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Nitrification at full-scale municipal wastewater treatment plants: Evaluation of inhibition and bioaugmentation of nitrifiers



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HIGHLIGHTS

• Revealed inhibitory factors of nitrification.

• Presented a successful side-stream nitrifiers bioaugmentation process.

• Discussed advantageous effects of bioaugmentation.

A R T I C L E I N F O

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ABSTRACT

Batch nitrification tests were conducted with sludge and wastewater streams obtained from field implementations to evaluate nitrification inhibition and efficiency of a nitrifiers bioaugmentation technology at full-scale municipal wastewater treatment plants (WWTPs). The results showed that the substrate organic carbon and pH of wastewater streams were inhibitory factors to nitrification and the low pH was the cause of the WWTP experiencing poor nitrification. An ammonia-nitrogen removal rate of 0.21 mg-N/g MLVSS-h was observed at pH 6.5, while the rate increased to 0.54 mg-N/g MLVSS-h with an introduction of 6% bioaugmented nitrifiers, indicating that the integrated side-stream nitrifiers bioaugmentation process was beneficial in improving nitrification efficiency, even under low pH conditions not conducive to nitrification. The study provides new insights into effective upgrading of municipal WWTPs exposed to poor nitrification.

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1. Introduction

Biological nitrification represents an essential operation of municipal wastewater treatment plants (WWTPs) in order to produce good effluent quality. It is a two-step autotrophic process, carried out by two categories of chemolithotrophic microorganisms: ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB). However, the nitrification process can be inhibited by various factors, such as temperature (Gu et al., 2012; Zhang et al., 2014), pH, dissolved oxygen (DO, Fitzgerald et al., 2015), solids retention time (SRT), ammonia and nitrite concentrations (Yang et al., 2010), carbon to nitrogen ratio, and the presence of inhibitory compounds (Hu et al., 2004). Moreover, the characteristics of nitrifying bacteria such as their slow growth rates and high sensitivity to inhibitory compounds (Dvořák et al., 2013) compared to the heterotrophs add to the difficulty of reducing the inhibition. The breakdown of nitrification in WWTPs may cause severe damages of the activated sludge and reduce the safe function of the plants significantly, leading to negative ecological effects in the aquatic environment. It is therefore necessary to protect nitrifying WWTPs absolutely reliable.

Although the nitrification as a key process for advanced wastewater treatment has to be carefully protected from negative influences (Schweighofer et al., 1996), it turns out that unstable nitrification due to inhibition at some full-scale municipal WWTPs has become a widespread phenomenon, and measures have to be taken in order to identify and reduce the inhibitory effects. For example, before the upgrading of a municipal WWTP can take place, a comprehensive program of examination of the incoming wastewater and of the sludge is required for identifying sources of inhibition in order to address the problems and their consequences (Jönsson et al., 1996). Since the inhibiting substances in municipal wastewater are often very diluted and difficult to detect (Jönsson and Jansen, 1999), laboratory batch methods are typically used to have some model inhibitory compounds tested







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in advance to understand the nitrifying processes developing under stress (Figuerola and Erijman, 2010). It is also known that since the ability of an activated sludge system to nitrify is dependent on SRTs, if a WWTP is operated at lower SRTs than the required values, the growth rate of nitrifiers is lower than the rate of their daily wasting with the excess activated sludge, causing nitrifiers to be washed out of the system. Therefore, upgrading of a WWTP for nitrification usually requires enlargement of aeration basins to allow operation at higher SRTs. Bioaugmentation of nitrifiers as a process of adding acclimatized nitrifiers to assist in the nitrification process, represents a promising approach to eliminate the needs for enlargement of tanks and allows nitrification at short SRT values. To date, there is less information on the discussion of identifying and solving nitrification inhibition issues at full-scale municipal WWTPs. Reports on bioaugmentation in WWTPs also show frequent failures and inclusive outcomes (Herrero and Stuckey, 2014). The inhibitory factors of nitrification and the bioaugmentation approach to reduce the inhibition on full-scale basis need to be evaluated.

In this work, a series of batch nitrification tests with various combinations of wastewater streams and sludge obtained from field implementations of full-scale municipal WWTPs (including a side-stream nitrifiers bioaugmentation plant) were conducted. The objectives of this research were twofold: (1) to examine the potential nitrification-inhibitory factors for a municipal WWTP experiencing poor nitrification; and (2) to evaluate an integrated side-stream nitrifiers bioaugmentation process for improving nitrification efficiency. The results of this study provide insights into effective upgrading of municipal activated sludge systems exposed to poor nitrification.

2. Methods

2.1. Process description of WWTPs

The biological treatment processes of the municipal WWTPs under investigation were schematically shown in Fig. 1. The Harrisburg WWTP (Harrisburg, Pennsylvania, USA), denoted as "P-1" in this research, employed a highly pure oxygen activated sludge process (Fig. 1a) to achieve nitrification. The Elizabethtown WWTP (Elizabethtown, Pennsylvania, USA), denoted as "P-2", used oxidation ditches (Fig. 1b) to achieve nitrogen removal. An InNitriTM system (Mixing & Mass Transfer Technologies LLC, State College, Pennsylvania, USA), denoted as "P-3", was designed as a nitrifiers bioaugmentation plant (Fig. 1c) to provide supplemental nitrifiers for P-1. As a side-stream process, P-3 used a small fraction of the P-1 primary effluent as the inflow to the bioaugmentation reactor for acclimatization purpose and was able to produce a high nitrifiers population (>97%) in its sludge by operating under "non-limiting" conditions.

It is noted that only P-1 was found to have poor nitrification according to the facility management. Because identifying the sources of nitrification inhibition and proposing inexpensive upgrading alternatives were highly desirable, P-1 was evaluated for inhibitory factors before the upgrading can take place. P-1 accepted an average flow of approximately 1650 L/s, about 90% of which was domestic sewage received by pipeline. P-1 also received and treated three to five pump-truck loads (30,000–50,000 L) of domestic septic-tank waste daily and primary sludge from nearby smaller and less advanced treatment plants. The trucked-in sludge was mixed with the sewer-line influent, followed by being separated into sludge and supernatant and then processed. The effluent was aerated with highly pure oxygen, neutralized, and chlorinated before being discharged into the Susquehanna River.

2.2. Experimental design

Table 1 shows the experimental design of four series of batch nitrification tests. Each series of experiment used four parallel reactors which were filled with different substrate and seed combinations and operated under non-limiting conditions. Series 1 was designed to assess the impact of P-1 streams on a good nitrifying sludge from P-2, as P-2 was a plant that was able to achieve stable nitrification at all times. The P-2 sludge was used as seed while the P-1 primary influent, primary effluent, secondary effluent, and P-2 primary influent were used as substrate. Series 2 was designed to assess the impact of P-1 streams on a poor nitrifying sludge. The P-1 sludge was used as seed while the four above-mentioned streams were used as the substrate. To evaluate the integrated side-stream bioaugmentation process on nitrification performance. the supplemental nitrifiers were cultured in a side-stream P-3 followed by mixing with activated sludge of P-1 to "reinforce" the nitrifiers populations. For evaluating the application for field implementations, a volume ratio of 94:6 between centrifuge thickened P-1 and P-3 sludge was used. It is noted that during the experiment, P-3 was in the phase of nitrifiers enrichment and acclimatization, and its excess sludge was not diverted to P-1 yet. Therefore, the sampled P-1 sludge was not under the influence of bioaugmentation. Series 3 was designed to assess the effect of nitrifiers bioaugmentation under low pH conditions. The P-1 secondary effluent was used as substrate to avoid the limitation of organic carbon while the use of P-1 sludge with 6% P-3 sludge as seed was evaluated, especially under pH 6.5 and 6.8. Series 4 was also used to evaluate the effect of nitrifiers bioaugmentation with respect to nitrifiers populations. The P-1's returned activated sludge (RAS) supernatant was used as substrate. The P-1 sludge combined with different volume percentages (0%, 6%, and 100%) of P-3 sludge was compared.

2.3. Batch nitrification tests

Field sludge from the RAS lines of the above-mentioned three plants and wastewaters from P-1 and P-2 were sampled and used for the same-day analysis. The sludge samples remained undisturbed for 30 min, following by supernatant decanting and further thickening of residues by centrifugation at 1000 rpm $(78 \times g)$ for 3 min. The pretreatment methodology maximally removed the organics in wastewater while preserving the characteristics of the field sludge. An experimental setup of 4 parallel reactors was used for the batch nitrification tests. The working volume of each reactor was 3.0 L and the operational temperature was approximately 22 °C. Each reactor was started with approximately 0.5 L seed sludge and 2.5 L substrate wastewater. DO and pH probes (Vernier Software & Technology, Oregon, USA) were inserted into all reactors for measurements, and the other ends of the probes were connected to a programmable logic controller module, which was linked to a computer for continuous recording of the readings. To ensure sufficient amount of ammonia-N (NH₃-N), 4.5 mL of ammonium chloride (NH₄Cl, 10 g N/L) solution was added into each reactor to boost the NH₃-N concentration by 15 mg/L at the beginning of the tests. During the tests, the pH was controlled above 7.2 by dosing with 0.5 N HCl or NaOH solutions when needed. All reactors were intensively mixed with a magnetic mixer and aerated to result in a DO of approximately 5.0-5.5 mg/L. Grab samples (50 mL) were taken every 10 min for 1 h from each reactor and filtered through 0.45 µm Millipore membrane filters for the measurements of water quality parameters.

2.4. Analytical methods

The analysis of mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), and NH₃-N were done



Fig. 1. Schematic diagrams of the biological treatment processes of the municipal WWTPs: (a) Harrisburg WWTP; (b) Elizabethtown WWTP; (c) M²T InNitri™ side-stream bioaugmentation WWTP.

Table 1

The experimental design of the batch nitrification tests.

Series	Sets	Seed ^a	Substrate
Series 1	Set 1.1	100% P-2	P-1 primary influent
	Set 1.2	100% P-2	P-1 primary effluent
	Set 1.3	100% P-2	P-1 secondary effluent
	Set 1.4	100% P-2	P-2 primary influent
Series 2	Set 2.1	100% P-1	P-1 primary influent
	Set 2.2	100% P-1	P-1 primary effluent
	Set 2.3	100% P-1	P-1 secondary effluent
	Set 2.4	100% P-1	P-2 primary influent
Series 3	Set 3.1	100% P-1	P-1 secondary effluent @ pH 6.5
	Set 3.2	94% P-1 and 6% P-3	P-1 secondary effluent @ pH 6.5
	Set 3.3	94% P-1 and 6% P-3	P-1 secondary effluent @ pH 6.8
	Set 3.4	94% P-1 and 6% P-3	P-1 secondary effluent
Series 4	Set 4.1	100% P-1	P-1 RAS supernatant
	Set 4.2	94% P-1 and 6% P-3	P-1 RAS supernatant
	Set 4.3	100% P-3	P-1 RAS supernatant
	Set 4.4	100% P-3	P-3 RAS supernatant

^a Seed sludge samples were prepared based on volume percentages after centrifugation.

by using the methodology described in Standard Methods 2540 and 4500-NH₃ D (APHA, 2005), respectively. The HACH test kits TNT 839 and 835 were used to measure the nitrite-N (NO₂-N) and nitrate-N (NO₃-N) concentrations, respectively. In batch nitrification tests, the oxidation of NH₃-N was considered as a zeroorder reaction since the NH₃-N concentration was made sufficient (Liu and Wang, 2012). The nitrification kinetics were calculated based on linear regression of data (Yang et al., 2015) by plotting the NH₃-N concentrations against time, and the slope of each graph corresponded to the nitrification kinetics of each preset test. The NH₃-N removal rate was given as mg N removed per g MLVSS per hour. To express the relative degree of inhibition, the relative % inhibition unit was used, and it could be estimated by comparing evaluated rate with the "non-inhibited" rate from the tests or literature (Schweighofer et al., 1996):

$$\frac{R_{\rm ref} - R_{\rm sample}}{R_{\rm ref}} \times 100 = \% \text{ inhibition}$$

where R_{ref} is the NH₃-N removal rate of the "non-inhibited" reference using seed sludge only, while R_{sample} is the evaluated NH₃-N removal rate of the sample tested (Kim et al., 2006).

3. Results and discussion

3.1. Inhibitory effects of substrate organic carbon and seed sludge

3.1.1. Effect of substrate organic carbon

Batch tests with various P-1 streams were evaluated to explore the effects of substrate organic carbon on nitrification inhibition. As shown in Fig. 2, while the P-2 sludge was used as the sole seed, the resulting % inhibition profile showed an initial high value (25% for the P-1 primary influent), which decreased with sequential degradation of organics afterwards (14% for the P-1 primary effluent and 11% for the P-1 secondary effluent), reflecting that lower substrate organic carbon could benefit nitrification. It was suspected that the P-1 primary influent might contain inhibitory substances to nitrification. As the inhibitory substances were degraded during the treatment processes, the level of inhibition was expected to decrease. The trend of the % inhibition curve against substrate organic carbon concentration was quite characteristic and similar to the one obtained in another series of batch tests using the P-1 sludge as the sole seed at the same substrate settings. The % inhibition profile dropped down to 11% for the P-1 primary influent (the initial high value), where those of the P-1 primary effluent and secondary effluent were decreased to 7% and 1%. respectively. It was deduced that as the available biodegradable organic carbon was depleted from the wastewater streams, the nitrification process would be less inhibited.

3.1.2. Effect of seed

Nitrification characteristics of seed were evaluated by batch tests with the P-1 and P-2 sludge as seeds while the above-mentioned P-1 streams were used as substrate. P-2 was known as a plant with good nitrification and sufficient nitrifiers were expected to be present in the P-2 sludge, while P-1 was a plant with poor nitrification and the nitrifying capability of P-1 sludge remained largely unknown before the experiment. However, for the batch tests carried out with the P-1 sludge, results showed that a significant reduction of the inhibitory effects took place (Fig. 2). Apparently, biological degradation of the inhibitory substances by the P-1 sludge was not only feasible but also at a higher rate due to acclimatization of the sludge to the P-1 streams - possibly with the help of an enzyme system (Schweighofer et al., 1996). Jönsson et al. (2000) also reported variations in nitrification rates for sludge taken from different WWTPs. Suárez-Ojeda et al. (2010) reported that the exposure time between the inhibitory compounds and the enriched AOB sludge was a crucial factor when dealing with inhibition. Since the P-2 sludge took time to be adapted to inhibitory substances present in P-1 streams, the

nitrification rates of P-2 sludge were lower compared to that of P-1 sludge when treating P-1 streams. The results were in agreement with Schweighofer et al. (1996) who reported that the inhibitory effect of a wastewater could not be reduced in a short two hour contact time in the course of pilot investigations.

3.2. Application of nitrifiers bioaugmentation

3.2.1. Effect of pH

Fig. 3 presents the effect of pH on nitrification inhibition and the effect of nitrifiers bioaugmentation under low pH conditions. The batch tests used the P-1 secondary effluent as substrate to minimize the impact of inhibitory substances, while the reference NH₃-N removal rate was based on the P-1 sludge. At pH 6.5, the results showed an NH₃-N removal rate of 0.21 mg-N/g MLVSS-h and a 95% inhibition. With 6% bioaugmented nitrifiers in P-1 sludge, the rate increased to 0.54 mg-N/g MLVSS-h, implying an 86% inhibition. A further increase of pH to 6.8 and 7.2 led to a continuous increase of NH₃-N removal rate to 1.04 and 2.58 mg-N/g MLVSS-h, respectively. It was deduced that pH was a critical inhibitory factor for the poor nitrification at P-1 and the incorporation of P-3 sludge introduced more nitrifiers which increased the nitrification. As the operational data from the P-1 facility management and the sampling data from this research both revealed that the pH of the secondary effluent could be as low as 6.5, the inhibitory effect of pH at the full-scale municipal WWTP was significant. The use of highly pure oxygen instead of air enabled higher DO concentration being maintained in the reaction vessel of P-1, resulting in greater transfer efficiency (Salvetti et al., 2006). However, the process also led to a low pH due to recarbonation with the produced CO₂. The low pH may also be attributed to the consumption of alkalinity caused by the trucked-in load. It can be concluded that the bioaugmentation of nitrifiers were beneficial in improving the nitrification performance, even under low pH conditions not conducive to nitrification.

3.2.2. Effect of nitrifiers populations

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Fig. 4 illustrates the effect of nitrifiers populations on improving nitrification efficiency. Batch tests with the P-1 sludge in its supernatant were used to minimize potential inhibitory effects. With zero addition of the bioaugmented nitrifiers, the NH₃-N removal rate was 4.00 mg-N/g MLVSS-h. The results were in agreement with Kim et al. (2006) who reported a NH₃ removal rate of 2–6.5 mg NH₃/g MLSS-h under a steady-state, non-inhibitory condition. The batch test evaluated the full-scale upgrading implementation by including 6% of the bioaugmented nitrifiers in the

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Fig. 2. Effect of substrate organic carbon and seed sludge on nitrification inhibition.

Primary

Effluent

Secondary

Effluent

Primary

Influent

Fig. 3. Effect of pH on nitrification (P-1 secondary effluent as substrate and P-1 sludge as seed).



Fig. 4. Effect of nitrifiers bioaugmentation on nitrification (P-1 RAS supernatant as substrate and P-1 sludge as seed).

activated sludge of P-1, and the results implied the NH₃-N removal rate increased to 6.13 mg-N/g MLVSS-h. With 100% bioaugmented nitrifiers, the rate further increased to 9.29 mg-N/g MLVSS-h. Therefore, the bioaugmentation option chosen to upgrade the existing facilities was found to be effective. It has a potential to improve nitrification efficiency without decreasing the daily wasted sludge (increasing the apparent SRT) (Head and Oleszkiewicz, 2004). In addition, feeding the bioaugmentation reactor with extra NH₃-N would lead to high nitrification potential due to sludge acclimatization to high nitrogen load (Salem et al., 2003). The successful bioaugmentation strategies can be beneficial to achieve better nitrification.

Table 2

The overall characteristics of the batch nitrification tests

3.3. Implications

3.3.1. Troubleshooting of P-1

There were debates over the causes of nitrification inhibition issues experienced at P-1. As P-1 periodically accepted pump-truck loads from other sources, it was regarded as a possible cause of inhibition. However, the experiment revealed that the impact of pump-truck loads was not necessarily causal because the P-1 primary influents under the influence of pump-truck loads were able to nitrify by both P-1 and P-2 sludge at high rates. Instead, pH was found to be an impact factor. The experiment reported a significant decrease of nitrification rate for the P-1 sludge (0.21 mg-N/ g MLVSS-h) at pH 6.5, while the low pH value was frequently observed by the facility management during the plant operation. The experiment also revealed recovered nitrifying capability of P-1 sludge (4.0 mg-N/g MLVSS-h) with pH raised (Table 2, Set 4.1), indicating that the nitrification inhibition issue could be solved by raising the pH. For this plant, because of the special design of the process with undersized reactors, there were additional considerations. The plant was operated with an SRT of less than 4 days and an MLSS of approximately 1700 mg/L, because the undersized clarifier would have settling problems if higher MLSS and longer SRT were maintained. These operational data led to practical concerns on the growth of nitrifiers. Although the experiment found the P-1 sludge was able to nitrify, indicating the presence of nitrifiers under the scheme of plant operation at an F/M of 0.18, it would be beneficial to have another secure and inexpensive approach for plant upgrading.

Further discussions on other potential causes during the plant operation excluded organic carbon, ammonification, and free ammonia as inhibitory factors: (1) Although the instability of

Series	Sets	pH of substrate	pH during the tests	Temperature [°C]	MLSS [mg/L]	MLVSS [mg/L]	Initial NH ₃ -N [mg/L]	Initial NO _x -N [mg/L]	Final NH3-N [mg/L]	Final NO _x -N [mg/L]	NH3-N removal rate [mg-N/g MLVSS-h]
Series 1	Set	7.2	7.7	23.1	2900	1890	23.0	1.3	19.3	4.2	1.8
	Set	7.0	7.6	23.2	3835	2445	25.3	1.0	20.1	6.1	2.0
	Set	6.9	7.7	23.1	4030	2650	19.2	7.0	13.9	12.3	2.1
	Set 1.4	6.9	7.6	23.1	4225	2860	37.8	1.8	30.6	7.4	2.3
Series 2	Set	7.1	7.6	23.2	2265	1245	22.7	3.8	16.1	9.7	5.1
	Set	7.1	7.5	23.2	2695	1470	23.9	2.3	16.1	9.8	5.4
	Set	7.0	7.5	23.2	2525	1425	20.5	5.8	11.9	13.3	5.8
	2.5 Set 2.4	7.1	7.7	23.5	2675	1645	37.2	2.8	27	10.7	6.0
Series 3	Set	6.5	6.5	21.4	2830	1720	20.5	2.9	19.8	2.3	0.21
	Set	6.5	6.5	22.2	2865	1820	21.1	3.4	19.8	4.7	0.54
	Set	6.5	6.8	22.2	2850	1830	20.5	3.5	18.6	5	0.88
	Set 3.4	6.5	7.2	22.3	2980	1870	21.7	3.1	15.6	9.9	2.6
Series 4	Set 4 1	6.4	7.2	19.8	3755	2615	18.4	4.1	7.9	11.8	4.0
	Set	6.8	7.9	20.9	2080	1660	20.2	19.4	10.8	31	6.1
	Set	7.1	7.6	20.3	123	99	18.4	11.8	17.5	12.5	9.3
	Set 4.4	7.5	7.7	20.3	164	123	18.1	219	15.6	222.2	19.7

nitrification process could be attributed to washing out of nitrifiers by fast growth of competitive heterotrophic microorganisms under increased concentrations of inhibitory organics during plant operation (Kim et al., 2007), the heterotrophic respiration and elimination of organic carbon were not affected in case of nitrification inhibition (Pagga et al., 2006) and the plant had no observed issues with the removal of organic carbon according to the facility management. (2) It was true that extra ammonia could be released through microbial break-down of organic nitrogen and might impact nitrification. However, ammonification would not be rate limiting for municipal wastewater due to lower hydrolysis kinetics (Katipoglu-Yazan et al., 2012). (3) It was true that nitrification might be incomplete or even ceased if free ammonia level is high, because the NOB are more sensitive to free ammonia in a range of 0.1–1.0 mg/L while AOB are inhibited by free ammonia in a range of 10–150 mg/L (Kim et al., 2008; Li et al., 2011). Calculation of free ammonia concentrations in the P-1 scenario led to values well below the sensitive levels, and hence free ammonia could not imply an inhibitory effect either

[Free NH₃] =
$$\frac{[\text{Total NH}_3] \times 10^{\text{pH}}}{e^{[6334/(273+T)]} + 10^{\text{pH}}}$$
 (Li et al., 2011)

3.3.2. Advantages and disadvantages of nitrifiers bioaugmentation

Bioaugmentation, as a process of adding selected strains or mixed cultures to wastewater treatment reactors, is a promising approach to inexpensively solve practical problems of P-1. Increasing the nitrification in mainstream aerobic reactors can be obtained by two types of bioaugmentation schemes: (1) in situ augmentation, which provides internal process enhancements that add nitrification capability in the aerobic compartment by addition of immobilized nitrifiers; and (2) external bioaugmentation, which augments the sludge with external nitrifiers grown in a separate reactor. The research showed that the external nitrifiers bioaugmentation scheme had a potential in increasing the nitrification in the mainstream aerobic reactor. The advantages of the bioaugmentation are obvious: it cultivates the endogenous population rather than adding potential non-representative type of nitrifiers (Salem et al., 2003); further, it stabilizes biological processes that would otherwise be unsustainable at the SRT allowed by the available reactor volume; and finally, a system with a short SRT allows simple construction and low investment and operational costs. The disadvantageous effects were largely reported being associated with bioaugmentation failures caused by the lack of acclimation for microorganisms to survive under harsh environmental conditions (Herrero and Stuckey, 2014). This research, however, adds to the successful cases of external nitrifiers bioaugmentation for main-stream systems operated under low pH conditions not conducive to nitrification.

4. Conclusions

The research led to conclusions that the substrate organic carbon and pH of wastewater were inhibitory factors to nitrification and the low pH was the cause of the full-scale municipal WWTP experiencing poor nitrification. An integrated side-stream nitrifiers bioaugmentation process was found beneficial in reducing the inhibition, even under low pH conditions not conducive to nitrification. The research successfully presents a promising approach to solve practical nitrification problems when the existing facilities become insufficient to meet the demands of the increasingly strict regulations.

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