ASSESSMENT OF BIOLOGICAL NITROGEN REMOVAL (BNR) IN POULTRY PROCESSING FACILITIES

BNR SYSTEM OPTIMIZATION FOR RESILIENCE AND EFFICIENCY

Spyros G. Pavlostathis and Malek G. Hajaya

School of Civil and Environmental Engineering
Georgia Institute of Technology
Atlanta, GA 30332-0512

Final Project Report – Project # 660

Date of Project Completion: April 30, 2011

Funded by U. S. Poultry & Egg Association

U.S. Poultry & Egg ASSOCIATION
"The All Feather Association"

Georgia Institute of Technology

Table of Contents

SUMMARY

Sufficient wastewater (pre)treatment capacity is needed at poultry processing facilities to keep up with the increased broiler production. Controlling effluent quality (e.g., biochemical oxygen demand, suspended solids, ammonia and phosphorus) is mandatory. Although the combination of nitrification and denitrification leads to efficient nitrogen removal, not well understood factors periodically lead to system upset and incomplete nitrogen removal. Ammonia is a poultry processing wastewater component that requires a high degree of removal before the final disposal of the treated wastewater. As discharge effluent limits are being established for total nitrogen, not just ammonia, nitrogen removal will become an even more pressing issue for poultry processing facilities, especially those with direct effluent discharge. The overall objective of this research project was to systematically assess the effect of conditions/parameters which can affect the efficiency of biological nitrogen removal (BNR) in poultry processing facilities by conducting targeted sampling in such facilities as well as long-term bench-scale testing.

Nitrification and denitrification tests conducted with mixed liquor samples collected at a poultry processing wastewater treatment plant in the Southeastern US during warm (Fall) and cold (Winter) seasons confirmed previous reports that low temperature conditions are more detrimental to nitrification than to denitrification. A laboratory-scale, multi-stage BNR system maintained at room temperature (22-24^{o}C) was continuously fed with poultry processing wastewater amended with a mixture of three benzalkonium chlorides (BAC), a class of quaternary ammonium compounds (QACs). The nitrogen removal efficiency initially deteriorated at a BAC feed concentration of 5 mg/L due to severe inhibition of nitrification in the unacclimated system. However, the system recovered after 27 days of operation achieving high nitrogen removal efficiency, even after the feed BAC concentration was stepwise increased up to120 mg/L. The same high nitrogen removal efficiency was retained when the system was operated at 10^{o}C with BAC-amended poultry processing wastewater. Microbial acclimation to and degradation of BAC was responsible for the successful operation of the BNR system with the BAC-amended poultry processing wastewater. Batch assays performed before, during, and post BAC exposure showed that the development of BAC biotransformation capacity and the acquisition of resistance to BAC, especially by the nitrifiers, contributed to the recovery of nitrification and led to a high nitrogen removal efficiency.

Simulations using a comprehensive mathematical BNR model developed for this research accurately described the fate and effect of BAC in the BNR system when the interactions between adsorption, inhibition, and resistance/biotransformation were considered within the conditions prevailing in each reactor of the BNR system. Adsorption determines the level of the inhibitory effect of BAC, while BAC biotransformation and resistance determine the extent of exposure of the microbial communities to BAC. Finally, the inhibitory effect of BAC is reduced, if not completely removed, by the development of BAC resistance and biotransformation capacity.

Overall, the results of this study will enable the rational design and operation of BNR systems for the efficient treatment of QAC-bearing wastewater. The outcome of this research provides information presently lacking, supporting the continuous use of QACs as antimicrobial agents in poultry processing facilities, when and where needed, while avoiding any negative impacts on biological treatment systems and the environment. Given the benefits of using QACs as effective sanitation chemicals in poultry processing facilities, the effectiveness of biological processes for the degradation of QACs in order to avoid process upsets, especially for the nitrification step, should be further evaluated using alternative process configurations (e.g., sequential batch reactors, fixed-film reactors) in order to capture process variability across the entire poultry processing industry.

1.0 INTRODUCTION

1.1 Problem Statement

Poultry processing is characterized by high water usage and the generation of high strength wastewater. Recent increases in volume, complexity and strength of poultry processing wastewater have resulted in the need to achieve higher treatment levels and more efficient removal of by-products in order to meet environmental regulatory limits for effluent discharges. In most plants, after the recovery of secondary poultry nutrients (SPN) by dissolved air flotation (DAF), the resulting wastewater is typically treated in a series of biological units ranging from one-step lagoon to multi-step biological units (Kiepper, 2003). Nutrient discharges (N and P) in poultry processing wastewater at a number of poultry plants were estimated in a previous study (Merka et al., 2001). Ammonia is a poultry processing wastewater component that requires a high degree of removal before the final disposal of the treated wastewater. As discharge effluent limits are being established for total nitrogen, not just ammonia, nitrogen removal will become an even more pressing issue for poultry processing facilities, especially those with direct effluent discharge.

The combination of nitrification and denitrification leads to efficient biological nitrogen removal (BNR) and has been practiced for many years, especially in municipal wastewater treatment plants. In the case of poultry processing plants however, not well understood factors periodically lead to system upset and incomplete nitrogen removal. It is not clear whether these factors are environmental conditions (e.g., low temperature), operational parameters (e.g., high organic loadings, low residence time), or chemical constituents used in the poultry processing plants (e.g., disinfectants), such as quaternary ammonium compounds (QACs). In the case of incomplete nitrogen removal, in addition to the low performance efficiency, a more serious consideration is the production of nitrous oxide (N_2O) as opposed to nitrogen gas (N_2). Nitrous oxide is a green-house gas considered to be more deleterious than carbon dioxide as it has 298 times more impact per unit weight than carbon dioxide. Thus, efficient and complete removal of nitrogen as N_2 is a must in BNR systems.

The fate and effect of QACs on BNR processes utilized in high-strength wastewater treatment systems (nitrification, denitrification, fermentation, and methanogenesis) have been studied (Shcherbakova et al., 1999; Tezel et al., 2006; Kreuzinger et al., 2007; Sutterlin et al., 2007; Yang, 2007; Pavlostathis et al., 2008; Tezel et al., 2008). However, all previous studies were conducted on individual biological processes within the confinement of a single environmental condition (anaerobic, anoxic, or aerobic) and did not assess the effect of QACs on multiple reactions under alternating environmental conditions (e.g., sequence of nitrification/denitrification). The latter is typical in engineered BNR systems in which multiple environmental conditions are specifically created to sustain different microbial groups which mediate the continuous treatment process. Moreover, different physiological groups participate in the biological nitrogen removal from poultry processing wastewater, and the response of each species to QAC inhibition is expected to be different in terms of both degree and extent.

The fate and effect of QACs in a continuous-flow, multi-stage BNR system can be divided into three main sub-processes: adsorption, inhibition, and biotransformation. The contribution of each sub-process will differ depending on the prevailing environmental conditions. Having a clear understanding of the degree and extent chemical and physical interactions of QACs is of vital importance when faced with the challenge of maintaining an adequate treatment capacity while handling QACs-bearing poultry processing wastewater. This information is currently lacking, which became the impetus of the present research.

1.2 Project Objectives

The overall objective of this research project was to systematically assess the effect of conditions/parameters which can affect the efficiency of biological nitrogen removal in poultry processing facilities by conducting targeted sampling in such facilities as well as long-term bench-scale testing.

The specific objectives/tasks of the project were:

1) Field activities: Visit representative poultry processing facilities, conduct targeting sampling, characterize wastewater, as well as obtain data related to wastewater treatment processes.

2) Laboratory activities: Evaluate the biological nitrogen removal through sequential nitrification/denitrification with respect to: a) unit process configuration; b) residence time; c) organic loading; d) temperature; and e) quaternary ammonium compounds (QACs).

3) Guidance and Recommendations: Combine field observations and the results of the bench-scale testing to document possible reoccurring problems relative to biological nitrogen removal processes (i.e., nitrification/denitrification) and provide solutions towards maintaining system resilience and a high nitrogen removal capacity in poultry processing facilities.

Although field samplings were performed in both warm and cold seasons, long-term sampling in poultry processing facilities as well as additional bench-scale testing is necessary before a comprehensive methodology in the form of a guidance document can be developed towards avoiding serious system upsets, increasing system resilience and consistently achieving high nitrogen removal efficiency. Detailed results and discussion related to the above-stated first two objectives are included in this report.

2.0 MATERIALS & METHODS

2.1 Field Sample Collection and Characterization

In continuation of the field activities conducted for a previously funded US Poultry & Egg Association project (Project #650; Pavlostathis et al., 2009), field activities focused on a single poultry processing facility (Plant B). After dissolved air flotation (DAF), this facility uses a combination of three reactors in series (anaerobic, aerobic, anoxic/microaerophilic) and a clarifier; sludge is recycled from the clarifier to the aerobic reactor, while mixed liquor from the anoxic/microaerophilic reactor is recycled back to the anaerobic reactor. Two sampling campaigns were made, one in the Fall (November 13, 2009) and one in Winter (January 15, 2010). The first visit was during a period of warm weather (ambient, air temperature above 15^{o}C) in which the temperature of the collected samples ranged between 22 and 24^{o}C. The second visit was conducted after cold weather persisted for one week (ambient, air temperature below 5^{o}C) and the temperature of the collected samples ranged between 11 and 15^{o}C. The samples were collected and immediately transported to the laboratory where they were analyzed for the following parameters: pH, total and volatile suspended solids (TSS and VSS), total and soluble chemical oxygen demand (COD), volatile fatty acids (VFAs), total Kjeldahl nitrogen (TKN), ammonia, nitrite, nitrate, and quaternary ammonium compounds (QACs). In addition to

sample characterization, batch nitrification and denitrification tests were performed in the laboratory to quantify the rates of both processes achieved in poultry processing plant B.

2.1.1 Batch Nitrification Tests

Batch nitrification tests were conducted with mixed liquor obtained from the aerobic reactor of poultry processing plant B. The objective of these tests was to assess the nitrification efficiency of mixed liquor samples collected at the poultry processing plant without any further processing of these samples in the laboratory. Aliquots of 1.5 L of mixed liquor samples were placed in 2-L glass reactors, maintained at room temperature (22 to 24^{o}C), and mixed with magnetic stirrers. Pre-humidified compressed air passed through a water tap was supplied through fine pore diffusers in order to maintain the dissolved oxygen (DO) concentration at or above 6 mg/L. The nitrification rate was measured in the absence of QACs at a single initial ammonia concentration (ca. 100 mg ammonia-N/L) using a stock NH_4Cl solution. The pH was monitored and periodically adjusted to around 7 using a 1 N $NaHCO_3$ solution. In addition, the following parameters were monitored throughout the test: DO, ammonia, nitrite, and nitrate, TSS, and VSS.

Nitrification was modeled as a two-step process, ammonia oxidation to nitrite followed by nitrite oxidation to nitrate. Assuming that the nitrogen species were the only limiting substrates, no inhibition, and a constant biomass concentration during the test period, the nitrification process was modeled using the following three differential equations:

$$\frac{dS_{nh3}}{dt} = -\left(\frac{k_{nh3}S_{nh3}}{K_{snh3}+S_{nh3}}\right)X \qquad \text{(Equation 1)}$$

$$\frac{dS_{no2}}{dt} = \left[\left(\frac{k_{nh3}S_{nh3}}{K_{snh3}+S_{nh3}}\right)-\left(\frac{k_{no2}S_{no2}}{K_{sno2}+S_{no2}}\right)\right]X \qquad \text{(Equation 2)}$$

$$\frac{dS_{no3}}{dt} = \left(\frac{k_{no2}S_{no2}}{K_{sno2}+S_{no2}}\right)X \qquad \text{(Equation 3)}$$

where S_{nh3}, S_{no2}, and S_{no3} are ammonia, nitrite, and nitrate concentration mg N/L, respectively; t is time in days; k is maximum specific utilization rate (MSUR) mg N/g VSS·day; K_s is the half saturation constant mg N/L; and X is the concentration of active autotrophic biomass g VSS/L. Half saturation constants were chosen based on literature values (Wett and Rauch, 2003; Magri and Flotats, 2008) and calibrated to the experimental data as 0.056, and 0.255 (mg N/L) for ammonia and nitrite oxidation, respectively. Ammonia, nitrite, and nitrate concentration data were fitted using Berkeley- Madonna Software Version 8.3 (Macey and Oster, 2006) to equations 1, 2, and 3, above. MSUR for ammonia and nitrite were estimated using the same software by minimizing the deviation between the model output and the experimental data. For the simulation, 4th order Runge-Kutta with a step size of 0.01 day was used. The root mean square deviation (RMSD) was used as a measure of the goodness of fit for each simulation.

2.1.2 Batch Denitrification Tests

Batch denitrification tests were conducted with mixed liquor obtained from the anoxic reactor of plant B. The objective of these tests was to assess the denitrification efficiency of mixed liquor samples collected at the poultry processing plant without any further processing of these samples

in the laboratory. Aliquots of 1 L of mixed liquor samples were placed in 2-L glass reactors, maintained at room temperature (22 to 24°C), and mixed with magnetic stirrers. In order to assess the maximum nitrate removal rates without any electron donor limitations, aliquots of 0.5 L of DAF underflow poultry processing wastewater were added to serve as the carbon and electron donor and the reactors were then sealed and flushed with helium gas. The nitrate removal rate was measured in the absence of QACs at a single initial nitrate concentration (between 65 and 100 mg nitrate-N/L) using a $NaNO_3$ stock solution. The following parameters were monitored: pH, soluble COD, TSS, VSS, ammonia, nitrite, and nitrate.

Denitrification was modeled as a two-step process, nitrate reduction to nitrite followed by nitrite reduction to dinitrogen (N_2). Assuming that the nitrogen species were the only limiting substrates, no inhibition, and a constant biomass concentration during the test period, the denitrification process was modeled using the following two differential equations:

$$\frac{dS_{no3}}{dt} = -\left(\frac{k_{no3}S_{no3}}{K_{sno3}+S_{no3}}\right)X \qquad \text{(Equation 4)}$$

$$\frac{dS_{no2}}{dt} = \left[\left(\frac{k_{no3}S_{no3}}{K_{sno3}+S_{no3}}\right)-\left(\frac{k_{no2}S_{no2}}{K_{sno2}+S_{no2}}\right)\right]X \qquad \text{(Equation 5)}$$

where S_{no3} and S_{no2} are nitrate and nitrite concentration mg N/L, respectively; t is time in days; k is maximum specific reduction rate (MSRR) mg N/g VSS·day; K_s is the half saturation constant mg N/L; and X is the concentration of active denitrifying biomass g VSS/L. Half saturation constants were chosen based on literature values (Tugtas et al., 2006) and calibrated to the experimental data as 4.5 and 10 (mg N/L) for nitrate and nitrite, respectively. Nitrate and nitrite concentration data were fitted using Berkeley- Madonna Software Version 8.3 (Macey and Oster, 2006) to equations 4 and 5, above. MSRR for nitrate and nitrite were estimated using the same software by minimizing the deviation between the model output and the experimental data. For the simulation, 4th order Runge-Kutta with a step size of 0.01 day was used. The root mean square deviation (RMSD) was used as a measure of the goodness of fit for each simulation.

2.2 Continuous-flow Biological Nitrogen Removal (BNR) System

This part of the study investigated the performance of a laboratory-scale, multi-stage BNR system treating a poultry processing wastewater without and with quaternary ammonium compounds (QACs).

2.2.1 BNR System Description

The laboratory-scale, multi-stage BNR system consisted of an anaerobic reactor (R1), used to ferment the influent wastewater and convert it to VFAs, an anoxic reactor (R2) for the purpose of denitrification, and an aerobic reactor (R3) for nitrification, with a sludge settler and internal sludge recycle as well as mixed liquor recycle from R3 to R2 and back to R3 (Figure 1). R1 and R2 were 4-L sealed glass bottles mixed with magnetic stirrers. Gas produced in R2 was collected in a graduated burette filled with an acid brine solution connected to a reservoir. R3 and its internal settler were made from Plexiglas with working volume of 5 and 1.5 L, respectively. Mixing was achieved by an overhead, variable speed mechanical mixer. Aeration of R3 was achieved with pre-humidified compressed air passed through a flow meter with a flow rate between 0.5-1.0 standard cubic feet per minute and fine pore stones. The feed reservoir was a 5-

L glass bottle which was housed in a refrigerator at 4°C and its contents were continuously mixed with a magnetic stirrer. Feeding and wasting of R1, recycle from R3 to R2 and back to R3 was achieved with peristaltic pumps controlled by an electronic timer. The clarified effluent was removed by gravity. Waste biomass was manually removed directly from the aerobic reactor, daily, after the settler baffle was lifted and the mixed liquor in the reactor and the bottom of the settler was allowed to mix for 30 minutes.

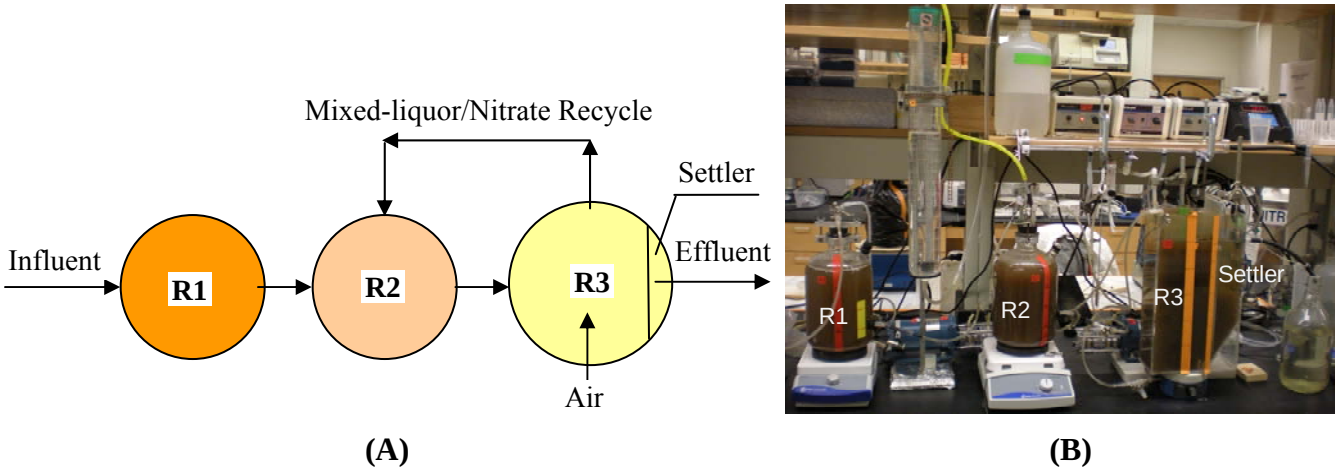


Figure 1. Bioreactor system. (A) Schematic representation; (B) Photograph (R1, anaerobic reactor; R2, anoxic reactor; R3, aerobic reactor).

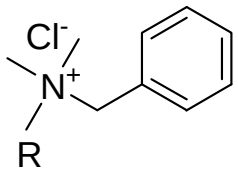
2.2.2 BNR System Operation – Phase 1

During Phase 1, the BNR system was operated at room temperature (22 to 23°C) with a poultry processing wastewater without quaternary ammonium compounds (QACs). The hydraulic and solids residence times (θ and θ_c, respectively) and the R2/R3 recycle ratio were determined based on theoretical calculations for one-sludge system to accomplish total nitrogen removal, i.e., total nitrification and denitrification (Rittmann and McCarty, 2001; Hajaya, 2011). The chosen values (θ/θ_c in days) were: 2/2, 2/10, and 2.5/15 for R1, R2, and R3, respectively. The seed used for the three reactors was mixed liquor samples obtained from two poultry processing wastewater treatment plants. The influent wastewater flow rate (Q) was 2 L/day and the recycle ratio (r = recycle flow rate/feed flow rate) between the anoxic and aerobic reactors (R2/R3) was initially chosen as 4Q. The influent wastewater for the laboratory-scale BNR system was DAF underflow collected periodically from a poultry processing facility (plant B) which was stored in 5-gallon plastic containers at 4°C.

Preliminary batch assays were conducted with mixed liquor samples collected from the aerobic and anoxic reactors in order to assess the effect of benzalkonium chlorides (BAC), a class of quaternary ammonium compounds (QACs), on nitrification and denitrification, respectively, before the BNR system was fed with a BAC mixture (Phase 2, section 2.2.3, below). These batch nitrification and denitrification assays were conducted following procedures described in sections 2.2.3.2 and 2.2.3.3, below, respectively. BAC was selected for this study due to its extensive use among all QACs. The general formula of BAC is $C_{n+9}H_{2n+14}NCl$, where n refers to the number of carbon in the alkyl chain (12, 14, or 16). Figure 2 shows the structure of the BAC homologs. The commercial sanitizer Barquat MB-80™, which was used in this study, is a mixture of BAC with C_{12}, C_{14}, and C_{16} alkyl group lengths, and contains ethanol and water

(10% each, w/w). Stock BAC solutions of 5,000 mg/L of active ingredient (as the C_{16} equivalent) were prepared in deionized (DI) water and further diluted as needed in the various parts of this research.

Figure 2. Structure of alkyl benzyl dimethyl ammonium chloride (R = C_{12}, C_{14}, or C_{16}).



After the performance of the BNR system with the above-described conditions was evaluated (QACs-free, baseline performance), the system was operated with the following changes to further evaluate its efficiency. Recycle ratio: Its value was changed from 4 to 6, then to 2 and back to 4. The change in the recycle ratio was made by adjusting the pumping time in the electronic timer for the dual-head recycle pump. Organic and nitrogen loading: Concentrated poultry processing wastewater was obtained from Plant B resulting from gravity dewatering of DAF skimmings collected in a holding tank. The concentrated wastewater had the following composition: total COD 24.3±0.6 g/L, soluble COD 22.5±0.5 g/L, and ammonia 924±19 mg N/L. The concentrated wastewater was diluted 11-fold with tap water to yield an ammonia concentration of 84 mg N/L, which was about double the DAF underflow ammonia concentration (46 ± 3 mg N/L). Modified configuration: In order to assess the system's performance without a pre-fermentation step, the anaerobic reactor was removed from the BNR system by connecting the feed line directly to the anoxic reactor.

2.2.3 BNR System Operation – Phase 2

During Phase 2, the BNR system was operated at room temperature (22 to 23°C), fed with QACs-amended poultry processing wastewater. The hydraulic and solids residence times were as those in Phase 1 and the R2/R3 recycle ratio was 4Q. The system was initially operated with QAC-free wastewater. Then, in order to test the effect of QACs on the BNR performance, the feed wastewater was amended with a stock mixture of benzalkonium chlorides (BAC; alkyl benzyl dimethyl ammonium chlorides) step-wise to arrive at target influent BAC concentrations 5 to 120 mg/L.

The fate and effect of BAC in a continuous-flow BNR system can only be understood by following three processes, adsorption, biotransformation, and inhibition, throughout the entire system. Therefore, the objective of the research reported in this section was to investigate the performance of a continuous-flow BNR system while treating BAC-bearing wastewater. This objective was attained through evaluation of the interactions between BAC adsorption, inhibition, and biotransformation in the four BNR system components (feed wastewater, and the anaerobic, anoxic, and aerobic reactors) by conducting a series of batch assays as described below.

2.2.3.1 Batch Anaerobic Assay

A batch assay utilizing the mixed liquor of the anaerobic reactor was performed to examine the fate and effect of the BAC mixture on feed hydrolysis, volatile fatty acids (VFAs) production, and ammonia release from the feed organic nitrogen (ammonification). The assay was conducted in 160-mL serum bottles (100 mL liquid volume) sealed with rubber stoppers and aluminum crimps, and flushed with helium gas for 15 min before any liquid addition. An aliquot of 45 mL of the anaerobic reactor mixed liquor was introduced into each bottle, followed by 50 mL of poultry processing DAF underflow wastewater. Then, 5 mL of BAC stock solution were

introduced to reach a final BAC concentration of 5, 15, 30, 45, and 60 mg/L in the BAC-amended culture series. Two more culture series were prepared: seed blank and reference, which consisted of seed and DI water, and seed, poultry processing wastewater, and DI water, respectively. The initial soluble COD (sCOD) and VFAs concentration was 388 ± 55 and 174 ± 36 mg COD/L, respectively. Each culture series, including the seed blank and the reference, was prepared in duplicate. Throughout the incubation period, the bottle contents were mixed using an orbital shaker. sCOD, VFAs, pH, and ammonia concentrations were measured throughout the incubation period. At the end of the incubation, pH, ammonia, sCOD, VFAs, TSS/VSS, as well as total and liquid-phase BAC concentrations were measured.

2.2.3.2 Batch Nitrification Assays

Two batch nitrification assays were performed to investigate the effect of the BAC mixture on the nitrifying activity of the aerobic reactor (R_3) mixed liquor during and after the BNR system operation with BAC-bearing poultry processing wastewater, respectively. In these assays, BAC-amended and BAC-free culture series were prepared in 250-mL Erlenmeyer flasks (200 mL liquid volume). Aliquots of 100 mL of R_3 mixed liquor (collected during the daily wasting time) were introduced to each flask, followed by 100 mL of BAC-amended poultry processing wastewater to arrive at target initial BAC concentrations. The poultry processing wastewater was the only source of ammonia and organic carbon in order to simulate the operational conditions of the aerobic reactor. The assay was conducted at room temperature (22 to 23^{o}C) and the cultures were aerated with compressed, pre-humidified air, and continuous mixing was provided by an orbital shaker. sCOD, ammonia, nitrite, and nitrate were monitored throughout the incubation period. The pH was monitored throughout the assay, and manually adjusted to between 6.5 and 7.5 by the addition of $NaHCO_3$. Total and liquid-phase BAC concentrations were measured to evaluate BAC phase distribution and degradation. The initial specific ammonia removal rate (SARR) and initial specific sCOD utilization rate (SCUR) were calculated by performing a linear regression of the initial time course ammonia and sCOD concentrations data, respectively, and normalizing the resulting rates to the mean value of the initial and final VSS concentrations of each culture series.

2.2.3.3 Batch Denitrification Assay

A batch assay was performed to evaluate the fate and effect of BAC on denitrification in the anoxic reactor (R_2). The assay was conducted in 160-mL serum bottles (100 mL liquid volume) sealed with rubber stoppers and aluminum crimps, flushed with helium gas for 15 min before any liquid addition. The carbon source for this assay was the effluent of a BAC-unexposed anaerobic reactor with a sCOD and VFAs concentrations of 745 ± 134 and 297 ± 38 mg COD/L, respectively. The assay included seven culture series amended with the BAC mixture solution resulting in initial total BAC concentrations of 5, 10, 15, 20, 25, 30, and 45 mg/L. Two additional BAC-free culture series were prepared: seed blank and reference, which consisted of seed, treated poultry processing wastewater, and DI water, and seed, treated poultry processing wastewater, DI water, and nitrate, respectively. Aliquots of 100 mL of R_2 mixed liquor (collected during the daily wasting time) were introduced to each bottle, followed by 50 mL of treated poultry processing wastewater. Nitrate was then added (35 mg N/L) followed by the BAC mixture, and the total liquid volume was adjusted to 100 mL with DI water. Each culture series, including the seed blank and the reference, was prepared in duplicate bottles, one used for liquid analyses and the other for gas analyses. All culture series were incubated in the dark at 22^{o}C and

the bottles were agitated daily by hand. Throughout the incubation period, the headspace pressure and the nitric oxide, nitrous oxide, dinitrogen, and carbon dioxide content were measured. Nitrate and nitrite measurements were carried out by removing liquid samples from the bottles at the same time intervals with the gas measurements. At the end of the incubation period, nitrate, nitrite, pH, ammonia, sCOD, TSS and VSS, as well as total and liquid-phase BAC concentrations were measured. The initial specific nitrate removal rate (SNRR) was calculated by performing a linear regression of the initial ammonia concentration data, and normalizing the resulting rate to the mean value of the initial and final VSS concentrations of each culture series.

2.2.3.4 BAC Biotransformation Assay

A batch assay was performed to investigate the biotransformation of BAC using mixed liquor of the aerobic reactor (R_3) during the BNR system operation with BAC-bearing poultry processing wastewater. The assay used the BAC mixture at an initial BAC concentration of 12 μM (5.6 mg/L) and was conducted using 250-mL Erlenmeyer flasks (200 mL liquid volume). An aliquot of 150 mL R_3 mixed liquor was collected during the daily wasting time, introduced into the flask, and then aerated for 24 hours to remove any residual BAC and possible metabolites. Then, BAC was introduced into the flask at the aforementioned initial concentration. The extraction and HPLC analysis mentioned in section 2.5, below, were used to follow the concentration of BAC and four possible BAC metabolites: benzyl trimethyl amine (BTMA), benzyl dimethyl amine (BDMA), benzyl methyl amine (BMA), and benzyl amine (BA).

2.2.3.5 BAC Adsorption Assay

The equilibrium adsorption behavior of BAC in the poultry processing wastewater, anaerobic, anoxic, and aerobic reactors mixed liquors was tested by 24-hour equilibration assays. The adsorption assays were performed at a BAC concentration range between 5 and 60 mg/L and a fixed initial solids concentration. Triplicate series were prepared in 250-mL Erlenmeyer flasks with azide-amended wastewater and mixed liquor aliquots (1 g NaN_3/L), amended with the BAC mixture at initial concentrations of 5, 10, 15, 30, 45, 60, 75 and 60 mg/L. The flasks were sealed with stoppers and agitated with an orbital shaker for 24 h at 22°C. The phase distribution of BAC was determined at the end of each batch adsorption assay by quantifying both the total and liquid-phase BAC concentration, and then the BAC mass adsorbed on the solids was calculated by difference.

The Freundlich isotherm was used to describe the BAC adsorption equilibrium data. The Freundlich model, which was originally developed as an empirical expression that accounts for surface heterogeneity and exponential distribution of sites and their energies, is an appropriate model when more than one sorption mechanism apply (Ismail et al., 2010), which is the case with BAC (Ren et al., 2011). The Freundlich isotherm equation is as follows:

$$q_e = K_F C_e^n \qquad \text{(Equation 6)}$$

where q_e is BAC concentration on the biomass at equilibrium (mg/g VSS); C_e is BAC concentration in the liquid-phase at equilibrium (mg/L); K_F is the adsorption capacity factor ((mg/g VSS)(L/mg)^n); and n is the Freundlich intensity parameter. The BAC concentration data were fitted to the Freundlich isotherm equation and both adsorption parameter values (K_F and n)

were estimated by non-linear regression analysis performed using SigmaPlot, Version 10 software (Systat Software Inc., San Jose, CA, USA).

2.2.4 BNR System Operation – Phase 3

At the completion of Phase 2, the entire BNR system was moved to a controlled temperature room and operated at 22°C with a QAC-free poultry processing wastewater. The room temperature was then step-wise dropped to 10°C within one week to simulate operation in cold season. The system was operated at 10°C with QAC-free poultry processing wastewater for an additional period of 50 days. Then, in order to assess the effect of low temperature on BNR performance while treating BAC-bearing poultry processing wastewater, BAC was introduced into the feed while the entire BNR system was maintained at 10°C. The poultry processing wastewater feed BAC concentration was initially 5 mg/L and then step-wise increased to 15, 45, and 120 mg/L. The hydraulic and solids residence times were as those in Phase 1 and 2, and the R2/R3 recycle ratio was 4Q.

2.2.5 Nitrogen Removal Calculations

The system's performance with regards to nitrogen removal (denitrification in R2 and nitrification in R3) can be defined based on the mass of nitrogen found in each reactor influent and effluent as shown in Figure 3.

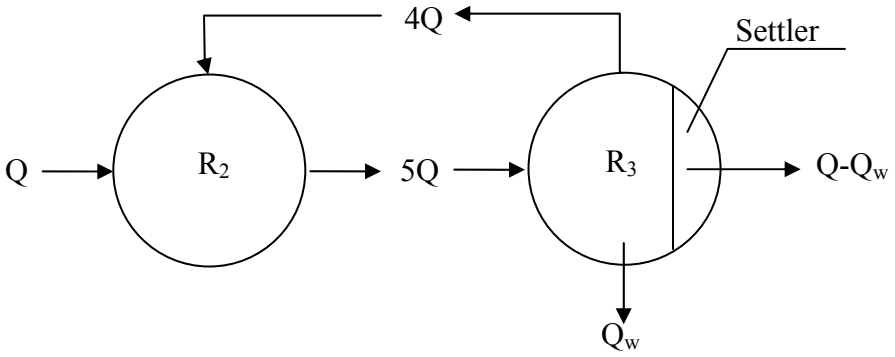


Figure 3. Schematic showing the flow rates of the different streams around the anoxic (R2) and aerobic (R3) reactors of the BNR system for a recycle ratio equal to 4Q.

Based on the above-shown flow diagram, the system removal efficiency for ammonia and nitrogen oxides are defined as follows:

$$r_{NH_3} = \frac{5Q \times C_{R2,NH3} - 5Q \times C_{R3,NH3}}{5Q \times C_{R2,NH3}} \times 100\% \qquad \text{(Equation 7)}$$

$$r_{NO_X} = \frac{4Q \times C_{R3,NOx} - 5Q \times C_{R2,NOx}}{4Q \times C_{R3,NOx}} \times 100\% \qquad \text{(Equation 8)}$$

where r_{NH_3} and r_{NO_X} are ammonia and nitrogen oxides (nitrate and nitrite) removal efficiency (%), Q is the influent flow rate (L/d), $C_{R2,NH3}$ and $C_{R3,NH3}$ are the ammonia concentrations (mg

N/L) in R2 and R3 effluents, respectively, and $C_{R2,NOx}$ and $C_{R3,NOx}$ are the nitrogen oxides concentrations (mg N/L) in R2 and R3 effluents, respectively.

2.3 Analytical Methods

TSS, VSS, pH, DO, TKN, and ammonia were measured following procedures outlined in *Standard Methods* (APHA, 2005). Total gas production was measured with a digital pressure transducer. The gas composition and VFAs were determined by gas chromatography thermal conductivity detection and flame ionization detection, respectively, as previously reported (Tugtas and Pavlostathis, 2007). Nitrate and nitrite concentrations were determined using a Dionex DX-100 ion chromatography unit (Dionex Corporation, Sunnyvale, CA) equipped with a conductivity detector, a Dionex IonPac AG14A (4x50 mm) precolumn, and a Dionex IonPac AS14A (4x250 mm) analytical column. The unit was operated in autosuppression mode with 1 mM $NaHCO_3$/8 mM Na_2CO_3 eluent with a flow rate of 1 mL/min. Calibration curves were generated using standards prepared by dissolving reagent-grade sodium salts of each compound in deionized (DI) water. Samples were prepared by centrifugation at 10,000 rpm for 20 min. All standards and samples were filtered through 0.2 μm membrane filters prior to injection. The minimum detection limits for nitrate and nitrite were 0.05 and 0.1 mg N/L, respectively.

Total carbohydrates were measured by the anthrone method using glucose as the standard (Gaudy, 1962). Lipids were measured gravimetrically following a modified chloroform-methanol extraction procedure (Loehr and Rohlich, 1962). Crude protein was estimated based on organic nitrogen measurements (i.e., TKN, minus ammonia), multiplied by a conversion factor of 6.25 (equivalent to 0.16 g nitrogen per gram of protein).

A previously reported disulfine blue method (HMSO, 1981), modified by Tezel et al. (2007) was used for the detection of quaternary ammonium compounds in poultry processing wastewater samples as well as the quantification of BAC in the laboratory assays. The minimum method detention limit was 0.2 mg QAC/L. Benzalkonium chlorides as well as benzyl trimethyl amine (BTMA), benzyl dimethyl amine (BDMA), benzyl methyl amine (BMA) and benzyl amine (BA), which are possible BAC transformation products, were measured using HPLC (Tezel, 2009). The method used a HP 1100 Series HPLC (Hewlett Packard, Palo Alto, CA) unit equipped with a Phenomenex Luna SCX column (250 x 4.6 mm, 5μ) (Phenomenex, Inc., Torrance, CA) followed by a Polaris C_{18} A column (50 × 4.6 mm, 3.2 μ) (MetaChem Technologies Inc., Torrance, CA). A Phenomenex SCX SecurityGuard cartridge (4 × 3.0 mm) was used as a precolumn. A 60:40 (v/v) mixture of acetonitrile and 50 mM phosphate buffer (pH 2.5) was used as the mobile phase at a flow rate of 1.0 ml/min and the columns were maintained at 35^{o}C. Detection was achieved with a HP 1100 series UV-Vis diode array detector at a wavelength of 210 nm. The minimum detection limit for C_{12}BDMA-Cl, C_{14}BDMA-Cl, C_{16}BDMA-Cl, BA, BMA, and BDMA was 1.57, 2.55, 4.36, 1.13, 1.46 and 1.21 μM, respectively. Prior to the HPLC analysis, 2.5 mL sample was extracted with a mixture of 1 mL of 100 mM $AgNO_3$, 1.5 mL of acetonitrile and 2.5 mL of ethylacetate (Tezel, 2009) and the extract used for the HPLC analysis.

3.0 RESULTS & DISCUSSION

3.1 Characterization of Field Samples

The results of analyses of samples collected at the poultry processing Plant B in two different seasons, conducted in the laboratory, are shown in Tables 1 and 2, below.

Table 1. Characteristics of samples collected at four locations at the study poultry processing Plant B (samples collected in Fall 2009; sample temperature 22 to 24°C).

Parameter	DAF Underflow	Anaerobic Reactor	Aerobic Reactor	Anoxic Ditch
pH	6.1	6.7	6.1	6.5
TSS (mg/L)	160±6[a]	1547±133	1340±35	1267±23
VSS (mg/L)	147±6	1123±92	953±38	883±21
Total COD (mg/L)	1813±403	1296±395	1070±141	867±129
Soluble COD (mg/L)	722±60	327±103	413±103	55±17
VFAs (mg COD/L)	ND[b]	ND	ND	ND
Ammonia (mg N/L)	23.1	22.4	2.1	2.8
Nitrate (mg N/L)	ND	ND	9.3	10.6
Nitrite (mg N/L)	ND	ND	ND	ND
TKN (mg N/L)	104±12	91±11	126±9	82±6

[a] Mean ± standard deviation (n = 3); [b] ND, not detected

Table 2. Characteristics of samples collected at four locations at the study poultry processing Plant B (samples collected in Winter 2010; sample temperature 11 to 15°C).

Parameter	DAF Underflow	Anaerobic Reactor	Aerobic Reactor	Anoxic Ditch
pH	6.1	6.4	6.0	6.1
TSS (mg/L)	71±6[a]	1219±135	1495±81	1153±106
VSS (mg/L)	59±8	987±13	993±5	976±28
Total COD (mg/L)	1092±101	2237±107	2209±56	2268±107
Soluble COD (mg/L)	790±43	169±19	97±31	45±13
VFAs (mg COD/L)	ND[b]	ND	ND	ND
Ammonia (mg N/L)	44.8	50.4	8.4	5.6
Nitrate (mg N/L)	ND	ND	ND	11.9
Nitrite (mg N/L)	ND	ND	12.9	ND
TKN (mg N/L)	91±1	100±8	146±4	95±9

[a] Mean ± standard deviation (n = 3); [b] ND, not detected

The values of all analytes varied within expected, typical ranges for poultry processing facilities. Although there is significant variability from plant to plant reflecting the plant configuration, as well as operational conditions, several overall conclusions can be drawn. The DAF underflow total COD ranged from 1092 to 1813 mg/L and its soluble COD fraction ranged from 40 to 72% of the total COD. The ammonia content of the DAF underflow ranged from 23 to 45 mg N/L, while its TKN content ranged from 91 to 104 mg N/L. Thus, the ammonia content

of the DAF underflow represented 22 to 49% of its TKN value. Significant nitrate concentrations (9 to 13 mg N/L) were detected in the aerobic reactor mixed liquor samples of Plant B. Nitrite was not detected during the warm season, but it was detected in significant levels during the cold season. It is noteworthy that the aerobic reactor ammonia concentration was higher during the cold season as compared to the warmer season. VFAs were not detected in any samples. QACs were not detected in any of the samples, consistent with reports indicating that QACs are not used in the test poultry processing facility (Plant B).

3.1.1 Batch Nitrification Tests

The results of the batch nitrification tests conducted in the laboratory at room temperature (22 to 24°C) with mixed liquor samples collected from the aerobic unit of poultry processing Plant B are shown in Figure 4. Throughout these tests, the DO was maintained between 6 and 8 mg/L in all reactors. With the exception of the NH_4Cl solution added at the beginning of the test and periodic addition of $NaHCO_3$ to keep the pH above 6.5, these reactors did not receive any feed during these tests. Table 3 summarizes the estimated MSUR values. Thus, as the mixed liquor temperature decreased from 22 to 15°C, the ammonia and nitrite specific utilization rates decreased by 39.5 and 32%, respectively.

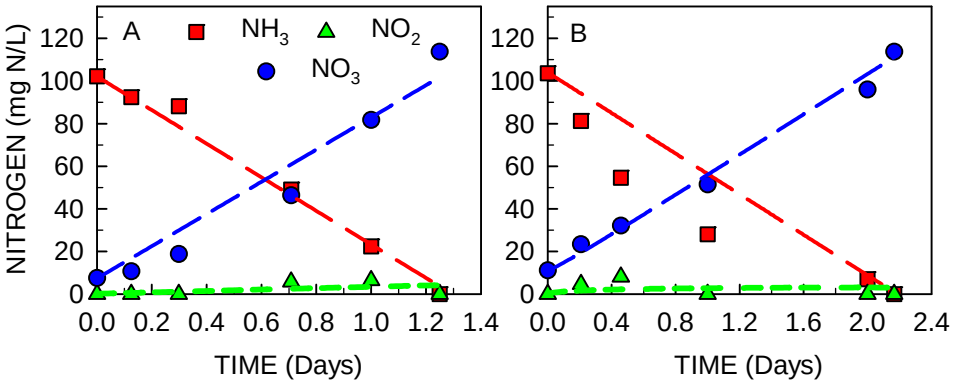


Figure 4. Measured and simulated (lines) nitrogen species during the batch nitrification tests with Plant B aerobic mixed liquor samples (laboratory tests conducted at 22 to 24°C; A and B denote Fall and Winter sampling at Plant B, see text).

Table 3. Batch nitrification test conditions and maximum specific nitrification utilization rates for Plant B aerobic reactor mixed liquor.

Parameter	Fall 2009	Winter 2010
Temperature (°C)[a]	22	15
Average TSS (mg/L)	1340±35[b]	1418±58
Average VSS (mg/L)	953±38	973±21
k_{nh3} (mg N/g VSS·day)	82.8	50.0
k_{no2} (mg N/g VSS·day)	79.3	53.9
RMSD	16.2	24.4

[a] Temperature of mixed liquor at the time of sampling; [b] Mean ± standard deviation (n = 3)

3.1.2 Batch Denitrification Tests

The results of batch denitrification tests conducted in the laboratory at room temperature (22 to 24°C) with mixed liquor samples collected from the anoxic unit at the study poultry processing plants (Plant B) are shown in Figure 5. DAF underflow wastewater was added at the beginning of these tests. Throughout these tests, the pH varied between 7 and 8. Nitrate removal proceeded relatively fast in both cases. Very low nitrite concentrations were observed in the test conducted with mixed liquor obtained in the Fall, whereas significant nitrite concentrations were detected in the second test conducted with mixed liquor obtained in Winter. The observed decrease in total nitrogen is attributed to N_2 losses as a result of denitrification. Table 4 summarizes the estimated MSRR values.

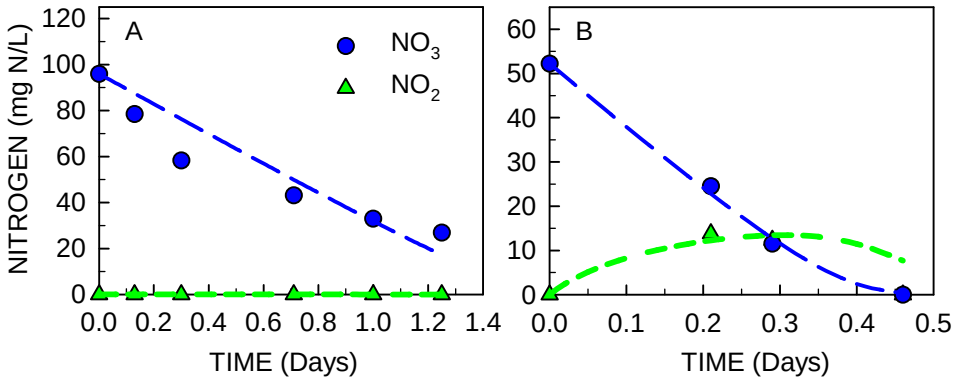


Figure 5. Measured and simulated (lines) nitrogen species during the batch denitrification tests with Plant B anoxic mixed liquor samples (laboratory tests conducted at 22 to 24°C; A and B denote Fall and Winter sampling at Plant B, see text).

Table 4. Batch denitrification test conditions and maximum specific reduction rates for Plant B anoxic reactor mixed liquor.

Parameter	Fall 2009	Winter 2010
Temperature (°C)[a]	23	14
Average TSS (mg/L)	1267±23[b]	1210±65
Average VSS (mg/L)	883±21	929±24
k_{no3} (mg N/g VSS·day)	82.4	160.5
k_{no2} (mg N/g VSS·day)	3552.4	202.5
RMSD	9.6	2.3

[a] Temperature of mixed liquor at the time of sampling; [b] Mean ± standard deviation (n = 3)

The nitrate MSRR was higher for the second sample collected during the cold season, while the nitrite MSRR was significantly lower compared to the values obtained with the sample collected during the warmer season. Nitrate reductase is believed to have an optimal temperature of 15°C (Kristiansen, 1983), compared to 35°C for the nitrite reductase (Snape et al., 1997). Enzymes retain their maximum activity near their optimal temperature, and temperatures lower or higher lead to reduced activity (Rittmann and McCarty, 2001; Madigan and Martinko, 2006).

3.2 BNR System Operation – Phase 1

The BNR system was operated with QAC-free wastewater and the baseline conditions (see Section 2.2.2) for over 5 months before changes in its operational conditions were applied as discussed below. Table 5 summarizes the influent wastewater characteristics. Most COD was soluble (at least 72%) and 96% of the suspended solids were volatile. The solids were comprised mainly of lipids (28%), carbohydrates (18%) and crude protein (54%). The sum of lipids, carbohydrates, and crude protein COD accounted for 85% of the measured total COD. Most of the total nitrogen content was soluble, 63% crude protein, and 37% ammonia. Nitrate and nitrite were not detected. BAC was never detected in any of the collected poultry processing wastewater. The poultry processing wastewater characteristics were comparable to previously reported values (Pierson and Pavlostathis, 2000; Tezel et al., 2007; Avula et al., 2009), with some variation related to sampling time and poultry processing plant operation. The feed solids are mainly composed of lipids, carbohydrates and proteins, which based on their ionic and hydrophobic properties, provide perfect media for BAC adsorption. BAC has a high affinity to accumulate on solids via both ionic and hydrophobic interactions (Ismail et al., 2010; Ren et al., 2011), rendering the poultry processing wastewater a "perfect medium" by which BAC is introduced into the BNR treatment system. Ultimately, these interactions define the fate of BACs in engineered and natural biological systems.

Table 5. Characteristics of Plant B poultry processing DAF underflow wastewater used as the feed to the BNR system.

Parameter	Value
pH	6.9 ± 0.2[a]
TSS (mg/L)	125 ± 20
VSS (mg/L)	120 ± 23
Total COD (mg/L)	1275 ± 16
Soluble COD (mg/L)	920 ± 111
VFAs (mg COD/L)	ND[d]
Carbohydrates (mg COD/L)[b]	26 ± 6
Lipids (mg COD/L)[c]	680 ± 28
Crude protein (mg COD/L)[e]	381 ± 10
NH_3 (mg N/L)	46 ± 13
NO_3^- (mg N/L)	ND
NO_2^- (mg N/L)	ND
TN (mg N/L)[f]	133 ± 5

[a] Mean ± standard deviation ($n \geq 3$); [b] As glucose; [c] As palmitic acid; [d] ND, not detected; [e] As alanine; [f] Total nitrogen, i.e., sum of organic, ammonia, nitrite, and nitrate nitrogen.

Table 6 summarizes the characteristics of the three reactors and effluent during 20 days of continuous, QAC-free operation. The actual solids retention time in the anoxic and aerobic reactors was recalculated after taking into account the solids lost in the effluent. The actual θ_c for the anoxic and aerobic reactors was 10.1 and 12.6 days, respectively. Volatile fatty acids (VFAs) were detected in the R_1 effluent and accounted for 27% of the reactor's effluent soluble COD concentration. The COD removal efficiency was 71.3±15.2%. The ammonia concentration in the

R_1 effluent was almost double the feed's ammonia concentration, and accounted for 93% of its total nitrogen content. As for the BNR reactors, the R_2 ammonia concentration was 28±9 mg N/L and nitrate was never detected. On the other hand, the R_3 nitrate concentration was 28±7 mg N/L and ammonia was never detected. Nitrite was never detected in both R_2 and R_3 during the QAC-free operation. The effluent nitrate concentration was 27±3 mg N/L, while ammonia was never detected. Overall, during the BAC-free operation period the system achieved 100% ammonia removal efficiency. In terms of nitrogen balance, 206 mg N/day was fed into the system, of which 109 mg N/day was removed as dinitrogen from the anoxic reactor, 54 mg N/day was removed with the mixed liquor waste from the aerobic reactor, and 50 mg N/day in the effluent (nitrate and escaping biomass), yielding a total nitrogen removal of 75.9%. The total nitrogen removal was less than the 90% removal efficiency reported for the Barnard process (Rittmann and McCarty, 2001). Unlike the original Barnard process, which uses two denitrification stages, one denitrification stage was used in the laboratory-scale BNR system, which resulted in lower total nitrogen removal efficiency. Nevertheless, the BNR operation was stable and provided a baseline system performance against which the performance of the BNR system treating a QAC-bearing poultry processing wastewater could be compared with (Phase 2 and 3).

Table 6. Performance of the BNR system during continuous, QAC-free operation (Data from day 10 to 30; recycle ratio 4).

Parameter	R_1	R_2	R_3	Effluent
pH	6.7 ± 0.1[a]	7.5 ± 0.3	7.1 ± 0.4	7.1 ± 0.4
TSS (mg/L)	171 ± 29	1428 ± 336	1557 ± 342	33 ± 22
VSS (mg/L)	155 ± 14	1157 ± 236	1251 ± 242	25 ± 20
Soluble COD (mg/L)	650 ± 248	485 ± 144	289 ± 179	273 ± 169
VFAs (mg COD/L)	168 ± 107	ND	ND	ND
NH_3 (mg N/L)	94 ± 34	28 ± 9	ND	ND
NO_3^- (mg N/L)	ND[b]	ND	28 ± 7	27 ± 3
NO_2^- (mg N/L)	ND	ND	ND	ND
DO (mg/L)	-	-	5.0 ± 1.5	-

[a] Mean ± standard deviation ($n \geq 6$); [b] ND, not detected

Based on oxygen uptake rate (OUR) analysis on the aerobic reactor mixed liquor performed at the 25th day of operation, the corresponding ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) biomass fractions in the aerobic reactor population were 2.8% and 2.2%, respectively. A wide variation in AOB and NOB fractions in similar microbial communities has been reported. These fractions varied between 0.34% in activated sludge, 2.5–6.2% in sequencing batch reactors, and 6–18% in combined activated sludge and rotating biological contactor (Li et al., 2007). The wide variation could be attributed mainly to wastewater type, system configuration, and operational conditions.

3.2.1 Preliminary BAC Effect on Nitrification and Denitrification

Biological nitrification and denitrification are both inhibited by QACs, but with a varying degree of susceptibility (Tezel et al., 2008; Yang, 2007; Tezel and Pavlostathis, 2009; Hajaya et al., 2011). Among the two processes, nitrification is more susceptible to the inhibitory effect of QACs. BNR efficiency deterioration can result from an adverse impact on either nitrification or denitrification. The two batch assays discussed below aimed to identify the effect of BAC on these **two** processes.

3.2.1.1 Nitrification Batch Assay

The assay testing the effect of BAC on nitrification lasted for 82 hours. Figure 6 shows the time course of the nitrogen species and pH variation during the batch incubation period for all eight culture series. For all BAC-amended culture series a decrease in the final biomass concentration was observed with increasing BAC concentration, resulting from BAC-induced growth inhibition and cell lysis brought about by the BAC antimicrobial effect (Cross and Singer, 1994).

Figure 7A shows the extent of ammonia removal in all culture series at the end of the incubation period. Among all culture series used in this assay, only the BAC-free and the 5 mg/L BAC-amended culture series achieved complete ammonia removal and oxidation to nitrate within 24 and 60 h, respectively. The initial SARR for both culture series was 75.3±4.5 and 46.1±11.8 mg N/g VSS · day for the BAC-free and 5 mg/L BAC-amended culture series, respectively. The 10 and 15 mg/L BAC-amended culture series achieved 43 and 28% ammonia removal by the end of the incubation period, with an initial SARR of 4.7±1.2 mg N/g VSS · day for both culture series. For the remaining BAC-amended culture series (25 to 100 mg/L BAC), complete inhibition of nitrification was observed (≤ 5% ammonia removal) with an initial SARR ≤ 1.4±0.1 mg N/g VSS · day. Nitrite was never detected in any culture series, i.e., all removed ammonia was fully oxidized to nitrate. Figure 7B shows the relative SARR (i.e., SARR normalized to the BAC-free culture series) for all culture series.

Based on total BAC measurements, at 60 h of incubation, ≥ 90% of the initially added BAC was removed in all culture series, except in the 100 mg/L BAC-amended culture series, where only 14% BAC removal was observed. Further BAC removal was not observed by the end of the incubation period (82 h). Figure 8 shows the recovered BAC at the end of the incubation period. BAC was degraded by the heterotrophic population which constituted a large fraction of the mixed liquor used in this assay (almost 95% based upon OUR analysis). However, further ammonia removal was not observed in the inhibited culture series (10 to 100 mg/L) despite the observed BAC degradation, which indicates that the inhibitory effect of BAC on nitrification was irreversible within the duration of the batch assay. In a recent study, Pavlostathis et al. (2008) reported a long-term nitrification inhibition in a QAC-exposed BNR plant treating poultry processing wastewater, and that nitrification recovered after 30 days of the initial BAC exposure.

Among the BAC-amended culture series, nitrification was complete in the 5 mg/L culture series, albeit with a reduced initial SARR value. Previous studies have shown that the toxicity of BAC in biological systems depends on the extent of BAC adsorption (Zhang et al., 2011). At low BAC concentrations, most of BAC will be adsorbed to biomass leading to reduced bioavailability and inhibition of the microbial activity. Therefore, the observed complete nitrification in the 5 mg/L BAC-amended culture series was attributed to low BAC exposure due to limited BAC bioavailability.

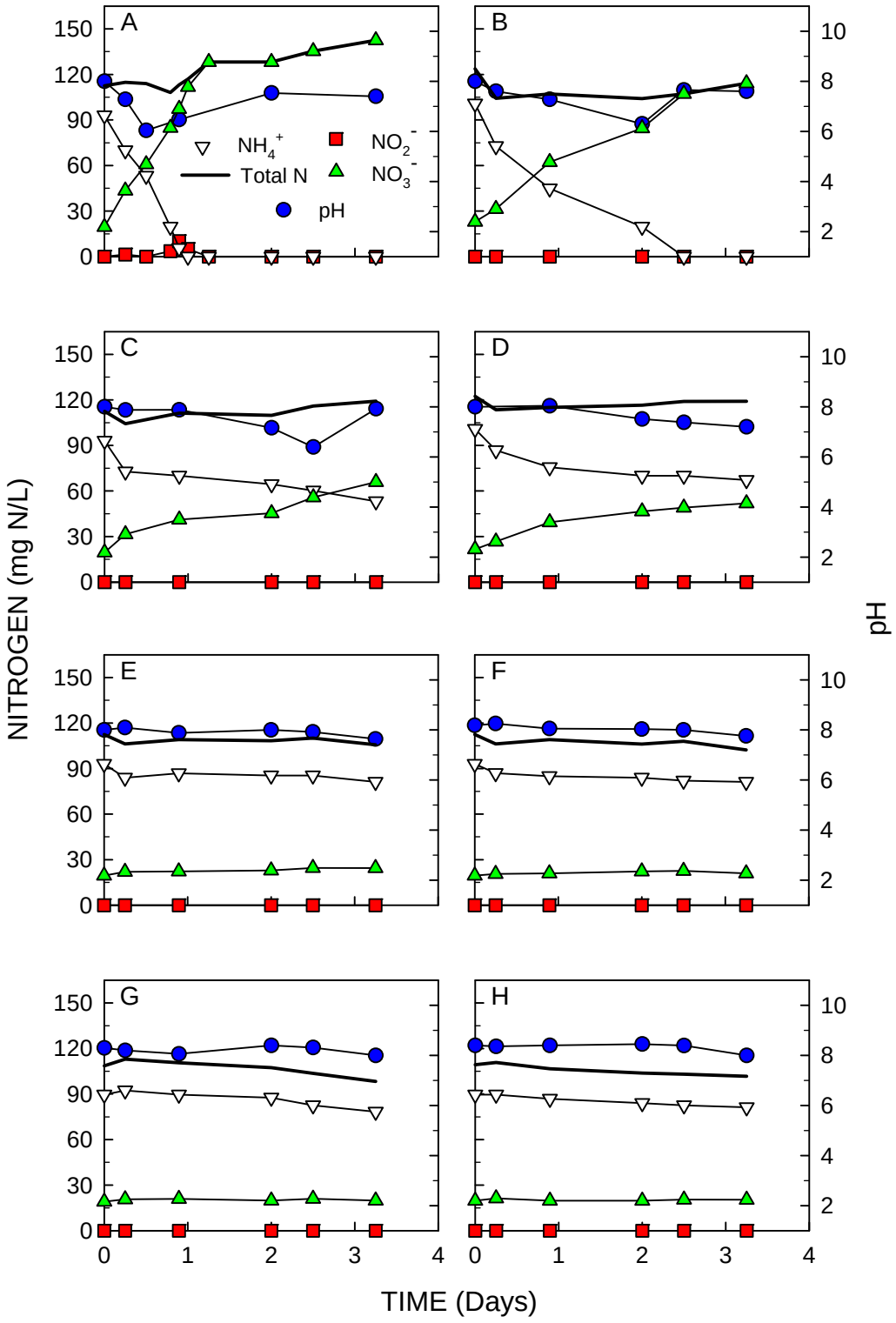


Figure 6. Time course of nitrogen species and pH variation during the batch nitrification assay conducted with BAC-free mixed liquor collected from the aerobic reactor (R_3). Initial BAC concentrations of 0 (A), 5 (B), 10 (C), 15 (D), 25 (E), 50 (F), 75 (G), and 100 mg/L (H).

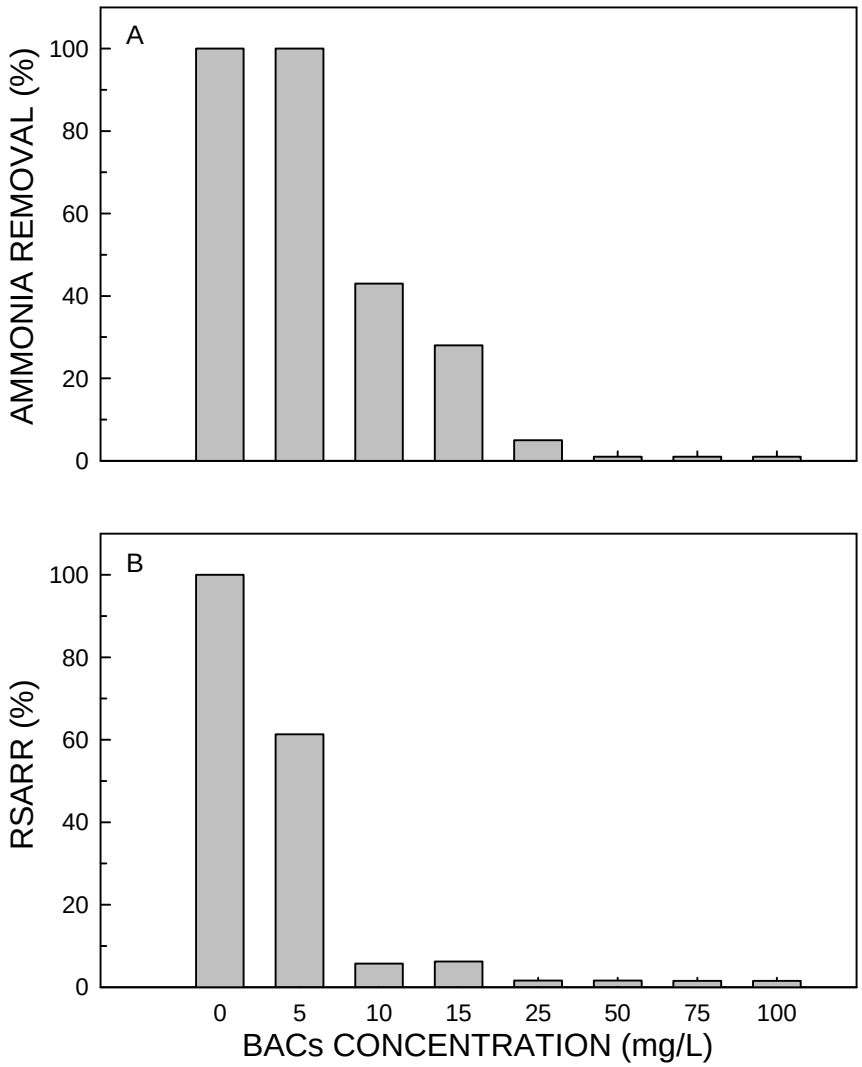


Figure 7. Extent of ammonia removal (A) and relative specific ammonia removal rate (RSARR) (B) for the culture series used in the batch nitrification assay conducted with BAC-free mixed liquor collected from the aerobic reactor (R_3).

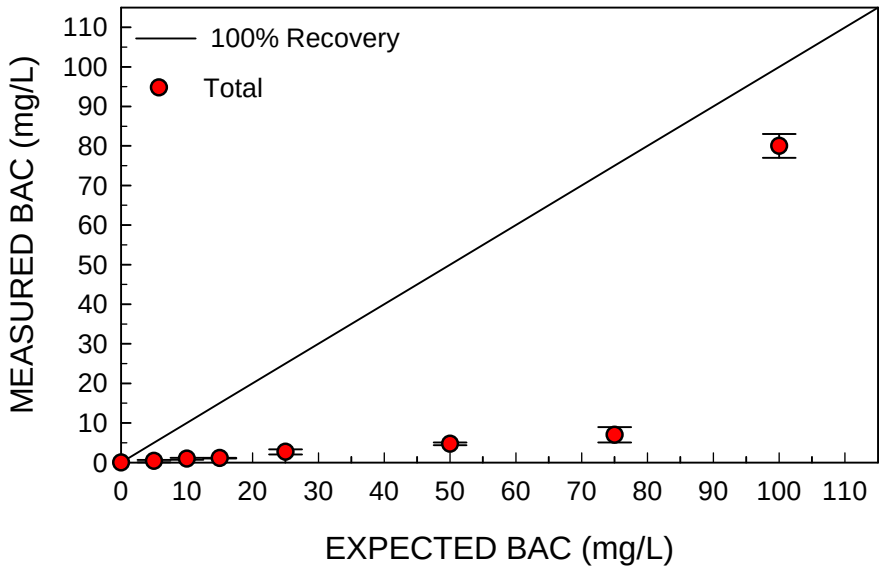


Figure 8. BAC recovery at the end of the incubation period in the culture series of the batch nitrification assay conducted with BAC-free mixed liquor collected from the aerobic reactor (R_3). Error bars represent one standard deviation of the means (n = 3).

3.2.1.2 Denitrification Batch Assay

The assay testing the effect of BAC on denitrification lasted for 9 days. Figure 9 shows the time course of nitrogen species in all five culture series. All added nitrate was fully reduced to either dinitrogen gas (>90%) or ammonia, which indicates that the dissimilatory nitrogen reduction to ammomia (DNRA) process was also active in these culture series. Neither nitric oxide nor nitrous oxide was detected in any culture series. Figure 10A shows the nitrogen species distribution at the end of the incubation period. Similar to the nitrification assay, a decrease in the final biomass concentration was observed with increasing BAC concentration, which resulted from BAC-induced growth inhibition and cell lysis.

The initial SNRR of the BAC-free culture series was 117.9 ± 4.8 mg N/g VSS · day, while BAC decreased the initial SNRR in the BAC-amended culture series to 106.1±4.0, 90.0±11.5, 83.5±16.2, 78.4±11.7, and 16.0±0.6 mg N/g VSS · day at an initial BAC concentration of 10, 25, 50, 75, and 100 mg/L, respectively. Figure 10B shows the relative SNRR in all culture series used in the denitrification assay. At BAC concentrations of 50 mg/L and above, a substantial decrease in the nitrate reduction rate was observed (Figure 10B). However, this decrease was less than the one observed for nitrite reduction, where, compared to the BAC-free culture series, transient accumulation of nitrite was apparent as the BAC concentration increased. The highest measured nitrite concentrations were 9.6, 120, and 123 mg NO_2^--N/L in the cultures amended with 50, 75, and 100 mg BAC/L. Transient accumulation of nitrite suggests that the nitrite reduction was inhibited by BAC and nitrite reduction is more sensitive to BAC compared to nitrate reduction. Inhibition of denitrification by BAC was previously reported to take place at BAC concentrations equal to or higher than 50 mg/L (Tezel et al., 2008; Tezel and Pavlostathis, 2009).

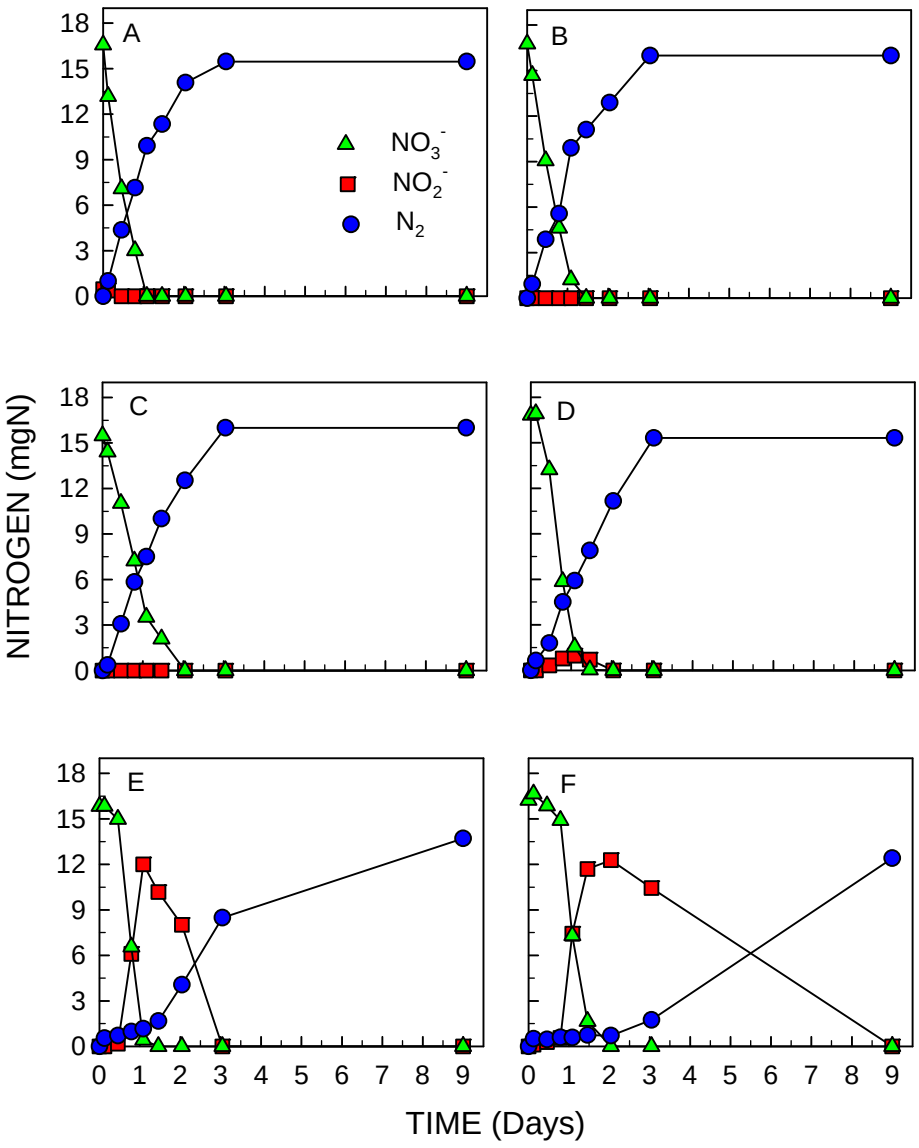


Figure 9. Time course of nitrogen species during the batch denitrification assay conducted with BAC-unexposed, denitrifying mixed liquor and initial BAC concentrations of 0 (A), 10 (B), 25 (C), 50 (D), 75 (E) and 100 mg/L (F).

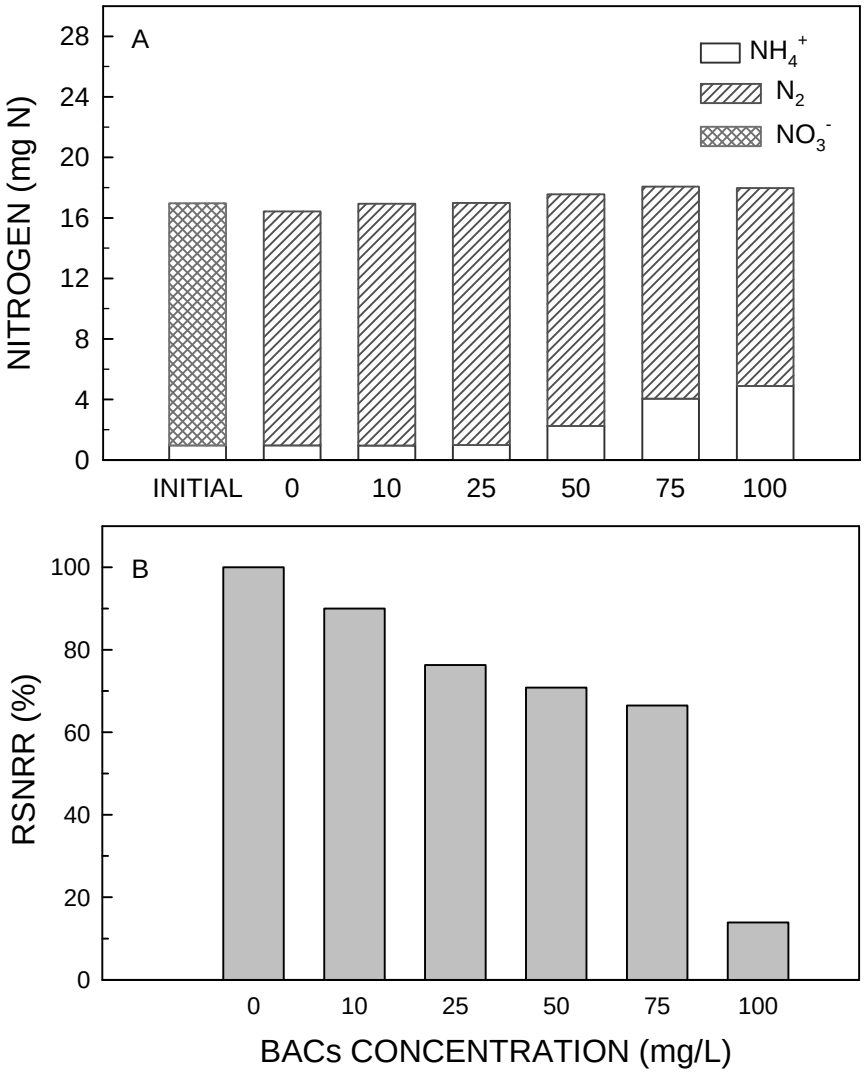


Figure 10. Distribution of the nitrogen species at the end of the incubation period (A) and relative specific nitrate removal rate (RSNRR) (B) in the batch denitrification assay conducted with BAC-unexposed, denitrifying mixed liquor.

Figure 11 shows the recovered BAC concentration at the end of the incubation period for all culture series. All added BAC was recovered at the end of the incubation period, which indicates that BAC did not degrade under the anoxic conditions of this study. BAC has been considered to be recalcitrant under anoxic and anaerobic conditions, as previously reported (Garcia et al., 2006; Tezel et al., 2006; Tezel et al., 2007; Tezel et al., 2008). BAC transformation to alkyl dimethyl amines under nitrate reducing conditions was only recently reported by means of an abiotic, yet biologically initiated, reaction in which nitrite was utilized in a nucleophilic substitution reaction with BAC in a modified Hofmann reaction (Tezel and Pavlostathis, 2009). The researchers also found that BAC transformation required elevated nitrite concentrations (70 mg N/L). More importantly, the BAC liquid-phase concentration was the

limiting factor for the abiotic transformation, as the BAC transformation did not occur at liquid-phase concentrations below a range of 7.8-10.8 mg/L (Tezel and Pavlostathis, 2009).

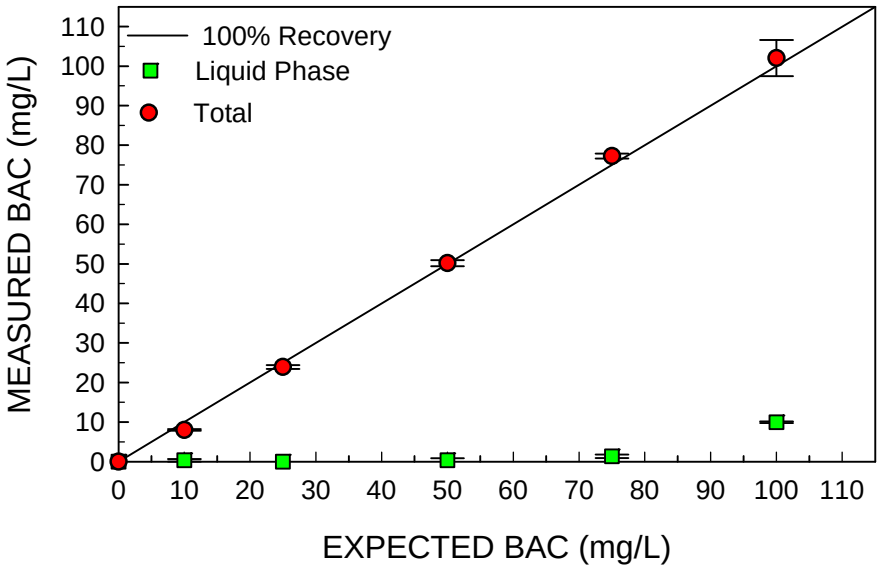


Figure 11. BAC recovery at the end of the incubation period in the batch denitrification assay conducted with BAC-unexposed, denitrifying mixed liquor. Error bars represent one standard deviation of the means (n = 3).

In the denitrification assay conducted in the present study with denitrifying mixed liquor unacclimated to BAC, nitrite concentrations exceeded 70 mg N/L at initial BAC concentrations of 75 and 100 mg/L. Nevertheless, the BAC liquid-phase concentration in all culture series was below the above-mentioned concentration required for the abiotic transformation. In fact, the highest measured BAC liquid-phase concentration was 11.8 ± 0.3 mg/L in the 100 mg/L culture series, which was comparable to the BAC concentration that did not facilitate the abiotic transformation reaction to take place as reported by Tezel and Pavlostathis (2009).

Nitrification vs. Denitrification: Between nitrification and denitrification, the former is by far the process most susceptible to BAC. Denitrification was inhibited by BAC, but complete nitrate reduction was achieved in all denitrification culture series. On the other hand, inhibition of nitrification by BAC was persistent, despite the fact that BAC was largely removed from most of the nitrification culture series by the end of the incubation period. This result leads to the conclusion that nitrogen removal in BNR systems will dramatically and irreversibly deteriorate when treating BAC-bearing poultry processing wastewater without prior acclimation. The validity of this conclusion was further investigated (see Phase 2 and 3, sections 3.3 and 3.4, below).

3.2.2 Effect of Recycle Ratio

Figure 12 shows the BAC-free BNR system performance during its operation at three recycle ratios. At a recycle ratio of 4, the system achieved 100% ammonia removal and 77.6% nitrogen removal. Nitrate was undetected in the anoxic reactor and reached 23.3±2.8 mg N/L in the aerobic reactor. Ammonia was detected at 25.3±1.2 mg N/L in the anoxic reactor and was not detected in the aerobic reactor. Nitrite was never detected in either reactor.

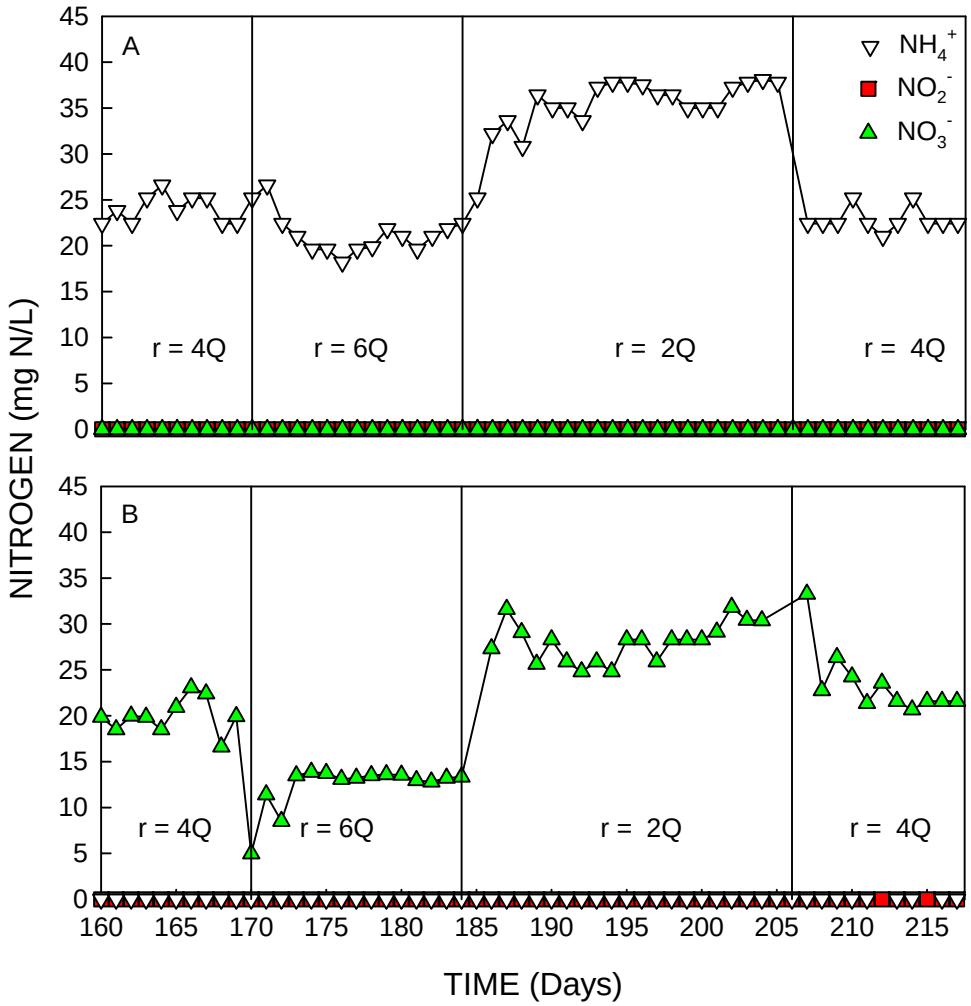


Figure 12. Nitrogen species in the anoxic (A) and aerobic (B) reactors at three mixed liquor recycle ratios during QAC-free BNR system operation.

At a recycle ratio of 6, the ammonia concentration decreased marginally in the anoxic reactor reaching 20.9±2.1 mg N/L while nitrate and nitrite remained undetected. On the other hand, the nitrate concentration decreased significantly in the aerobic reactor reaching 12.3±2.4 mg N/L, while ammonia and nitrite remained undetected. A higher recycle ratio corresponds to a higher anoxic residence time for the mixed liquor, thus enhancing nitrate removal in the system. The TSS/VSS concentrations in the anoxic and aerobic reactors were 1328±120/1115±290 and 1483±32/1197±25, respectively, and did not change during operation with a recycle ratio of 6 (14 days). The nitrogen removal efficiency was 85.8%.

At a recycle ratio of 2, the ammonia concentration increased in the anoxic reactor reaching 35.3±3.1 mg N/L while nitrate and nitrite remained undetected. On the other hand, the

nitrate concentration increased significantly in the aerobic reactor reaching 28.0±2.1 mg N/L, while ammonia and nitrite remained undetected. A lower recycle ratio corresponds to a lower anoxic residence time for the mixed liquor, thus reducing nitrate removal in the system, leading to higher nitrate concentrations. The TSS/VSS concentrations in the anoxic and aerobic reactors decreased at a recycle ratio of 2 to 912±86/715±90 and 1003±32/907±25, respectively. The nitrogen removal efficiency for the BNR system was 52.9%. After 22 days, the recycle ratio was returned to 4, where the system reestablished its original, pre-recycle ratio change performance (nitrogen removal efficiency of 78.2%).

3.2.3 Effect of Increased Organic and Nitrogen Loading

By using a concentrated poultry processing wastewater, the ammonia concentration in the BNR system feed was doubled (on day 273). Figure 13 shows the QAC-free BNR system performance before, during, and after the feed change.

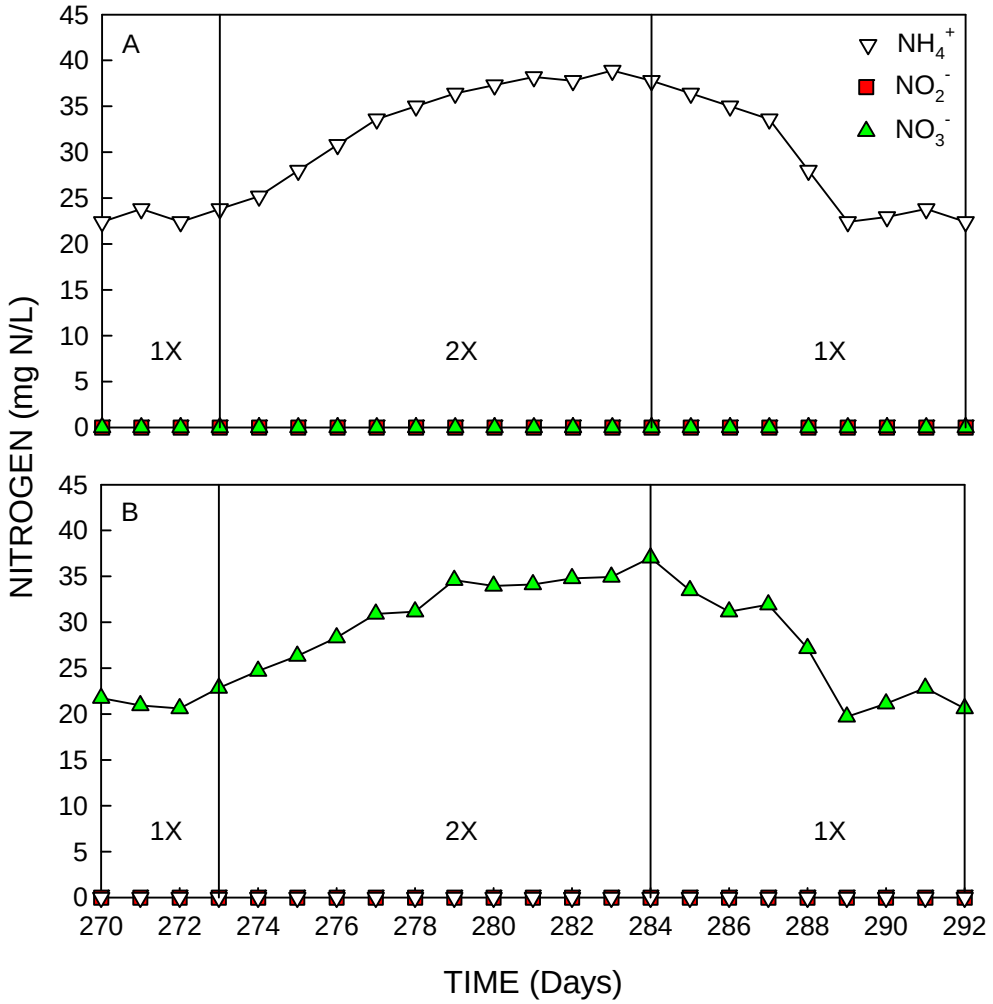


Figure 13. Nitrogen species in the anoxic (A) and aerobic (B) reactors of the QAC-free BNR system while operating with the normal poultry processing wastewater (1X) and concentrated poultry processing wastewater feed (2X).

The anaerobic reactor effluent ammonia concentration increased from 75.1±2.1 to 110.6±5.2 mg N/L (47.4% increase). The ammonia concentration in the anoxic reactor nearly

doubled (increased from 22.7 to 38.9 mg N/L), while nitrate and nitrite were never detected. The nitrate concentration in the aerobic reactor increased by 70% (from 21.8 to 37.04 mg N/L), while ammonia and nitrite were never detected. These observations indicate that both the anoxic and aerobic reactors were capable of complete denitrification and nitrification, respectively, under elevated nitrogen loading conditions. The enhanced denitrification in the anoxic reactor is attributed to the increase in the organic carbon concentration in the concentrated feed as its soluble COD concentration increased from 919±11 to 2045±14 mg/L. The increase in nitrate concentration in the aerobic reactor is reflected in the system nitrogen removal efficiency, which decreased to 66.7%. Nonetheless, increasing the mixed liquor recycle ratio will reduce the nitrate concentration in the aerobic reactor and ultimately the effluent. The BNR system was switched back to the regular feed on day 284 and on day 288 the system regained its original performance (Figure 13).

3.2.4 Effect of Process Configuration – Elimination of Pre-Fermentation Step

On day 295, the anaerobic reactor was removed from the system and the feed was directed to the anoxic reactor. Figure 14 shows the QAC-free BNR system performance during its operation with and without the anaerobic reactor (R1).

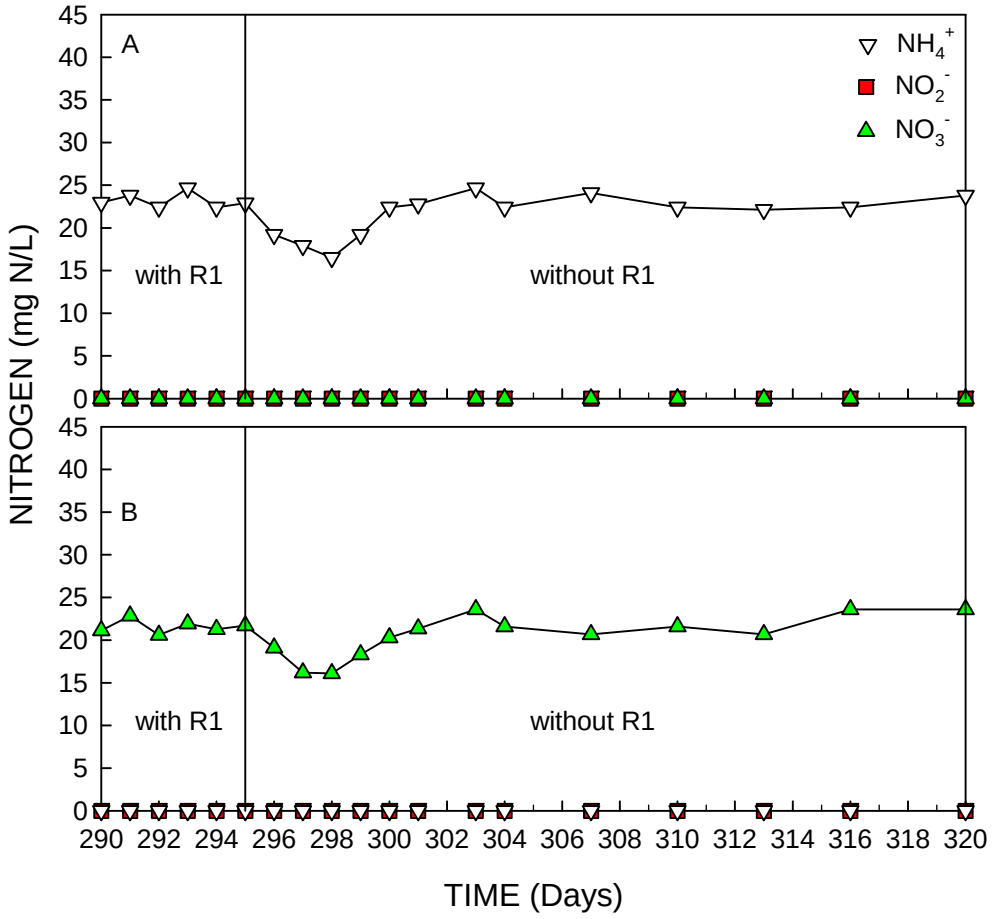


Figure 14. Nitrogen species in the BAC-free BNR system anoxic (R_2) (A) and aerobic (R_3) (B) reactors while operating with regular feed, with and without the anaerobic reactor (R_1).

Initially, the ammonia concentration decreased in the anoxic reactor reflecting the difference between ammonia concentration in the feed and the anaerobic reactor effluent (46±3 and 94±34 mg N/L, respectively). On day 298, the ammonia concentration in the anoxic reactor started to increase, more likely due to enhanced hydrolysis and ammonification in this reactor. On day 302, the ammonia concentration stabilized and reached levels similar to those observed during operation as a three-step process (23±3 mg N/L). Similar to the normal operation, VFAs were never detected in the anoxic reactor effluent. The nitrate concentration in the aerobic reactor initially decreased (16.1 mg N/L), but eventually increased and reached levels similar to the normal operation (21.8±1.4 mg N/L). These results indicate that a multi-stage BNR system could be operated successfully at 22^{o}C without a pre-fermentation step. The system nitrogen removal efficiency was 77.9%.

3.3 BNR System Operation – Phase 2

The BNR system was operated at room temperature (22 to 24^{o}C) with QAC-amended wastewater and the baseline conditions (see Section 2.2.2) for over 510 days, during which the influent BAC concentration was increased, step-wide from 5 to 120 mg/L. Subsequently, the BNR system was operated for over 60 days with QAC-free influent wastewater while maintained at room temperature (22 to 24^{o}C). The system performance and dynamics in terms of nitrogen species, the effect of BAC on the nitrification and denitrification, BAC biotransformation, as well as BAC sorption and phase distribution in the BNR system components are described below.

3.3.1 Initial BAC Exposure

After establishing stable BNR system operation regarding nitrogen removal (see Section 2.2), BAC was introduced into the system's poultry processing wastewater feed at a concentration of 5 mg/L. This concentration was chosen based on observations gathered from the nitrification assay conducted with mixed liquor prior to BAC exposure (Phase 1; see section 3.2.11, above), where complete nitrification took place at a BAC concentration of 5 mg/L, albeit with reduced SARR, at a mixed liquor VSS concentration comparable to that of the BNR system. Figure 15 shows the nitrogen species concentration in the BNR system while treating the BAC-bearing poultry processing wastewater at a feed BAC concentration of 5 mg/L.

Six days after BAC introduction to the poultry processing wastewater feed, the ammonia concentration in R_3 gradually increased and reached a maximum of 53 mg N/L (Figure 15). The simultaneous drop of the nitrate concentration in the aerobic reactor rather than the accumulation of nitrite, suggests that the ammonia oxidizing bacteria, not the nitrite oxidizing bacteria, were inhibited by BAC. The aerobic reactor VSS concentration dropped from 1250±242 to 1098±21 mg VSS/L. At the highest concentration of ammonia in the aerobic reactor the total nitrogen removal of the system dropped from 75.9% to 35.3%, the latter achieved by the daily waste of the aerobic reactor mixed liquor.

On the other hand, nitrate was never detected in the effluent of R_2, which suggests that BAC did not affect denitrification at the 1.1±0.2 mg/L BAC concentration detected in this reactor. Nevertheless, similarly to R_3, the VSS concentration in R_2 dropped from 1157±236 to 859±53 mg VSS/L. The drop in VSS concentration in both reactors is attributed to decreased microbial growth as well as to induced cell lysis associated with the antimicrobial action of BAC (Cross and Singer, 1994). As discussed in section 3.3, nitrification is more susceptible to BAC compared to denitrification, which explains the previous observations regarding nitrification and

denitrification. Nevertheless, the observed nitrification inhibition occurred at a far less BAC concentration compared to the batch assay, i.e., 0.8 vs. 5 mg/L BAC in the aerobic reactor and nitrification batch assay, respectively.

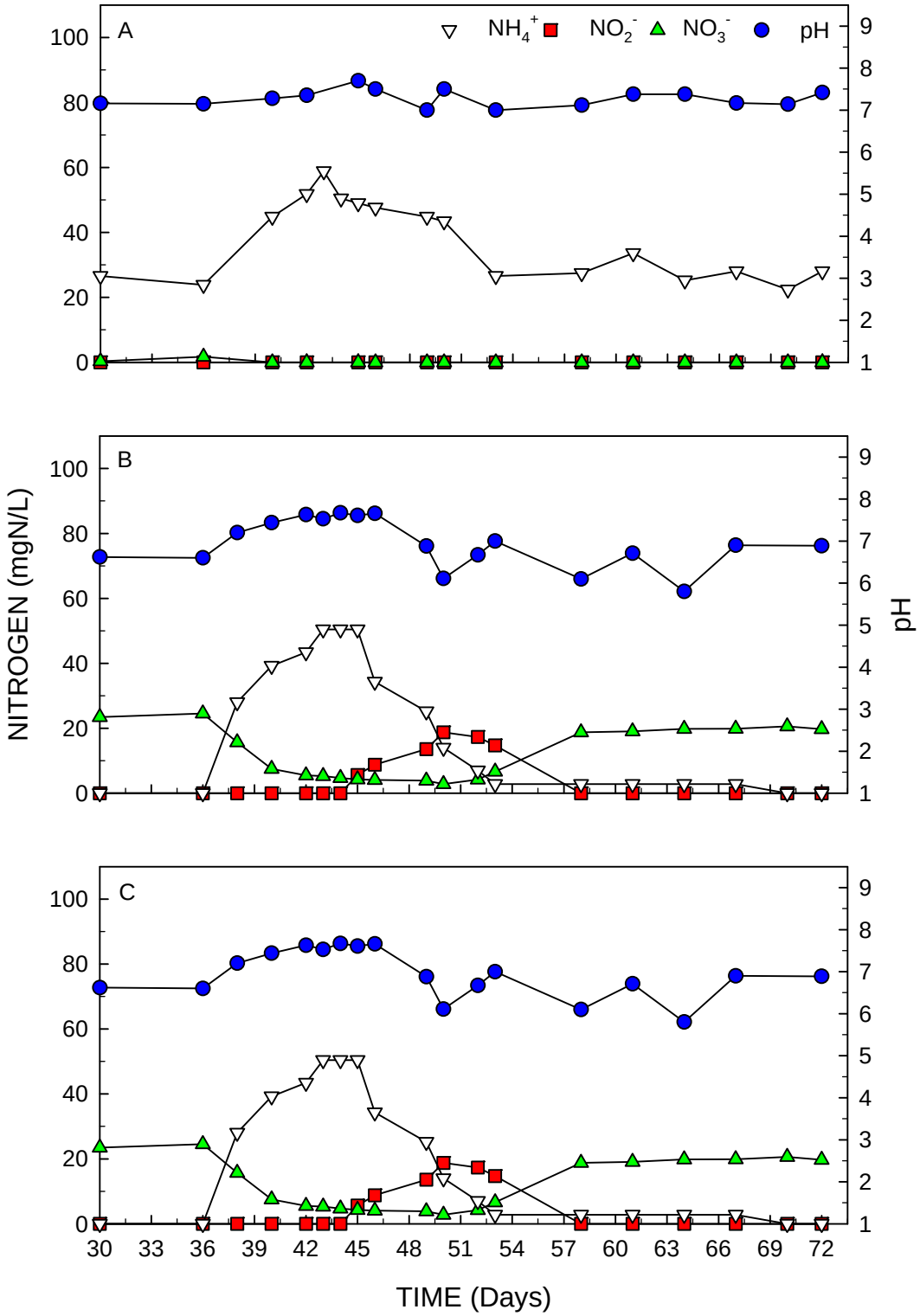


Figure 15. Nitrogen species and pH in the BNR system while treating the BAC-bearing poultry processing wastewater at a feed BAC concentration of 5 mg/L (A, R_2; B, R_3; and C, Effluent).

After 8 days of operation with a feed BAC concentration of 5 mg/L, the BAC concentration reached 5.1±0.1 mg/L in R_1, while in the R_2, R_3 and effluent reached a maximum of 1.1±0.2, 0.8±0.2, and 0.5±0.1 mg BAC/L, respectively. After 15 days of operation with the BAC-bearing wastewater at a feed BAC concentration of 5 mg/L (45 days continuous operation), the BAC concentration in both the aerobic reactor and effluent decreased to non detectable levels, indicating complete BAC biotransformation by the heterotrophic population in the aerobic reactor. Figure 16 shows the BAC concentration in the BNR system while treating the BAC-bearing poultry processing wastewater at a feed concentration of 5 mg/L.

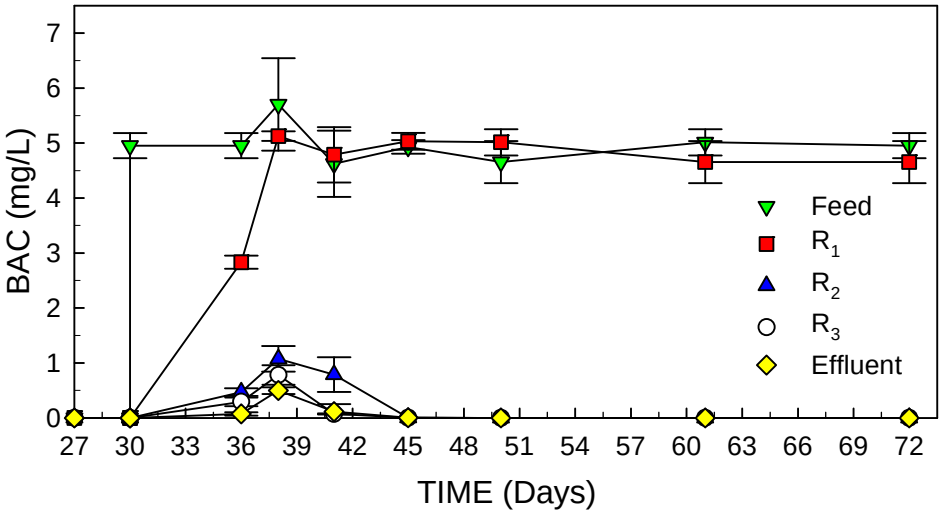


Figure 16. Total BAC concentration in the BNR system while treating the BAC-bearing poultry processing wastewater at a feed BAC concentration of 5 mg/L.

After the complete biotransformation of BAC, the ammonia concentration in R_3 gradually decreased (Figure 15). The subsequent transient increase in nitrite concentration suggests that the ammonia oxidizing bacteria (AOB) were the first to recover from the initial BAC inhibition. After 27 days of operation with BAC-bearing poultry processing wastewater feed at 5 mg/L, the system performance stabilized to nitrogen removal levels similar to those achieved during the BAC-free operation, reaching an ammonia and nitrogen removal efficiency ≥99% and 74% (on day 57 and onward; see Figure 15). Based on OUR analysis of the aerobic reactor mixed liquor performed at the 100th day of operation, the AOB and NOB biomass fractions were 2.0 and 3.1%, respectively, of the aerobic reactor biomass. These AOB and NOB biomass fractions are similar to the values obtained during the BAC-free operation period (Phase 1, section 3.2, above).

Table 7 summarizes the performance of the BNR system operated with BAC-bearing poultry processing wastewater at a feed concentration of 5 mg /L, after recovering from the initial inhibitory effect of BAC. The recovery of nitrifiers more likely resulted from BAC removal in R_3 through aerobic biotransformation by the heterotrophic population as previously documented (Nishihara et al., 2000; Patrauchan and Oriel, 2003; Tezel, 2009; Zhang et al., 2011). BAC was recalcitrant under the anoxic conditions of the anoxic reactor (R_2) as discussed in section 3.2, above. In addition, the BAC-bearing R_1 effluent is diluted as it is mixed with R_2 mixed liquor (i.e., CSTR effect), thus resulting in a significantly lower BAC concentration in

both R_2 and R_3, which in turn results in reducing the extent of BAC exposure of the microbial populations in these reactors.

An increase in ammonia and sCOD concentrations was observed in the anaerobic reactor effluent while the BNR system feed was maintained at 5 mg/L. The surface active properties of BAC favor its adsorption to organic particles found in the anaerobic reactor, resulting in enhanced lysis and particulate matter solubilization, which in turn causes the release of organic nitrogen (as ammonia) and soluble organics (detected as sCOD).

Table 7. Performance of the BNR system during continuous operation with BAC-bearing poultry processing wastewater at a feed BAC concentration of 5 mg /L (Data from day 58 to 72).

Parameter	R_1	R_2	R_3	Effluent
pH	6.6 ± 0.2[a]	7.2 ± 0.1	6.8 ± 0.3	6.5 ± 0.5
TSS (mg/L)	156 ± 56	1140 ± 167	1324 ± 8	81 ± 71
VSS (mg/L)	144 ± 36	859 ± 53	1098 ± 21	58 ± 39
Soluble COD (mg/L)	772 ± 144	408 ± 40	381 ± 38	211 ± 54
VFAs (mg COD/L)	268 ± 99	ND	ND	ND
NH_3 (mg N/L)	100 ± 5	24 ± 4	0.5 ± 0.8	2 ± 1
NO_3^- (mg N/L)	ND[b]	ND	17 ± 5	20 ± 0.7
NO_2^- (mg N/L)	ND	ND	0.9 ± 0.7	0.8 ± 0.6

[a] Mean ± standard deviation ($n \geq 3$); [b] ND, not detected;

3.3.2 Operation at Increasing Feed BAC Concentrations

In order to assess the BNR system's response to a range of BAC concentrations, the poultry processing wastewater feed BAC concentration was increased stepwise to 10, 15, 30, 45, and 60 mg/L. Figure 17 shows the nitrogen species throughout the BNR system while treating the BAC-bearing poultry processing wastewater at stepwise increased feed BAC concentrations from 5 to 60 mg/L and Table 8 summarizes the BNR system performance during the same period.

During the stepwise increased poultry processing wastewater feed BAC concentration, a high nitrification efficiency was sustained at all BAC concentrations tested indicated by nitrogen species levels in the R_3 similar to those achieved during the BAC-free operation (Figure 17). The sustained high nitrification efficiency is attributed to an increased BAC degradation rate by the heterotrophic population in the aerobic reactor, which in turn reduced the extent of nitrifying population exposure to BAC. OUR analysis showed that the AOB and NOB biomass fractions of the aerobic reactor population were comparable to those found after the system recovery from the initial BAC exposure (2.21 ± 0.2 % and 2.85 ± 0.3 % for AOB and NOB, respectively; mean ± standard deviation, $n = 3$).

Table 9 shows the steady-state BAC concentration throughout the BNR system during the 530 days of operation with the BAC-bearing poultry processing wastewater and Figure 18 shows the BAC concentration throughout the BNR system during the same period. As seen in Table 9, beginning at a poultry processing wastewater feed BAC concentration of 30 mg/L, the BAC concentration in R_3 was higher than that observed during the initial exposure to BAC (1.5 ± 0.4 to 1.8±0.2 mg/L vs. 0.8±0.2 mg/L). In spite of the higher BAC concentration, nitrification was not affected, which indicates that the nitrifiers in the aerobic reactor became more resistant to BAC over time. This hypothesis was later examined as discussed in section 3.3.4.3, below.

Moreover, the effluent BAC concentration never exceeded 1.2±0.5 mg/L at a poultry processing wastewater feed BAC concentration of 60 mg/L, thus achieving a continuous BAC removal efficiency ≥ 98% at all feed BAC concentrations.

Table 8. Performance of the BNR system during continuous operation with BAC-bearing poultry processing wastewater at a feed BAC concentration from 10 to 60 mg/L (Data from day 87 to day 342).

Parameter	R_1	R_2	R_3	Effluent
pH	6.7 ± 0.1[a]	7.0 ± 0.1	7.0 ± 0.4	7.0 ± 0.4
TSS (mg/L)	167 ± 60	1187 ± 32	1309 ± 124	52 ± 2
VSS (mg/L)	154 ± 48	994 ± 54	1073 ± 80	42 ± 2
Soluble COD (mg/L)	668 ± 258	295 ± 138	273 ± 74	282 ± 15
VFAs (mg COD/L)	279 ± 21	ND	ND	ND
NH_3 (mg N/L)	90 ± 7	19 ± 3	0.9 ± 0.2	1 ± 0.5
NO_3^- (mg N/L)	ND [b]	1 ± 0.9	23 ± 5	20 ± 7
NO_2^- (mg N/L)	ND	ND	1.9 ± 0.9	1 ± 4

[a] Mean ± standard deviation ($n \geq 6$); [b] ND, not detected;

Table 9. Steady-state BAC concentration (mg/L) throughout the BNR system during operation with stepwise increased poultry processing wastewater feed BAC concentrations (Data from day 33 to day 381).

Feed	R_1	R_2	R_3	Effluent
5	4.8 ± 0.1[a]	0.5 ± 0.4	0.4 ± 0.3	0.3 ± 0.3
10	10.5 ± 0.3	0.5 ± 0.4	0.4 ± 0.3	0.3 ± 0.3
15	15.2 ± 0.5	1.7 ± 0.3	0.6 ± 0.2	0.6 ± 0.2
30	46.1 ± 1.6	2.4 ± 0.6	1.8 ± 1.1	1.1 ± 0.4
45	46.1 ± 1.5	6.7 ± 1.5	1.5 ± 0.4	0.5 ± 0.2
60	59.8 ± 2.1	9.7 ± 0.6	1.8 ± 0.2	0.4 ± 0.3

[a] Mean ± standard deviation ($n \geq 8$)

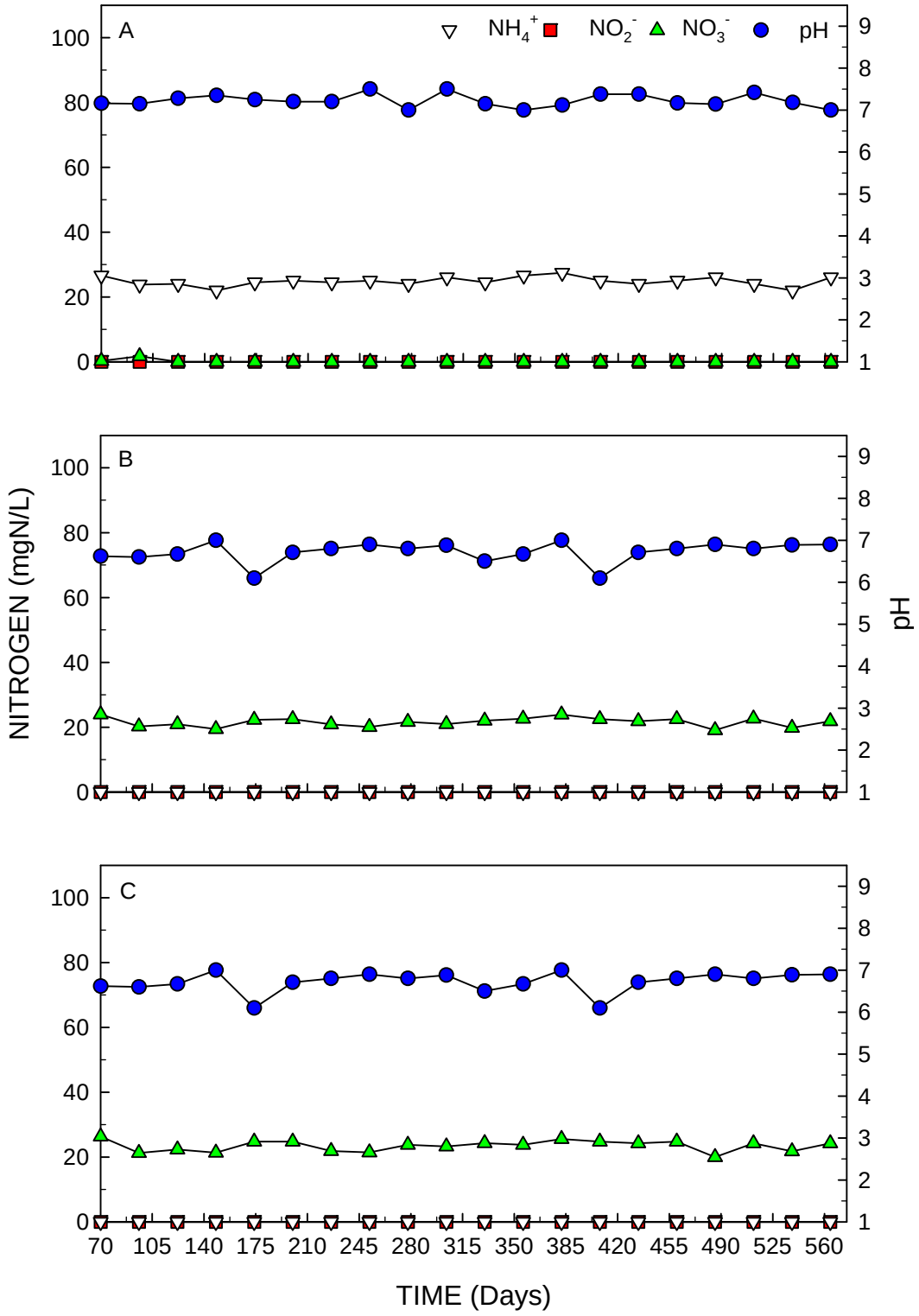


Figure 17. Nitrogen species and pH in the BNR system while treating the BAC-bearing poultry processing wastewater at stepwise increased feed BAC concentrations from 5 to 60 mg/L (A, R_2; B, R_3; and C, Effluent).

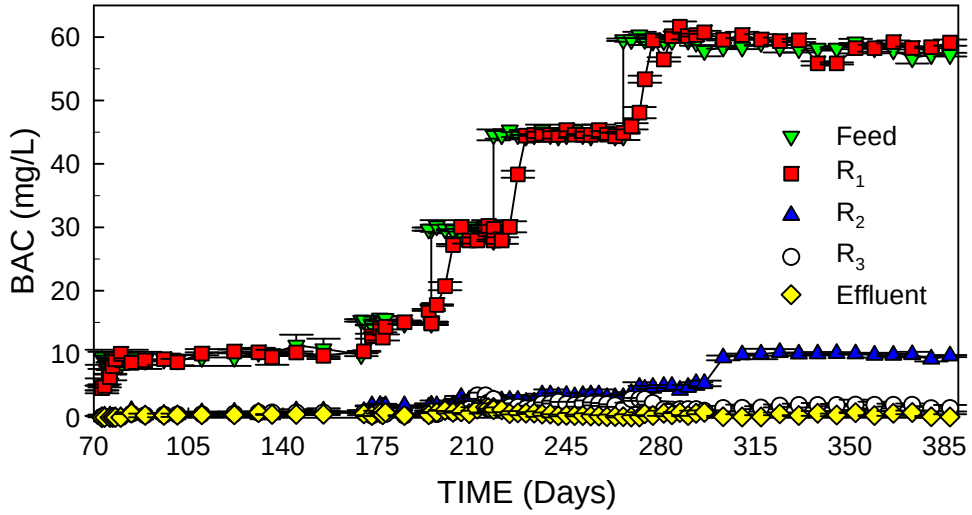


Figure 18. Total BAC concentration in the BNR system while treating the BAC-bearing poultry processing wastewater at stepwise increased feed BAC concentrations from 5 to 60 mg/L.

3.3.3 BNR System Resilience Test

To further examine the BNR operation and performance while treating a BAC-bearing wastewater, a step increase in the poultry processing wastewater feed BAC concentration was made to simulate an accidental spill in a poultry processing plant. This scenario is more likely to happen during upstream cleaning procedures in food processing facilities.

The poultry processing wastewater feed BAC concentration was increased from 60 to 120 mg/L, then back to 0 in 6.5 days, which is equal to one hydraulic retention time of the system. Figure 19 shows the BAC concentration throughout the BNR system during operation at a feed BAC concentration of 120 mg/L and the subsequent BAC-free feed period. The highest BAC concentrations, detected in the BNR system were after seven days from the time the feed BAC concentration was increased to 120 mg/L, were 22.6 ± 1.9, 2.2 ± 0.3, and 1.2 ± 0.5 mg/L in the R_2 and R_3 reactors, and the effluent, respectively. The BNR system performance remained identical to that attained before the step change in the feed BAC concentration, achieving ≥ 97% ammonia removal. The BAC concentration in R_3 was less than the previously identified limit for efficient nitrification (only reached 2.2±0.3 mg/L vs. 15 mg/L), which may explain the sustained nitrogen removal efficiency even at such a high BAC concentration in the poultry processing wastewater feed.

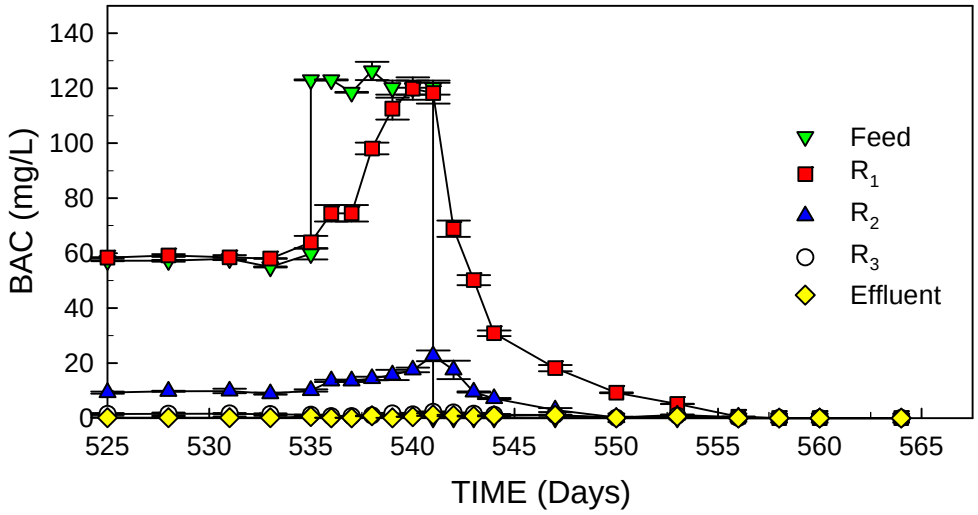


Figure 19. Total BAC concentration in the BNR system while treating the BAC-bearing poultry processing wastewater at a stepwise increase of the feed BAC concentrations from 60 to 120 mg/L.

3.3.4 Effect of BAC on the Performance of BNR System Components

In order to understand the degree and extent of BAC's (or QACs in general) fate and effect on the BNR performance, a series of batch assays were conducted using the mixed liquor of the laboratory-scale BNR system. The batch assays allow for independent examination of the performance of each BNR system component in the presence of BAC, thus contributing to a better understanding of the fate and effect of BAC in a BNR system.

3.3.4.1 Anaerobic Batch Assay

The assay testing the fate and effect of BAC in the anaerobic reactor (R_1) lasted for 25 hours. Three processes were followed in this assay: organic carbon solubilization, VFAs production, and ammonia release (ammonification). The sCOD concentration increased by 70, 81, 75, 77, 81, and 79 % for the culture series at 0, 5, 15, 30, 45, and 60 mg/L BAC, respectively, while the VFAs concentration increased by 30, 29, 24, 24, 27, and 29% for the same culture series. Acetate was the predominant VFA, followed by propionate and i- and n-butyric acid. The ammonia release in all BAC-amended culture series was higher than in the BAC-free culture series. The final ammonia concentration in the BAC-amended culture series was relatively the same, reaching 19.7 ± 0.4 mg N/L (mean ± standard deviation, n = 5), while the ammonia concentration in the BAC-free culture series was 16.9 mg N/L. The ammonia release in all culture series followed the same trend of sCOD production.

Under anaerobic conditions, the release of organic material and nitrogen from particulate matter is achieved through two solubilization steps: disintegration (non-biological) and hydrolysis by extracellular enzymes (Batstone et al., 2002). The increased sCOD production and ammonia release in the BAC-amended culture series is attributed to enhanced solubilization through BAC induced lysis. On the other hand, as previously reported (Tezel et al., 2006; Tezel et al., 2007), the production of VFAs in the BAC-amended culture series was not inhibited by BAC. The uninhibited VFAs production in the anaerobic reactor more likely resulted from metabolic activities unaffected by BAC. The BAC inhibitory effect is associated with inhibition

of respiratory enzymes present in microbial cellular membranes (Zhang et al., 2011). However, VFAs are produced through fermentative, non-respiratory activities (Rittmann and McCarty, 2001; Madigan and Martinko, 2006), which explains the observed lack of inhibition of the VFAs production.

BAC was completely recovered in all BAC-amended culture series at the end of the incubation period as shown in Figure 20. Therefore, BAC did not degrade under the fermentative conditions of reactor R_1. BAC is considered to be recalcitrant under anoxic and anaerobic conditions, as previously reported (Garcia et al., 2006; Tezel et al., 2006; Tezel et al., 2007; Tezel et al., 2008). Two exceptions are the modified Hofmann nucleophilic substitution reaction and the activation reaction by fumarate addition (Tezel and Pavlostathis, 2009; Tezel, 2009).

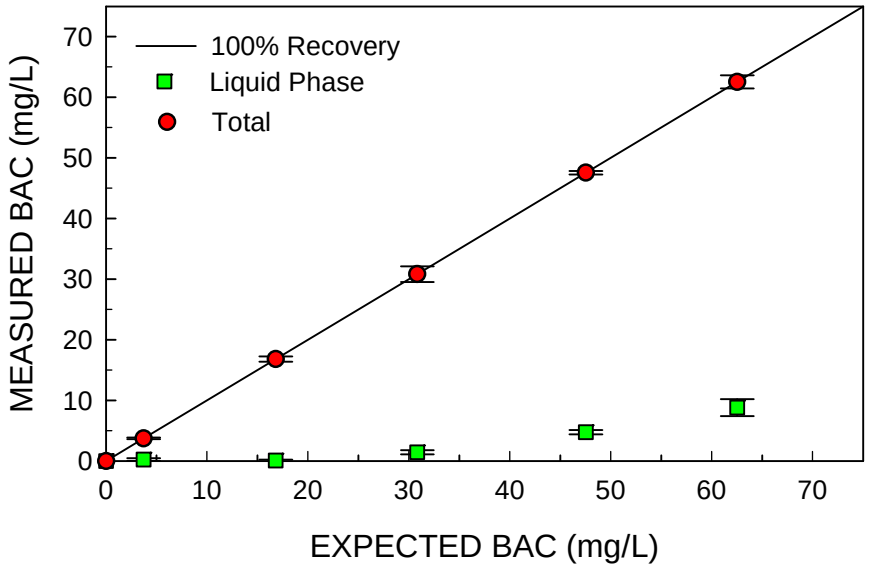


Figure 20. BAC phase distribution at the end of the anaerobic batch assay.

3.3.4.2 Batch Nitrification Assay

Using mixed liquor from the aerobic reactor (R_3) after 370 days of continuous operation when the poultry processing wastewater feed BAC concentration was 60 mg/L, a second batch assay was performed to assess the effect of BAC on nitrification. The assay lasted for 33 hours. Similarly to the previous nitrification assay which was conducted with BAC-unacclimated mixed liquor (Phase 1; section 3.2.11), a decrease in the final biomass concentration was observed in all BAC-amended culture series with increasing BAC concentration resulting from BAC-induced growth inhibition and cell lysis brought about by the BAC effect as an antimicrobial agent (Cross and Singer, 1994).

Figure 21A shows the extent of ammonia removal in all culture series at the end of the incubation period. Among all the culture series, the BAC-free, and the 5 and 15 mg/L BAC-amended culture series achieved complete ammonia removal and oxidation to nitrate within 21 h of incubation. The initial SARR was 60.2 ± 1.9, 58.9 ± 6.2, and 49.9 ± 7.4 mg N/g VSS · day for the BAC-free, and the 5 and 15 mg/L BAC-amended culture series, respectively. Complete inhibition of nitrification (≤ 2.5% ammonia removal) and a low initial SARR (≤ 4.4±5.2 mg N/g VSS · day) was observed in the remaining four BAC-amended culture series (20 to 45 mg/L BAC). Similarly to the previous nitrification assay, nitrite was never detected in any of the culture series. Figure 21B shows the relative SARR (i.e., SARR normalized to the BAC-free

culture series) for all culture series. Overall, after a long-term acclimation period, efficient nitrification was sustained in a BNR system while treating BAC-bearing poultry processing wastewater as long as the BAC concentration in the aerobic reactor was kept below 15 mg/L. Yang (2007) found that an acclimated, fed-batch aerobic reactor, treating a synthetic wastewater (dextrin and peptone) was capable of efficient nitrification at an initial BAC concentration of 20 mg/L.

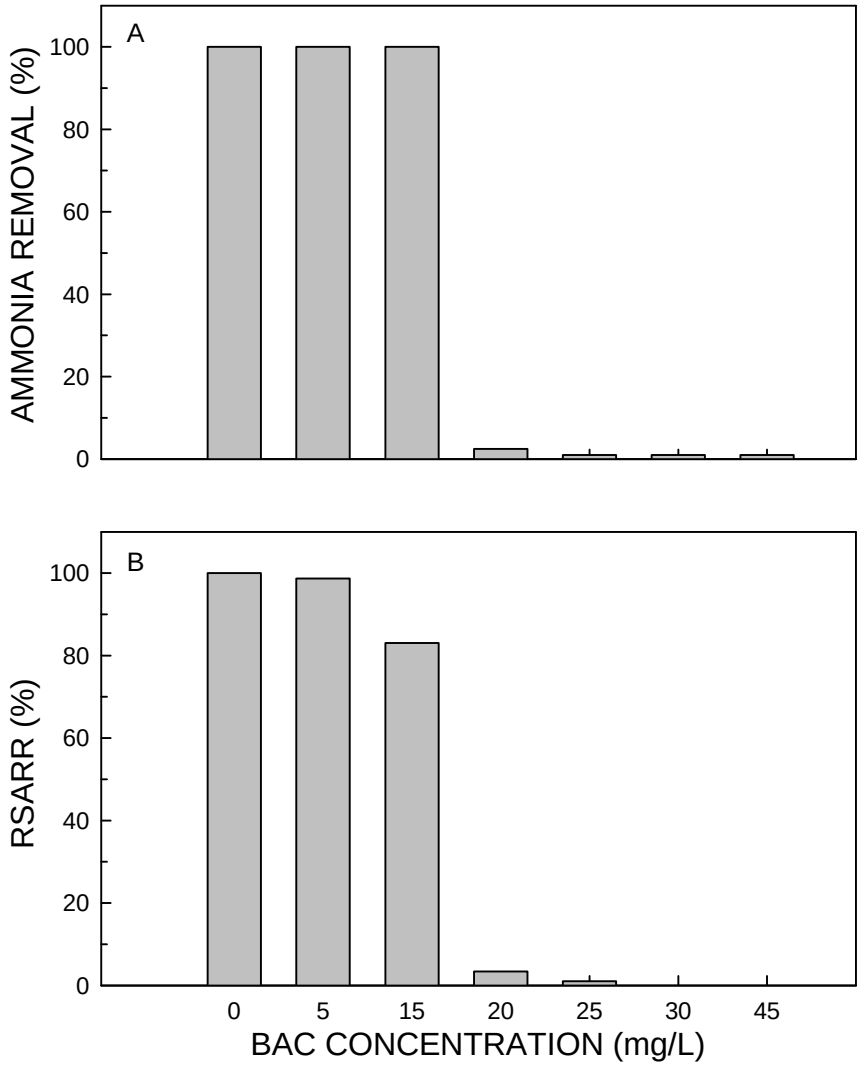


Figure 21. Extent of ammonia removal (A) and relative specific ammonia removal rate (RSARR) (B) for the culture series in the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 370 and a range of initial BAC concentration 0 – 45 mg/L.

An increase in the sCOD concentration was observed in the BAC-amended culture series at an initial BAC concentration ≥ 20 mg/L. Similarly to the anaerobic assay (see section 3.3.4.1, above), the increase in the sCOD is attributed to BAC-induced inhibition of cell growth and cell lysis. Figure 22 shows the relative SCUR (i.e., SCUR normalized to the BAC-free culture series) for all culture series. BAC inhibited sCOD utilization by the heterotrophic population in all BAC-amended culture series. The BAC inhibitory effect on the organic carbon utilization by activated sludge mixed cultures was previously reported (Sutterlin et al., 2008; Zhang et al.,

2011). For comparison purposes, Table 10 summarizes the results of the nitrification inhibition assays performed with the aerobic reactor (R_3) mixed liquor before, during, and after BAC exposure of the BNR system.

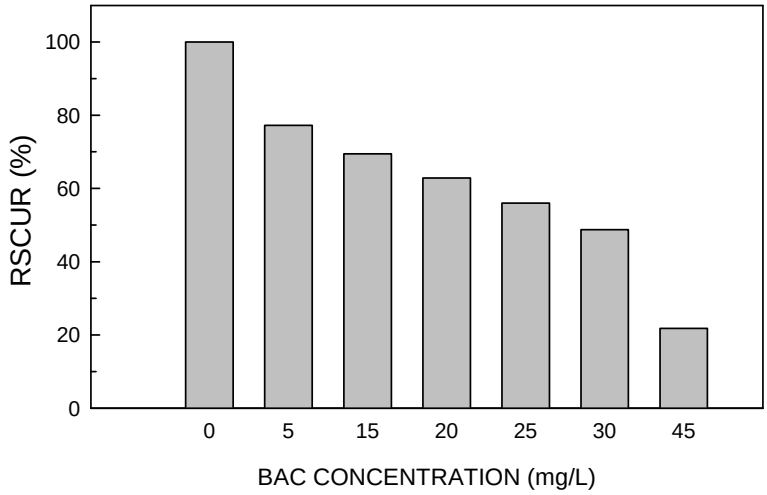


Figure 22. Relative specific sCOD utilization rate (RSCUR) for the seven culture series used in the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 370 and a range of initial BAC concentration 0 – 45 mg/L.

Table 10. Results of nitrification inhibition assays performed with the aerobic reactor mixed liquor before, during, and after BAC exposure (AR, ammonia removal; SARR, initial specific ammonia removal rate; RSARR, SARR relative to the BAC-free culture series).

BAC Exposure	Initial BAC (mg/L)	AR (%)	SARR (mg N/g VSS-day)	RSARR (%)	Recovered BAC (mg/L)
Before	0	100	75.3 ± 4.5[a]	100	-
	5	100	46.1 ± 11.8	61.2	ND[b]
	10	43	4.3 ± 0.4	5.7	1.0 ± 0.2
	15	28	4.7 ± 1.2	6.2	1.1 ± 0.1
	25	5	1.2 ± 0.4	1.6	2.7 ± 0.7
	50	-	1.1± 0.2	1.6	4.7 ± 0.3
	75	-	-	-	7.0 ± 2.0
	100	-	-	-	89.6 ± 17.4
During	0	100	60.2 ± 1.9	100.0	-
	5	100	58.9 ± 6.2	98.7	0.5 ± 0.1
	15	100	33.7 ± 7.4	83.1	1.3 ± 0.3
	20	3	4.4 ± 5.2	3.4	1.18 ± 0.2
	25	2	1.4 ± 2.2	1.0	1.32 ± 0.3
	30	0	-	-	2.2 ± 0.1
	45	0	-	-	2.6 ± 0.3
After	0	100	40.7 ± 6.2	100	-
	5	100	37.3 ± 1.1	91.6	ND
	10	100	33.6 ± 2.1	82.6	3.7 ± 0.3
	15	100	22.7 ± 3.3	55.7	8.3 ± 0.4

[a] Mean ± standard deviation ($n \geq 3$); [b] ND, not detected;

Based on total BAC measurements performed at 20 h of incubation, more than 95% of the initially added BAC was removed and further BAC removal was not detected by the end of the incubation period. Figure 23 shows the time course of total and liquid-phase BAC concentrations during the batch incubation period for all six BAC-amended culture series. Similar to the previous nitrification assay, nitrification did not recover in the inhibited culture series after BAC removal during the last 10 h of incubation. BAC biotransformation rates in the BAC-exposed mixed liquor were much higher than those observed with the unexposed aerobic reactor mixed liquor (i.e., ≥ 95% BAC removal within 20 h vs. 60 h), which confirms that the heterotrophic population indeed achieved high BAC biotransformation rates after a long-term exposure to BAC, which is attributed to both acclimation and enrichment.

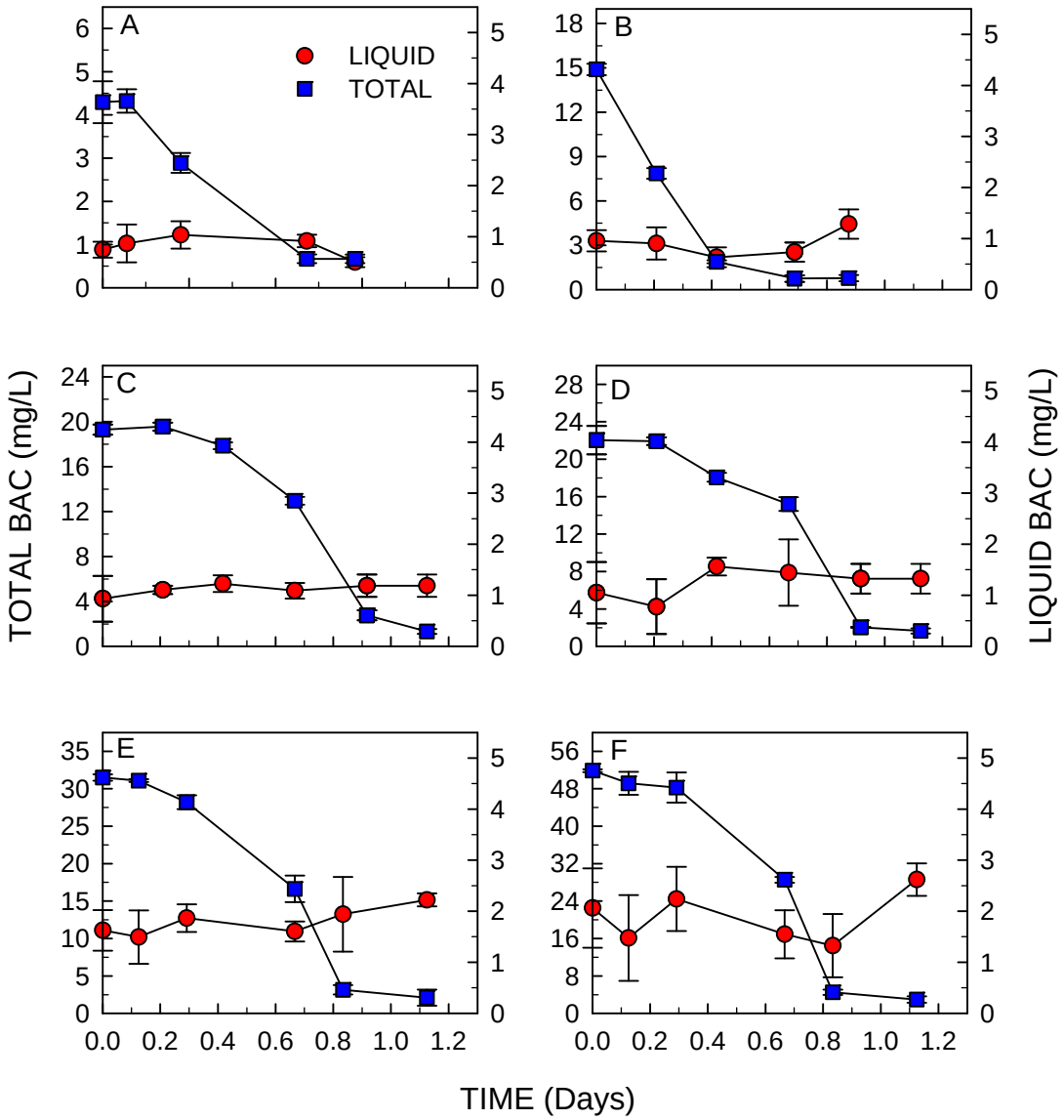


Figure 23. Time course of total and liquid-phase BAC concentration during the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 370 and at initial BAC concentrations of 5 (A), 15 (B), 20 (C), 25 (D), 30 (E), and 45 (F) mg/L. Error bars represent one standard deviation of the means (n = 3).

As a result of fast BAC biotransformation, the extent of nitrification inhibition by BAC in the 5 and 15 mg/L BAC-amended culture series was limited, evident by the complete ammonia removal and the higher SARR values obtained as compared to those achieved in the first batch nitrification assay conducted with BAC-unacclimated mixed liquor (Table 10). However, the higher BAC removal rates did not prevent nitrification inhibition at initial BAC concentrations ≥ 20 mg/L.

3.3.4.3 Post BAC Exposure Batch Nitrification Assay

As discussed in the previous section, after an acclimation period, efficient nitrification was sustained in the BNR system while treating the BAC-bearing wastewater. High BAC biotransformation rates in the aerobic reactor only indicate an acclimated and enriched heterotrophic population in the system. Until this point, the only indication that the nitrifying population was also acclimated, perhaps by acquiring resistance to BAC, is the fact that nitrification took place even at BAC concentrations in the aerobic reactor higher than those observed during the initial exposure to BAC (1.5 ± 0.4 to 1.8±0.2 mg/L vs. 0.8±0.2 mg/L). In order to confirm this observation, a batch nitrification assay was conducted using the mixed liquor of the aerobic reactor collected at day 660 of continuous operation, when the BNR system was treating BAC-free poultry processing wastewater for 100 days.

The assay lasted for 28 hours. Figure 24 shows the time course of nitrogen species in the BAC-free and BAC-amended cultures series and Figure 25A shows the extent of ammonia removal in all culture series. Similarly to the nitrification batch assay conducted during the system operation with BAC-bearing poultry processing wastewater, the BAC-free and the 5 and 15 mg/L BAC-amended culture series achieved complete ammonia removal and oxidation to nitrate within 28 hours of incubation. The initial SARR was 40.7 ± 6.2, 37.3 ± 1.1, 33.6 ± 2.1, and 22.7 ± 3.3 mg N/g VSS · day for the BAC-free and the 5, 10 and 15 mg/L BAC-amended culture series, respectively (Table 10).

Figure 25B shows the relative SARR (i.e., SARR normalized to the BAC-free culture series) for all culture series. What was different in this batch assay is the rate of BAC biotransformation, which was drastically reduced as shown in Figure 26 and was comparable to the initial BAC biotransformation rates obtained with the BAC-unexposed aerobic reactor mixed liquor (see section 3.2). Moreover, the 10 and 15 mg/L BAC-amended culture series in this assay achieved 100% ammonia removal in the presence of BAC, while the 10 and 15 mg/L BAC-amended culture series in the previous nitrification assay conducted with BAC-unacclimated mixed liquor (see section 3.2.1.1) achieved only 43 and 28% ammonia removal, respectively, in spite the fact that BAC was completely removed in these culture series (Table 10).

The above observations indicate that the nitrifying population in the aerobic reactor did indeed acquire BAC resistance as a result of prolonged exposure to BAC in the BNR system. Additionally, although BAC exposure was terminated, the nitrifiers remained acclimated to BAC longer than the heterotrophs.

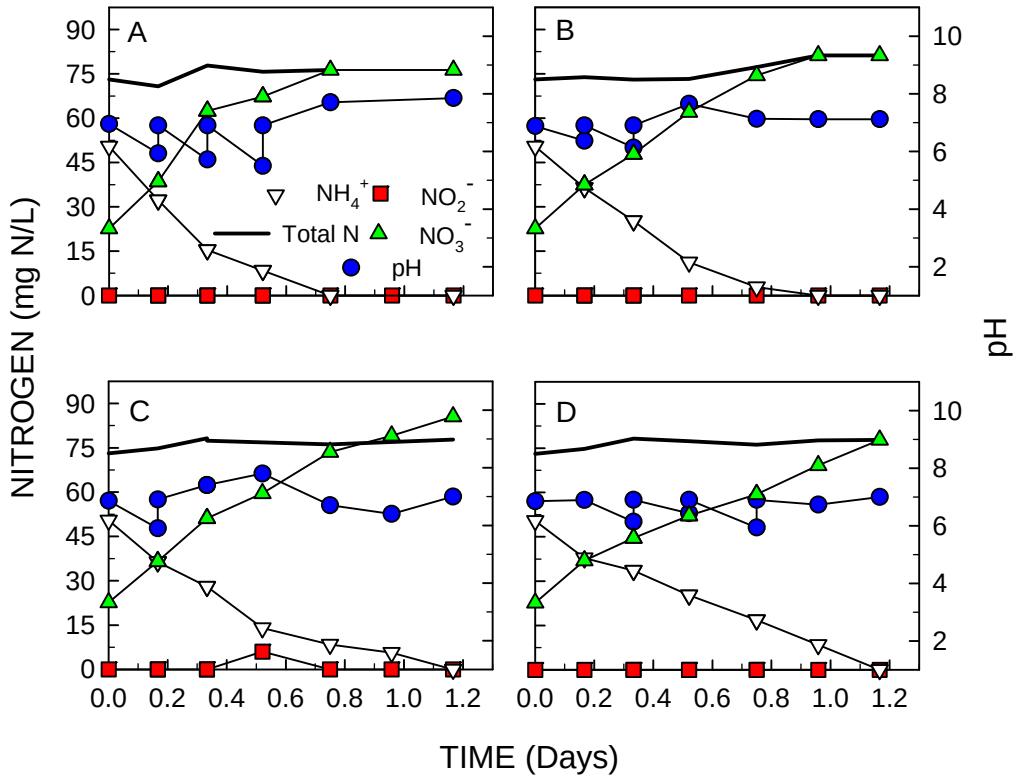


Figure 24. Time course of nitrogen species and pH variation during the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 660 and at initial BAC concentrations of 0 (A), 5 (B), 10 (C), and 15 (D) mg/L.

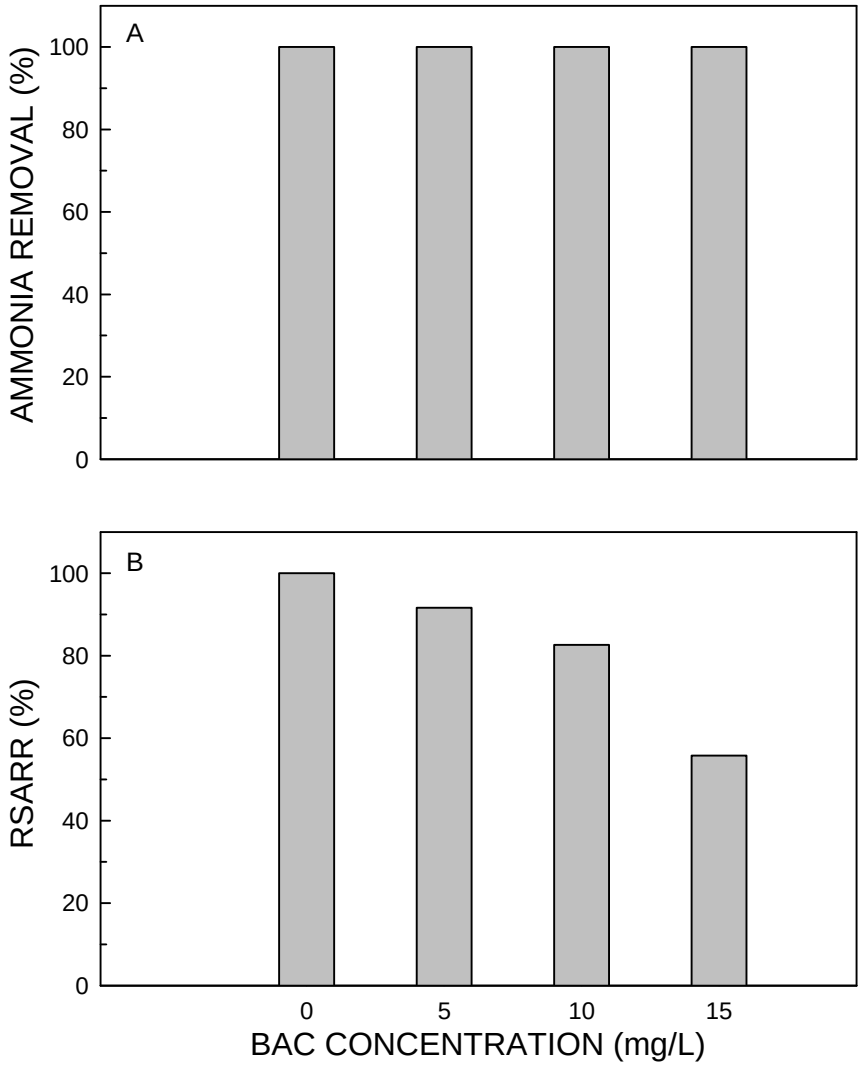


Figure 25. Extent of ammonia removal (A) and relative specific ammonia removal rate (RSARR) (B) for the culture series in the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 660 and a range of initial BAC concentration 0 – 15 mg/L.

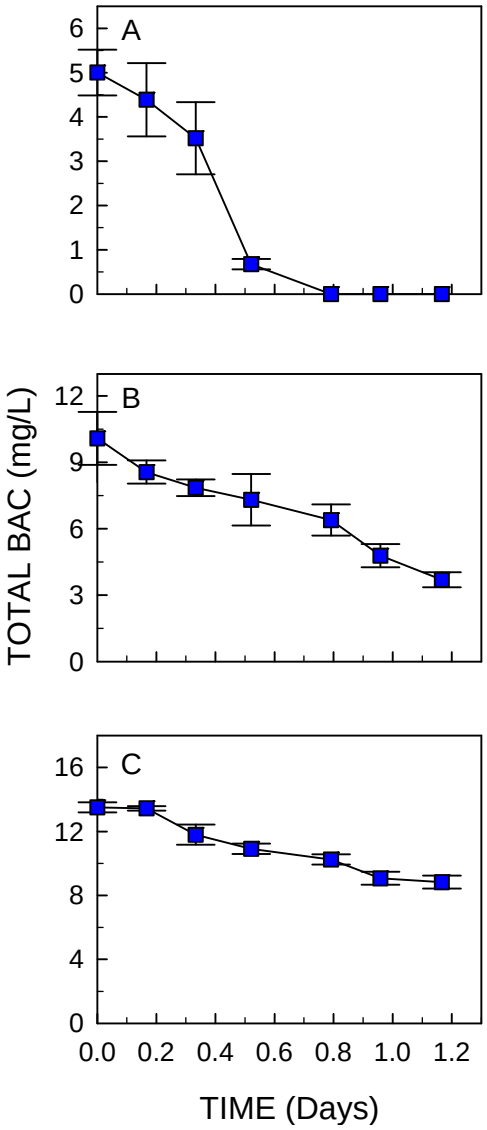


Figure 26. Time course of total BAC concentration during the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 600 at initial BAC concentrations of 5 (A), 10 (B), and 15 (C) mg/L. Error bars represent one standard deviation of the means (n = 3).

3.3.4.4 Batch Denitrification Assay

The assay testing the effect of BAC on denitrification lasted for 1.8 days (42 hours). Figure 27 shows the time course of nitrogen species in all eight culture series. All added nitrate was fully reduced to either dinitrogen gas (≥95%) or ammonia, which indicates that the DNRA process was also active in these cultures. Neither nitric oxide nor nitrous oxide was detected in any culture series.

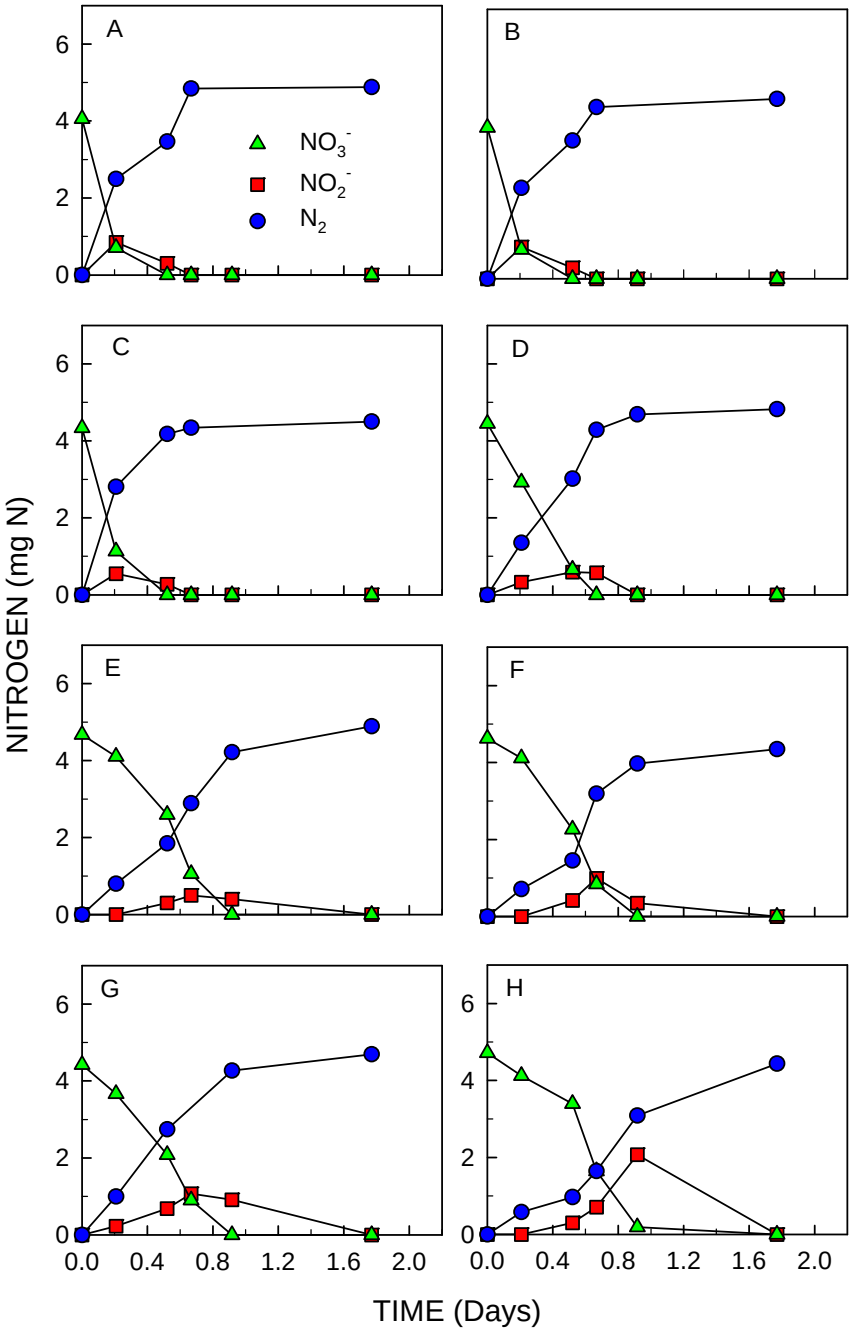


Figure 27. Time course of nitrogen species mass per bottle during the batch denitrification assay conducted with the anoxic reactor (R_2) mixed liquor collected at day 400 and at initial BAC concentrations of 0 (A), 5 (B), 10 (C), 15 (D), 20 (E), 25 (F), 30 (G), and 45 (H) mg/L.

Figure 28A shows the nitrogen species distribution at the end of the incubation period. As observed in the nitrification assay, for all BAC-amended culture series a decrease in the final biomass concentration was observed with increasing BAC concentration, resulting from BAC-induced microbial growth inhibition and cell lysis.

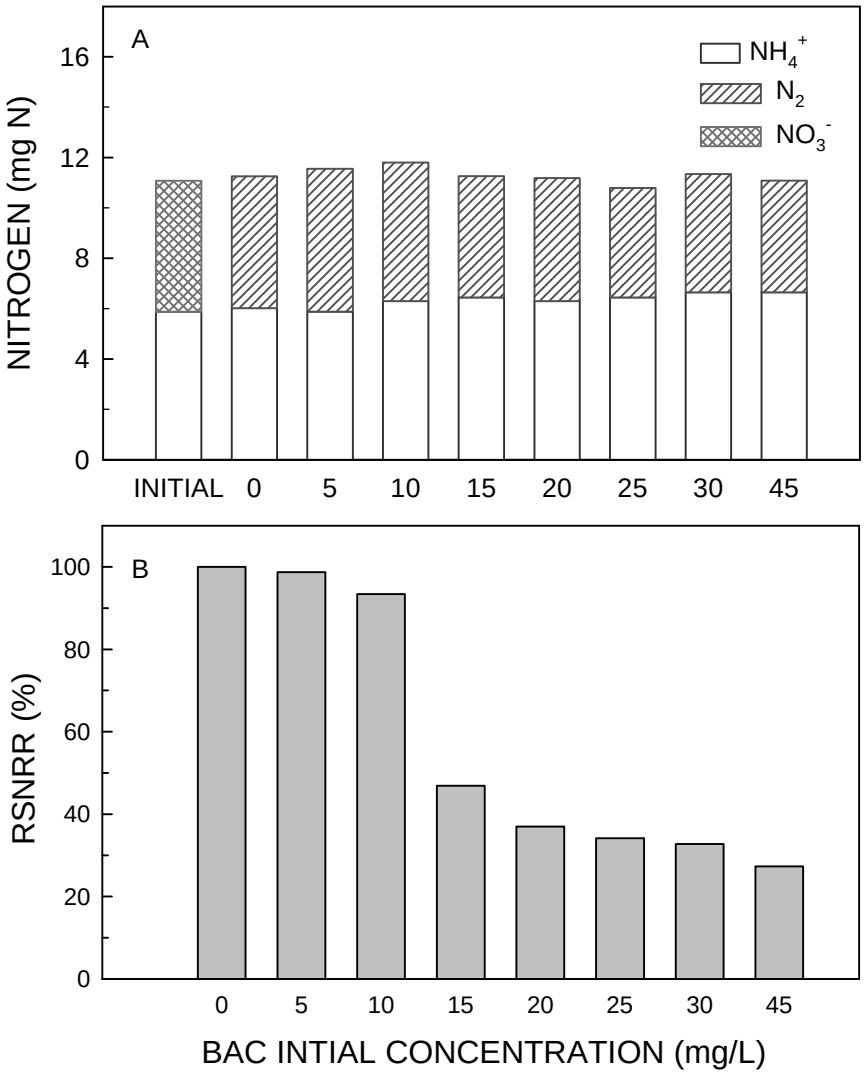


Figure 28. Nitrogen species distribution at the end of the incubation period (A) and relative specific nitrate removal rate (RSNRR) (B) in the batch denitrification assay conducted with anoxic reactor (R_2) mixed liquor, collected on day 400, and a range of initial BAC concentration 0 – 45 mg/L.

The initial SNRR of the BAC-free culture series was 198.1 ± 5.1 mg N/g VSS-day, while BAC decreased the initial SNRR in the BAC-amended culture series to 195.6 ± 5.3, 185.0 ± 9.1, 92.8 ± 0.2, 73.3 ± 11.6, 67.7 ± 11.2, 64.9 ± 6.4, and 54.1 ± 15.0 mg N/g VSS-day at an initial BAC concentration of 5, 10, 15, 20, 25, 30, and 45 mg/L, respectively. Figure 28B shows the relative SNRR of the culture series in this assay. At BAC concentrations of 15 mg/L and above, the decrease in the nitrate reduction rate was substantial (Figure 28B). Similarly to the initial

denitrification assay (i.e., before exposure to BAC; see section 3.2.1.2, above), transient accumulation of nitrite was observed at initial BAC concentrations ≥ 25 mg/L. The SNRR values of the BAC-exposed denitrifying mixed liquor were higher than the SNRR values of the BAC-unexposed denitrifying mixed liquor cultures (section 3.2.1.2) at BAC concentrations ≤ 20 mg/L, and the remaining culture series had marginally lower SNRR values.

Similarly to the previous denitrifying assay, all added BAC was fully recovered at the end of the incubation period, which indicates that BAC did not degrade under the conditions of the denitrification assay.

3.3.4.5 BAC Biotransformation Assay

The assay examining BAC biotransformation under aerobic conditions lasted for 2 days. At the time this assay was conducted, the BNR system was operating with BAC-bearing poultry processing wastewater at a feed BAC concentration of 60 mg/L (day 373). The collected aerobic reactor mixed liquor was aerated for 24 hours to fully remove any residual BAC and/or possible BAC metabolites present before BAC was reintroduced and sample collection for BAC metabolites analysis commenced. Figure 29 shows the time course of BAC and detected BAC metabolites during the course of the assay.

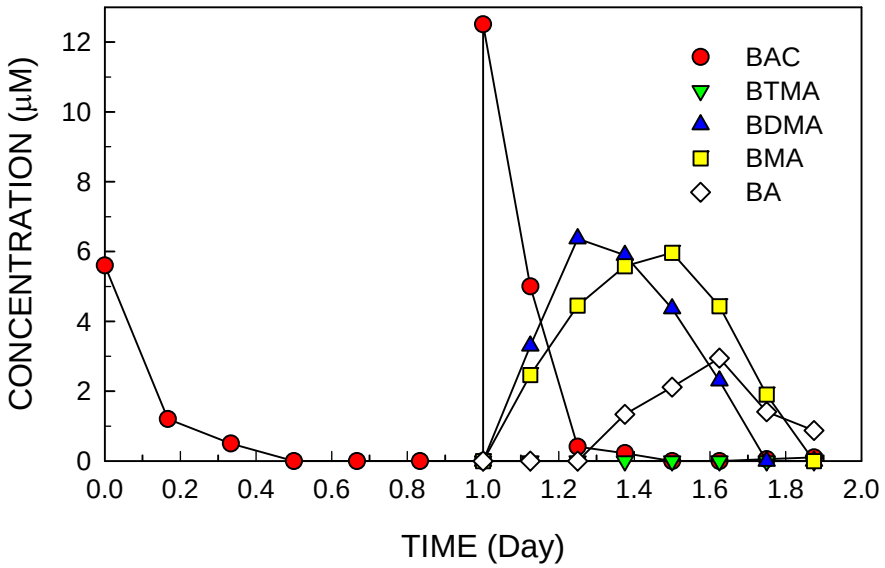


Figure 29. Time course of BAC and detected BAC metabolites during the BAC biotransformation assay conducted with aerobic reactor (R_3) mixed liquor collected at day 373.

BAC was transformed without lag yielding benzyl dimethyl amine (BDMA), benzyl methyl amine (BMA), and benzyl amine (BA), while benzyl trimethyl amine (BTMA) was not detected in any of the collected samples. The detected simultaneous production and transformation of BAC and the three intermediates suggests that the BAC biotransformation reactions were taking place simultaneously. Based upon previous observations, the BAC biotransformation followed the pathway reported by Patrauchan and Oriel (2003), where BAC biotransformation begins by the fission of the central C_{alkyl}-N bond resulting in BDMA, which in turn undergoes successive N-demethylations to yield BMA and BA. Tezel (2009) reported a different pathway for BAC aerobic biotransformation, where tetradecyl benzyl dimethyl ammonium (C_{14}BDMA) underwent a dealkylation reaction resulting in tetradecanoate and

BDMA, which was further transformed to dimethyl amine and benzoic acid by a debenzylation reaction. It should be noted that BAC biotransformation reported by Tezel (2009) was achieved with a highly-enriched culture which was sustained fed-batch with BAC as the sole carbon and energy source for over two years, supplemented with stoichiometric levels of NH_4NO_3. Figure 30A shows the proposed pathways for BAC in the BNR system aerobic reactor, while Figure 30B shows an alternative pathway reported by Tezel (2009) for a highly enriched BAC-degrading culture.

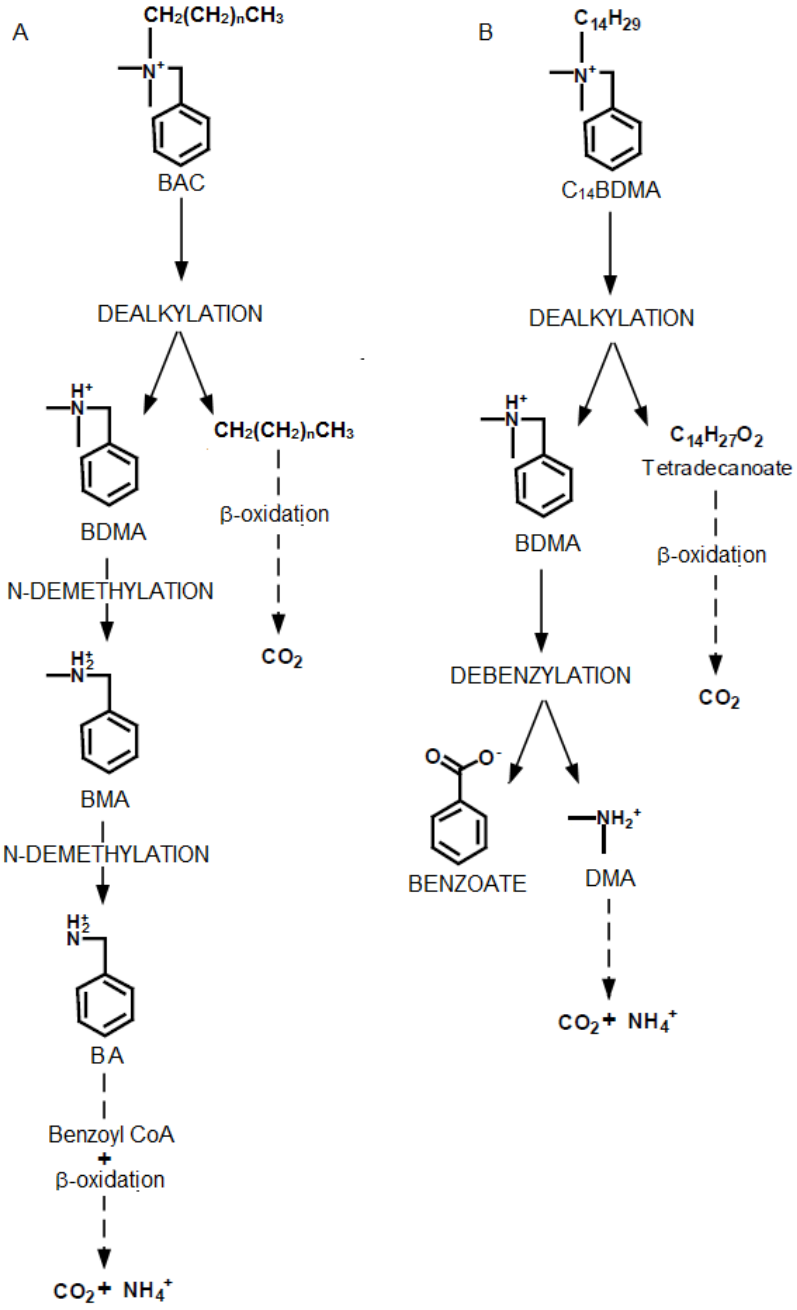


Figure 30. Proposed BAC biotransformation pathways as observed in the BNR system aerobic reactor (A) and reported by Tezel (2009) (B).

3.3.4.6 BAC Phase Distribution

BAC phase distribution data from the adsorption assays conducted with the poultry processing wastewater feed and the anaerobic, anoxic and aerobic reactors mixed liquors are shown in Figure 31. The BAC phase distribution at the end of the anaerobic assay was used to assess the BAC phase distribution in the anaerobic reactor (R_1). The biomass concentration was not altered for the adsorption assay in order to simulate the operational conditions in the BNR system. The VSS concentrations were 92 ± 20, 406 ± 17, 938 ± 71, and 1073 ± 94 mg/L (mean ± standard deviation, $n \geq 6$) for the poultry processing wastewater feed and the anaerobic, anoxic, and aerobic reactors mixed liquor, respectively.

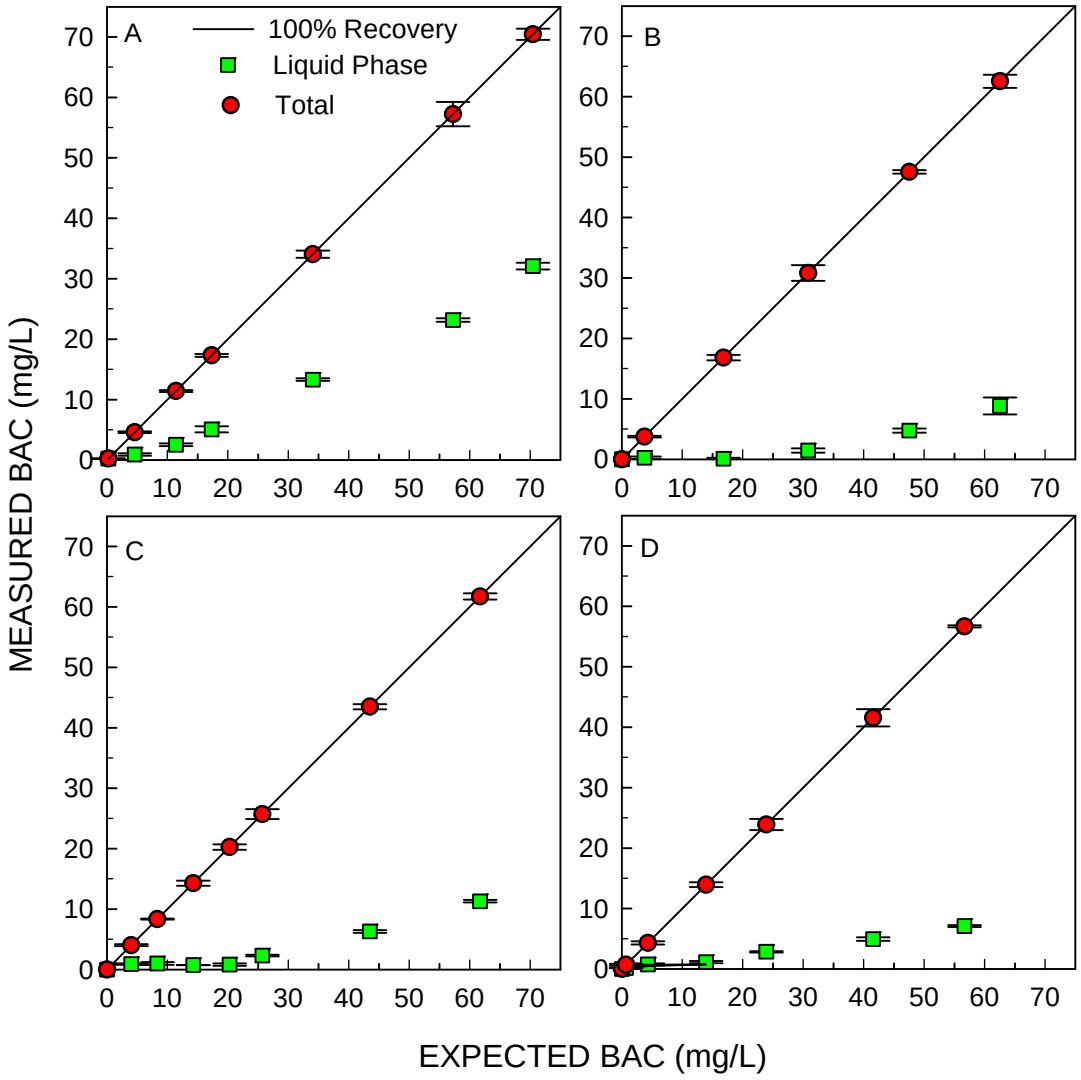


Figure 31. BAC phase distribution in the poultry processing wastewater feed (A), and the anaerobic reactor (B), anoxic reactor (C), and aerobic reactor (D) mixed liquors after 24-hours equilibration. Error bars represent one standard deviation of the means (n = 3).

The poultry processing wastewater feed had the highest liquid-phase BAC concentration because it had the lowest VSS concentration. On the other hand, the anaerobic reactor mixed liquor had a liquid-phase BAC concentration similar to that in the anoxic and aerobic reactors despite the much lower VSS concentration in the anaerobic reactor, a result that indicates a much higher sorption capacity of the anaerobic reactor biomass.

BAC phase distribution data were fitted to the Freundlich isotherm and the values of both the adsorption capacity (K_F) and intensity parameter (exponent; n) are listed in Table 11, while Figure 32 shows the BAC phase distribution in the four BNR system components. The values of both the adsorption capacity and exponent are comparable to previously reported values for BAC adsorption (Tezel, 2009; Ismail et al., 2010), and differ from others (Zhang et al., 2011; Ren et al., 2011). The BAC used in this study contained three homologs with different alkyl chain length (see section 2.2.2), whereas single BAC compounds were used by Zhang et al. (2011) and Ren et al. (2011).

Table 11. Freundlich isotherm equation coefficients[a].

Adsorbent	K_F ((mg/g VSS)(L/mg)n)	n (-)	r^2 (-)
Feed	63.9 ± 9.0[a]	0.54 ± 0.05	0.968
R_1 Mixed Liquor	125.3 ± 22	0.42 ± 0.09	0.931
R_2 Mixed Liquor	11.4 ± 2.4	0.69 ± 0.09	0.989
R_3 Mixed Liquor	11.0 ± 1.8	0.69 ± 0.07	0.975

[a] Best fit ± standard error; number of data points ≥ 5.

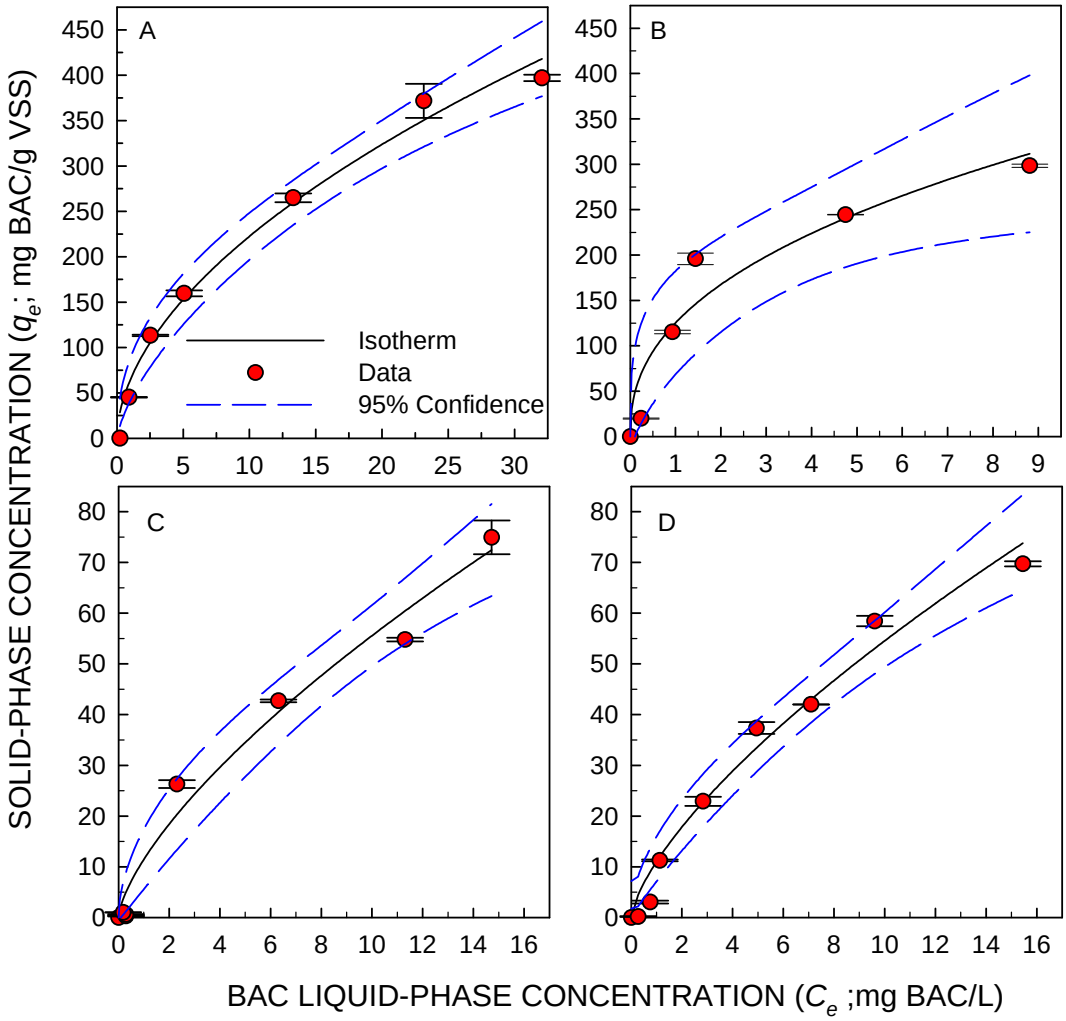


Figure 32. Solid- and liquid-phase BAC concentration in the poultry processing wastewater feed (A), and the anaerobic reactor (B), anoxic reactor (C), and aerobic reactor (D) mixed liquors after 24-hours equilibration. Error bars represent one standard deviation of the means (n = 3). Broken lines represent 95% confidence intervals.

The difference in BAC adsorption behavior between the poultry processing wastewater feed and the anaerobic, anoxic, and aerobic reactors mixed liquors is evident from the values of both the adsorption capacity (K_F) and intensity parameter (exponent; n). The adsorption capacity for the anaerobic reactor mixed liquor is almost double that obtained with the poultry processing wastewater feed, which indicates that the anaerobic reactor biomass was more heterogeneous than the surface of the poultry processing wastewater feed solids (Aksu et al., 2002). On the other hand, the intensity parameter was slightly lower for the anaerobic reactor compared to the poultry processing wastewater feed indicating that adsorption to the anaerobic reactor biomass is more favorable compared to the poultry processing wastewater feed solids (Aksu et al., 2002; Babakhouya et al., 2010).

For the anoxic and aerobic reactors, both the adsorption capacity and exponent values were almost identical because both reactors share the same mixed liquor and supernatant, albeit under different environmental conditions. Compared to the poultry processing wastewater feed and anaerobic reactor, the anoxic/aerobic biomass surface is more homogenous, indicated by a lower Freundlich adsorption capacity. The largest fraction of solids in the anoxic/aerobic reactors is comprised of microbial biomass, which explains the higher surface homogeneity. On the other hand, the higher value of the Freundlich exponent for the anoxic/aerobic reactors indicates that BAC adsorption is less favorable to the reactors biomass compared to the poultry processing wastewater feed solids and anaerobic reactor biomass.

BAC adsorption plays an important role in the degree and extent of BAC inhibition. The adsorption behavior determines the distribution of BAC in the system between the solid and liquid phases, changing the microbial population exposure level and ultimately their susceptibility to BAC (Hajaya, 2011).

3.4 BNR System Operation – Phase 3

3.4.1 BAC-Free Operation

The BAC pre-exposed system continuously treated BAC-amended poultry processing wastewater feed at increasing concentrations of BAC from 5 to 120 mg BAC/L for 511 days (Phase 2). BAC was removed from the system feed, and the system was operated with BAC-free poultry processing wastewater for 145 days at 22°C. The system maintained its nitrogen removal efficiency during this time. On day 691 the operating temperature of the system was dropped step-wise to 10°C within 8 days and the system was maintained at 10°C till the end of Phase 3 (Figure 33).

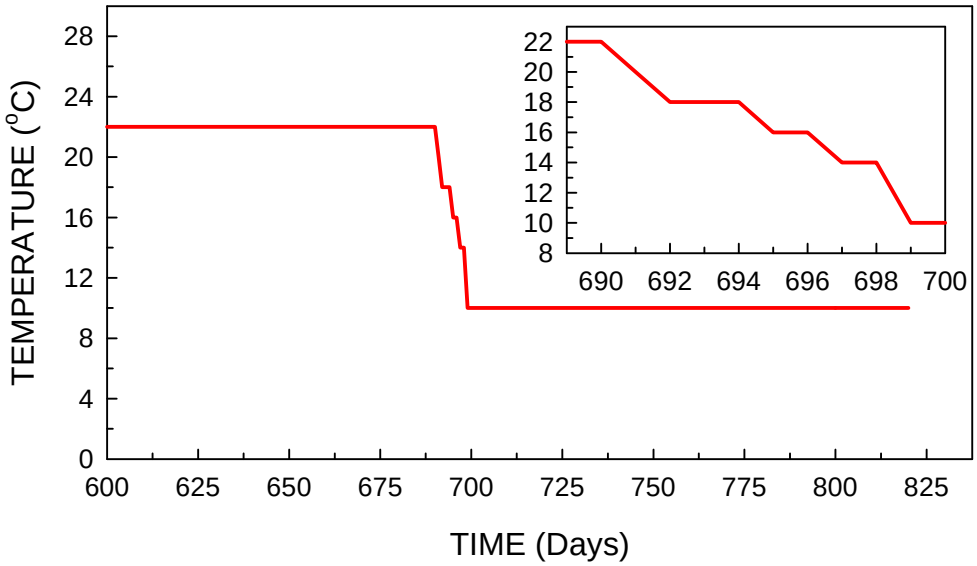


Figure 33. Temperature profile of the BNR system housed in a controlled temperature room.

Figure 34 shows the nitrogen species in the BAC pre-exposed BNR system anoxic and aerobic reactors during the transition in operating temperature from 22 to 10°C. On day 708, a decrease in ammonia concentration in the anoxic reactor (R2) was detected and continued to drop reaching a steady state value of 10.5 ± 1.6 mg N/L. Simultaneously, nitrate was detected in the anoxic reactor at concentration levels reaching 41.3 mg N/L. Nitrite was never detected. The

decrease in ammonia concentration is attributed to a reduced fermentation rate in the aerobic reactor (R1) brought about by the reduction in operational temperature as the VFAs concentration in the anaerobic reactor effluent dropped from 279 ± 21 mg COD/L to undetected levels. The reduction in readily degradable organic electron donor in the anaerobic reactor (highlighted by reduced levels of VFAs and ammonia concentrations) more likely caused incomplete denitrification in the anoxic reactor and ultimately led to nitrate accumulation. Similarly to the behavior of the anoxic reactor, the nitrate concentration increased in the aerobic reactor and reached 55.2 mg N/L, while nitrite was never detected. The BNR system nitrogen removal efficiency dropped to 52.5% while operating at 10°C with a QAC-free poultry processing wastewater feed.

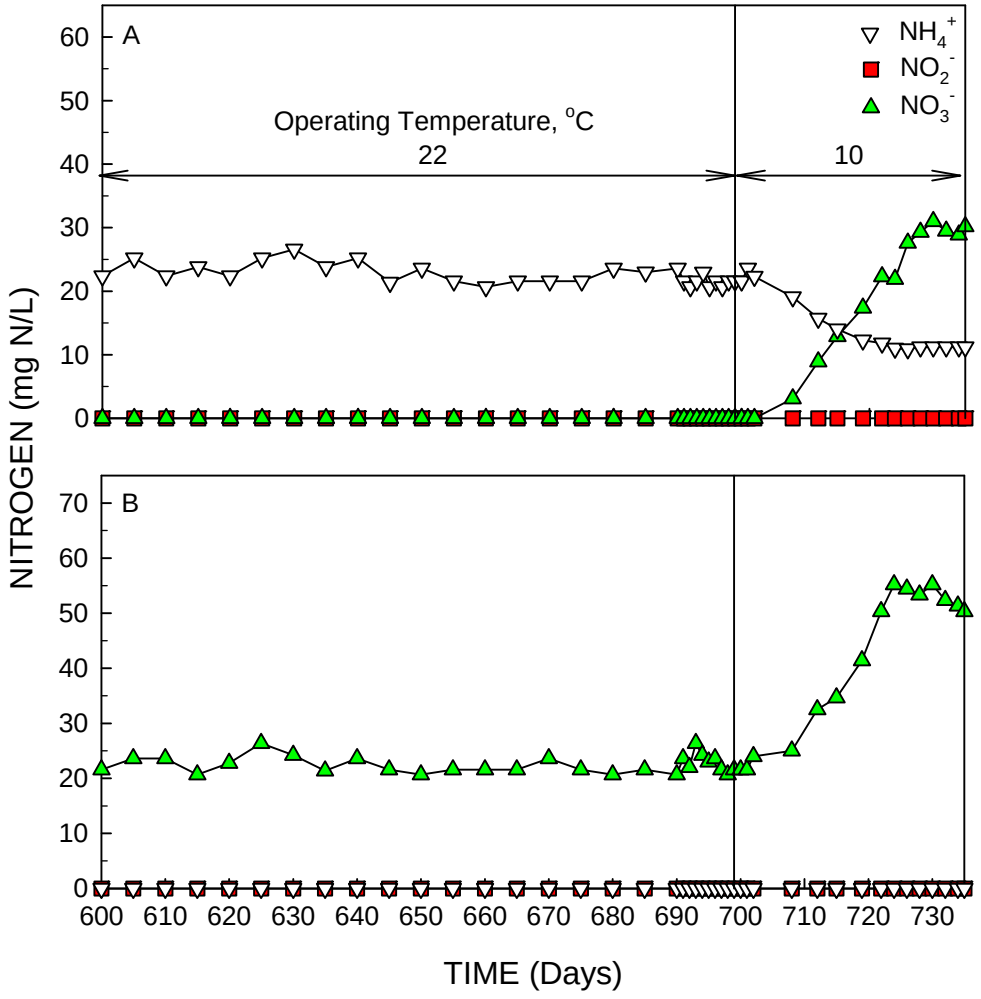


Figure 34. Nitrogen species in the BAC pre-exposed BNR system anoxic (A) and aerobic (B) reactors during the transition in operating temperature from 22 to 10°C (BAC-free feed).

3.4.2 BAC-Amended Feed Operation

On day 750, BAC was introduced into the BNR system poultry processing wastewater feed at a concentration of 5 mg/L. Figure 35 shows the nitrogen species in the BNR system anoxic and aerobic reactors while operating with BAC-amended feed. The BNR system performance did not change in the 16 days of operation with BAC-amended feed at 5 mg/L BAC, except for a period of time when for 2 days an old stored feed was used which had an elevated ammonia concentration (99.4 mg N/L) and was more likely rich in VFAs. During this period, the nitrate concentration in the anoxic reactor decreased significantly to 7.4 mg N/L and ammonia was only temporally detected (Figure 35), which might indicate reduced ammonia removal rates brought about by the reduction in operational temperature.

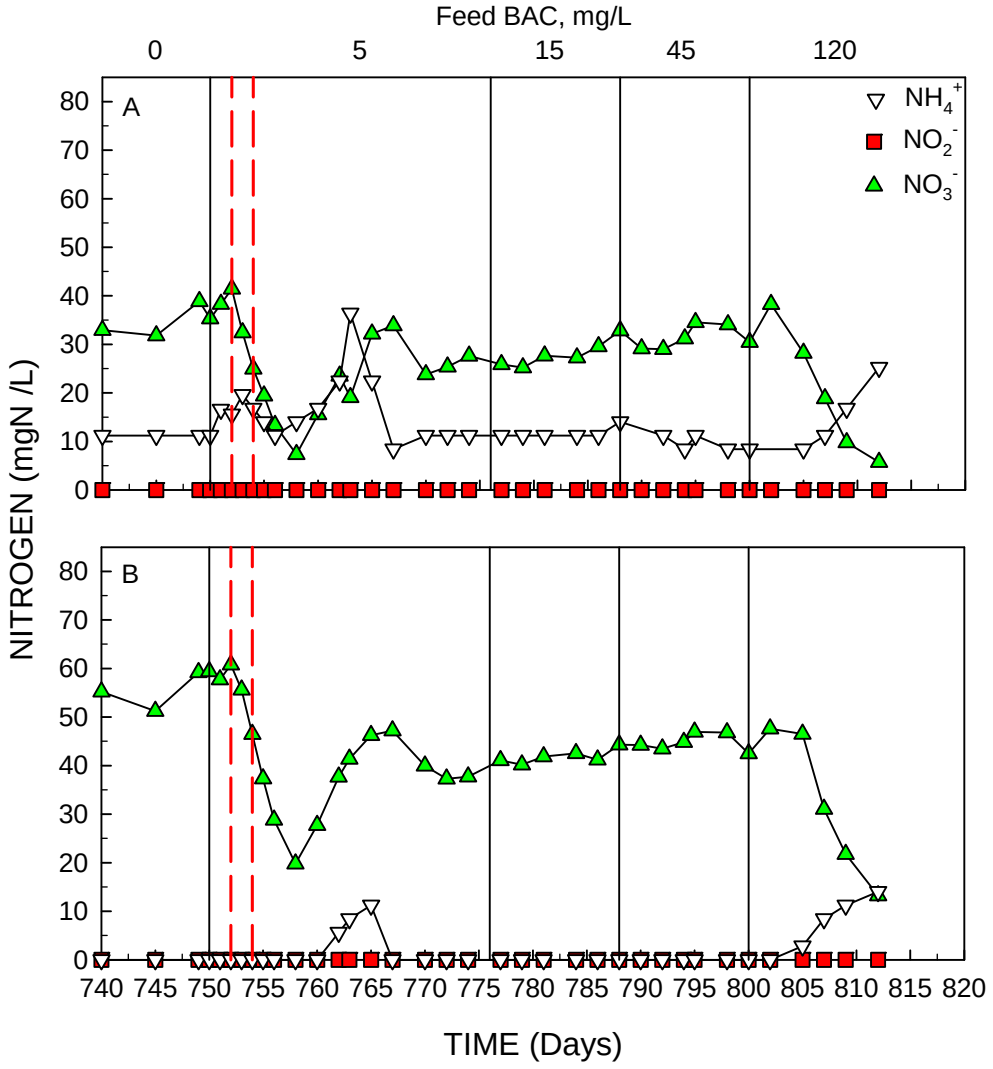


Figure 35. Nitrogen species in the BNR system anoxic (A) and aerobic (B) reactors while operating with BAC-free and BAC-amended feed. The dashed vertical lines show the period when the system was fed with the old poultry processing wastewater.

The system maintained its performance levels after the BAC concentration in the poultry processing wastewater feed was step-wise increased to 15 and 45 mg/L, indicating that the BNR system was successful in maintaining its resilience towards BAC in spite of the fact that the system operating temperature was 10°C. Table 12 shows the mean BAC concentration in the BNR system components while treating BAC-bearing poultry processing wastewater at 10°C.

Table 12. Steady-state BAC concentration (mg/L) in the BNR system during operation with stepwise increased BAC concentration in the poultry processing wastewater feed (system temperature, 10°C).

Feed	R_2	R_3	Effluent
5	0.7 ± 0.3[a]	1.1 ± 0.4	0.6 ± 0.4
15	1.3 ± 0.5	1.6 ± 0.6	1.0 ± 0.5
45	2.5 ± 1.2	1.4 ± 0.5	0.7 ± 0.3
120	18.3 ± 15.8[b]	2.5 ± 1.4	0.7 ± 0.3

[a] Mean ± standard deviation ($n \geq 5$); [b] Maximum concentration 45 ± 1.2 mg/L

As shown in Figure 36, the BNR system was successful in removing BAC while treating BAC-bearing wastewater at a concentration of 45 mg/L. The solids concentration (TSS/VSS; g/L) in the anoxic reactor decreased while treating the BAC-bearing feed from 2.08 ± 0.01/1.86 ± 0.03 to 1.80 ± 0.03/1.61 ± 0.05 and 1.47 ± 0.1/1.4 ± 0.0 (TSS/VSS; g/L) at a feed BAC concentration of 5 and 15 mg/L BAC, respectively. Likewise, the solids concentration (TSS/VSS; g/L) in the aerobic reactor decreased from 2.64 ± 0.02/2.34±0.01 to 2.5 ±0.05/2.24 ± 0.05 and 1.85 ± 0.2/1.72 ± 0.2 at a feed BAC concentration of 5 and 15 mg/L BAC, respectively. The observed decrease in solids concentration is attributed BAC-induced cell lysis.

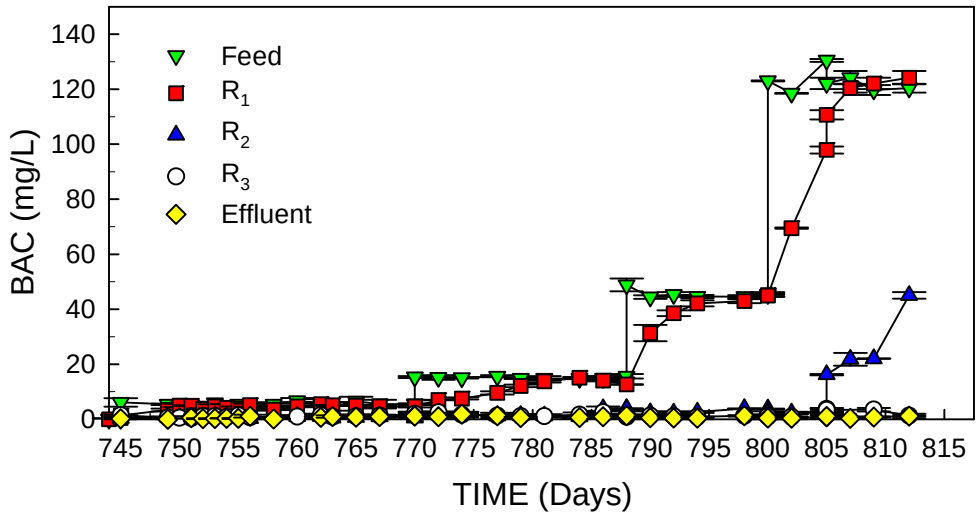


Figure 36. Total BAC concentration in the BNR system components while maintained at 10°C.

On day 800, the system resilience was retested by introducing a step increase in the feed BAC concentration from 45 to 120 mg/L. The same test was performed on the system at 22^{o}C, and lasted for 7 days (the hydraulic retention time of the system). At 10^{o}C, the system performance did not change initially (until day 805). At day 805, the BAC concentration in the anoxic reactor abruptly increased from 2.6 ± 0.3 to 16.2 ± 0.3 mg/L, while the BAC concentration in the aerobic reactor remained relatively low and stable (Table 12). The relatively higher BAC concentration in the anoxic reactor more likely caused cell lysis, increasing the readily degradable organic carbon concentration in the anoxic reactor, which explains the drop in nitrate concentration in this reactor (Figure 35), and resulted in enhanced nitrogen removal efficiency of the BNR system (72.6%). Furthermore, ammonia was detected in the aerobic reactor at the same time (day 805), and continued to increase until day 812, when the operation of the system was terminated, despite the relatively low BAC concentration in the aerobic reactor (Table 12). On day 811, the liquid-phase BAC concentration was 1.3 ± 0.8 and 0.8 ± 0.04 mg/L in the anoxic and aerobic reactors, respectively. The increase in ammonia concentration coexisted with an operational malfunction in the mixing of the anoxic reactor, which lasted for 1 day (from day 805 to day 806). During this incident, the solids concentration in the anoxic reactor significantly increased from 1.47 ± 0.01/1.4 ± 0.0 to 3.52 ± 0.19/3.28 ± 0.19 (TSS/VSS, g/L), while significantly decreased in the aerobic reactor from 1.85 ± 0.02/1.72 ± 0.02 to 0.56 ± 0.01/0.50 ± 0.01 (TSS/VSS, g/L). The sharp decrease in solids concentration in the aerobic reactor more likely influenced BAC bioavailability in this reactor, bringing the BAC concentration to inhibitory levels; the solids-associated BAC concentration was 0.85 ± 0.50 mg BAC/g VSS. The increase in ammonia concentration is attributed to BAC-induced nitrification inhibition, which occurred after the drastic reduction in the aerobic reactor solids concentration. However, if a high solids concentration was maintained in the aerobic reactor, the observed nitrification inhibition would have more likely been avoided. These results show the significance of maintaining relatively high solids concentration in cold season and/or when relatively high BAC concentrations are either expected or occur as a result of an accidental spill of QAC sanitizer mixtures.

Overall, the results of this phase of the study demonstrate the high resilience of multi-step BNR systems towards QACs even under relatively low temperature conditions, providing that prior system acclimation to both QACs and low temperature has been acquired.

3.5 Modeling and Simulation of the Fate and Effect of BAC in a BNR System

Kinetic sub-models for the three reactors of the BNR system were successfully developed and used to develop a comprehensive overall model of a BNR system fed with BAC-bearing wastewater (Hajaya, 2011). The anaerobic reactor sub-model simulated sCOD release, VFAs production, and ammonification. The anoxic reactor sub-model simulated heterotrophic growth and denitrification in a two-step reaction scheme, while the aerobic reactor sub-model simulated heterotrophic growth, and autotrophic, two-step nitrification. Kinetic parameters for the three sub-models were evaluated using the excremental data obtained from independent batch assays. BAC degradation was modeled with a mixed-substrate Monod equation. The inhibitory effect of BAC was modeled by a competitive inhibition equation for readily degradable COD utilization and denitrification, and a non-competitive inhibition equation for nitrification. The inhibitory effect of BAC was correlated with its total concentration, liquid-phase concentration, and solids-associated concentration for heterotrophic COD utilization, denitrification, and nitrification, respectively. Competitive and non-competitive inhibition coefficients were also evaluated.

Sensitivity analysis on the three sub-models showed that the hydrolysis rate is the most sensitive parameter in the anaerobic sub-model, while the nitrate and nitrite maximum specific reduction rates were the most sensitive parameters in the anoxic reactor sub-model. The heterotrophic population fraction and the maximum specific ammonia oxidation rate were the most sensitive parameters in the aerobic reactor sub-model. Kinetic analysis of the three sub-models showed that the rate of BAC removal and the level of nitrification inhibition by BAC were dynamic and depended on the duration of the exposure of the BNR system to BAC.

The three sub-models were used in a comprehensive overall model which was based on the Activated Sludge Model 1 (ASM1)(Henze et al., 2000). The model simulated the BNR system performance treating a poultry processing wastewater with and without BAC. Model simulations showed that the dynamic behavior of BAC degradation and the level of nitrification inhibition by BAC needed to be incorporated in the model in order to reflect the observed acclimation/enrichment of microbial population over time. Additionally, the model showed that reduced BAC degradation rates will result in BAC accumulation in the BNR system to concentrations much higher than those in the BAC-amended poultry processing wastewater influent and will adversely impact the BNR system performance.

Overall, the developed BNR system model was capable of simulating the performance of the laboratory-scale BNR system. The predictive power of the model could be used to further explore the effect of operational and environmental conditions on the performance of BNR systems treating QAC-bearing wastewater and guide both the rational design and operation of such systems.

4.0 SUMMARY & CONCLUSIONS

Analyses of samples collected at a poultry processing wastewater treatment plant in the Southeastern US resulted in values of all analytes within expected values for typical poultry processing facilities. Quaternary ammonium compounds were not detected in any field samples. Specific ammonia removal rates were 83 and 50 mg ammonia-N/g VSS-day and specific nitrate removal rates were 82 and 161 mg nitrate-N/g VSS-day during warm (Fall) and cold (Winter) seasons, respectively (mixed liquor temperature 22-23 and 14-15^{o}C, respectively). Thus, as the mixed liquor temperature decreased, the specific ammonia removal rate decreased, but the specific nitrate removal rate increased. These results confirm previous reports which state that low temperature conditions are more detrimental to nitrification than to denitrification.

A laboratory-scale, continuous-flow, multi-stage BNR system, consisting of an anaerobic, anoxic, and aerobic reactor, was constructed and tested. At room temperature (22 to 24^{o}C), the system achieved 100 and 75% ammonia and total nitrogen removal efficiency, respectively, during 30 days of continuous operation with a QACs-free poultry processing wastewater that was comprised mainly of protein, lipids, and carbohydrates. The effect of BAC was independently tested on both nitrification and denitrification up to a concentration of 100 mg/L, using nitrifying and denitrifying microbial populations unexposed to BAC. BAC substantially decreased the nitrification rate by 39, 94, and 94% at an initial BAC concentration of 5, 10, and 15 mg/L and the ammonia removal was completely inhibited at BAC concentrations equal to and higher than 10 mg/L. In the BAC-amended culture series, ≥ 90% of the initially added BAC was removed in all culture series, except in the 100 mg/L BAC-amended culture series, where only 14% BAC removal was observed. Nitrification did not recover despite the apparent BAC degradation in the BAC-amended culture series, which indicates acute, irreversible nitrification inhibition. In the batch denitrification assay, BAC significantly decreased the nitrate reduction rate, especially a BAC concentration equal to and higher than 50 mg/L, but did not affect the extent of nitrate removal. Nitrate was mostly and completely reduced to dinitrogen in all BAC-amended culture series. Nitrite reduction was more susceptible to BAC compared to nitrate reduction, evident by transient accumulation of nitrite. No other denitrification intermediates were detected during the incubation period. The initially added BAC was fully recovered at the end of the incubation period of the denitrification assay, indicating that BAC was not degraded under the anoxic conditions of the assay. Thus, among the two bioprocesses related to nitrogen removal, nitrification is far more sensitive than denitrification to relatively low temperature values (i.e., below 15^{o}C). The presence of QACs further exacerbates the otherwise negative effect of low temperature, especially for systems without prior acclimation.

The nitrogen removal efficiency of the BNR system was examined while treating BAC-bearing poultry processing wastewater at a range of BAC concentrations, maintained at room temperature (22 to 24^{o}C). The laboratory-scale, multi-stage BNR system was continuously fed with poultry processing wastewater amended with a mixture of three BAC homologs. The nitrogen removal efficiency initially deteriorated at a poultry processing wastewater feed BAC concentration of 5 mg/L due to severe inhibition of nitrification. However, the system recovered after 27 days of operation achieving high nitrogen removal efficiency, even after the feed BAC concentration was stepwise increased up to120 mg/L, resulting in effluent BAC concentration below 1 mg/L. The same high nitrogen removal efficiency was retained when the system was operated at 10^{o}C with BAC-amended poultry processing wastewater. Batch assays performed using mixed liquors of the BNR system reactors, during and post BAC exposure, showed that

microbial biotransformation, acclimation/enrichment, and acquisition of resistance to BAC limited the extent and degree of BAC inhibition in the system. Compared to the unexposed BNR system, nitrifiers achieved higher specific ammonia removal rates and complete nitrification at higher BAC concentrations and acquired resistance to BAC, while denitrifiers achieved higher specific nitrate removal rates with lower transient nitrite accumulation. BAC was also found to be inhibitory to soluble COD utilization by the heterotrophic population in the aerobic reactor. BAC biotransformation occurred only in the aerobic reactor and began by the fission of the central C_{alkyl}-N bond resulting in benzyl dimethyl amine, which in turn underwent successive N-demethylations to yield benzyl methyl amine and benzyl amine. BAC phase distribution data with the poultry processing wastewater feed and the anaerobic, anoxic, and aerobic reactor mixed liquors was fitted to the Freundlich isotherm equation. BAC adsorption to the biomass of the four aforementioned BNR system components was found to be favorable, but with the following descending order: anaerobic reactor biomass > poultry processing wastewater feed > anoxic/aerobic reactors biomass.

Kinetic analysis based on sub-models representing BNR processes showed that BAC inhibition of denitrification and nitrification is correlated with BAC liquid-phase and solid-phase concentrations, respectively. Simulations using a comprehensive mathematical BNR model developed for this research showed that BAC degradation and the level of nitrification inhibition by BAC are dynamic, brought about by acclimation and enrichment of the heterotrophic and nitrifying microbial populations, respectively. The fate and effect of BACs in the BNR system were accurately described when the interactions between adsorption, inhibition, and resistance/biotransformation were considered within the conditions prevailing in each reactor of the BNR system. Adsorption determines the level of the inhibitory effect of BAC, while BAC biotransformation and resistance determine the extent of exposure of the microbial communities to BAC. Finally, the inhibitory effect of BAC is reduced, if not completely removed, by the development of BAC resistance and biotransformation capacity.

Overall, this study has shown that, after acclimation to both QACs and low temperature, multi-stage BNR systems can achieve effective nitrogen removal even at an influent BAC concentration of 120 mg/L and at 10^{o}C. The results of this study will enable the rational design and operation of BNR systems for the efficient treatment of QAC-bearing wastewater. The outcome of this research provides information presently lacking, supporting the continuous use of QACs as antimicrobial agents in poultry processing facilities, when and where needed, while avoiding any negative impacts on biological treatment systems and the environment. Given the benefits of using QACs as effective sanitation chemicals in poultry processing facilities, the effectiveness of biological processes for the degradation of QACs in order to avoid process upsets, especially for the nitrification step, should be further evaluated using alternative process configurations (e.g., sequential batch reactors, fixed-film reactors) in order to capture process variability across the entire poultry processing industry.

5.0 REFERENCES

Aksu Z., F. Gonen, and Z. Demircan. 2002. Biosorption of chromium(VI) ions by Mowital (R) B30H resin immobilized activated sludge in a packed bed: Comparison with granular activated carbon. *Process Biochemistry* 38 (2): 175-186.

American Public Health Association (APHA). 2005. Standard Methods for the Examination of Water and Wastewater, 21th Ed. APHA-AWWA-WEF, Washington, D.C.

Avula R.Y., H. M. Nelson, and R. K. Singh. 2009. Recycling of poultry process wastewater by ultrafiltration. *Innovative Food Science & Emerging Technologies* 10 (1): 1-8.

Babakhouya N., H. Aksas, S. Boughrara, and K. Louhab. 2010. Adsorption of Ed(II) ions from aqueous solution using mixed sorbents prepared from olive stone and date pit. *Journal of Applied Sciences* 10 (19):2316-2321.

Batstone D.J., J. Keller, I. Angelidaki, S. V. Kalyuzhnyi, S. G. Pavlostathis, A. Rozzi, W. T. M. Sanders, H. Siegrist, and V. A. Vavilin. 2002. The IWA Anaerobic Digestion Model No 1 (ADM1). *Water Science and Technology* 45 (10):65-73.

Cross J., and E. J. Singer. 1994. Cationic Surfactants: Analytical and Biological Evaluation. Marcel Dekker, Inc. New York.

Garcia M. T., E. Campos, J. Sanchez-Leal, and F. Comelles. 2006. Sorption of alkyl benzyl dimethyl ammonium compounds by activated sludge. *Journal of Dispersion Science and Technology* 27 (5):739-744.

Gaudy A. F. 1962. Colorimetric determination of protein and carbohydrate. *Ind. Water Wastes* 7:17-27.

Hajaya M. G., U. Tezel, and S. G. Pavlostathis. 2011. Effect of temperature and benzalkonium chloride on nitrate reduction. *Bioresource Technology* 102:5039–5047.

Hajaya M. G. 2011. Fate and Effect of Quaternary Ammonium Antimicrobial Compounds on the Biological Nitrogen Removal within High-strength Wastewater Treatment Systems. Ph.D. Thesis, Georgia Institute of Technology, Atlanta, GA.

Henze M., W. Gujer, T. Mino, and M. C. M. Loosdrecht. 2000. Activated Sludge Models ASM1, ASM2, ASM2d, and ASM3. IWA Scientific and Technical Report 9. IWA Publishing, London, UK.

HMSO. 1981. Methods for the Examination of Waters and Associated Materials, SCA, Her Majesty's Stationery Office, London, UK.

Ismail Z. Z., U. Tezel, and S. G. Pavlostathis. 2010. Sorption of quaternary ammonium compounds to municipal sludge. *Water Research* 44:2303-2313.

Kiepper B. H. 2003. Characterization of Poultry Processing Operations, Wastewater Generation, and Wastewater Treatment. M.S. Thesis, The University of Georgia, Athens, GA.

Kreuzinger N., M. Fuerhacker, S. Scharf, M. Uhl, M., O. Gans, and B. Grillitsch. 2007. Methodological approach towards the environmental significance of uncharacterized substances -Quaternary ammonium compounds as an example. *Desalination* 215 (1-3):209-222.

Kristiansen S. 1983. The temperature optimum of the nitrate reductase assay for marine-phytoplankton. *Limnology & Oceanography* 28:776-780.

Li B., S. Irvin, and K. Baker. 2007. The variation of nitrifying bacterial population sizes in a sequencing batch reactor (SBR) treating low, mid, high concentrated synthetic wastewater. *Journal of Environmental Engineering and Science* 6 (6):651-663.

Loehr R. C., and G. A. Rohlich. 1962. A wet method for grease analysis. Proc. 17th Ind. Waste Conf., Purdue Univ., Engineering Extension Series No. 12, Lafayette, Indiana, pp. 215-232.

Macey R. I., and G. F. Oster. 2006. Berkeley Madonna, Berkeley Madonna Inc.

Madigan M. T., and J. M. Martinko. 2006. Brock Biology of Microorganisms, 11th edition, Pearson Prentice Hall, Upper Saddle River, NJ.

Magri A., and X. Flotats. 2008. Modelling of biological nitrogen removal from the liquid fraction of pig slurry in a sequencing batch reactor. *Biosystems Engineering* 101 (2):239-259.

Merka W. C., A. E. Reynolds, J. Sellers, and B. Kiepper. 2001. Profile and Production Process Determination of Phosphorus and Nitrogen Wastewater Discharges from Poultry Processing Plants. Final Report – Project No. 558; U.S. Poultry & Egg Association, Tucker, GA.

Nishihara T., T. Okamoto, and N. Nishiyama. 2000. Biodegradation of didecyldimethylammonium chloride by *Pseudomonas fluorescens* TN4 isolated from activated sludge. *J. Applied Microbiology* 88:641-647.

Patrauchan M.A., and P. J. Oriel. 2003. Degradation of benzyldimethylalkylammonium chloride by *Aeromonas hydrophila* sp. K. *J. Applied Microbiology* 94:266-272.

Pavlostathis S. G., K. Li, U. Tezel, and J. A. Pierson. 2008. Preliminary Assessment of Biological Nitrogen Removal in Poultry Processing Facilities. Final Report – Project No. 0607-E004; US Poultry & Egg Association, Tucker, GA.

Pavlostathis S. G., J. A. Pierson, K. Li, and M. G. K. Hajaya. 2009. Assessment of Biological Nitrogen Removal in Poultry Processing Facilities. Final Report – Project No. 650; US Poultry & Egg Association, Tucker, GA.

Pierson J. A., and S. G. Pavlostathis. 2000. Real-time monitoring and control of sequencing batch reactors for secondary treatment of a poultry processing wastewater. *Water Environment Research* 72 (5):585-592.

Ren R., K. Li, C. Zhang, D. Liu, and J. Sun. 2011. Biosorption of tetradecyl benzyl dimethyl ammonium chloride on activated sludge: Kinetic, thermodynamic and reaction mechanisms. *Bioresource Technology* 102 (4):3799–3804.

Rittmann B.E., and P. L. McCarty. 2001. Environmental Biotechnology: Principles and Applications. McGraw-Hill. New York.

Shcherbakova V.A., K. S. Laurinavichius, and V. K. Akimenko. 1999. Toxic effect of surfactants and probable products of their biodegradation on methanogenesis in an anaerobic microbial community. *Chemosphere* 39 (11):1861-1870.

Snape J. R., N. A. Walkley, A. P. Morby, S. Nicklin, and G. F. White. 1997. Purification, properties, and sequence of glycerol trinitrate reductase from *Agrobacterium radiobacter*. *Journal of Bacteriology* 179 (24):7796-7802.

Sutterlin H., R. Trittler, S. Bojanowski, E. A. Stadbauer, and K. Kummerer. 2007. Fate of benzalkonium chloride in a sewage sludge low temperature conversion process investigated by LC-LC/ESI-MS/MS. *Clean-Soil Air Water* 35 (1):81-87.

Sutterlin H., R. Alexy, and K. Kummerer. 2008b. The toxicity of the quaternary ammonium compound benzalkonium chloride alone and in mixtures with other anionic compounds to bacteria in test systems with *Vibrio fischeri* and *Pseudomonas putida*. *Ecotoxicology and Environmental Safety* 71 (2):498-505.

Tezel U. 2009. Fate and Effect of Quaternary Ammonium Compounds in Biological Systems. Ph.D. Thesis, Georgia Institute of Technology, Atlanta, GA.

Tezel U., and S. G. Pavlostathis. 2009. Transformation of benzalkonium chloride under nitrate reducing conditions. *Environmental Science & Technology* 43:1342-1348.

Tezel U., J. A. Pierson, and S. G. Pavlostathis. 2006. Fate and effect of quaternary ammonium compounds on a mixed methanogenic culture. *Water Research* 40 (19):3660-3668.

Tezel U., J. A. Pierson, and S. G. Pavlostathis. 2007. Effect of polyelectrolytes and quaternary ammonium compounds on the anaerobic biological treatment of poultry processing wastewater. *Water Research* 41:1334-1342.

Tezel U., J. A. Pierson, and S. G. Pavlostathis. 2008. Effect of didecyl dimethyl ammonium chloride on nitrate reduction in a mixed methanogenic culture. *Water Science & Technology* 57:541-546.

Tugtas A. E., and S. G. Pavlostathis. 2007. Effect of sulfide on nitrate reduction in mixed methanogenic cultures. *Biotechnology & Bioengineering* 97:1448-1459.

Tugtas A. E., U. Tezel, and S. G. Pavlostathis. 2006. An extension of the Anaerobic Digestion Model No. 1 to include the effect of nitrate reduction processes. *Water Science and Technology* 54 (4):41-49.

Wett B., and W. Rauch. 2003. The role of inorganic carbon limitation in biological nitrogen removal of extremely ammonia concentrated wastewater. *Water Research* 37 (5):1100-1110.

Yang J. 2007. Fate and Effect of Alkyl Benzyl Dimethyl Ammonium Chloride in Mixed Aerobic and Nitrifying Cultures. MS Thesis, School of Civil and Environmental Engineering, Georgia Institute of Technology, Atlanta, GA.

Zhang C., U. Tezel, K. Li, D. Liu, R. Ren, J. Du, and S. G. Pavlostathis. 2011. Evaluation and modeling of benzalkonium chloride inhibition and biodegradation in activated sludge. *Water Research* 45:1238-1246.

Presentations

Hajaya M. G., J. A. Pierson, and S. G. Pavlostathis. "Assessment of Biological Nitrogen Removal in Poultry Processing Facilities," 13th Annual Symposium, Association of Environmental Engineers and Scientists, Georgia Institute of Technology, Atlanta, GA, April 2009 (poster).

Tezel U., R. Martinez, M.Tandukar, P. A. Sobecky, and S. G. Pavlostathis. "Biotransformation of Benzalkonium Chloride by a Mixed Heterotrophic Culture under Oxic and Anoxic Conditions," American Society of Microbiology 109th General Meeting, Philadelphia, PA, May 2009 (poster).

Tezel U., J. Yang, Z. Z. Ismail, and S. G. Pavlostathis. "Fate and Biotransformation of Quaternary Ammonium Compounds in Biological Treatment Systems," Department of Microbiology and Environmental Change Institute, National University of Ireland, Galway, Ireland, May 2009.

Tezel U., H. Giles, A. deRussy, and S. G. Pavlostathis. "Effect of Counter Ions and Natural Organic Matter on the Toxicity of Benzalkonium Chloride," 82nd Annual Water Environment Federation Technical Exhibition and Conference (WEFTEC 2008) – Research Symposium: Trace Constituents, Orlando, FL, October 2009.

Hajaya M. G., J. A. Pierson, and S. G. Pavlostathis. "Assessment of Biological Nitrogen Removal in Poultry Processing Facilities," Industrial Conference & Expo, Georgia Association of Water Professionals, Callaway Gardens, Pine Mountain, GA, March 2010.

Tezel U., and S. G. Pavlostathis. "Quantitative Structure Activity Relationships (QSAR) for Determining the Mobility and Toxicity of Quaternary Ammonium Compounds," ACS 240th National Meeting, Division of Environmental Chemistry, Boston, MA, August 2010.

Tezel U., and S. G. Pavlostathis. "Fate and Biotransformation of Quaternary Ammonium Compounds in Biological Treatment Processes," 83rd Annual Water Environment Federation Technical Exhibition and Conference (WEFTEC 2010), New Orleans, LA, October 2010.

Hajaya M. G., U. Tezel, M. Tandukar, and S. G. Pavlostathis. "Fate and Effect of Benzalkonium Chlorides in a Continuous-flow Biological Nitrogen Removal System Treating Poultry Processing Wastewater," 8th IWA International Symposium on Waste Management Problems in Agro-Industries, Çeşme, Turkey, June 2011.

Publications

Tezel U., and S. G. Pavlostathis. 2009. Transformation of benzalkonium chloride under nitrate reducing conditions. *Environmental Science & Technology* 43:1342-1348.

Ismail Z. Z., U. Tezel, and S. G. Pavlostathis. 2010. Sorption of quaternary ammonium compounds to municipal sludge. *Water Research* 44:2303-2313.

Zhang C., U. Tezel, K. Li, D. Liu, R. Ren, J. Du, and S. G. Pavlostathis. 2011. Evaluation and modeling of benzalkonium chloride inhibition and biodegradation in activated sludge. *Water Research* 45:1238-1246.

Hajaya M. G., U. Tezel, and S. G. Pavlostathis. 2011. Effect of temperature and benzalkonium chloride on nitrate reduction. *Bioresource Technology* 102:5039–5047.