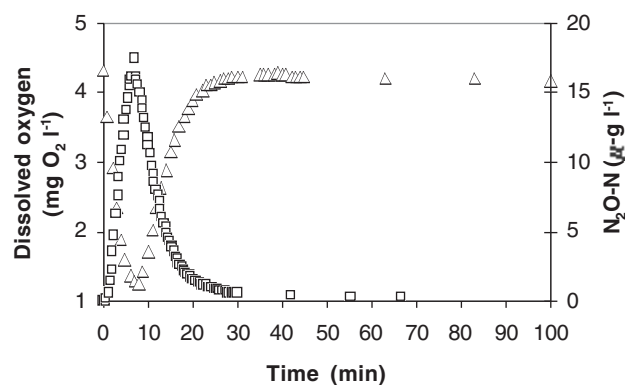


Figure 2 Example data set showing aeration tank DO and exhaust gas N_2O concentrations when shock loaded with $1.60 \text{ mgNH}_3\text{-N gTSS}^{-1}$. (Δ) DO; (\square) N_2O .

infrared gas analyser (Model VIA-510, Horiba UK Ltd, Northampton, UK) fitted with a mist trap and sampler unit (Model ES-C510SS, Horiba UK Ltd) was used for online determination of N_2O concentrations (ppm_v) in the exhaust gas from the aeration tank. The N_2O analyser was calibrated using nitrogen gas (99.99% N_2 ; Air Products, Walton-on-Thames, UK) for zero calibration and 160 ppm_v N_2O in nitrogen gas (Air Products) for endpoint calibration. The volume of exhaust gas from the aeration tank was determined by channelling all of the exhaust gas through a flow meter. The total amount of N_2O in the exhaust gas and the rate of N_2O production were calculated from the concentration and flow data.

Results

In order to determine whether the presence of N_2O in the exhaust gas from the aeration tank could be used as an indicator for monitoring the nitrification process, several experiments were carried out in which the activated sludge system was either shock-loaded with increasing amounts of ammonia ($0.53\text{--}3.30 \text{ mgNH}_3\text{-N gTSS}^{-1}$) or was subjected to aeration failure. The effect of ammonia shock loading and aeration failure on the presence of N_2O in the exhaust gas was monitored together with build-up of ammonia and nitrite in the aeration tank.

Table 1 N_2O production by a mixed culture of nitrifiers when shock-loaded with ammonia

$\text{NH}_3\text{-N}$ load (mg gTSS^{-1})	$\text{N}_2\text{O}_{\text{MAX}}^a$ (ppm_v)	$\text{N}_2\text{O}_{\text{TOT}}^b$ (μg)	$r_{\text{N}_2\text{O}}^c$ ($\mu\text{g min}^{-1}$ gTSS^{-1})	$Y_{\text{N}_2\text{O}}^d$ (%)
0.48	1.4	28	0.35	0.08
0.53	2.3	44	0.53	0.11
1.03	7.4	155	1.79	0.33
1.06	8.2	175	1.65	0.28
1.39	16.8	414	2.50	0.70
1.60	17.4	402	2.42	0.62
3.30	19.0	779	2.75	1.17

^a $\text{N}_2\text{O}_{\text{MAX}}$, maximum concentration of N_2O detected in the exhaust gas.

^b $\text{N}_2\text{O}_{\text{TOT}}$, total amount of N_2O in the exhaust gas.

^c $r_{\text{N}_2\text{O}}$, the rate at which the N_2O concentration increased in the exhaust gas.

^d $Y_{\text{N}_2\text{O}}$, the percentage of ammonia load converted into N_2O .

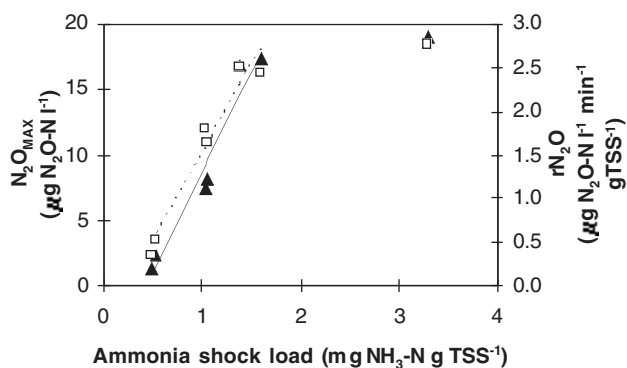


Figure 3 The maximum N_2O concentration and rate of increase of the N_2O concentration in the exhaust gas in relation to the ammonia shock loading. (\blacktriangle) N_2O_{MAX} ; (\square) rN_2O .

Ammonia shock loading

The nitrification process was shock-loaded by adding 5–25 ml of feed directly in the aeration tank of the activated sludge system running at the conditions stated previously. This gave a shock loading in the range of 0.53–3.30 $mgNH_3-N gTSS^{-1}$. The air supply was kept constant at $1.53 (\pm 0.03 l min^{-1})$, which gave a starting DO of $4.9 \pm 0.3 mg l^{-1}$. DO and pH in the aeration tank were logged once a minute, and the concentration of N_2O in the exhaust gas was logged every 30 s during each experiment. The nitrifying culture in the aeration tank was sampled at 1- to 4- min intervals during an experiment, and NH_3-N , NO_2-N and NO_3-N concentrations were determined. The pH in the aeration tank was between 7.2 and 7.5 during all experiments.

Shock loading the nitrifiers with ammonia was followed by a decrease of DO in the aeration tank and an increase in the concentration of N_2O in the exhaust gas from the aeration tank (Figure 2). The extent of the decreases in DO and increases in N_2O were dependent on the size of the shock load, with N_2O being produced at DO concentrations between 1 and 4 $mg l^{-1}$. When shock loading the system with $1.60 mgNH_3-N gTSS^{-1}$ (Figure 2), minimum DO and maximum N_2O concentrations were reached approximately 8 min after addition of the shock load, after which the DO started to increase and the N_2O concentration to decrease. Approximately 40 min after applying the shock load, both the DO in the aeration tank and N_2O concentration in the exhaust gas had recovered.

The exhaust gas flow rate and N_2O concentrations were used to calculate the total amount of N_2O in the exhaust gas (N_2O_{TOT}), the rate at which the N_2O concentration in the exhaust gas increased (rN_2O) and the yield of N_2O (Y_{N_2O}) (Table 1). In Table 1, the relationship between ammonia shock load and the above calculated parameters is shown together with the maximum concentration of N_2O (N_2O_{MAX}) determined in the exhaust gas during an experiment. The calculated values for N_2O_{TOT} , rN_2O , Y_{N_2O} and N_2O_{MAX} all increased with increasing level of shock load (Table 1). Regression analysis showed that strong linear correlations were found between ammonia shock loads up to $1.60 mgNH_3-N gTSS^{-1}$ and N_2O_{MAX} (correlation coefficient (CC)=0.98), N_2O_{TOT} (CC=0.96) and rN_2O (CC=0.95), whereas the CC for the correlation with Y_{N_2O} was only 0.87. The ammonia shock loads and increases in rN_2O and N_2O_{MAX} showed linear relationships up to $1.60 mgNH_3-N gTSS^{-1}$ shock load, after which the relationship appears to reach saturation (Figure 3).

Accumulation of NO_2-N was observed for shock loads greater than $1.03 mgNH_3-N gTSS^{-1}$, with the concentration increasing for increasing shock load. Figure 4 shows the concentration of N_2O in the exhaust gas and NO_2-N in the aeration tank after shock loading with 1.39 and $1.60 mgNH_3-N gTSS^{-1}$. At shock loads of less than $1.60 mgNH_3-N gTSS^{-1}$ (Figure 4a), only small amounts of NO_2-N accumulated ($>0.6 mg l^{-1}$). By contrast, at high levels of shock loading rN_2O and N_2O_{MAX} had reached saturation and the accumulation of NO_2-N followed a similar profile to the concentration of N_2O in the exhaust gas (CC=0.86). The maximum concentration of NO_2-N in the aeration tank was $2.5 mg l^{-1}$ (Figure 4b).

Aeration failure

The aeration rate of the nitrifying activated sludge system was lowered from 3.7 to $0.3 l min^{-1}$ and the concentrations of aeration tank NO_2-N and DO and exhaust gas N_2O were recorded. DO and pH in the aeration tank were logged once a minute and the concentration of N_2O in the exhaust gas was logged every 30 s. The nitrifying culture in the aeration tank was sampled at 1- to 4- min intervals during the experiments, and NH_3-N , NO_2-N and NO_3-N concentrations were determined.

Figure 5a shows the decrease in aeration tank DO concentration after reducing the air flow rate. When the DO reached a concentration of less than $1 mg l^{-1}$, N_2O started to appear in the exhaust gas and NO_2-N started to accumulate in the aeration tank.

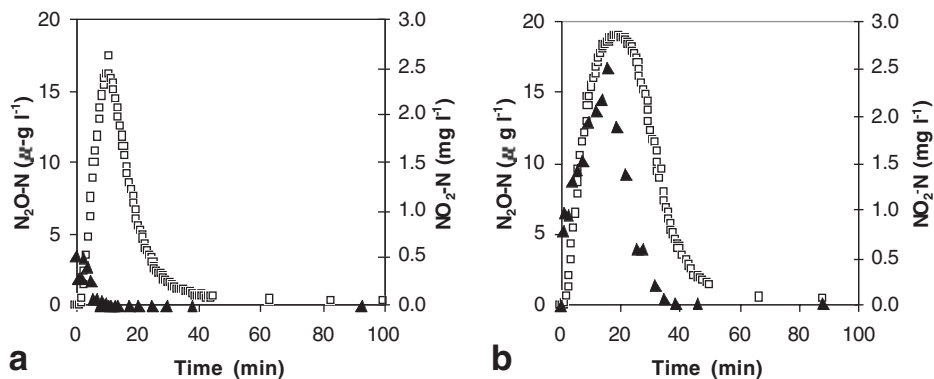


Figure 4 Nitrite in the aeration tank and N_2O in the exhaust gas from the aeration tank. (a) Ammonia shock load of $1.39 mgNH_3-N gTSS^{-1}$; (b) ammonia shock load of $1.60 mgNH_3-N gTSS^{-1}$. (\blacktriangle) N_2O ; (\square) NO_2-N .

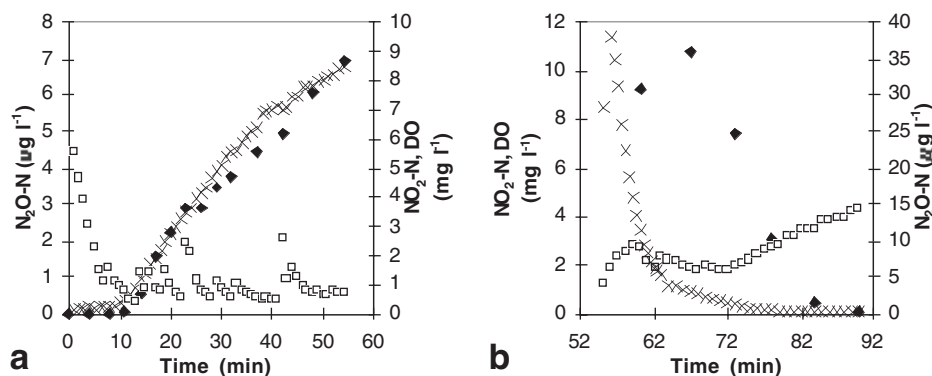


Figure 5 Aeration tank DO and NO_2^- concentrations and exhaust gas N_2O concentration during aeration failure. (a) The aeration was turned down from 3.7 to 0.3 l min^{-1} at time zero; (b) the aeration was turned up from 0.3 to 3.7 l min^{-1} at time 53 min. (\square) DO; (\times) N_2O ; (\blacklozenge) $\text{NO}_2\text{-N}$.

It can be observed that the profile for $\text{NO}_2\text{-N}$ accumulation in the aeration tank followed the profile for N_2O concentration in the exhaust gas, with the concentration increasing with time. Regression analysis again showed that a strong linear correlation was found between the concentrations of $\text{NO}_2\text{-N}$ in the aeration tank and N_2O in the exhaust gas ($CC=0.99$). After 53 min, the aeration was returned to 3.7 l min^{-1} (Figure 5b). After the aeration was restored, the concentration of N_2O in the exhaust gas decreased rapidly, whereas it took another 20 min for the concentration of $\text{NO}_2\text{-N}$ in the aeration tank to return to pre-experimental levels. The DO concentration started to increase after 53 minutes, another small decrease was observed between 65–72 minutes and at 67 minutes the $\text{NO}_2\text{-N}$ concentration reached its maximum (Figure 5b). Approximately 90 min from the start of the experiment, the DO, N_2O and $\text{NO}_2\text{-N}$ concentrations had recovered to preexperimental values.

Discussion

A laboratory-scale activated sludge system inoculated with a mixed culture of nitrifying bacteria was used to study the production of N_2O during ammonia shock loading and aeration failure. The results from the ammonia shock loading experiments showed that N_2O was produced at DO concentrations as high as 4 mg l^{-1} . Both the rate at which the N_2O concentration increased in the exhaust gas and the maximum N_2O concentration observed in the exhaust gas correlated with to the size of the ammonia shock load (for ammonia shock loads less than 1.60 $\text{mgNH}_3\text{-N gTSS}^{-1}$). However, with ammonia shock loads greater than 1.60 $\text{mgNH}_3\text{-N gTSS}^{-1}$, both the maximum and the rate of increase of N_2O in the exhaust gas appeared to reach saturation, which indicated that N_2O production by the nitrifiers had reached its maximum. This may have been due either to DO reaching zero or to the oxidation rate reaching its maximum at this concentration. Similar results were reported by Nogita *et al* [15] and Satio *et al* [21], who found that the rate of N_2O production by activated sludge receiving municipal wastewater was related to both the COD and nitrogen loading rates. However, they did not observe the N_2O production rates reaching a maximum.

Previous authors have reported that ammonia-oxidising bacteria under both oxygen-limited and abundantly oxygenated conditions reduce nitrite arising from ammonia oxidation to N_2O and further to N_2 [25]. The increase in N_2O is a result of the decrease in DO,

which causes the bacteria to use nitrite as the terminal electron acceptor. Other studies have shown that the principal mechanisms for N_2O production by ammonia oxidisers are simultaneous reduction of ammonia and oxidation of nitrite, also causing nitrite to be used as the terminal electron acceptor instead of oxygen [1,4,17,19]. The large amounts of nitrite that accumulated when shock loads were above 1.60 $\text{mgNH}_3\text{-N gTSS}^{-1}$ (Figure 4a and b) could be explained by the possibility that the rate at which N_2O increased in the exhaust gas had reached its maximum and nitrite was produced in excess. However, it could also be due to the nitrite-oxidising bacteria being unable to oxidise the influx of nitrite fast enough, owing to lack of oxygen. Laanbroek and Gerards [14] suggested that the ability of ammonia oxidisers to replace oxygen with nitrite as the terminal electron acceptor at low oxygen concentrations gives them a competitive edge over nitrite oxidisers at low oxygen concentrations.

The response to aeration failure was similar to that of high level ammonia shock loading, with simultaneous decreases in the aeration tank DO, increases in exhaust gas N_2O concentration and increased nitrite in the aeration tank (Figure 5a). However, during this experiment, the presence of N_2O first occurred at DO concentrations below 1 mg l^{-1} .

Conclusions

- The concentration of N_2O in the exhaust gas from the nitrifying activated sludge system and the rate at which it increases were correlated to the size of the ammonia shock load for shock loads less than 1.60 $\text{mgNH}_3\text{-N gTSS}^{-1}$.
- For ammonia shock loads greater than 1.60 $\text{mgNH}_3\text{-N gTSS}^{-1}$, the concentration of N_2O in the exhaust gas was related to the build-up of $\text{NO}_2\text{-N}$ in the aeration tank.
- During aeration failure, the increase of N_2O in the exhaust gas was closely related to the build-up of nitrite in the aeration tank.

Both the concentration and the rate of increase of N_2O may be good indicators for monitoring the nitrification process, as they are correlated to both ammonia shock load and the build-up of nitrite in the aeration tank. If the emergence of N_2O in the exhaust gas of the first stage of a nitrification process precedes the appearance of ammonia in the final effluent by approximately one HRT, as

expected, these results illustrate the potential of monitoring N₂O for predicting operational failure and providing an opportunity to take remedial action.

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