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Effects of pH and substrate availability on the half saturation coefficient and kinetics of ammonium oxidizing bacteria

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ABSTRACT*: The controversial arguments on the true substrate in nitritation kinetics might be due to the cells' dual substrate-transport system. Our experiments revealed that, under ammonia-rich environments, it diffused into the membrane (ammonia was the direct substrate); but, under oligotrophic, ammonium ion was actively transported (ammonium was the direct substrate). Facilitating this change-over, the bacterial composition in the sludge was altered, although the predominant was Nitrosomonaseutrophain most of the six chemostats. Also, the substrate affinity constant (K_s) fell resulting in partial compensation for the reduced availability of substrate. When the environmental ammonia concentration was lower than the cytoplasmic one, a backward diffusion appeared to take place, which probably had the cells accelerate its energy-consuming ammonium transport. The %AOB to the total number of bacteria in the sludge remarkably decreased when cells were grown under oligotrophic environments. This could be evidence of the cellular energy dissipation caused by ammonia loss and recovery. Intracellular TAN accumulations were observed, which gradually increased from a basal value of ~1M (for AOB grown under copious environments) to much higher values (grown under oligotrophic environment). It not affected the reaction kinetics but potentially served as a reserve against famine.*

KEYWORDS: *Ammonia oxidizing bacteria (AOB),True substrate, Dual ammonia transport, Ammonia leakage,Intracellular TAN reserve, Affinity constant*

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I. INTRODUCTION

Nitrification is an essential step in the biological nitrogen removal from waste water. There have been contradictory views on the direct substrate between ammonium and un-ionized free ammonia (NH_3) that is taken up by ammonium oxidizing bacteria (AOB). Although several researchers (Suzuki et al. 1974; Van Hulle et al. 2007) reported that free ammonia was the direct form of substrate, a critical question was not answered: Why do cells not utilize ionized ammonium which present at much higher concentration under most natural environmental conditions.

Depending on the form of substrate to take up, there is a significant difference in the removal rate prediction, when the medium pH varies. The purpose of this study was to investigate the true substrate that was taken up by AOB.

II. MATERIALS AND METHODS

Sludge was taken from six lab scale nitrifying continuous stirrer tank reactor (CSTRs). The reactors were operated at different substrate concentrations such as, ~1 mg-N/L(R1), ~10 mg-N/L, ~100 mg-N/L, 200 mg-N/L, 500 mg-N/L, and 1000 mg-N/L. Batch tests were performed to obtain the specific oxygen uptake rate (SOUR) at different initial ammonium concentrations. The tests were run for a short period of time (~10 min), and no nitrite oxidation was observed. The Lineweaver-Burk plot method was used to estimate the half saturation coefficient (Ks) of ammonium oxidation at four different pH values (6.5, 7, 8, and 8.5).

III. RESULTS AND DISCUSSION

Figure 1 shows dynamic changes in the observed Ks values when the direct substrate was considered as Free Ammonia (FA) fig. 1a, when ammonium fig. 1b. The observed Ks values at different pH (6.5, 7, 8, & 8.5) were close to each other for R1, R10, and R80 when the truesubstrate was considered as ammonium and much scattered Ks values were observed for R200, R500 and R1000. In terms of FA the Ks values for lower substrate CSTRs were much scattered and were less scattered for high concentrated CSTRs, variation of Ks at different pH was quite system. This suggest that for lower concentrated reactor the true substrate for AOB was observed ionic form of ammonia, but at higher concentrated CSTR the Free Ammonia was the true substrate for AOB.

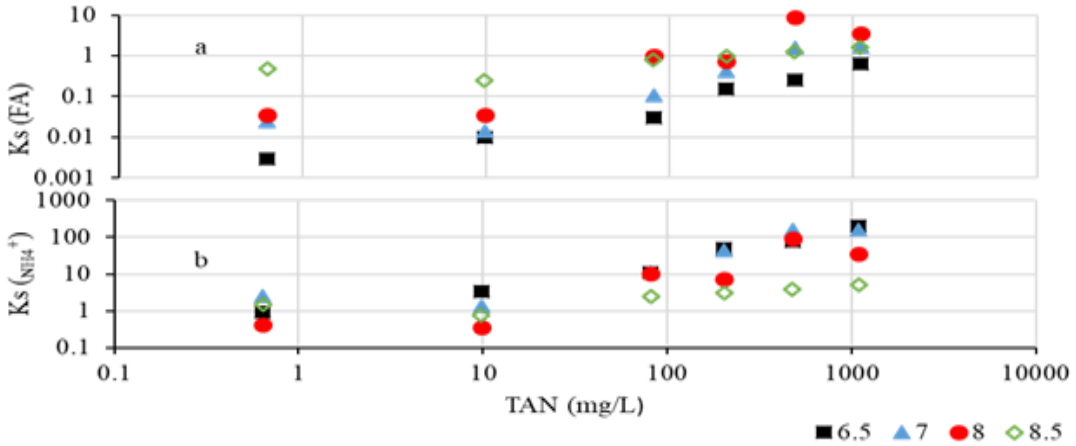

Fig. 1. Observed Ks values at different pH when the substrate was considered (a) Free Ammonia (NH_3) (b) Ammonium(NH_4^+). TAN concentration in the CSTRs where the sludge samples were taken.

IV. CONCLUSION

1. At lower substrate concentration ≤80 mg-/L; the Ks values looked much closer to each other when substrate considered as ammonium (NH_4^+), but Ks values looks much scatter when substrate considered a Free Ammonia (FA). Reverse condition was observed at higher substrate concentration ≥200 mg-/L.
2. If rector operated at lower concentration ≤80 mg-/L ionic form of ammonia was observed the true substrate for AOB. At higher concentration ≥200 mg-/L the FA was observed the direct substrate for AOB.

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