FATE AND EFFECT OF QUATERNARY AMMONIUM ANTIMICROBIAL COMPOUNDS ON BIOLOGICAL NITROGEN REMOVAL WITHIN HIGH-STRENGTH WASTEWATER TREATMENT SYSTEMS

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SUMMARY

High strength wastewater (HSWW) generated in food processing industries is characterized by high organic carbon and nitrogen content, and thus high oxygen demand. Biological nitrogen removal (BNR) is a technology widely used for the treatment of HSWW. Food processing facilities practice sanitation to keep food contact surfaces clean and pathogen-free. Benzalkonium chlorides (BACs) are cationic quaternary ammonium antimicrobial compounds (QACs) common in industrial antimicrobial formulations. BAC-bearing wastewater generated during sanitation applications in food processing facilities is combined with other wastewater streams and typically treated in BNR systems. The poor selectivity and target specificity of the antimicrobial BACs negatively impact the performance of BNR systems due to the susceptibility of BNR microbial populations to BAC.

The objectives of the research were: a) assessment and quantification of the inhibitory effect of QACs on the microbial groups, which mediate BNR in HSWW treatment systems while treating QAC-bearing HSWW; b) evaluation of the degree and extent of the contribution of QAC adsorption, inhibition, and biotransformation on the fate and effect of QACs in BNR systems. A laboratory-scale, multi-stage BNR system was continuously fed with real poultry processing wastewater amended with a mixture of three benzalkonium chlorides. The nitrogen removal efficiency initially deteriorated at a BAC feed concentration of 5 mg/L due to complete 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.

Batch assays performed using the mixed liquors of the BNR system reactors, before, during, and post BAC exposure, showed that the development of BAC biotransformation capacity and the acquisition of resistance to BAC contributed to the recovery of nitrification and nitrogen removal. 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 were 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.

This work is the first study on the fate and effect of antimicrobial compounds, such as BACs, in a continuous-flow, multi-stage BNR system, and the first study to quantify and report parameter values related to BAC inhibition of nitrification and denitrification. The results of this study 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 food processing facilities, when and where needed, while avoiding any negative impacts on biological treatment systems and the environment.

CHAPTER 1

INTRODUCTION

1.1. Preface

High strength wastewaters (HSWW) generated by food industries, such as poultry and beef processing, are characterized by high organic carbon and nitrogen content. Biological nitrogen removal (BNR) is a technology widely employed for the treatment of high strength wastewater (Casani et al., 2005). BNR is achieved by two processes mediated by two distinct microbial populations: nitrification, where ammonia is oxidized to nitrate in the presence of oxygen by autotrophic nitrifying bacteria, and denitrification, where nitrate is reduced to nitrogen gas, with sufficient carbon and energy sources, under anoxic conditions by facultative heterotrophic denitrifying bacteria (Rittmann and McCarty, 2001; Madigan and Martinko, 2006). Achieving efficient BNR is only possible through sustaining the two aforementioned microbial populations and biological processes at their optimum physiological and environmental conditions.

Food processing facilities practice sanitation to clean food contact surfaces. Quaternary ammonium compounds (QACs) are surface active organic compounds common in industrial sanitation formulations, which are widely used in various sanitation applications. While acting as effective biocides against a wide range of pathogenic microorganisms, QACs lack selective toxicity and often have poor target specificity, which when carried over with the wastewater to the treatment plant may have adverse effects. QACs negatively impact the physiological groups responsible for wastewater treatment and thus the performance of the biological treatment systems.

The fate and effect of QACs on different biological processes utilized in HSWW 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 typically encountered conditions (e.g., sequence of nitrification/denitrification). The latter is typical in engineered wastewater treatment systems in which multiple environmental conditions are specifically created to sustain different groups of microorganisms, which biologically mediate the overall treatment process.

Relative to modeling and simulation of the fate and effect of QACs on BNR systems, although the International Water Association Activated Sludge Model 1 (ASM1) is considered an excellent benchmark for nitrogen-removing activated sludge systems (Henze et al., 2000), it does not deal with inhibitory substances such as QACs. In addition, a QAC inhibition model that can predict the degree and extent of QAC effect on nitrification and denitrification is currently lacking.

1.2. Research Objectives

The overall objective of the research was to systematically assess the fate and effect of QACs, mainly benzalkonium chloride (BAC), on the efficiency of biological nitrogen removal within poultry processing wastewater (categorized as HSWW) systems. A thorough understanding of the fate and effect of QACs will provide effective, practical solutions towards maintaining the system's high nitrogen removal capacity while treating

QACs-bearing poultry processing wastewater. The specific objectives of the research were:

1. Assessment and quantification of the inhibitory effect of QACs on the microbial groups, which mediate BNR in poultry processing wastewater treatment systems (fermentative, carbon-oxidizing aerobic, nitrifying and denitrifying bacteria), independently and collectively, while treating QAC-bearing poultry processing wastewater.
2. Evaluation of the degree and extent of contribution of adsorption, inhibition, and biotransformation/biodegradation on the fate and effect of QACs in a BNR system in order to maintain system resiliency and high nitrogen removal capacity while treating QACs-bearing poultry processing wastewater.

1.3. Significance

QACs have unique characteristics (partitioning/sorption, potency/inhibition, and bioavailability/biodegradation), which vary under different conditions. Therefore, information derived solely from studies on a single environmental condition, while important, fails to accurately describe the fate and effect of QACs in multi-stage, engineered treatment systems, as is the case in real-world applications. How a sequential change in prevailing conditions would influence the fate and effect of QACs is crucial information that is currently lacking. Such information is imperative to support the continuous usage of QACs as sanitizing agents, where and if needed, without any negative implications on the biological treatment systems and through them to the environment. Recent studies have connected QACs and antimicrobial resistance, a consequence of their high-volume use and discharge into the environment. A well

engineered system will not only insure adequate performance in terms of QACs-bearing wastewater treatment, but will also facilitate their management prior to discharge in order to avoid unwanted consequences. The present research enhances our understanding relative to the fate and effect of QACs in HSWW treatment systems and thus contributes to the effective management of QACs-bearing wastewaters.

1.4. Thesis Organization

The overall objective of this work was to assess the effect of QACs, mainly the antimicrobial benzalkonium chloride (BAC), on the efficiency of BNR within poultry processing wastewater treatment systems, by developing convenient and practical solutions to maintain the system's resiliency and high nitrogen removal efficiency while treating QACs-bearing poultry processing wastewater. In order to satisfy the objectives of this work, the research was divided into two parts.

The first part of the research focused on experimentally assessing the effects of BAC physical and chemical interactions (sorption, inhibition, and biotransformation) within a multi-stage BNR system treating real poultry processing wastewater. For the experimental part, a laboratory-scale, continuous-flow BNR system which simulated three environmental conditions typically encountered in BNR systems (i.e., anaerobic, anoxic, and aerobic), was constructed (Chapter 4). The system nitrogen removal performance was examined while treating real BAC-free poultry processing wastewater (Chapter 4), and BAC-bearing poultry processing wastewater (Chapter 5). Bioassays were conducted using the mixed liquors of the laboratory-scale BNR reactors before (Chapter 4) and after (Chapter 5) BAC exposure to independently assess the fate and effect of BAC on nitrification and denitrification, as well as quantifying BAC adsorption,

inhibition, and biotransformation/biodegradation (Chapter 5). An additional bioassay was conducted to assess the effect of BAC on fermentation, hydrolysis, and ammonification of poultry processing wastewater (Chapter 5).

The second part of the research focused on constructing a mathematical model which simulates the BNR operation and performance while treating BAC-free and BAC-bearing poultry processing wastewater. Time series data resulting from the bioassays conducted before and after BAC exposure were used to evaluate kinetic parameters for sub-models which simulate fermentation/hydrolysis/ammonification, nitrification, and denitrification (Chapter 6). BAC biotransformation kinetics and inhibition sub-models were also evaluated (Chapter 6). The sub-models were then integrated in a comprehensive, ASM1-based model which simulates the BNR system operation and performance (Chapter 6). A sensitivity analysis was performed on the model parameters to identify how each parameter affects the overall model outcome (Chapter 6).

Finally, it is recommended that the work presented here be expanded to consider the effect of temperature resulting from seasonal variations, the evolution of antimicrobial resistance and its effect on the proliferation of antibiotic resistance genes, as well as the use of the mathematical model developed in this study to further optimize the design and operation of BNR systems treating QAC-bearing wastewater (Chapter 7).

CHAPTER 2

BACKGROUND

2.1 High Strength Wastewater

High strength wastewaters (HSWWs) are characterized by high levels of chemical oxygen demand (COD) and nitrogenous compounds (mainly ammoniac nitrogen), with specific characteristics depending on the wastewater source. Food and agro-industries, such as livestock operations, poultry processing, canning and slaughterhouses, consume a substantial amount of water and are considered among the main sources of HSWWs (Mosquera-Corral et al., 2003; Salminen and Rintala, 2003; Choi et al., 2004; Tezel et al., 2007). Meat processing facilities use water up to 1700 L/ton of meat for chiller showers (Casani et al., 2005) and poultry processing facilities use water between 26.5 and 150 L/bird (Northcutt, 2007; Avula et al., 2009). A substantial increase in water consumption came with the implementation of Hazard Analysis and Critical Control Point (HACCP) in 1998, when poultry processing facilities doubled, and in some cases tripled water consumption (Northcutt and Jones, 2004; Northcutt, 2007). HACCP was developed by the National Advisory Committee on Microbiological Criteria for Foods (NACMCF) and imposed by the U. S. Food and Drug Administration (USFDA) (USFDA, 1997). HACCP is widely employed as a method for hazards control and risk reduction in poultry processing facilities (Mul and Koenraadt, 2009).

HSWW COD levels can reach up to 8000 mg COD/L for waste slurries generated by livestock operations (Anceno et al., 2009), 1300-1500 mg COD/L for tannery waste (Carrera et al., 2003), 400-1600 mg COD/L for poultry slaughterhouse waste (after

recovery of blood and viscera to lower the organic load) (Del-Pozo and Diez, 2003), while nitrogen levels can be between 200-500 mg ammonia-N/L for tanneries waste (Carrera et al., 2003), and 105-320 mg ammonia-N/L for poultry slaughterhouse wastewater (Del-Pozo and Diez, 2003).

2.2 Biological Nitrogen Removal (BNR)

Despite high levels of COD and nitrogen, biological processes are successfully employed for HSWW treatment, achieving both COD and nitrogen removal levels that satisfy effluent discharge limits (Casani et al., 2005). In these treatment systems, emphasis is placed on biological nitrogen removal (BNR), which is achieved through a combination of sequential nitrification and denitrification processes. The BNR process is part of the naturally occurring nitrogen cycle (Figure 2.1)

2.2.1 Nitrification

Biological nitrification is considered the controlling factor in BNR, and its stability will affect the entire operation (Del-Pozo and Diez, 2003). Nitrification is an aerobic process carried out by two separate groups of chemolithotrophic bacteria. The first group (nitrosifiers) oxidizes ammonia (NH_4^+) to hydroxylamine (NH_2OH) by the membrane-bound enzyme ammonium monooxygenase (AMO). NH_2OH is then further oxidized by the periplasmic enzyme hydroxylamine oxidoreductase (HAO) to nitrite (NO_2^-). This group is usually called ammonia-oxidizing bacteria (AOB). The second bacterial group (nitrifiers), which oxidizes nitrite to nitrate (NO_3^-) by means of nitrite oxidoreductase (Madigan and Martinko, 2006), is called nitrite-oxidizing bacteria (NOB). Among the two aforementioned steps, ammonia oxidation is considered the rate-limiting

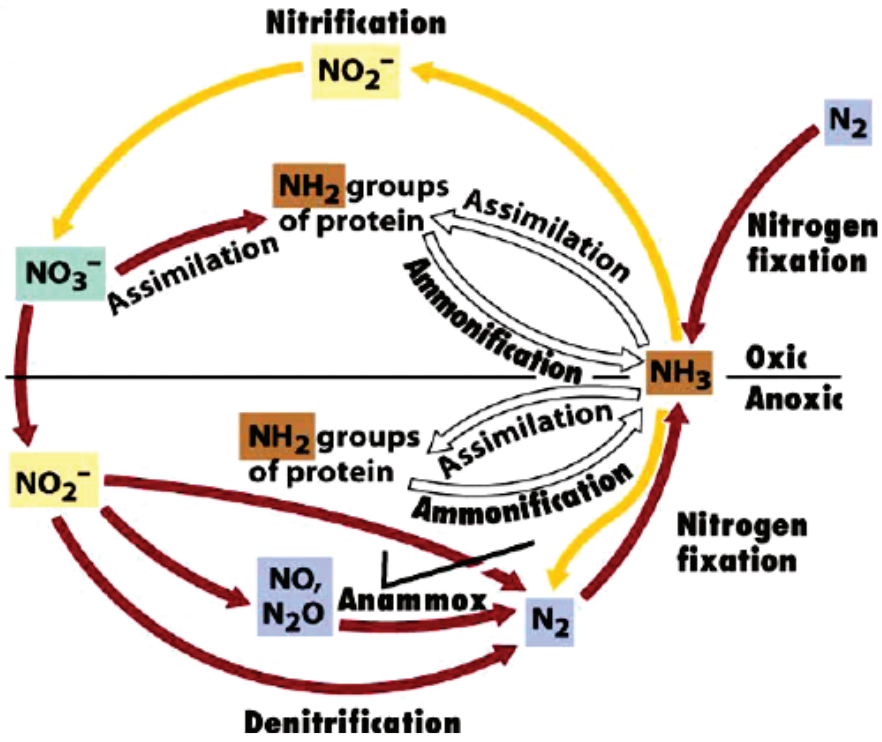

Figure 2.1. The natural nitrogen cycle within oxic (aerobic) and anoxic environments (Madigan and Martinko, 2006).

step (Zhang et al., 2010). Figure 2.2 shows the location of nitrification enzymes as well as the electron transport within AOB and NOB.

The most commonly recognized genus of bacteria among the first bacterial group is *Nitrosomonas*; however *Nitrosococcus*, *Nitrosovibro*, and *Nitrosolobus* posses the ability to nitrosify. *Nitrobacter* is the most common genus among the second bacterial group. In addition, *Nitrospira, Nitrospina, Nitrococcus*, and *Nitrocystis* can use nitrite as an electron donor (Rittmann and McCarty, 2001). Furthermore, until now, no autotrophic bacteria that can mediate both nitrification reactions (i.e., ammonia to nitrate) have been identified (Kowalchuk and Stephen, 2001; Costa et al., 2006).

Ammonia can also be oxidized under anoxic conditions in a process known as ANAMMOX (anaerobic ammonia oxidation). *Brocadia anammoxidans*, the organism which catalyzes the process, is a prokaryotic autotroph that utilizes a membrane-enclosed structure, called the anammoxosome, to perform the oxidation of ammonia with nitrite as an electron acceptor, yielding gaseous dinitrogen (N_2) (Madigan and Martinko, 2006).

2.2.2 Denitrification

In denitrification (i.e., dissimilatory nitrate reduction to nitrogen gas, DNRN), nitrate (NO_3^-) is reduced to dinitrogen gas in the absence of oxygen. This process consists of four reaction steps catalyzed by four enzymes: the molybdenum-containing, membrane-integrated, nitrate reductase (NAR) reduces nitrate to nitrite (NO_2^-), nitrite reductase (NIR) reduces nitrite to nitric oxide (NO), nitric oxide reductase (NOR) reduces nitric oxide to nitrous oxide (N_2O), and finally nitrous oxide reductase (N_2OR) reduces nitrous oxide to dinitrogen gas. Denitrifiers are prokaryotic facultative aerobes, metabolically diverse in terms of alternative energy generating mechanisms. In addition

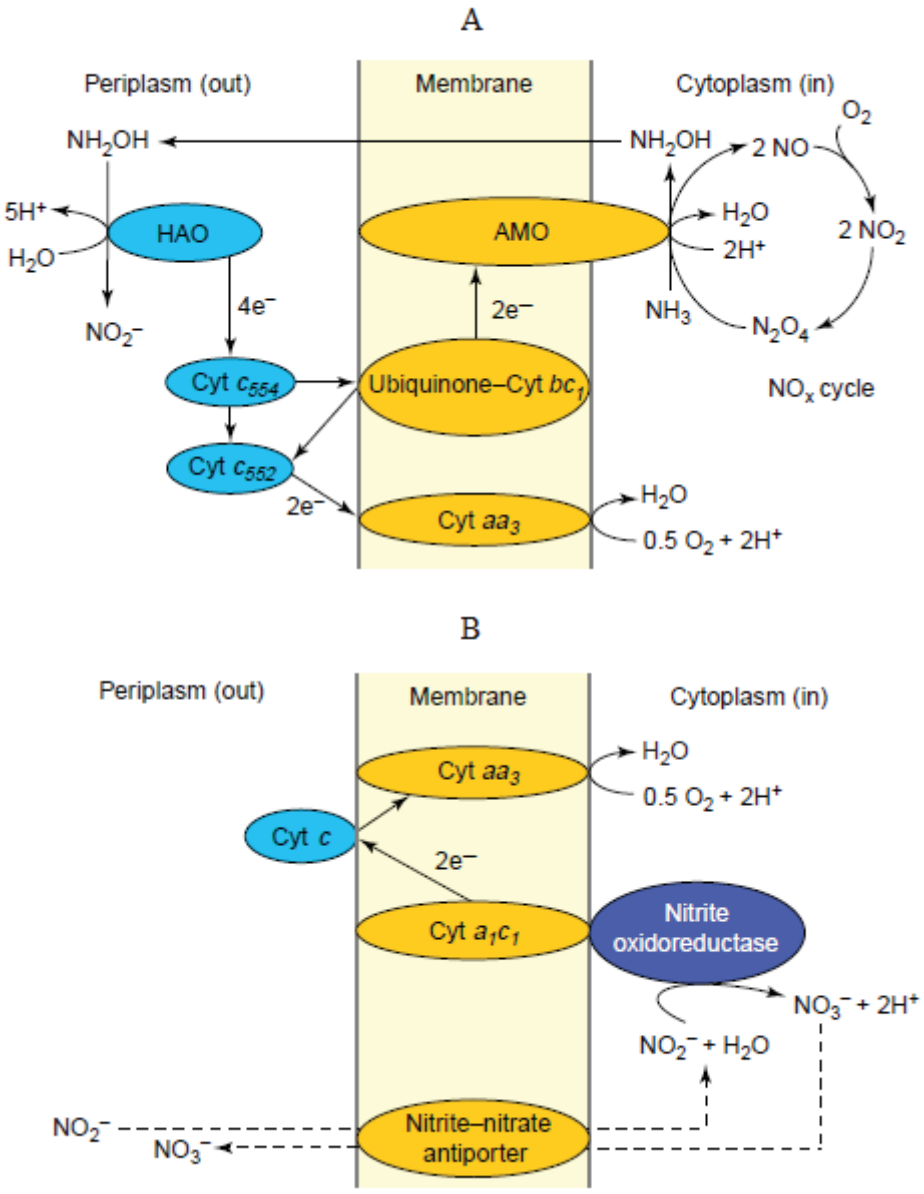

Figure 2.2. Placement of nitrification enzymes in AOB (A) and NOB (B) and electron flow (AMO, ammonium monooxygenase; HAO, hydroxylamine oxidoreductase)(Costa et al., 2006).

to nitrate reduction, aerobic respiration is favored when oxygen is present, and other electron acceptors (such as ferric iron, Fe_3^+) are reduced under anoxic/anaerobic conditions. Many denitrifiers can also grow by fermentation (Madigan and Martinko, 2006). The most common denitrifiers are members of Proteobacteria, and include the genera *Pseudomonas*, *Alcaligenes*, *Paracoccus*, and *Thiobacillus* (Rittmann and McCarty, 2001). Figure 2.3 shows the location of denitrification enzymes relative to the cell membrane and the electron transport during denitrification.

Most denitrifying bacteria have all four reductases (i.e., nitrate, nitrite, nitric oxide, and nitrous oxide reductase) and thus can mediate all four N-oxides reduction steps. Such bacteria include *Pseudomonas stutzeri*, *Paracoccus denitrificans*, and *Sinorhizobium meliloti* (Zumft, 1997). On the other hand, some bacteria have only one or two of the four enzymes. For example *Achromobacter* and *Alcaligenes odorans* lack nitrate reductase (termed as nitrite dependant), while *Escherichia coli* and *Hyphomicrobium vulgare* are only capable of nitrate reduction (Knowles, 1982).

In addition to denitrification, nitrate reduction is possible through dissimilatory nitrate reduction to ammonia (DNRA) (Madigan and Martinko, 2006). In DNRA, ammonia is produced by reducing nitrate and/or nitrite via membrane-bound nitrate and nitrite reductases (Brunet and Garcia-Gil, 1996). DNRA occurs at very low redox potential values, in the presence of sulfide, and in extremely carbon-rich environments (Christensen et al., 2000). DNRA takes place in anaerobic environments, such as the rumen, anaerobic sludge digesters, anoxic sediments and soils and is carried out primarily by anaerobic, fermentative microorganisms (Morkved et al., 2005). DNRA is used to

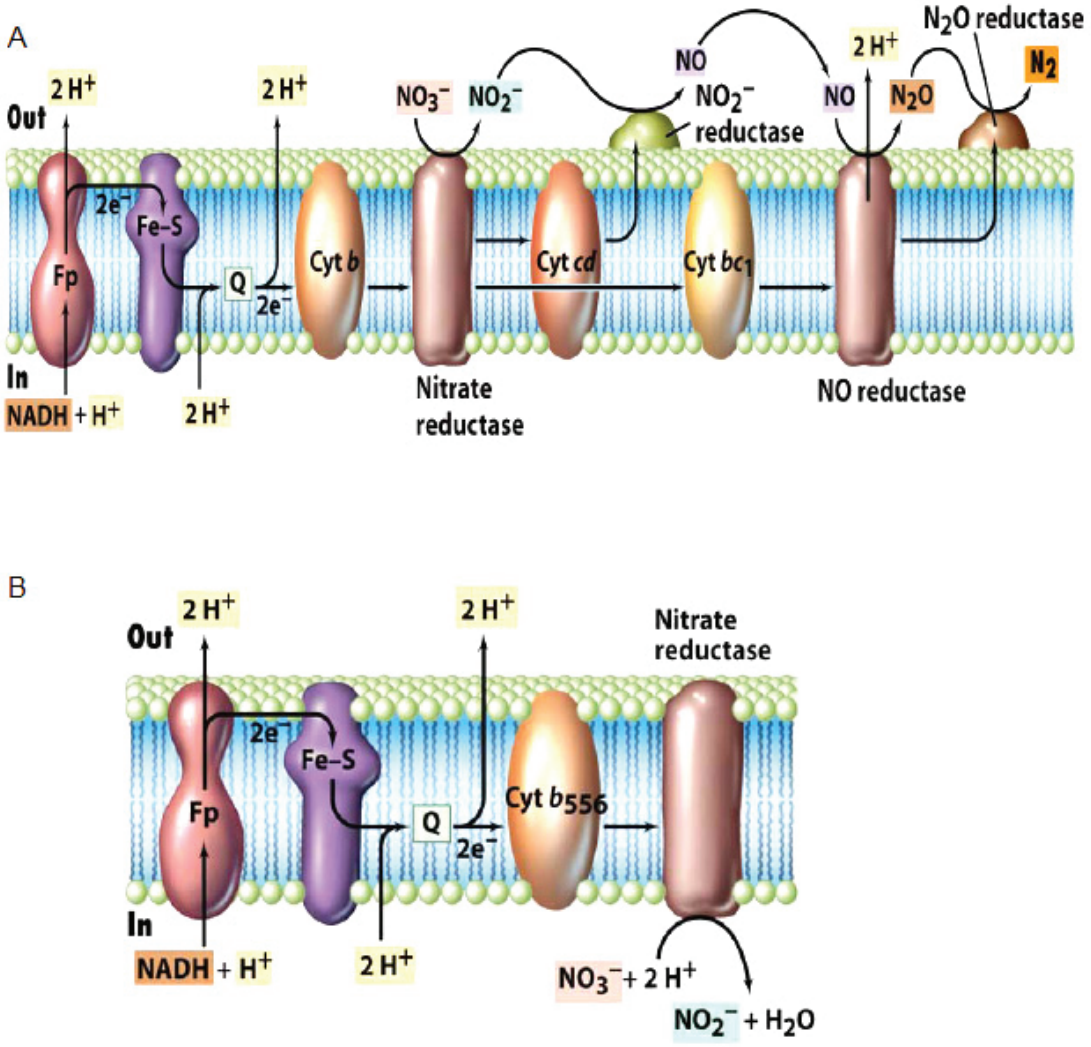

Figure 2.3. Location of denitrification enzymes relative to the bacterial cell membrane and electron flow (*Pseudomonas stutzeri* (A) and *Escherichia coli* (B))(Madigan and Martinko, 2006).

detoxify accumulated nitrite, serves as an electron sink oxidizing NADH, while its energy production is through electron transport phosphorylation (Tiedje, 1988).

2.3 HSWW Biological Nitrogen Removal (BNR) Systems

Wastewater treatment is a combination of unit operations and processes that target the removal of harmful constituents, ensuring the protection of public health in conjunction with environmental, economic, social, and political concerns (Tchobanoglous et al., 2007).

For HSWW, anaerobic pretreatment is widely employed to reduce organic loads resulting from high COD levels, overcoming complications that will otherwise arise in aerobic units when degrading the high COD waste, such as oxygen limitation, high heterotrophic growth, and elevation of the reactor temperature, all of which can inhibit nitrification (Mosquera-Corral et al., 2003; Choi et al., 2004; Kim et al., 2004; Botrous et al., 2004). In addition, free ammonia (NH_3) in the range of 0.1–1.0 mg N/L and 10–150 mg N/L inhibits AOB and NOB, respectively, resulting in nitrite or even ammonium (NH_4^+) accumulation (Terada et al., 2003; Kim et al., 2006). On the other hand, denitrification is sensitive to the carbon to nitrogen ratio (C/N), a factor critical for complete denitrification, and values of 3.5 - 4.5 are recommended (Henze, 1996; Choubert et al., 2009). High nitrogen levels can lower this ratio resulting in N_2O emissions during the treatment process as a result of incomplete denitrification (Itokawa et al., 2001; Kishida et al., 2004).

Different biological systems have been used for the efficient treatment of HSWWs, all of which utilize anoxic and aerobic steps, separated either temporally (sequencing batch reactor, SBR), or spatially (separate bioreactors with recycle), to

achieve both nitrogen and COD removal. To achieve the objective of nitrogen removal, both nitrification and denitrification must be favored, while ammonia removal alone requires favoring only the former process. Three treatment schemes are found to achieve nitrogen removal from wastewater streams (Sharma and Ahlert, 1977), as follows:

A) Three-stage process scheme. Stage 1 is used mainly for carbon oxidation to decrease the carbon-to-nitrogen ratio and the organic loading to values favorable for nitrification. In stage 2, oxidation of ammonia to nitrate takes place. In stage 3 reduction of nitrate to nitrogen gas takes place. The main drawback of such scheme is the difficulty of maintaining a nitrifying biomass inventory due to the very low biomass yields at low COD/N ratios. In addition, such systems produce biomass with poor settling characteristics.

B) Two-step, combined process schemes for nitrogen removal, where both carbon oxidation and nitrification are carried out in the same reactor, i.e., simultaneous carbon oxidation and nitrification in one stage and denitrification in a second stage. The denitrification step could be performed before the carbon and ammonia oxidation step, thus utilizing the organic content of the wastewater as carbon source. Shortcomings of such scheme are the production of relatively high amounts of waste sludge and the higher vulnerability of the combined carbon oxidation and nitrification to variations in organic loading and toxicants.

C) Single-stage process scheme, where both nitrification and denitrification take place in the same reactor. Physical (baffles) or temporal (on and off aeration) separation ensures that both processes take place. The organic content of the wastewater can be

utilized as a carbon source for denitrification. The main concern in this scheme is sludge bulking in the final clarifier, especially during winter.

Different nitrogen and ammonia removal efficiencies have been reported for HSWWs BNR systems. The SHARON (Single reactor system for High activity Ammonia Removal Over Nitrite) process, which operates at a high temperature (30-40^{o}C) and pH (7-8), was developed to treat centrifuged digested sludge effluent (up to 800 mg COD/L and 1000 mg ammonia-N/L). The higher temperature increases the specific growth rate of AOB compared to NOB, which eliminates the need for sludge retention and favors the prevention of nitrite oxidation. Denitrification is used to control the pH and up to 85% ammonium conversion is achievable (Hellinga et al., 1998). Pilot and full-scale results by a study which targeted nitrogen removal via nitrite for digester supernatant in a sequencing batch reactor (SBR) and a SHARON system, reported a nitrogen removal efficiency of over 90% with a C/N ratio of just 2.2, but resulted in N_2O emissions (Fux et al., 2003).

The Bardenpho process is a process in which pre- and post-denitrification is used in separate reactors. A study conducted using four stages in total (anoxic-aerobic-anoxic-aerobic), with external carbon and pH control, achieved 90% nitrogen removal from real waste containing up to 2200 mg ammonia-N/L (Ilies and Mavinic, 2001).

A combination of two fixed-film bioreactors (FFB), the first anaerobic and the second aerobic, connected in series with recirculation achieved 92% organic removal under 390 g COD/m^3·day loading and 95% nitrogen removal under 0.064 kg TKN/m^3·day loading (TKN, Total Kjeldahl Nitrogen) (Del-Pozo and Diez, 2003). Nitrogen and organic matter removal efficiencies up to 92% were obtained with an

aerobic, fixed-film bioreactor treating poultry slaughterhouse wastewater at loading rates up to 200 g TKN/m^3·day and 800 g COD/m^3·day (Del Pozo et al., 2004).

Two up-flow anaerobic sludge blanket (UASB) reactors were used to treat slaughterhouse wastewater, achieving total and soluble COD removal of 67% and 85%, respectively, but nutrients levels in the effluent were high and required further advanced treatment (Del Nery et al., 2007). A full-scale application of partial nitritation and anammox in a single, suspended-growth sequencing batch reactor (SBR) was used to remove nitrogen from an ammonium-rich wastewater with low concentrations of COD and suspended solids, achieving over 90% nitrogen conversion to N_2 while treating 200 mg ammonia-N/L, but high N_2O emissions were observed (Joss et al., 2009*).*

2.4 Quaternary Ammonium Compounds (QACs)

QACs are produced by a nucleophilic substitution reaction of α-olefins or tertiary amines by an alkyl halide or benzyl halide (Boethling, 1984). QACs can be classified in three major groups, depending on the type of the functional groups: monoalkonium, dialkonium and benzalkonium halides (Tezel, 2009). QACs are used in a multitude of applications, such as antimicrobials, cosmetics, pharmaceuticals, fabric softeners, and binding agents (Cross and Singer, 1994). The focus of this work is antimicrobial QACs.

2.4.1 QACs structure and properties

QACs are organic compounds that contain a central nitrogen atom covalently attached to four functional groups (R_4N^+). At least one group (R) is a long-chain alkyl and the other groups are either methyl or a benzyl group (Cross and Singer, 1994). Figure

2.4 shows the chemical structure of commonly used QACs. Both structure and chain length of the functional groups varies.

QACs are large molecules (molecular weights between 300 and 400 g/mole); they are odorless and have a benign effect on synthetic materials including plastics, rubber, ceramic, and stainless steel. QACs may be freely soluble or insoluble in water. The aqueous solubility of QACs decreases as the hydrophobicity or the alkyl chain length of the molecule increases (Boethling, 1984). Due to their structure, QACs are composed of two distinctly different moieties (Figure 2.5): hydrophobic alkyl groups and a hydrophilic, positively charged central N atom, which retains its cationic character at any pH value. The two moieties affect the physical/chemical properties of QACs (Cross and Singer, 1994) and explain the bioactivity of these compounds.

2.4.2 QACs as antimicrobial agents

QACs have a broad spectrum antimicrobial activity over a wide range of pH against a variety of bacteria, fungi and viruses (Sundheim et al., 1998). QACs have several advantages over other commonly used disinfectants, such as no corrosiveness, relatively low toxicity and high surface activity (Langsrud and Sundheim, 1997). QACs have three targets on viable bacterial cells: cell wall, cytoplasmic membrane and cytoplasm. The cytoplasmic membrane is where QACs are usually more bioactive (Ioannou et al., 2007). Figure 2.6 shows the three mechanisms of biocidal action of QACs, which are: physical disruption of the membrane, dissipation of the proton motive force, and inhibition of membrane-associated enzyme activity. The structural functionality of QACs, especially the role of the alkyl chain length, has an effect on their bioactivity. QACs with a hydrophobic tail (C_{16}) affect the outer membrane of gram-

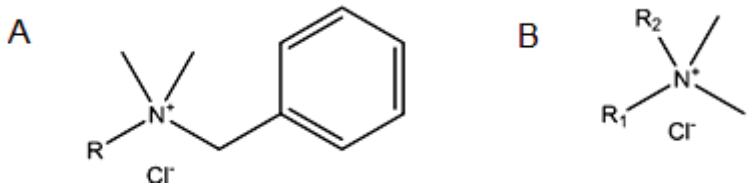

Figure 2.4. Chemical structure of QACs. Benzalkonium chloride (A) and Alkyl (R_1, R_2) dimethyl ammonium chloride (B).

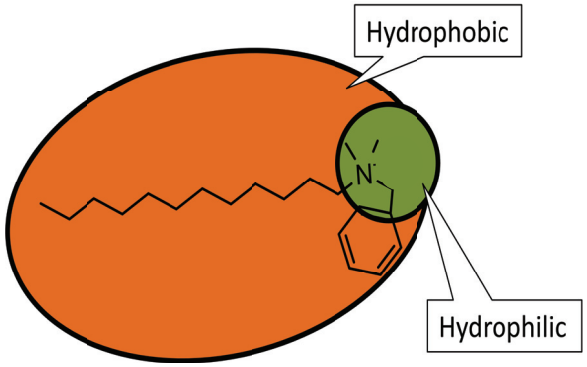

Figure 2.5. Hydrophobic and hydrophilic regions of a QAC (Example shown is benzalkonium chloride).

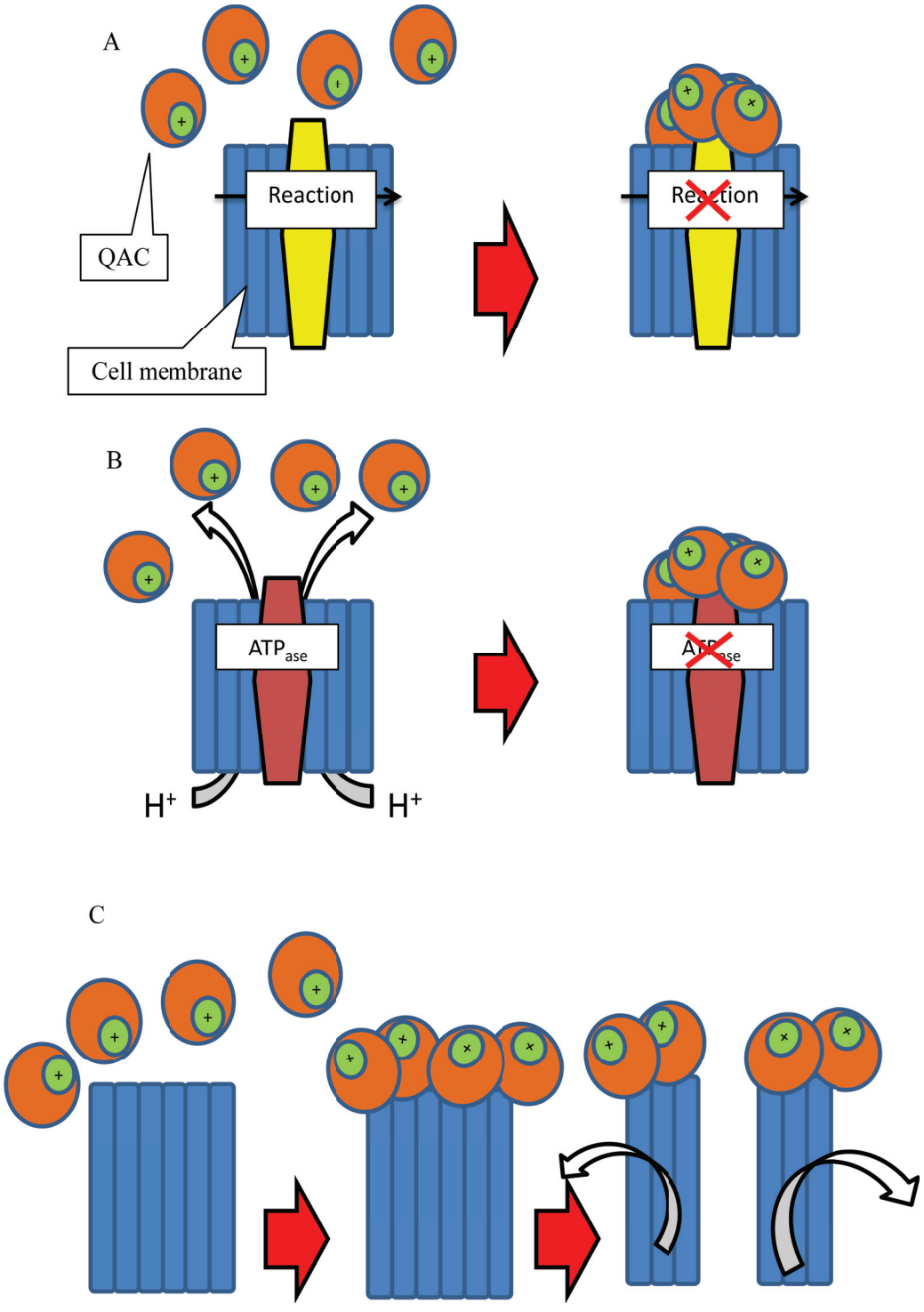

Figure 2.6. Modes of biocidal action of QACs: inhibition of membrane-associated enzyme activity (A), dissipation of the proton motive force (B), physical disruption of the membrane resulting in cell lysis (C).

negative bacteria more extensively than shorter-chain compounds, possibly due to strong interaction between the long chain and the fatty acid portion of lipids. In contrast, mono-alkyl QACs bind by ionic and hydrophobic interactions to microbial membrane surfaces, with the cationic head group facing outwards and the hydrophobic tails inserted into the lipid bi-layer, causing rearrangement of the membrane and subsequent leakage of intracellular constituents (Ioannou et al., 2007).

The most commonly used antimicrobial QACs are dialkonium and benzalkonium chlorides (BAC) (Tiedink, 2001). It has been reported that a mixture of three benzalkonium chloride homologues (C_{12}, C_{14}, and C_{16}) caused loss of membrane integrity of *Staphylococcus aureus* at approximately 9 μg/ml (0.0278 mM), as opposed to just above 4.5 μg/ml (0.0138 mM) of didecyl dimethyl ammonium chloride (DDAC). A catastrophic membrane collapse by both BAC and DDAC occurred above concentrations of 15 μg/ml (0.0463 mM) and 12 μg/ml (0.0368 mM), respectively (Ioannou et al., 2007). In addition, at relatively lower concentrations disruption of the cytoplasmic membrane can lead to potassium ion leakage thereby affecting intracellular homeostasis, which may initiate autolysis through activation of latent ribonucleases (Denyer and Stewart, 1998; Ioannou et al., 2007). Autolysis is an intracellular event where the cell enters a self-destruct state and becomes committed to death.

2.4.3 QACs adsorption

QACs adsorb strongly to suspended solids such as biomass and inorganic particles leading to their accumulation in wastewater biosolids, soil, and aquatic sediments (Cross and Singer, 1994). The adsorption affinity and characteristics differ based on the structure of the QAC, yet can be determined by adsorption isotherms, which

may also provide information on the availability of target sites (Giles et al., 1974; Ioannou et al., 2007). Equilibrium adsorption behavior of QAC antimicrobials is mostly expressed by the Freundlich isotherm, i.e., $q_e = K_F \times C_e^n$, where q_e is the QAC concentration on the adsorbent at equilibrium (mg QAC/g sorbent), C_e is the QAC concentration in the liquid-phase at equilibrium (mg QAC/L), K_f is the adsorption capacity factor ((mg QAC/g adsorbent)(L/mg QAC)^n), and n is the Freundlich intensity parameter (exponent). Table 2.1 shows the Freundlich isotherm parameters of a group of BACs with different adsorbents.

Relative to adsorption to biomass, QACs with a longer alkyl chain have a higher adsorption capacity compared to QACs with a shorter alkyl chain. The presence of a benzyl group enhances QACs adsorption, but this effect diminishes as the alkyl chain length increases. In addition, the extent of adsorption of QACs with relatively lower adsorption affinity decreases in binary mixtures of QACs with higher adsorption affinity, even at relatively low concentrations of the latter (Ismail et al., 2010). Temperature has a detrimental effect on QACs adsorption to biomass. For example, C_{14} BAC adsorption was more favored at low temperature (Ren et al., 2011), suggesting that QACs adsorption is an exothermic process. The adsorption behavior of QACs is also affected by the presence of electrolytes due to the presence of the positively charged nitrogen atom. The presence of sodium chloride and sodium sulfate in QACs aqueous solutions increased the hydrophobicity of QACs, resulting in higher adsorption capacity on activated carbon (Pahari and Sharma, 1993).

One aspect where adsorption could play an important, and some time critical, role is QACs bioavailability. In aqueous systems, the liquid-phase QACs concentration is

Table 2.1. Literature Freundlich isotherm parameters for different BACs.

QAC	Adsorbent	K_f	n	Reference
BTEA[a]	Course Activated Carbon	0.009	0.63	Pahari and Sharma, 1993
	Fine Activated Carbon	0.011	0.69	
C_{12}-C_{14}-C_{16}BDMA[b]	Activated Sludge	42.1	0.25	Zhang et al., 2011
C_{12}BDMA	Primary sludge	4.73	0.83	Ismail et al., 2010
C_{16}BDMA		20.73	0.76	
C_{12}BDMA	Waste activated sludge	3.81	0.86	
C_{16}BDMA		18.89	0.74	
C_{12}BDMA	Mesophilic digested sludge	10.76	0.58	
C_{16}BDMA		82.03	0.56	
C_{12}BDMA	Thermophilic digested sludge	9.76	0.63	
C_{16}BDMA		99.66	0.48	
C_{14}BDMA	Activated Sludge	70.2	0.39	Ren et al., 2011

[a] BTEA, benzyl triethyl ammonium chloride
[b] BDMA, benzyl dimethyl ammonium chloride

controlled by the extent of adsorption. At high adsorbent concentrations the QACs liquid-phase concentration will decrease dramatically, reducing their bioavailability, potency, and ultimately their effect on the system. Zhang et al. (2010) studied the adsorption behavior of a BAC mixture in activated sludge systems and found that increased biomass concentration (the adsorbent) reduced the QACs liquid-phase concentration resulting in lower toxicity.

2.4.4 QACs biotransformation

The alkyl chain length of QACs not only determines their physical/chemical properties, but also affects the fate and effect of these compounds in the environment. Under aerobic conditions, the biotransformation of QACs generally decreases as the number of the alkyl groups with more than one carbon increases. Substitution of a methyl group with a benzyl group further decreases QAC biotransformation potential (Ying, 2006; van Ginkel and Kolvenbach, 1991; Sutterlin et al., 2008a).

The general pathway of aerobic QAC biodegradation is a combination of hydrocarbon and N-methylamine biotransformation. Monooxygenase-catalyzed C-N bond cleavages take place resulting in dealkylation and demethylation, followed by β-oxidation, TCA cycle and final mineralization (Figure 2.7) (van Ginkel et al., 1992; Nishiyama et al., 1995; Kim et al., 2001). On the other hand, benzalkonium chloride C-N bond fission in aerobic biodegradation is believed to require an oxygen independent dehydrogenase (Figure 2.8) (Patrauchan and Oriel, 2003).

QACs are 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).

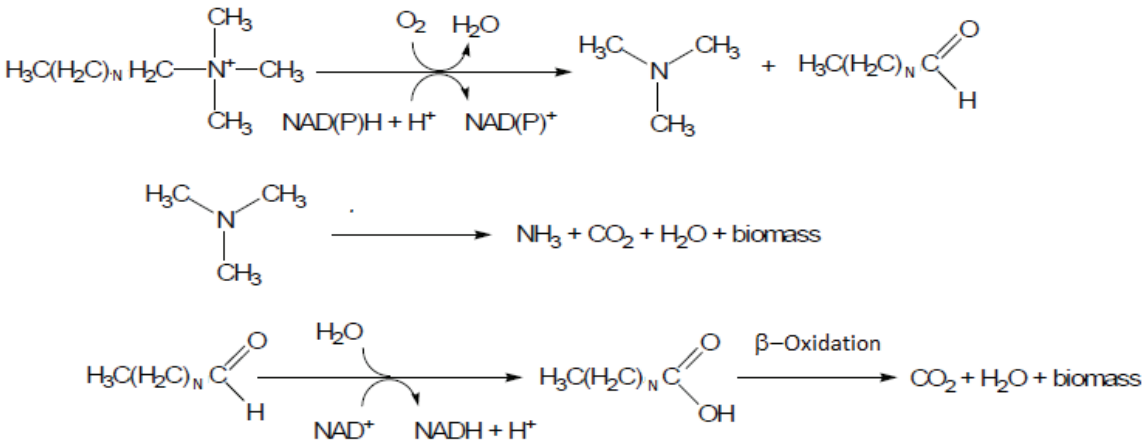

Figure 2.7. Monooxygenase-catalyzed C-N bond cleavages in QACs degradation (adapted from van Ginkel et al., 1992)

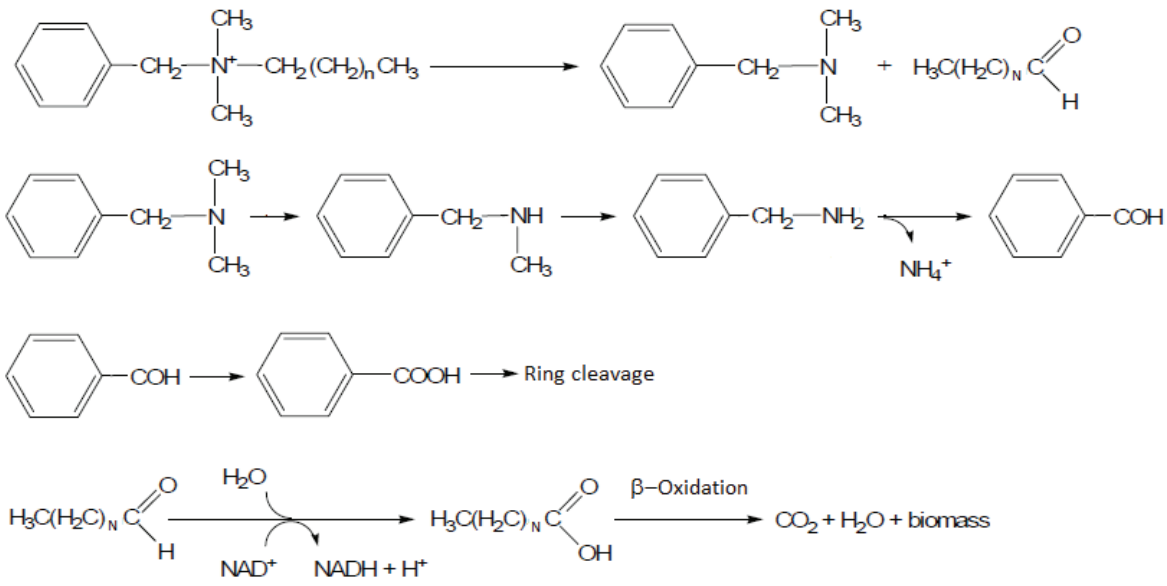

Figure 2.8. Dehydrogenase-catalyzed C-N bond cleavages in BAC degradation (adapted from Patrauchan and Oriel, 2003)

BAC transformation under anoxic/anaerobic (nitrate reducing) conditions was only recently reported by means of an abiotic (yet biologically initiated) reaction. This BAC transformation followed the modified Hofmann degradation pathway, producing alkyl dimethyl amines (tertiary amines) at equimolar levels to BAC transformed, which were not further degraded under the conditions of this previous study (Tezel and Pavlostathis, 2009).

2.4.5 QACs interaction with BNR systems

The main sources of HSWWs are industries which require a high level of sanitation during their production stages (food industries, slaughterhouses, poultry processing, and caning). Federal and state regulations require that equipment and facilities used for the first processing of all animal products prepared for human consumption be completely cleaned after every 8 hours of operation to maintain sanitary conditions (USEPA, 2004). Sanitary conditions are established within such establishments by using industrial sanitizers. The use of sanitizers is likely to increase as poultry processing plant operators face more stringent regulations related to water consumption and conservation, while complying with more strict sanitation rules and wastewater regulations (USEPA, 2004). QAC antimicrobial compounds, mainly BAC, are among the most widely used active ingredients in industrial sanitizers (Langsrud and Sundheim, 1997; Denyer and Stewart, 1998; Mullapudi et al., 2008; Sutterlin et al., 2008b).

Wastewater generated by QACs-using industries present a challenge to biological treatment systems, mainly because QACs, as potent biocides, lack selective toxicity and target specificity (Ioannou et al., 2007). Therefore, the bioactivity of QACs is carried

over with the wastewater targeting different microbial groups employed in biological treatment systems, thus affecting the key biological processes in the treatment system.

In anaerobic treatment, inhibition of methanogenesis by QACs is of great concern (Shcherbakova et al., 1999), where QACs at 25 mg/L or higher totally inhibited methanogenesis (Tezel et al., 2006). Nitrifying bacteria are severely affected by QACs (Kreuzinger et al., 2007; Yang, 2007), potentially by affecting the specific uptake mechanism for ammonium (Sutterlin et al., 2008a). BAC concentrations of 2 mg/L resulted in total inhibition of nitrification (Sutterlin et al., 2008a). In addition, the QACs inhibitory effect on nitrification is long-lasting. Pavlostathis et al. (2008) reported a long-term nitrification inhibition in the aerobic mixed liquor of a BAC-exposed BNR plant treating poultry processing wastewater, and that nitrification recovery was only possible after 30 days of the initial BAC exposure in the affected plant. Taking into account that nitrification is considered as the controlling process in BNR (Del-Pozo and Diez, 2003), QACs may have a pronounced, devastating effect on the overall BNR process performance.

QACs also inhibit denitrification. The effect of QACs on denitrification was previously investigated and was reported that QACs at and above 50 mg/L inhibited DNRA and caused incomplete denitrification resulting in the accumulation of nitrous oxide (Tezel et al., 2008). Regulations require low levels of total effluent nitrogen (USEPA, 2004), a goal achieved by maintaining a complete and efficient denitrification process in BNR systems. Furthermore, incomplete denitrification can result in nitrous oxide production rather than dinitrogen (Otte et al., 1996). Nitrous oxide is a potent greenhouse gas (Czepiel et al., 1995), and its emissions are strictly regulated (USEPA,

2010). Therefore, maintaining an efficient denitrification process during wastewater treatment is of paramount importance.

2.5 Kinetics and Modeling of BNR Processes

Kinetics of microbially-mediated reactions are generally modeled with one fundamental assumption: the microbial growth rate of a specific population (X) is stoichiometrically dependent on the utilization rate of a growth-limiting substrate(s):

$$\frac{dX_a}{dt} = \left(Y_1 \frac{dS_1}{dt} + Y_2 \frac{dS_2}{dt} + \ldots + Y_n \frac{dS_n}{dt} \right) \qquad \text{(Equation 2.1)}$$

where X_a and S_1 to S_n are the active microbial population and the limiting substrate(s) concentration in the system, respectively, and Y_1 to Y_n are the stoichiometric microbial yield coefficients for each substrate. Modeling of nitrification and denitrification kinetics follows the same approach.

2.5.1 Kinetics of nitrification

As discussed in section 2.2.1 above, biological autotrophic nitrification is achieved through a two-step process, $NH_4^+ \rightarrow NO_2^-$ and $NO_2^- \rightarrow NO_3^-$, mediated by two distinct microbial groups. For the first step of nitrification, mediated by AOB, both ammonia (Hellinga et al., 1999; Van Hulle et al., 2007) and ammonium (Hao et al., 2002; Moussa et al., 2005; Kampschreur et al., 2008) have been used as the initial substrate for AOB. In either case, the Monod equation is used to describe the substrate limitation on growth (Sin et al., 2008). Additional terms are also used for the first step, mainly to account for total ammonia and nitrous acid inhibition, pH effect on growth, and oxygen

and inorganic nitrogen limitation (Sin et al., 2008). Maximum specific growth rates for AOB values range between 1 and 2.1 d^{-1} (Hellinga et al., 1999; Moussa et al., 2005; Van Hulle et al., 2007), while half-saturation constant values range between 0.42 and 1.0 mg N/L (Hellinga et al., 1998; Henze et al., 2000; Sin et al., 2008; Fang et al., 2009)

In the second step of nitrification, mediated by NOB, both nitrite (Hao et al., 2002; Moussa et al., 2005) and nitrous acid (Hellinga et al., 1999; Pambrun et al., 2006) have been used as the initial substrate. Again, similar to the first step, the Monod equation is used to describe the substrate limitation on growth (Sin et al., 2008). Maximum specific growth rates for NOB values range between 0.56 and 1.05 d^{-1} (Hellinga et al., 1999; Moussa et al., 2005; Van Hulle et al., 2007), while half-saturation constant values range between 0.05 and 2.0 mg N/L (Hellinga et al., 1998; Henze et al., 2000; Sin et al., 2008; Fang et al., 2009). The wide variation of the reported parameter values is related to different conditions and factors, such as process characteristics and history, experimental technique, substrate diffusion, biomass adaptation, and finally model structure (Sin et al., 2008).

2.5.2 Kinetics of denitrification

As discussed in section 2.2.2 above, at the enzymatic level, denitrification is a four-step process, i.e., $NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$. However, denitrification may be carried out entirely by one group of denitrifying heterotrophic microorganisms leading to complete denitrification or by the activity of more than one group of denitrifying heterotrophic microorganisms leading to partial denitrification, i.e. different steps carried out by different organisms (Knowles, 1982; Zumft, 1997; Sin et al., 2008). As a result, in mixed culture systems, complete denitrification relies on several bacterial species.

Mathematically, denitrification can be described as if the reduction steps are carried out sequentially (i.e., by the use of mixed-substrate expressions) or simultaneously (i.e., by the use of single-substrate expressions). In either case, the Monod equation is commonly used to describe the substrate limitation on growth (Sin et al., 2008). A common assumption in denitrification models is that the last two steps of the denitrification process occur faster than the former two steps; hence, the kinetic description of nitric oxide (NO) and nitrous oxide (N_2O) formation/consumption is neglected.

As in the case of nitrification, denitrification models assume that the microbial growth rate is limited by the substrate utilization rate. Moreover, organic carbon utilization is included in the models to reflect the dependency of facultative denitrifiers on carbon. Finally, an anoxic growth factor is introduced alongside the substrate utilization rate to account for the reduction of growth rate under anoxic conditions (Sin et al., 2008). Not doing so may result in an overall denitrification rate that is higher than the aerobic respiration rate. Reported values for the maximum specific growth rate on nitrate range between 1.5 and 3 d^{-1}, while half-saturation constant values range between 0.14 and 0.5 mg N/L (Hellinga et al., 1999; Sin et al., 2008; Kaelin et al., 2009). Anoxic growth factor values range between 0.25 and 0.8. For nitrite, reported maximum specific growth rate values range between 1.5 and 3 d^{-1}, while half-saturation constant values range between 0.12 and 0.5 mg N/L (Hellinga et al., 1999; Sin et al., 2008; Kaelin et al., 2009). Discrepancies in reported values are related to process characteristics and history, type of organic carbon source, substrate diffusion, biomass adaptation, and finally model structure (Sin et al., 2008).

2.5.3 Modeling of BNR systems

BNR processes are generally modeled within activated sludge models. The benchmark for activated sludge models is the International Water Association (IWA) Activated Sludge Model (ASM), with its four variations: ASM1, ASM2, ASM2d, and ASM3. The ASM1 was initially developed to be a platform that can be used for future development of models for nitrogen-removing activated sludge processes (Henze et al., 2000). After its initial introduction, many development steps resulted in different variations of the ASM: ASM1, includes nitrogen removal processes, ASM2 includes biological phosphorus removal processes, ASM2d includes denitrifying, phosphate-accumulating organisms (PAOs), and ASM3, is based on the concept of internal storage of compounds, which in turn affect the metabolism of the organisms in activated sludge processes (Henze et al., 2000). Regarding nitrogen removal, activated sludge models are built to model single-step nitrification and denitrification. In reality, as mentioned above, both the nitrification and denitrification processes have multiple steps. The new aspects brought about by the newer ASM models, have yet to be fully embraced by the international community. Despite the limitations of ASM1, its universal appeal and practical verification overshadow its limitations (Alex et al., 2003).

The main structure of the ASM models is based upon utilizing multiple rate processes which connects a group of variables and parameters in a structured model. The processes mainly reflect microbial growth/decay, substrate utilization, and inhibition. Figure 2.9 shows how the different variables are connected in the ASM1 model. The processes included in the ASM1 are:

1. Aerobic growth of heterotrophs utilizing organic carbon, oxygen, and ammonia
2. Anoxic growth of heterotrophs utilizing organic carbon, nitrate, and ammonia
3. Aerobic growth of autotrophs utilizing oxygen and ammonia
4. Decay of heterotrophs
5. Decay of autotrophs
6. Ammonification of soluble organic nitrogen
7. Hydrolysis of entrapped organics
8. Hydrolysis of entrapped organic nitrogen

Both microbial growth and substrate utilization rates are modeled after Monod-type equations, while the remaining processes are modeled as first-order processes. All of the eight processes are taking place at the same time.

The ASM1 has some restrictions and limitations. The model assumes constant temperature, neutral pH, rate coefficients and parameters do not depend on concentration (i.e., they are constant), and there are no nutrient limitations (i.e., nitrogen, phosphorus, vitamins, and trace minerals are in excess relative to stoichiometry). Because of the above limitations, direct use of the ASM1 is almost non-existent, but ASM1 has been used as the core of numerous models with various modifications to fit almost every case (Henze et al., 2000).

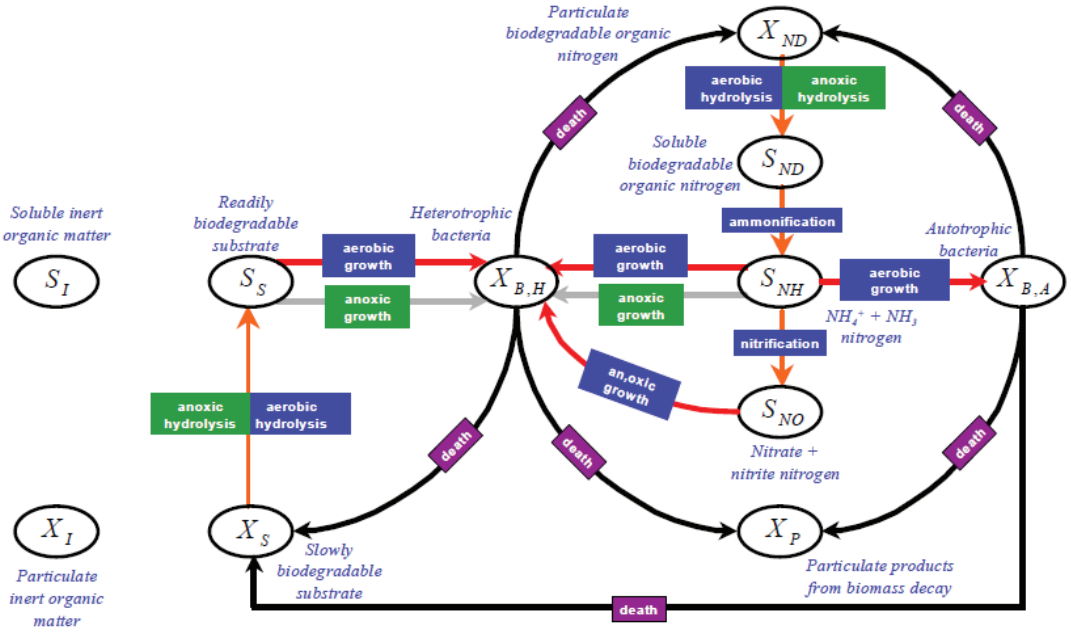

Figure 2.9. ASM1 general overview. Variables are encircled while arrows represent the different processes (Alex et al., 2008).

2.6 Problem Identification

QAC antimicrobial compounds present a challenge to BNR operation because of the inhibitory effect on both nitrification and denitrification. As discussed above, QACs is unlikely to inhibit municipal wastewater treatment at the levels normally expected in such systems. However, the extensive use of QACs in food-processing facilities, mainly poultry and meat processing plants, results in relatively high concentrations of QACs in the HSWW generated in such facilities. Moreover, sudden discharges of QACs resulting from accidental spills could upset the BNR plant function.

Previous studies related to the effect of QACs on BNR processes (i.e., nitrification and denitrification) were conducted on individual biological processes within the confinement of a single environmental condition (aerobic or anoxic) and did not assess the effect of QACs on multiple reactions under alternating 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.

As shown in Figure 2.10, 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 of QACs chemical and physical interactions is of vital importance when faced with

the challenge of maintaining an adequate treatment capacity while handling QACs-bearing HSWW (the poultry processing wastewater). This information is currently lacking, which became the impetus of the present research.

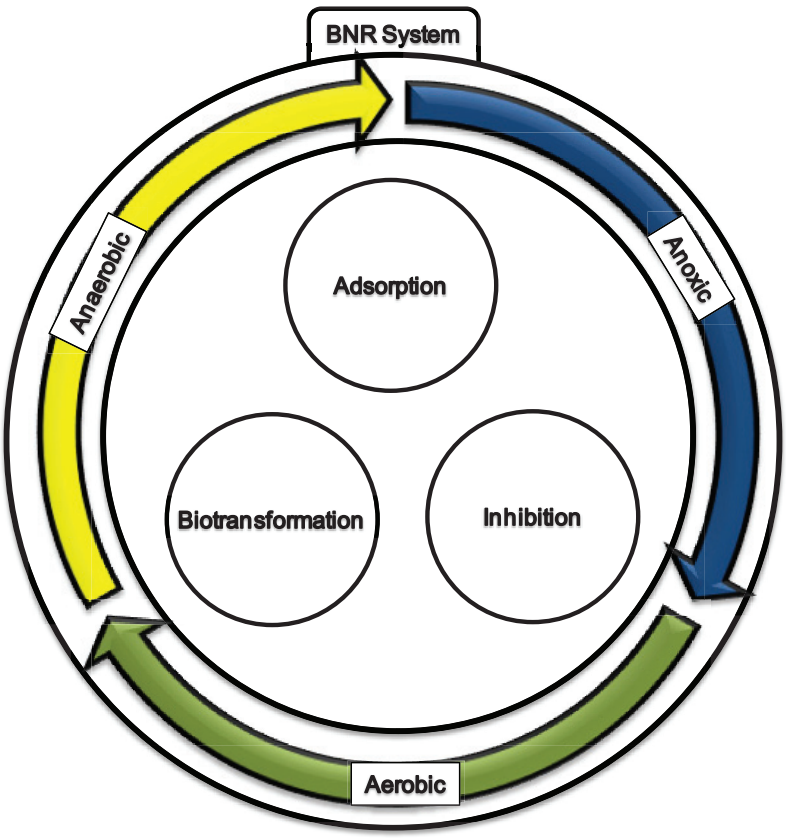

Figure 2.10. A schematic representation of three sub-processes related to the fate and effect of QACs in a biological treatment system. Circles represent the three sub-processes and arrows represent the sequential change of environmental conditions in the BNR system (anaerobic-anoxic-aerobic).

CHAPTER 3

MATERIALS AND METHODS

3.1 General Analytical Methods

3.1.1 pH

All pH measurements were performed using the potentiometric method with a ATI Orion Model 370 digital pH meter (Orion Research Inc., Boston, MA) and a gel-filled combination pH electrode (VWR International, West Chester, PA). The meter was calibrated weekly with pH 4.0, 7.0, and 10.0 standard buffer solutions (Fisher Scientific, Pittsburg, PA).

3.1.2 Ammonia

Ammonia was measured using the distillation method described in *Standard Methods* (Eaton et al., 2005). The samples were centrifuged at 10,000 rpm for 15 minutes and filtered through a 0.2 μm nitrocellulose membrane filter (Fisher Scientific, Pittsburgh, PA). Ammonia distillation was performed using a distillation apparatus (Labconco Corp., Kansas City, MO). The distillate was then titrated with 0.2 N H_2SO_4 and the ammonia was quantified.

3.1.3 Total and Soluble Chemical Oxygen Demand (tCOD and sCOD)

COD was measured using the closed reflux, colorimetric method as described in *Standard Methods* (Eaton et al., 2005). An aliquot of 3 mL digestion solution composed of 4.9 g $K_2Cr_2O_7$, 6 g $HgSO_4$, 6 g Ag_2SO_4 and 500 mL H_2SO_4 was transferred to HACH®

COD digestion vials (HACH® Company, Loveland, CO) and then 2 mL of sample was added to the vial. After tumbling the vial 4 to 8 times, the vial content was digested at 150^{o}C for 2 hours and then cooled down to room temperature. The absorbance was measured at 620 nm with a Hewlett-Packard Model 8453 UV/Visible spectrophotometer (Hewlett-Packard Co., Palo Alto, CA) equipped with a diode array detector, deuterium and tungsten lamps and a 1 cm path length. Samples were centrifuged and filtered through a 0.2 μm nitrocellulose membrane filter if the sCOD was measured; otherwise, well-mixed samples were used after appropriate dilution for tCOD measurements. All samples were prepared in triplicate and a calibration curve was prepared using 1 g/L standard solution of potassium hydrogen phthalate (KHP).

3.1.4 Total and Volatile Suspended Solids (TSS and VSS)

TSS and VSS were determined according to procedures described in *Standard Methods* (Eaton et al., 2005). Whatman GF/C glass fiber filters (47 mm diameter and 1.2 μm nominal pore size; Whatman, Florham Park, NJ) were washed with de-ionized (DI) water and ignited at 550^{o}C for 20 minutes in a Fisher Isotemp Model 550-126 muffle furnace before use. The filters were then cooled in a desiccator and weighed. Samples of known volume were filtered through the glass fiber filters. The filters were then rinsed with 10 mL DI water to remove dissolved organics and inorganic salts. The filters containing the samples were dried at 105^{o}C for 90 minutes. After cooling in a desiccator, the dry weight was recorded and the filters containing the dry samples were ignited at 550^{o}C for 20 minutes. After ignition, the samples were cooled down in a desiccator and the weight was measured. TSS and VSS concentrations were then calculated using the equations below.

$$\text{TSS (mg/L)} = \frac{\text{Filter weight after } 105^{o}\text{C (mg)} - \text{Filter tare weight (mg)}}{\text{Sample volume (L)}}$$

$$\text{VSS (mg/L)} = \frac{\text{Filter weight after } 105^{o}\text{C (mg)} - \text{Filter weight after } 550^{o}\text{C (mg)}}{\text{Sample volume (L)}}$$

3.1.5 Total Gas Production

Total gas production in closed assay bottles and large volume reactors was measured by either the gas-water displacement method or with a VWR Pressure/Vacuum transducer (resolution –1 atm to 1.974 atm with an accuracy of 0.002 atm).

3.1.6 Gas Composition

The gas composition was determined by a gas chromatography (GC) unit (Agilent Technologies, Model 6890N; Agilent Technologies, Inc., Palo Alto, CA) equipped with two columns and two thermal conductivity detectors. Dinitrogen (N_2) was separated with a 15 m HP-Molesieve fused silica, 0.53 mm i.d. column (Agilent Technologies, Inc.). Carbon dioxide (CO_2), nitric oxide (NO) and nitrous oxide (N_2O) were separated with a 25 m Chrompac PoraPLOT Q fused silica, 0.53 mm i.d. column (Varian, Inc., Palo Alto, CA). Helium was used as the carrier gas at a constant flow rate of 6 mL/min. The 10:1 split injector was maintained at 150^{o}C, the oven was set at 40^{o}C and the detector temperature was set at 150^{o}C. All gas analyses were performed by injecting a 100 µL gas sample. The minimum detection limit for CO_2, NO, N_2O and N_2 was, 800, 500, 7 and 50 ppmv, respectively.

3.1.7 Volatile Fatty Acids (VFAs)

VFAs (C_2 to C_7, i.e., acetic, propionic, iso-butyric, n-butyric, iso-valeric, n-valeric, iso-caproic, n-caproic and heptanoic acids) were measured after acidification of

filtered samples with a 2.5% H_3PO_4 solution containing 1.5 g/L acetoin as the internal standard (sample:acid, 2:1 volume ratio) using an Agilent 6890 Series GC unit equipped with a flame ionization detector and a 35-m Stabilwax-DA, 0.53-mm I.D. column (Restek, Bellefonte, PA). Samples used for the measurement of VFAs were prepared by centrifugation at 10,000 rpm for 30 minutes and filtration through 0.22-μm PVDF membrane filters before acidification. The minimum detection limit for each acid mentioned above was 0.25, 0.10, 0.03, 0.02, 0.10, 0.08, 0.02, 0.02, 0.05 mM, respectively.

3.1.8 Nitrite and Nitrate

Nitrite (NO_2^-) and nitrate (NO_3^-) concentrations were determined using a Dionex DX-100 ion chromatography unit (Dionex Coorporation, Sunnyvale, CA) equipped with a suppressed conductivity detector, a Dionex IonPac AG14A (4x50mm) precolumn, and a Dionex IonPac AS14A (4x250 mm) analytical column. The unit was operated in autosupression mode with 1 mM $NaHCO_3$/8 mM Na_2CO_3 eluent and a flow rate of 1 mL/min. All samples were filtered through 0.2 μm membrane filters prior to injection. The minimum detection limit for nitrite and nitrate was 0.02 and 0.04 mM, respectively.

3.1.9 Dissolved Oxygen (DO)

The DO concentration of the cultures used in this study was measured using the polarographic method (Eaton et al., 2005) with a YSI Model 58 oxygen meter in conjunction with a YSI 5750 oxygen probe (Yellow Springs Instrument, Yellow Springs, OH). The instrument was calibrated to water-saturated air (at a given temperature) before each use and the probe electrolytic solution and membrane were changed periodically.

3.1.10 Total Nitrogen (TN)

Total nitrogen was measured using the alkaline persulphate digestion method (HACH method NO. 10072). The nitrogen of 0.5 mL sample aliquot was digested and oxidized to nitrate in an alkaline persulphate solution at 104^{o}C in glass digestion vials for 30 minutes. Nitrate was then reduced to nitrite in an alkaline hydrazine sulphate solution, containing copper as a catalyst. The resulting nitrite was reacted with sulphanilamine under acidic conditions to form a diazo compound, coupling with naphthylethylenediamine forming an azo dye. The resulting color intensity was proportional to the sample nitrogen concentration. Absorbance was measured at 410 nm with a Hewlett-Packard Model 8453 UV/Visible spectrophotometer (Hewlett-Packard Co., Palo Alto, CA) equipped with a diode array detector, deuterium and tungsten lamps and a 1 cm path length. Well-mixed samples were used after appropriate dilution for TN measurements. All samples were prepared in triplicate and a calibration curve was prepared using 1 g/L standard solution of ammonium chloride. Figure 3.1 shows the calibration curve.

3.1.11 Total Carbohydrates

Total carbohydrates were measured using the anthrone method (Morris, 1948). The anthrone solution was prepared by dissolving 1 g of anthrone (Sigma Aldrich) in 500 mL 98% H_2SO_4. Each sample was digested in glass digestion vials with the 0.2% anthrone solution for 15 minute in a boiling water bath. Absorbance was measured at 620 nm with a Hewlett-Packard Model 8453 UV/Visible spectrophotometer (Hewlett-Packard Co., Palo Alto, CA) equipped with a diode array detector, deuterium and tungsten lamps and a 1 cm path length. Well-mixed samples were used after appropriate dilution for total

carbohydrates measurements. All samples were prepared in triplicates and a calibration curve was prepared using 1 g/L standard solution of glucose. Figure 3.2 shows the calibration curve.

3.1.12 Total Lipids

Total lipids were measured using a gravimetric method after liquid-liquid extraction with chloroform and methanol (Pavlostathis, 1985). A sample aliquot was pre-acidified using 1.0 N hydrochloric acid to pH ≤ 3, and then mixed with 40 and 100 mL chloroform and methanol, respectively, in a blender for 1 minute. An additional 60 and 40 mL of chloroform and methanol was added with further mixing. The blender contents were then filtered through a No.40 Whatman® filter, and the filtrate transferred into an extraction funnel and allowed to separate overnight. The chloroform bottom layer was transferred to a pre-weighed Erlenmeyer flask (initial flask weight) and the chloroform was evaporated by placing the flask on a heating plate set at 80^{o}C. The flask was then placed in a 100^{o}C oven in order to dry the remaining solvent. After being cooled in a desiccator, the flask was weighed again (final flask weight) and the total lipids concentration was calculated by the equation below.

$$\text{Total Lipids (mg/L)} = \frac{\text{Final flask weight (mg)} - \text{Initial flask weight (mg)}}{\text{Sample volume (L)}}$$

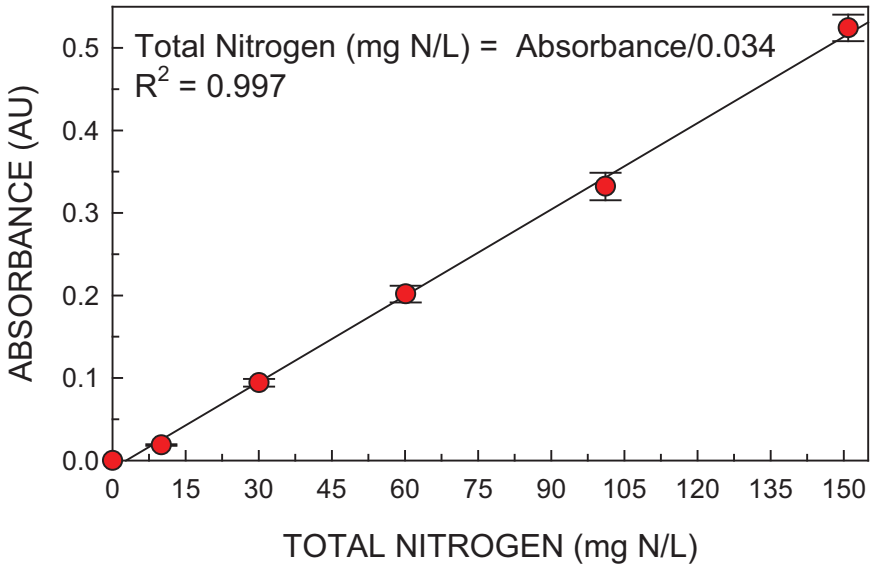

Figure 3.1. Calibration curve for the total nitrogen analysis by the alkaline persulphate digestion method.

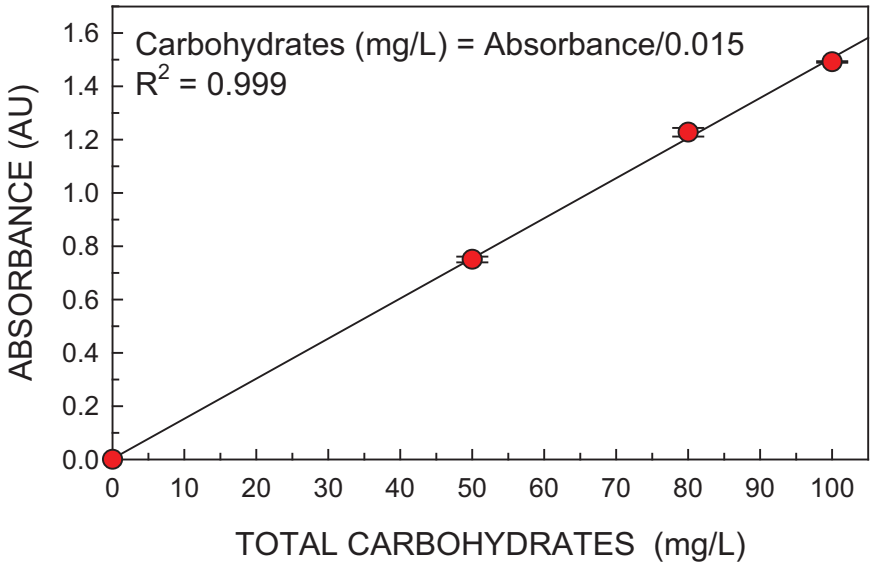

Figure 3.2. Calibration curve for the total carbohydrates analysis by the anthrone method.

3.2 Analysis of BACs

3.2.1 Disulfine Blue Pair-Ion Extraction Method (DSB-PIX)

The BACs concentration in whole and centrifuged culture samples was measured using a previously reported and modified disulfine blue pair-ion extraction (DSB-PIX) method (HMSO, 1981; Tezel et al., 2006). According to this method, an anionic dye-QAC ion pair is formed, which is then solvent extracted, and the color intensity in the solvent phase is measured spectrophotometrically. Analyses were carried out in 25-mL test tubes by adding 5 mL of acetate buffer, 2 mL of dye solution and 2 mL of the sample. The acetate buffer included 115 g anhydrous sodium acetate and 35 mL glacial acetic acid in 1 L DI water. The dye solution was prepared by dissolving 0.16 g of Patent Blue VF (Acros Organics, N.J., USA) in 2 mL ethanol and diluting to 250 mL with DI water. Addition of 10 mL of methylene chloride to the 25-mL test tube resulted in the formation of a biphasic solution that was tumbled for 24 hours in order to achieve complete transfer of the dye-QAC ion pair into the solvent phase. A portion of the bottom solvent layer was then transferred into 2-mL clear glass vials and the color intensity measured with a UV/Vis HP model 8453 spectrophotometer equipped with a diode array detector (Hewlett-Packard Co., Palo Alto, CA, USA). The QAC concentration was quantified based on sample absorbance at the characteristic maximum wavelength of 628 nm and previously prepared calibration curves for BAC at a concentration range 0 to 30 mg/L (Figure 3.3). Methylene chloride was used as the blank for all spectrophotometric analyses. The minimum method detection limit was 0.2 mg/L. Major BAC transformation products reported so far include tertiary and secondary amines which are positively

charged at neutral pH. Interference of these compounds with the quantification of BACs with the DSB-PIX method is insignificant (Tezel, 2009).

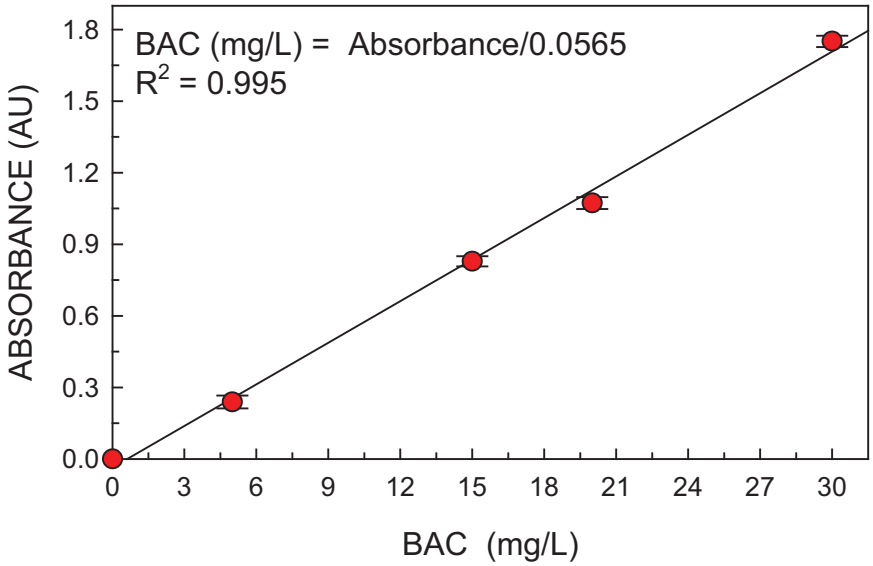

Figure 3.3. Calibration curve for the BAC analysis by the DSB-PIX method.

3.2.2 High Performance Liquid Chromatography (HPLC)

Benzalkonium chlorides as well as 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.

3.3 General Procedures

3.3.1 Denitrifying Culture Media

A mixed denitrifying culture used in this study was maintained with media that provided nutrients, trace metals, and vitamins. The composition of the culture media is shown in Table 3.1. Culture media was prepared by adding the first five ingredients in Table 3.1 to 8 L DI water in a 9 L Pyrex bottle. The bottle was then autoclaved at 121^{o}C

and 21 psi (1.43 atm) for 45 minutes. After autoclaving, the bottle contents were purged with helium for 1.5 hours in order to strip oxygen from the media. After purging, and while the media were still warm, the rest of the ingredients listed in Table 3.1were added.

Table 3.1. Composition of media for the mixed denitrifying culture used in this study

Compound/Solution	**Concentration g/L**
K_2HPO_4	0.9
KH_2PO_4	0.5
NH_4Cl	0.5
$MgCl_2 \cdot 6H_2O$	0.2
Trace metal stock solution	1 mL/L
Vitamin stock solution	1 mL/L
$CaCl_2 \cdot 2H_2O$	0.1
$FeCl_2 \cdot 4H_2O$	0.1
$NaHCO_3$	3.5
Trace metal stock solution	**Concentration g/L**
$ZnCl_2$	0.5
$MnCl_2 \cdot 4H_2O$	0.3
H_3BO_3	3.0
$CoCl_2 \cdot 6H_2O$	2.0
$CuCl_2 \cdot 2H_2O$	0.1
$NiSO_4 \cdot 6H_2O$	0.2
$Na_2MoO_4 \cdot 2H_2O$	0.3
Vitamin stock solution	**Concentration g/L**
Biotin	0.2
Folic Acid	0.2
Pyridoxine hydrochloride	1.0
Riboflavin	0.5
Thiamine	0.5
Nicotinic Acid	0.5
Pantothenic Acid	0.5
Vitamin B12	0.01
p-Aminobenzoic Acid	0.5
Thioctic Acid	0.5

CHAPTER 4

BIOLOGICAL NITROGEN REMOVAL SYSTEM – DEVELOPMENT AND BASELINE OPERATION

4.1 Introduction

Biological nitrogen removal (BNR) is widely employed for the treatment of high-strength wastewater as discussed in Chapter 2. Two distinct biological processes, mediated by two microbial populations, are responsible for BNR reactions: nitrification (ammonia oxidation to nitrate by nitrifying autotrophic population) and denitrification (nitrate reduction to dinitrogen by facultative heterotrophic denitrifying population), under aerobic and anoxic conditions, respectively. In engineered BNR systems compartmentalization is utilized to achieve the two aforementioned environmental conditions, either temporally (sequencing batch reactor), or spatially (multi-stage, separate bioreactors with recycle). BNR systems are ideally designed to provide complete nitrogen removal for a specific wastewater stream. Wastewater characteristics, such as flow rate, organic carbon and nitrogen content, and the existence of toxicants, determine the design criteria of the BNR system, including reactor mixed liquor concentration, solids and hydraulic retention times, supplementary organic carbon, dissolved oxygen concentration, and pH control.

As discussed in Chapter 2, the presence of QAC antimicrobials in HSWW streams presents a challenge to BNR by impacting the microbial populations responsible

for the treatment process, and ultimately resulting in reducing nitrogen removal efficiency of the BNR system. A QAC mixture of three benzalkonium chlorides (BACs) homologs widely used in industrial sanitizer formulations was chosen as the target QACs for this study. BACs were chosen because, as discussed in Chapter 2, they are the most commonly used antimicrobial QACs (Tiedink, 2001).

A modified pre-denitrification, one-sludge system, based upon the Barnard process (Rittmann and McCarty, 2001), was chosen as a model BNR system. Figure 4.1 shows the system schematic. The system was operated fed with real high-strength poultry processing wastewater. In the chosen BNR system, the wastewater is first utilized as a source of organic carbon for denitrification in an anoxic reactor, thus the designation pre-denitrification. Nitrification is performed in a subsequent aerobic reactor, while nitrate is recycled from the aerobic reactor to the anoxic reactor. The multi-stage system setup was specifically chosen for this study for two main reasons. The spatial separation allowed for a relatively fast establishment of stable BNR process, along with ease of maintenance and operation. In addition, it facilitated the examination of the fate and effect of QACs on the two main BNR processes independently, and eventually, collectively.

The main objective of the research presented in this chapter was to independently delineate the fate and effect of initial BAC exposure on the two main processes in a BNR system, nitrification and denitrification. In order to achieve this objective, a laboratory-scale, continuous-flow, multi-stage BNR system, treating BAC-free real wastewater was constructed and operated. Additionally, a mixed denitrifying culture, which was developed to study the effect of BAC on denitrification, was also used.

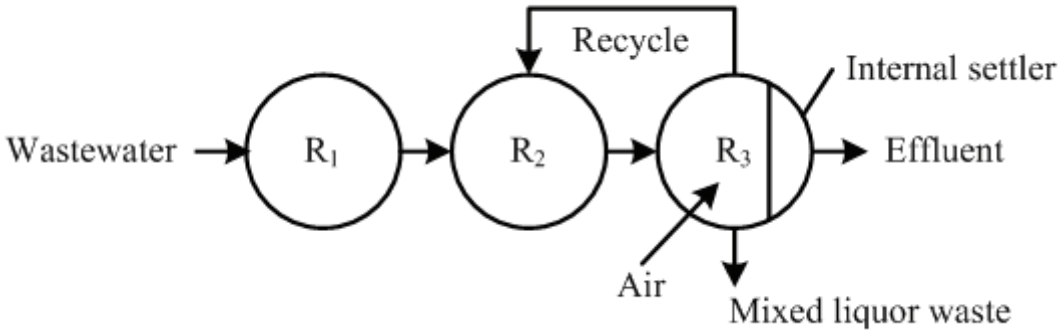

Figure 4.1. Schematic diagram of the continuous-flow, multi-stage, laboratory-scale BNR system (R_1, anaerobic; R_2, anoxic; and R_3, aerobic).

4.2 Materials and Methods

4.2.1 Theoretical BNR System Design

A modified, conservative, stoichiometrically-controlled approach was used in order to design the BNR system. This approach is based on establishing a stable and continuous nitrification. In a conservative design, kinetics do not control the overall performance in terms of nitrogen removal (stoichiometry does), as long as the solids retention time is sufficiently long (Rittmann and McCarty, 2001). The design assumes a one-step nitrification and denitrification reaction scheme, i.e., ammonia to nitrate and nitrate to dinitrogen, respectively, with complete ammonia oxidation in the aerobic reactor and complete nitrate reduction in the anoxic reactor. While being unrealistic, the one-step reaction scheme is widely used in the design of wastewater treatment systems (Henze et al., 2000; Alex et al., 2008; Sin et al., 2008). At steady-state, the synthesis rate of nitrifiers in the system is:

$$\left(\frac{\Delta X}{\Delta t}\right)_{nit} = Y_{n(nit)}\left[Q \cdot TN_{feed} - \left(\left(\frac{\Delta X}{\Delta t}\right)_{aer} + \left(\frac{\Delta X}{\Delta t}\right)_{den}\right) \cdot 0.124 \frac{g-N}{g-VSS}\right] \qquad \text{(Equation 4.1)}$$

where $\left(\frac{\Delta X}{\Delta t}\right)_{nit}$, $\left(\frac{\Delta X}{\Delta t}\right)_{aer}$, $\left(\frac{\Delta X}{\Delta t}\right)_{den}$ are the net mass production rate of nitrifiers, aerobic heterotrophs, and anoxic heterotrophic denitrifiers (g VSS/day), $Y_{n(nit)}$ is the nitrifiers observed yield (g VSS/g NH_4^+-N), Q is the feed flow rate (L/day), and TN_{feed} is the feed total nitrogen concentration. Aerobic heterotrophs grow utilizing the COD escaping the anoxic reactor:

$$\left(\frac{\Delta X}{\Delta t}\right)_{aer} = Y_{n(aer)} \cdot Q \cdot (1+r) \cdot COD_{anox} \qquad \text{(Equation 4.2)}$$

where $Y_{n(aer)}$ is the aerobic heterotrophs observed yield (g VSS/g COD), COD_{anox} is the anoxic reactor effluent COD (g COD/L), and r is the nitrate mixed liquor recycle ratio relative to the feed flow rate. Anoxic heterotrophic denitrifiers grow utilizing the COD in the feed to a degree determined by available nitrate:

$$\left(\frac{\Delta X}{\Delta t}\right)_{den} = Y_{n(den)} \cdot Q \cdot \left(COD_{feed} - (1+r) \cdot COD_{anox}\right) \qquad \text{(Equation 4.3)}$$

where $Y_{n(den)}$ is the anoxic heterotrophic denitrifiers observed yield (g VSS/g COD), and COD_{feed} is the feed COD concentration (g COD/L). Based on stoichiometry for the complete aerobic nitrification, complete anoxic denitrification, and aerobic growth of heterotrophs, the observed yield coefficients for each of the aforementioned microbial groups can be evaluated (Rittmann and McCarty, 2001):

$$f_s = f_s^o \left(1 - \frac{f_d \cdot b \cdot \theta_c}{1 + b \cdot \theta_c}\right) \qquad \text{(Equation 4.4a)}$$

$$Y_{n(nit)} = \frac{8.07 \cdot f_s}{2.5 + f_s} \qquad \text{(Equation 4.4b)}$$

$$Y_{n(den)} = \frac{5.65 \cdot f_s}{8} \qquad \text{(Equation 4.4c)}$$

$$Y_{n(aer)} = 5.65 \cdot f_s \qquad \text{(Equation 4.4d)}$$

where f_s^o and f_s are the true and net fractions of electron donor (based on electron equivalents) utilized in cell synthesis, f_d is the net biodegradable fraction of the biomass, and b is the microbial decay coefficient (day^{-1}).

Assuming total nitrogen oxidation in the aerobic reactor, the resulting nitrate concentration in the anoxic reactor effluent (S_{no3}^{anoxic}) will be:

$$S_{no3}^{anoxic} = \frac{Q \cdot TN_{feed} - \left[\left(\frac{\Delta X}{\Delta t} \right)_{aer} + \left(\frac{\Delta X}{\Delta t} \right)_{den} + \left(\frac{\Delta X}{\Delta t} \right)_{nit} \right] \cdot 0.124 \frac{g\,N}{g\,VSS}}{Q(1+r)} \qquad \text{(Equation 4.5)}$$

Finally, COD_{anox} is calculated by performing a mass balance on the anoxic reactor, and utilizing the stoichiometric carbon to nitrogen ratio $(g\ COD / g\ NO_3^-\ N)$:

$$COD_{anox} = \frac{COD_{feed} - r \cdot S_{no3}^{anoxic} \cdot (g\ COD / g\ NO_3^-\ N)}{(1+r)} \qquad \text{(Equation 4.6)}$$

Solving Equations 4.1 to 4.6 simultaneously for a predefined feed flow rate, both feed COD (COD_{feed}) and total nitrogen (TN_{feed}) concentrations, and recycle ratio (r), will yield the overall growth rate in the system ($\left(\frac{\Delta X}{\Delta t} \right)_{tot}$):

$$\left(\frac{\Delta X}{\Delta t} \right)_{tot} = \left(\frac{\Delta X}{\Delta t} \right)_{aer} + \left(\frac{\Delta X}{\Delta t} \right)_{den} + \left(\frac{\Delta X}{\Delta t} \right)_{nit} \qquad \text{(Equation 4.7)}$$

At steady-state, the overall growth rate in the system equals the sludge waste rate. Thus, this fact can be used to connect the total mixed liquor concentration (X_{tot}) and combined anoxic and aerobic solids (θ_c) and hydraulic (θ_h) retention times:

$$\theta_h = \frac{\theta_c}{X_{tot} \cdot Q} \left(\frac{\Delta X}{\Delta t} \right)_{tot} \qquad \text{(Equation 4.8)}$$

The previous analysis allows for choosing a solids retention time based on the volatile suspended solids concentrations. Equations 4.1 to 4.6 were solved

simultaneously for various solids retention times values and each iteration resulted in different volatile suspended solids concentrations in the system.

4.2.2 Oxygen Uptake Rate (OUR)

OUR measurements were conducted to establish the activity of nitrifying bacteria in the aerobic reactor mixed liquor. By selectively inhibiting individual bacterial groups responsible for nitrification, the ratio of nitrifying bacteria within the community can be estimated. Ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) biomass concentration can be correlated with the OUR of each bacterial group. Assuming that ammonia, nitrite, and organic carbon are not the rate-limiting substrate, and biomass decay is negligible during the test period, the OUR for AOB, NOB, and the heterotrophic bacteria can be represented as:

$$OUR_{AOB} = \left(\frac{\mu'_{nh3}}{Y_{nh3}} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{nh3}} \right) X_a^{AOB} \quad \text{(Equation 4.9)}$$

$$OUR_{NOB} = \left(\frac{\mu'_{no2}}{Y_{ano2}} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{no2}} \right) X_a^{NOB} \quad \text{(Equation 4.10)}$$

$$OUR_h = \left(\frac{\mu'_h}{Y_h} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{h}} \right) X_h \quad \text{(Equation 4.11)}$$

$$\frac{OUR_{AOB}}{OUR_h} = \frac{\left(\frac{\mu'_{nh3}}{Y_{nh3}} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{nh3}} \right)}{\left(\frac{\mu'_h}{Y_h} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{h}} \right)} \frac{X_a^{AOB}}{X_h} \quad \text{(Equation 4.12a)}$$

$$\frac{X_a^{AOB}}{X_h} = \frac{OUR_{AOB}}{OUR_h} \frac{\left(\frac{\mu'_h}{Y_h} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{h}} \right)}{\left(\frac{\mu'_{nh3}}{Y_{nh3}} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{nh3}} \right)} = R_{AOB} \quad \text{(Equation 4.12b)}$$

$$\frac{OUR_{NOB}}{OUR_h} = \frac{\left(\frac{\mu_{no2}^{'}}{Y_{ano2}} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{no2}}\right)}{\left(\frac{\mu_h^{'}}{Y_h} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{h}}\right)} \frac{X_a^{NOB}}{X_h} \qquad \text{(Equation 4.13a)}$$

$$\frac{X_a^{NOB}}{X_h} = \frac{OUR_{NOB}}{OUR_h} \frac{\left(\frac{\mu_h^{'}}{Y_h} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{h}}\right)}{\left(\frac{\mu_{no2}^{'}}{Y_{ano2}} \cdot \frac{S_{O2}^{SAT}}{S_{O2}^{SAT} + K_{SO2}^{no2}}\right)} = R_{NOB} \qquad \text{(Equation 4.13b)}$$

$$X_a^T = X_a^{AOB} + X_a^{NOB} + X_h \qquad \text{(Equation 4.14a)}$$

$$X_a^T = R_{AOB} \cdot X_h + R_{NOB} \cdot X_h + X_h = X_h(1 + R_{AOB} + R_{NOB}) \qquad \text{(Equation 4.14a)}$$

$$X_h = \frac{X_a^T}{(1 + R_{AOB} + R_{NOB})} \qquad \text{(Equation 4.14b)}$$

$$X_a^{AOB} = \frac{R_{AOB} \cdot X_a^T}{(1 + R_{AOB} + R_{NOB})} \qquad \text{(Equation 4.14c)}$$

$$X_a^{NOB} = \frac{R_{NOB} \cdot X_a^T}{(1 + R_{AOB} + R_{NOB})} \qquad \text{(Equation 4.14d)}$$

where OUR_{AOB}, OUR_{NOB}, and OUR_h are the oxygen uptake rate for AOB, NOB and heterotrophic bacteria while utilizing ammonia, nitrate, and organic carbon, respectively; μ' (day^{-1}) and Y (mg N/mg COD and mg COD/mg COD for nitrifiers and heterotrophs, respectively) are the maximum growth rate and yield coefficient, respectively; S_{O2}^{SAT} and K_{SO2} are dissolved oxygen (DO) saturation concentration and oxygen half-saturation coefficient (mg/L), respectively; and X_a^{AOB}, X_a^{NOB}, X_h, and X_a^T are AOB, NOB, heterotrophic bacteria, and total biomass concentrations (mg VSS/L), respectively. Table 4.1 summarizes literature values used for μ', Y, S_{O2}^{SAT}, and K_{SO2}.

Table 4.1. Parameter values used in the theoretical BNR system design.

Parameter	Value (Units)	Reference
μ'_{nh3}	2.0 (day^{-1})	Sin et al., 2008
μ'_{no2}	1.05 (day^{-1})	Sin et al., 2008
μ'_{h}	6.0 (day^{-1})	Henze et al., 2000
Y_{nh3}	0.15 (mg COD/mg N)	Sin et al., 2008
Y_{ano2}	0.04 (mg COD/mg N)	Sin et al., 2008
Y_{h}	0.67 (mg COD/mg COD)	Henze et al., 2000
K_{SO2}^{nh3}	1.45 (mg N/L)	Sin et al., 2008
K_{SO2}^{no2}	1.1 (mg N/L)	Sin et al., 2008
K_{SO2}^{h}	0.2 (mg COD/L)	Henze et al., 2000
S_{O2}^{SAT}	8 (mg/L)	Rittmann and McCarty, 2001

For OUR measurements, a 200 mL mixed liquor aliquot was collected at the end of the recycle cycle during the daily mixed liquor waste time. The mixed liquor was aerated for about 5 minutes to achieve oxygen saturation while mixing with a stirring bar and a magnetic stirrer. Meanwhile, 20 mL of wastewater was introduced to a 50 mL tapered-neck Erlenmeyer flask which was pre-baked at 350^{o}C to remove all organic carbon. Then, 2.5 mL of each ammonia and nitrite (for OUR_1), or ammonia and sodium azide (NaN_3) (for OUR_2), or NaN_3 and allylthiourea (ATU) (for OUR_3) stock solutions were added to the flask, bringing the flask liquid volume to 25 mL. The flask contents were then mixed with a stirring bar and a magnetic stirrer. Finally, 25 mL of aerated mixed liquor was introduced into the flask, the DO probe was inserted, and the DO concentration over time was recorded. The OUR was estimated by linear regression analysis performed using SigmaPlot, Version 10 software (Systat Software Inc., San Jose, CA, USA) using the DO concentration-time data.

The OUR_1 was measured with aerobic reactor mixed liquor sample amended with acetate (the predominant FVA in the anaerobic reactor effluent), ammonia, and nitrite; OUR_2 was measured with the same mixed liquor amended with wastewater, ammonia, and NaN_3 as an inhibitor to NOB (Ginestet et al., 1998); and OUR_3 was measured with the same mixed liquor amended with wastewater, NaN_3 and ATU as an inhibitor to AOB and NOB, respectively (Surmacz-Gorska et al., 1996). Figure 4.2 illustrates the action of NaN_3 and ATU on the mixed liquor activity and OUR. The OUR for AOB and NOB was calculated as follows:

$$OUR_{AOB} = OUR_2 - OUR_3 \quad \text{(Equation 4.15)}$$

$$OUR_{NOB} = OUR_1 - OUR_2 \quad \text{(Equation 4.16)}$$

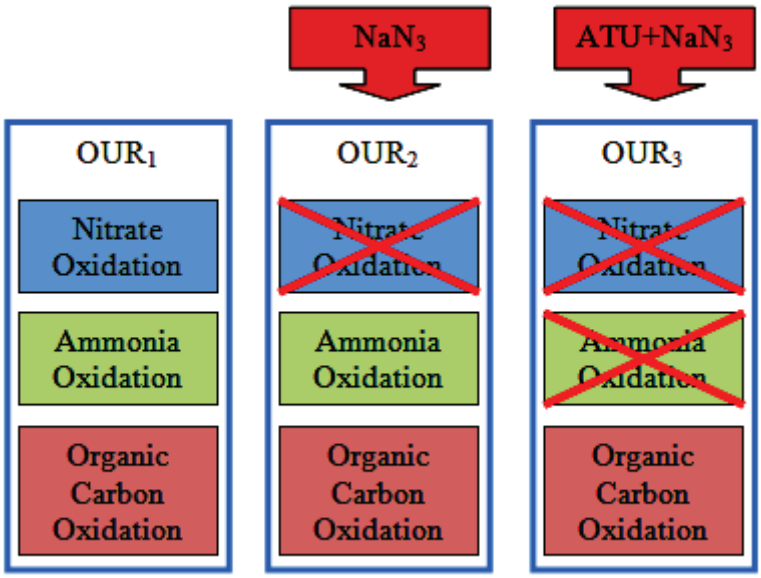

Figure 4.2. Schematic representation illustrating the action of NaN_3 and ATU on the mixed liquor activity and resulting OUR (Adapted from Surmacz-Gorska et al., 1996).

Using Equations 4.15 and 4.16 R_{AOB} and R_{NOB} were evaluated based on Equations 4.12a and 4.15b, and their values used in Equations 4.14c and 4.14d to evaluate the AOB and NOB biomass concentration.

4.2.3 Laboratory-Scale BNR System

The BNR system consisted of an anaerobic reactor (R_1), used to provide readily degradable organic carbon to be used in denitrification, an anoxic reactor (R_2) for the purpose of denitrification, and an aerobic reactor (R_3) for nitrification, with an internal biomass settler (Figure 4.1). Mixed liquor was recycled between R_3 and R_2 to provide nitrate to the anoxic reactor. R_1 and R_2 were 4-L sealed glass bottles mixed with magnetic stirrers. R_3 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 R_3 was achieved with pre-humidified compressed air passed through a flow meter with a flow rate between 2 and 4 standard cubic feet per minute (scfm) through a fine pore stone diffuser, insuring a dissolved oxygen concentration between 3 and 5 mg/L. The mixed liquor pH was controlled at a value of 7 ± 0.5 using a pH controller (Type HD PH-P, Barnant Company, Barrington, Illinois, USA) and a 42 g/L $NaHCO_3$ solution. Feeding and wasting of R_1, recycle from R_3 to R_2 and back to R_3 was achieved with three Masterflex® peristaltic pumps (Cole-Parmer, Vernon Hills, Illinois, USA) controlled by a model XT, table top, electronic timer (Chrontrol Corporation, San Diego, California, USA). The clarified effluent was removed by gravity.

The seed used for the three reactors was mixed liquor samples collected from a poultry processing wastewater treatment plants as follows: for R_1 the seed was from an anaerobic reactor mixed liquor; for R_2 the seed was from an anoxic reactor mixed liquor; and for R_3 the seed was from sequencing batch reactor (SBR) mixed liquor. Both seeds used of R_1 and R_2 were collected from a multi-stage treatment plant (anaerobic-aerobic-anoxic), while the seed for R_3 was collected from an SBR based treatment plant. Table 4.2 summarizes the characteristics of seed used in the BNR system reactors. To provide a continuous wastewater source, a 5-L glass bottle was filled with 4 L wastewater every two days. The glass bottle was housed in a refrigerator at 4^oC and its contents were continuously mixed with a stirring bar and magnetic stirrer.

In order to establish continuous-flow conditions for system operation, an electronic timer was used to activate and deactivate the three pumps for a specific duration of time every hour. The sequence of events was as follows: pump A (dual heads) was turned on to recycle mixed liquor between the anoxic (R_2) and the aerobic reactor (R_3) providing nitrate for the anoxic reactor and ammonia to the aerobic reactor. Pump B (dual heads) was then turned on and anaerobic reactor (R_1) effluent with readily degradable organics was pumped to the anoxic reactor (R_2) to supply the organic carbon required for denitrification (first pump head). At the same time, an equal volume of anoxic reactor mixed liquor was pumped to the aerobic reactor (second pump head), thus maintaining a constant anoxic reactor liquid volume, and overfilling the aerobic reactor to trigger the gravity effluent discharge. Finally, pump C (single head) was turned on to pump the feed from the refrigerated feed reservoir to the anaerobic reactor. Waste biomass was manually removed directly from the aerobic reactor, daily, after the settler

baffle was lifted and the mixed liquor in both the reactor and the bottom of the settler was allowed to mix for 30 minutes.

Table 4.2. Characteristics of the seed mixed liquors (ML) used in the continuous-flow, multi-stage, laboratory-scale BNR system.

Parameter	Units	Anaerobic ML	Anoxic ML	Aerobic ML
pH	-	6.65	6.87	6.61
TSS	mg/L	1023 ± 12[a]	2164 ± 53	1408 ± 12
VSS	mg/L	740 ± 10	1916 ± 50	993 ± 5
Total COD	mg COD/L	1504 ± 91	3583 ± 49	1833 ± 54
Soluble COD	mg COD/L	308 ± 208	218 ± 16	626 ± 38
VFAs	mg COD/L	ND[b]	27 ± 5.5	0.67 ± 0.6
NH_3	mg N/L	18 ± 1.6	4.7 ± 1.6	3.2 ± 0.8
NO^-_3	mg N/L	0.4	0.9	9.6
NO^-_2	mg N/L	ND	ND	ND
TN[c]	mg N/L	79 ± 10	148 ± 3.5	102 ± 3

[a] Mean ± standard deviation (n = 6); [b] ND, not detected; [c] Total nitrogen, i.e., sum of organic, ammonia, nitrite, and nitrate nitrogen.

4.2.4 Poultry Processing High-Strength Wastewater (HSWW)

Poultry processing HSWW contains suspended materials, mainly fat, oil and grease, which are removed by dissolved air flotation (DAF). DAF is a solid/liquid separation process where air is dissolved into water under pressure, forcing the formation of micro-bubbles as air escapes from the solution (Avula et al., 2009). DAF efficiency is typically enhanced by the addition of chemicals such as polyelectrolytes, which improve the removal of colloidal organic particles by charge neutralization (Tezel et al., 2007). The resulting solids (i.e., DAF skimmings) are commonly used by the rendering industry for the recovery of secondary processing nutrients (SPN), such as protein and lipids. The

liquid DAF underflow is usually treated using biological processes. DAF underflow wastewater was collected periodically from a local poultry processing wastewater treatment plant in 5 gallon plastic buckets, which were stored at 4^{o}C in an environmental chamber.

4.2.5 Benzalkonium Chloride

A mixture of three BAC homologues (Barquat MB-80^{TM}) obtained from Lonza Inc. (Williamsport, Pennsylvania, USA) was used in this study. The BAC mixture is composed of (%, w/w): C_{12}BAC, 32; C_{14}BAC, 40; C_{16}BAC, 8; ethanol, 10; and water, 10. Figure 4.3 shows the structure and Table 4.3 summarizes the main physical and chemical properties of the three BAC homologs. A 10 g/L BAC stock solution in de-ionized water (DI) was prepared, and used as needed throughout the study.

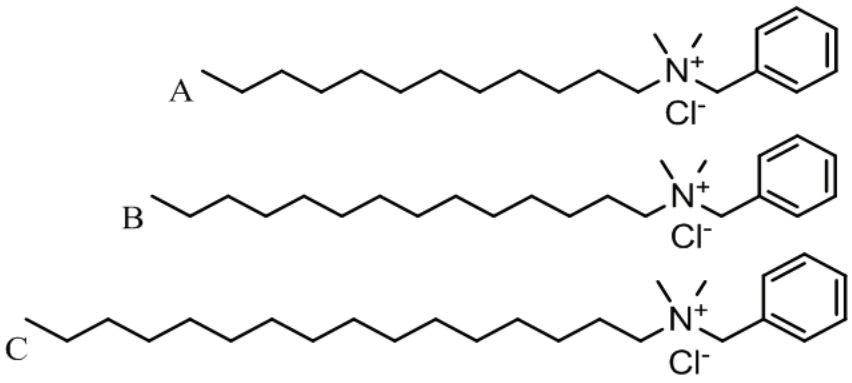

Figure 4.3. Molecular structure of the three BAC homologs used in this study: C_{12}-BAC (A), C_{14}-BAC (B), and C_{16}-BAC (C).

Table 4.3. Summary of physical and chemical properties of the three BAC homologs, used in this study (Tezel, 2009;Yang, 2007).

Parameter	C_{12} BAC	C_{14} BAC	C_{16} BAC
Boiling point (ºC)	537.6	560.8	584.0
Melting point (ºC)	230.2	241.0	251.9
Vapor pressure (mm Hg)	1.9E-11	3.53E-12	9.28E-11
Water solubility (g/L at 25 ºC)	22.5	2.203	0.215
Soil adsorption coefficient (K_{OC}, mL/g solid)	8.4E+05	2.865E+06	9.747E+06
Octanol/water partition coefficient (log K_{ow})	0.59 ± 0.04	1.67 ± 0.02	2.97 ± 0.03
Critical micelle concentration (mM)	3.8	1.72	0.58

4.2.6 Nitrification Batch Assay

A batch assay was performed to investigate the effect of the BAC mixture on the nitrification activity of the mixed liquor of the aerobic reactor (R_3) during the BNR system BAC-free operation. The BAC mixture (see section 4.2.5, above) was used at initial concentrations up to 100 mg/L (0, 5, 10, 15, 25, 50, 75, 100 mg/L). The seven BAC-amended and BAC-free culture series were prepared in 250-mL Erlenmeyer flasks (200 mL liquid volume). R_3 mixed liquor (100 mL), collected during the daily waste time (TSS and VSS of 1640±20 and 1273±32 mg/L, respectively), followed by 100 mL of BAC-amended poultry processing wastewater to arrive at the above-mentioned initial BAC concentrations were then added to the Erlenmeyer flasks. 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 culture series were aerated with compressed, pre-humidified air, while continuous mixing was provided by an orbital shaker. 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 $NaHCO_3$. Initial, intermediate, and final BAC total concentrations were measured to evaluate BAC degradation. The initial specific ammonia removal rate (SARR) 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.

4.2.7 BAC-Free Denitrifying Culture

A denitrifying culture, which was used to test the fate and effect of BAC on denitrification, was developed with a seed obtained from the anoxic reactor mixed liquor of a poultry processing wastewater treatment plant (same seed used for the BNR anoxic reactor). The culture was maintained with a hydraulic retention time of 3 days and a solids retention time of 12 days in a 5.5-L glass reactor, mixed with a stirring bar and a magnetic stirrer. The total liquid volume of the culture was 4 L. The culture was fed daily with culture media, 600 mg nitrate-N and 3,600 mg glucose (as electron donor and carbon source) resulting in an initial concentration of 150 mg nitrate-N/L and 900 mg glucose/L. The culture media contained (in mg/L): K_2HPO_4, 900; KH_2PO_4, 500; NH_4Cl, 500; $MgCl_2 \cdot 6H_2O$, 200; $CaCl_2 \cdot 2H_2O$, 100; and $FeCl_2 \cdot 4H_2O$, 100. In addition, 1.0 ml/L each of vitamin and trace metal stock solutions was added. The culture was maintained at 22±1^{o}C. Table 4.4 summarizes the culture characteristics.

4.2.8 Denitrification Batch Assay

A batch assay was performed to evaluate the inhibitory effect of BAC on denitrification. 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 assay included five culture series that were

amended with the BAC solution resulting in initial total BAC concentrations of 10, 25, 50, 75 and 100 mg/L. Two additional BAC-free culture series were prepared: seed blank and reference which consisted of seed, culture media and DI water, and seed, culture media, DI water, nitrate and glucose, respectively. Samples of 80 mL of the denitrifying culture were anaerobically transferred to each serum bottle along with 11 mL of culture media. Nitrate was then added, followed by the BAC mixture. Finally, a glucose solution, which served as carbon/energy source, was added and the total liquid volume was adjusted to 100 mL with DI water. The initial glucose and nitrate concentrations were 960 mg COD/L and 160 mg NO^-_3 N/L, which resulted in a COD/N ratio of 6/1. Each culture series, including the seed blank and 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, soluble COD, 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 nitrate concentration data, and normalizing the resulting rate to the mean value of the initial and final VSS concentrations of each culture series.

Table 4.4. BAC-free denitrifying culture characteristics.

Parameter	Units	Value
pH	-	7.0 ± 0.5
TSS	mg/L	2294 ± 17[a]
VSS	mg/L	1561 ± 13[a]
NH_3	mg N/L	239 ± 29[b]
NO_3^-	mg N/L	ND[c]
NO_2^-	mg N/L	ND
Nitrate Removal Rate	mg N/L · day	166 ± 6[d]

[a] Mean ± standard deviation ($n \geq 2$); [b] Mean ± standard deviation ($n \geq 6$); [c] ND, not detected at the end of the feeding cycle; [d] Based on the initial and final nitrate concentration during the feeding cycle

Table 4.5. Characteristics of the poultry processing DAF underflow poultry processing wastewater used as feed to the continuous-flow, multi-stage, laboratory-scale BNR system.

Parameter	Units	Value
pH	-	6.9 ± 0.2[a]
TSS/VSS	mg/L	125 ± 20/120 ± 23
Total/Soluble COD	mg COD/L	1275 ± 16/919 ± 111
Carbohydrates	mg/L (mg COD/L)[b]	24 ± 5 (26 ± 6)
Lipids	mg/L (mg COD/L)[c]	37 ± 10 (680 ± 28)
NH_3	mg N/L	46.4 ± 3
NO_3^-/ NO_2^-	mg N/L	ND[d]
TN[e]	mg N/L	103 ± 4.5
BAC	mg/L	ND

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

4.3 Results and Discussion

4.3.1 Poultry Processing Wastewater Characterization

DAF underflow wastewater was collected eight times during the course of this study. Each time, the collected poultry processing wastewater was characterized and Table 4.5 summarizes the characteristics of the poultry processing wastewater. As discussed above, solids are reclaimed from the raw poultry processing wastewater via DAF, which explains the low solids content in the DAF underflow wastewater.

Both the COD and nitrogen levels in the collected poultry processing wastewater were comparable to previously reported HSWW (Chapter 2). Most COD was soluble (at least 72%), with 96% of the suspended solids being volatile. The solids were comprised mainly of lipids (28%), carbohydrates (18%) and crude protein (54%). 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, which made it ideal for use in this study, since BAC can be introduced at any desired concentration in the feed. 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.

As seen above, 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 (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.

4.3.2 BNR Design

Information provided in section 4.2.1 above, was used to design the laboratory-scale BNR system. The volume of the anoxic and aerobic reactors (4 and 5 L respectively) was chosen based upon available equipment, with a chosen feed flow rate of 2 L/day. Utilizing the poultry processing wastewater COD and total nitrogen concentrations and a stoichiometric carbon to nitrogen ratio ($g\ COD / g\ NO_3^-\ N$) of 5.2, Microsoft Excel Solver was used to solve Equations 4.1 to 4.6 at various solids retention time values, yielding different volatile suspended solids concentrations. The end goal was a volatile suspended solids concentration of 1200 mg/L, an average value of both reactors' seed. A solids retention time of 25 days was chosen, giving X_{tot} concentration of 1196 mg/L. The theoretical effluent nitrate concentration was 18.2 mg N/L, while the net mass production rates were 280.9, 27.3, 122.3 (mg VSS/day) for aerobic, autotrophic, and anoxic growth, respectively. Figure 4.4 shows how the theoretical design variables changed with the overall solids retention time. The resulting values (θ/θ_c in days) were: 2/2, 2/11, and 2.5/14 for R_1, R_2, and R_3, respectively. The mixed liquor was wasted daily from R_3 at a rate of 0.35 L/day to establish the desired θ_c value.

4.3.3 BNR Startup and Baseline Performance

The BNR system was operated continuously for 30 days, treating BAC-free poultry processing wastewater. During the first 9 days of operation, a refrigerated 6-month old poultry processing wastewater was used as feed. Afterwards, fresh wastewater was used for the remainder of the study. Figure 4.5 illustrates the system performance

during the first 30 days of continuous, BAC-free, operation, while Table 4.6 summarizes the reactors' characteristics during the BAC-free continuous operation period. 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. The recycle ratio between R_2 and R_3 was initially set at 4 and then reduced to 3; however, the nitrate concentration in R_3 increased. Subsequently, the recycle ratio was set back to 4 on day 14 and remained constant for the duration of this study. Volatile fatty acids (VFAs) were detected in the R_1 effluent at a concentration of 175.1±4.6 mg COD/L, which accounted for 19% of the reactor's effluent soluble COD concentration.

Table 4.6. Performance of the BNR system during continuous, BAC-free operation (Data from day 10 to 30).

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	28.3 ± 7.2	26.9 ± 3.3
NO_2^- (mg N/L)	ND	ND	ND	ND

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

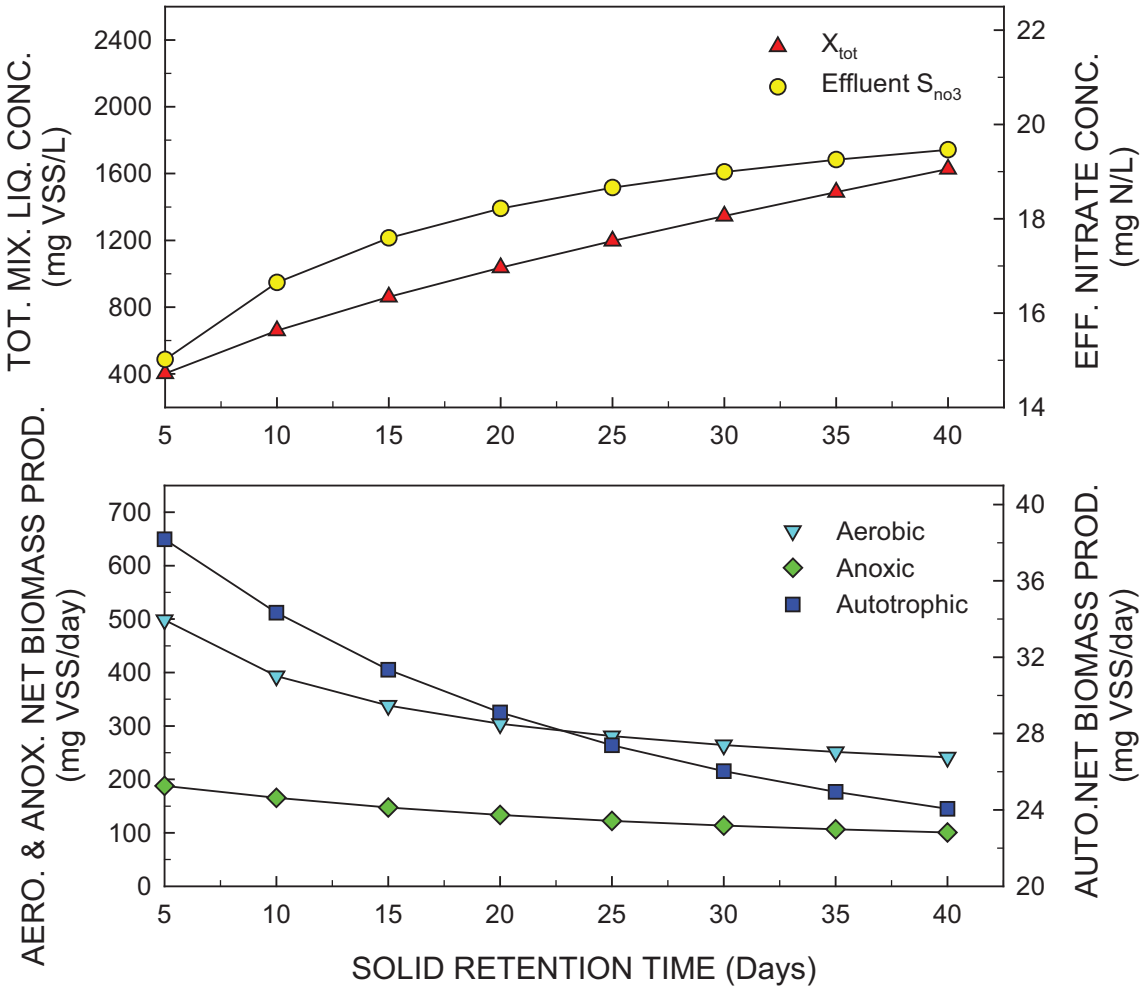

Figure 4.4. Effect of solids retention time on theoretical design variables for the BNR system: (A) total mixed liquor concentration and effluent nitrate; (B) net growth rates (B).

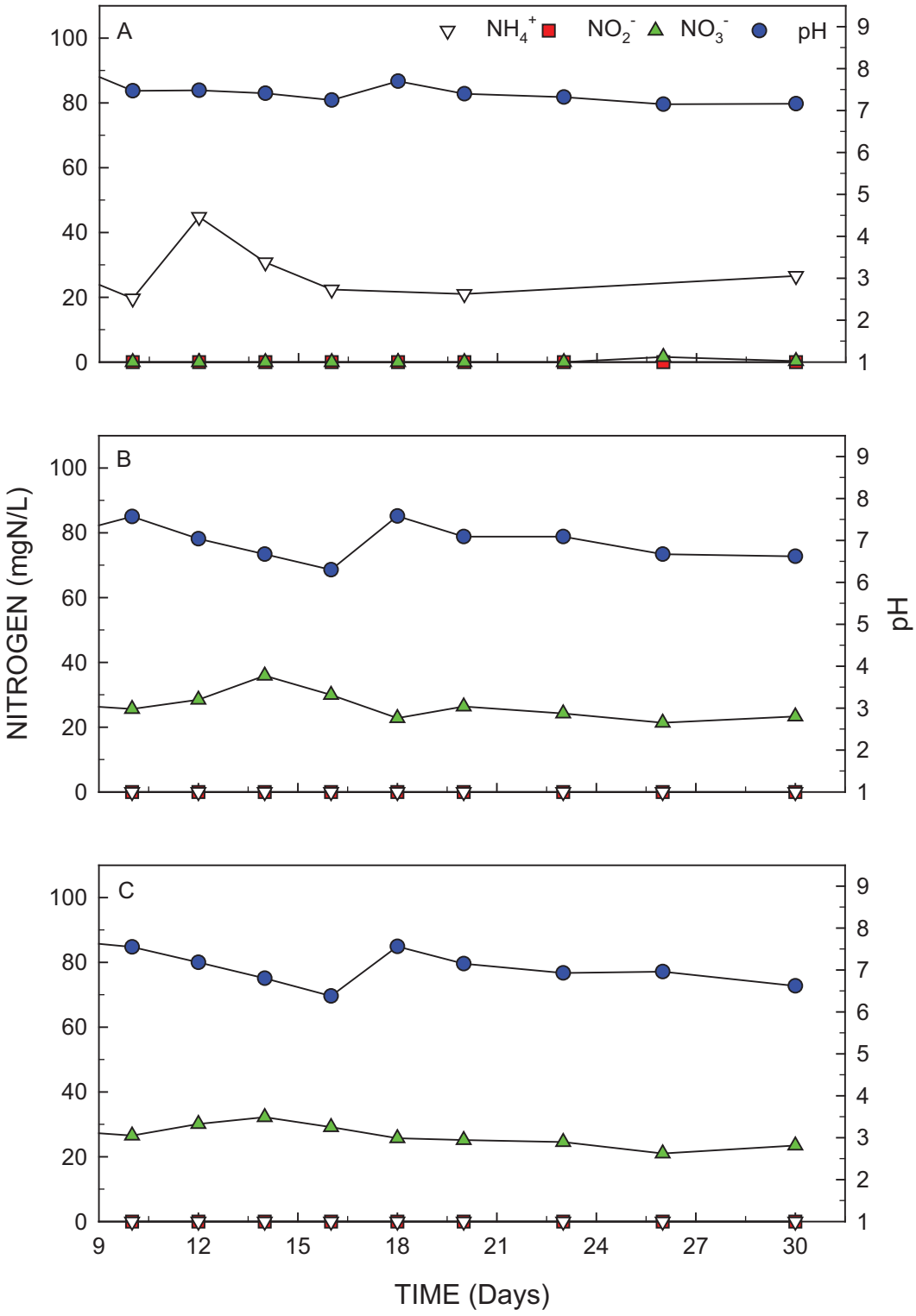

Figure 4.5. Nitrogen species and pH in the BNR system during the first 30 days of continuous, BAC-free operation (A, R_2; B, R_3; and C, Effluent).

The ammonia concentration in the R_1 effluent was 95.7±10.6 mg N/L, 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 26.1±7.4 mg N/L and nitrate was never detected. On the other hand, the R_3 nitrate concentration was 26.4±4.5 mg N/L and ammonia was never detected. Nitrite was never detected in both R_2 and R_3 during the BAC-free operation. The effluent nitrate concentration was 26.4±3.5 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 BAC-bearing poultry processing wastewater can be compared with.

OUR analysis on the aerobic reactor mixed liquor performed at the 25th day of operation resulted in OUR_1 18.6 ± 0.4, OUR_2 17.5 ± 0.4, and OUR_3 15.7 ±0.4 mg O_2/h (Figure 4.6). Based on these OUR values, the corresponding AOB and 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, 6.2–2.5% in an SBR, 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.

4.3.4 Preliminary BAC Effect on Nitrification and Denitrification

As discussed in Chapter 2, biological nitrification and denitrification are both inhibited by BAC, but with a varying degree of susceptibility. 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 processes.

4.3.4.1 Nitrification batch assay

The assay testing the effect of BAC on nitrification lasted for 82 hours. Figure 4.7 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 (Figure 4.8) brought about by the BAC antimicrobial effect (Cross and Singer, 1994).

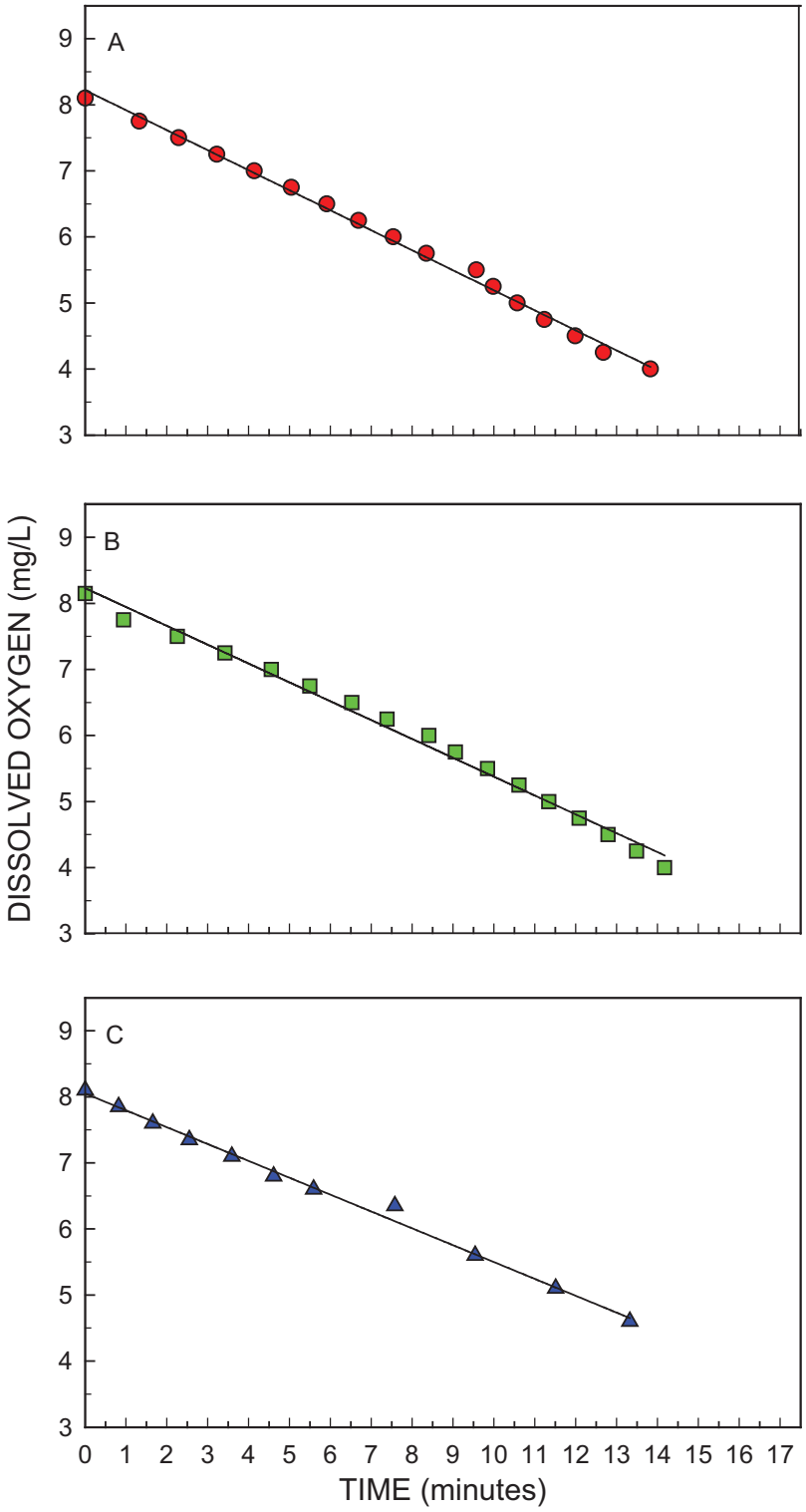

Figure 4.6. OUR measurements for the aerobic reactor mixed liquor conducted with acetate, ammonia, and nitrite (A), acetate and ammonia (B), and acetate (C).

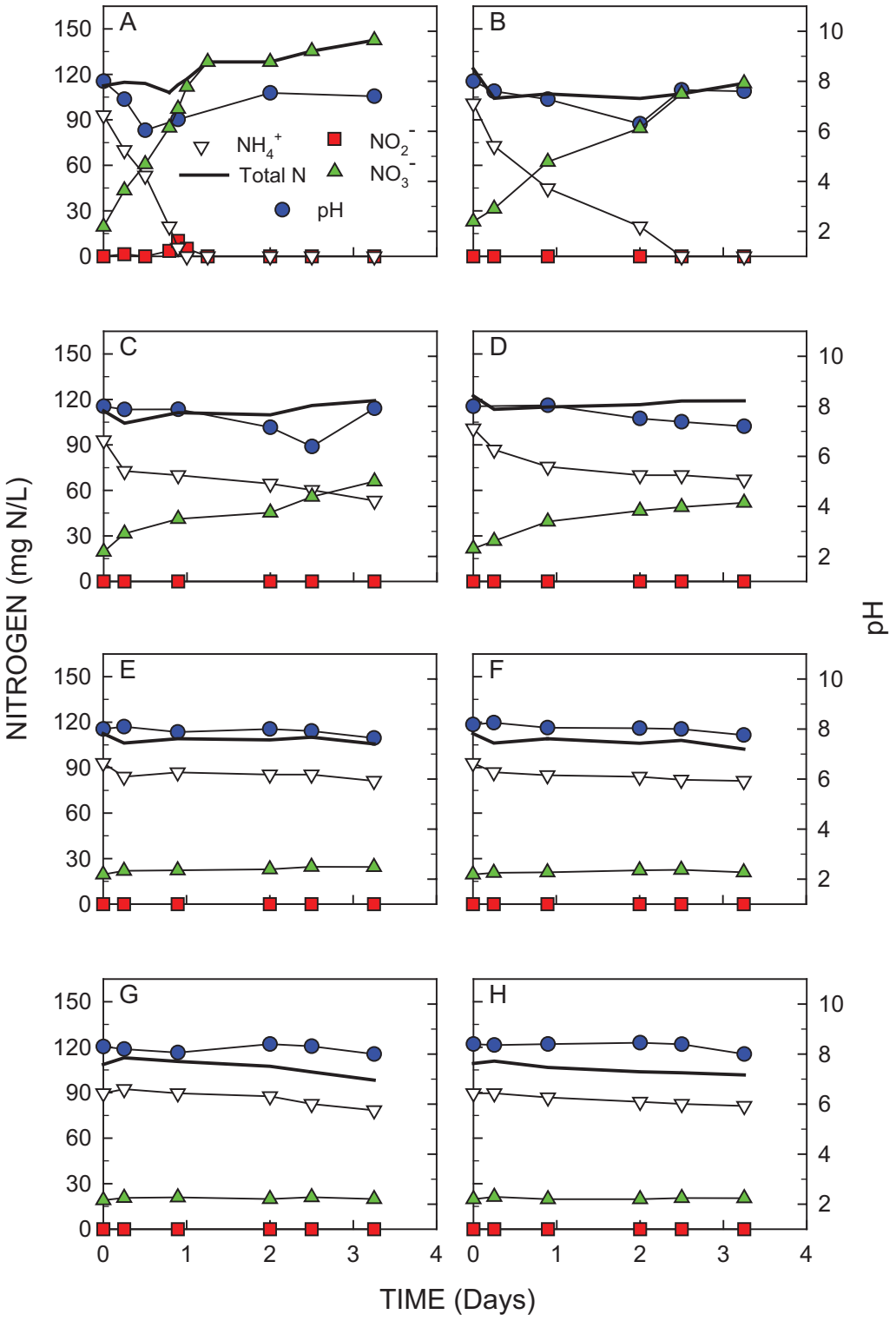

Figure 4.7. Time course of nitrogen species and pH variation during the batch nitrification assay conducted with BAC-free mixed liquor collected from the aerobic reactor (R_3). Initial BAC concentrations of 0 (A), 5 (B), 10 (C), 15 (D), 25 (E), 50 (F), 75 (G), and 100 mg/L (H).

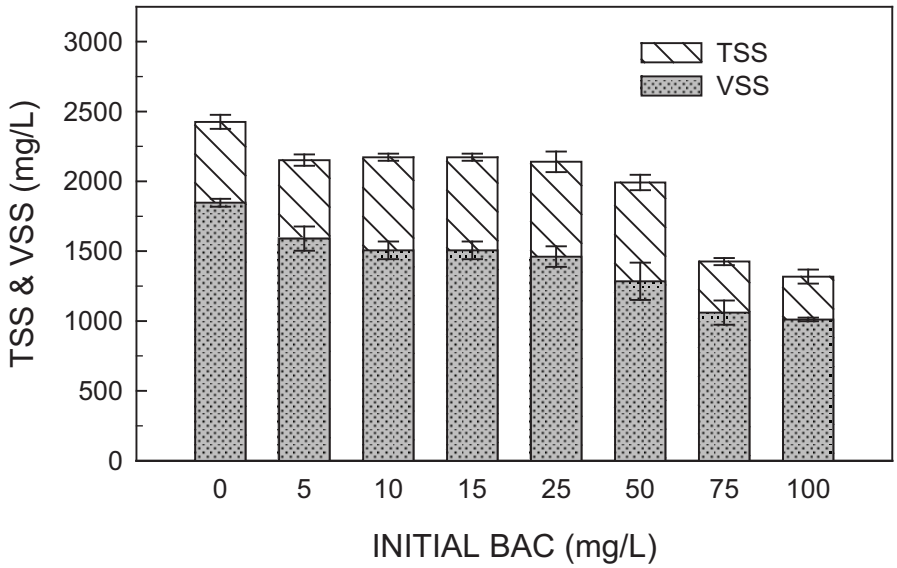

Figure 4.8. Total and volatile suspended solids (TSS and VSS) at the end of the incubation period for the culture series used in the batch nitrification assay conducted with BAC-free mixed liquor collected from the aerobic reactor (R_3). Error bars represent one standard deviation of the means (n = 3).

Figure 4.9A 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 4.9B 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 4.10 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.

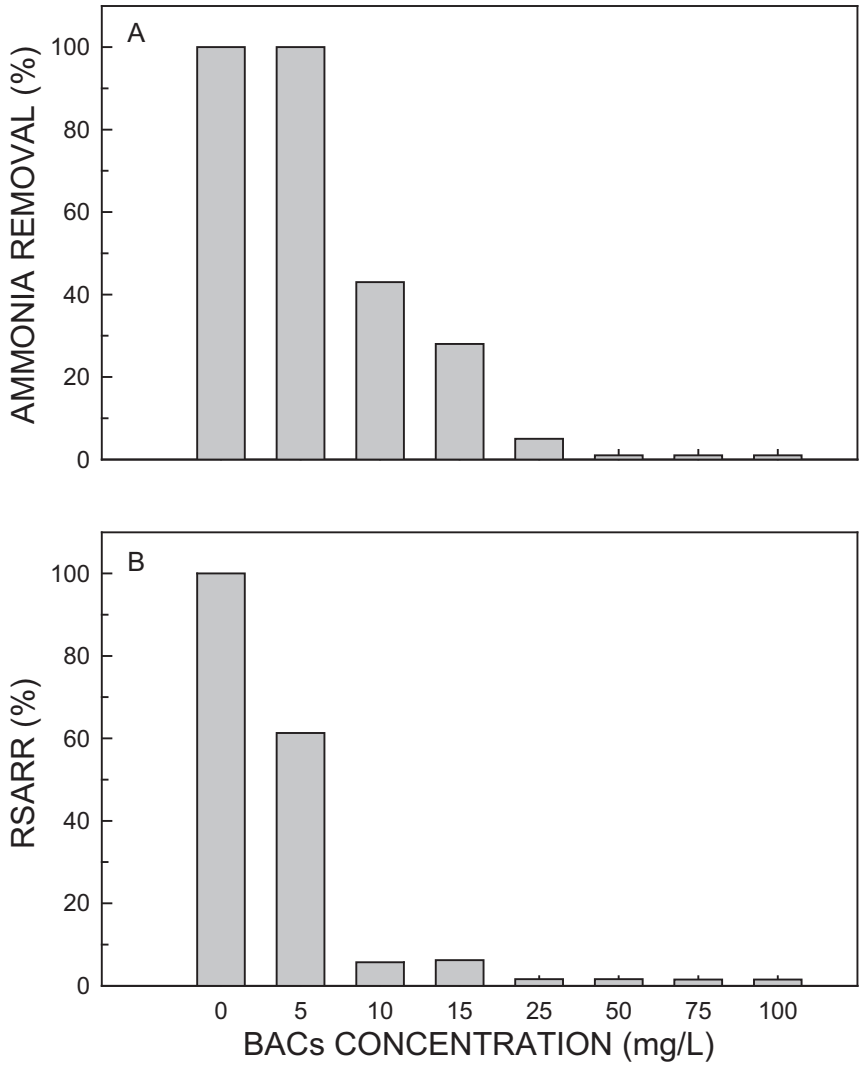

Figure 4.9. Extent of ammonia removal (A) and relative specific ammonia removal rate (RSARR) (B) for the culture series used in the batch nitrification assay conducted with BAC-free mixed liquor collected from the aerobic reactor (R_3).

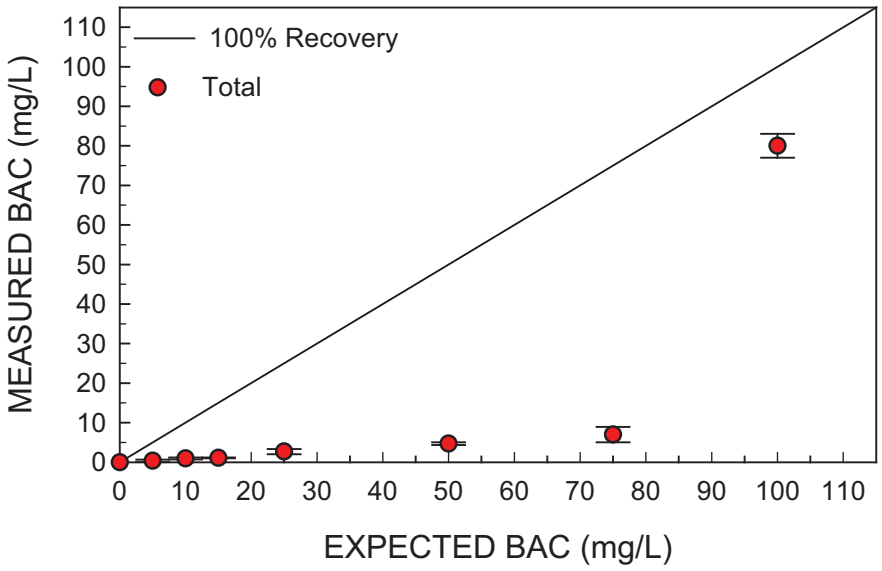

Figure 4.10. BAC recovery at the end of the incubation period in the culture series of the batch nitrification assay conducted with BAC-free mixed liquor collected from the aerobic reactor (R_3). Error bars represent one standard deviation of the means (n = 3).

In a recent study Pavlostathis etal (2008) reported a long-term nitrification inhibition in a BAC-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.

4.3.4.2 Denitrification batch assay

The assay testing the effect of BAC on denitrification lasted for 9 days. Figure 4.11 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 DNRA process was also active in these culture series. Neither nitric oxide nor nitrous oxide was detected in any culture series. Figure 4.12A 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 (Figure 4.13).

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 4.12B 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 4.12B). 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.

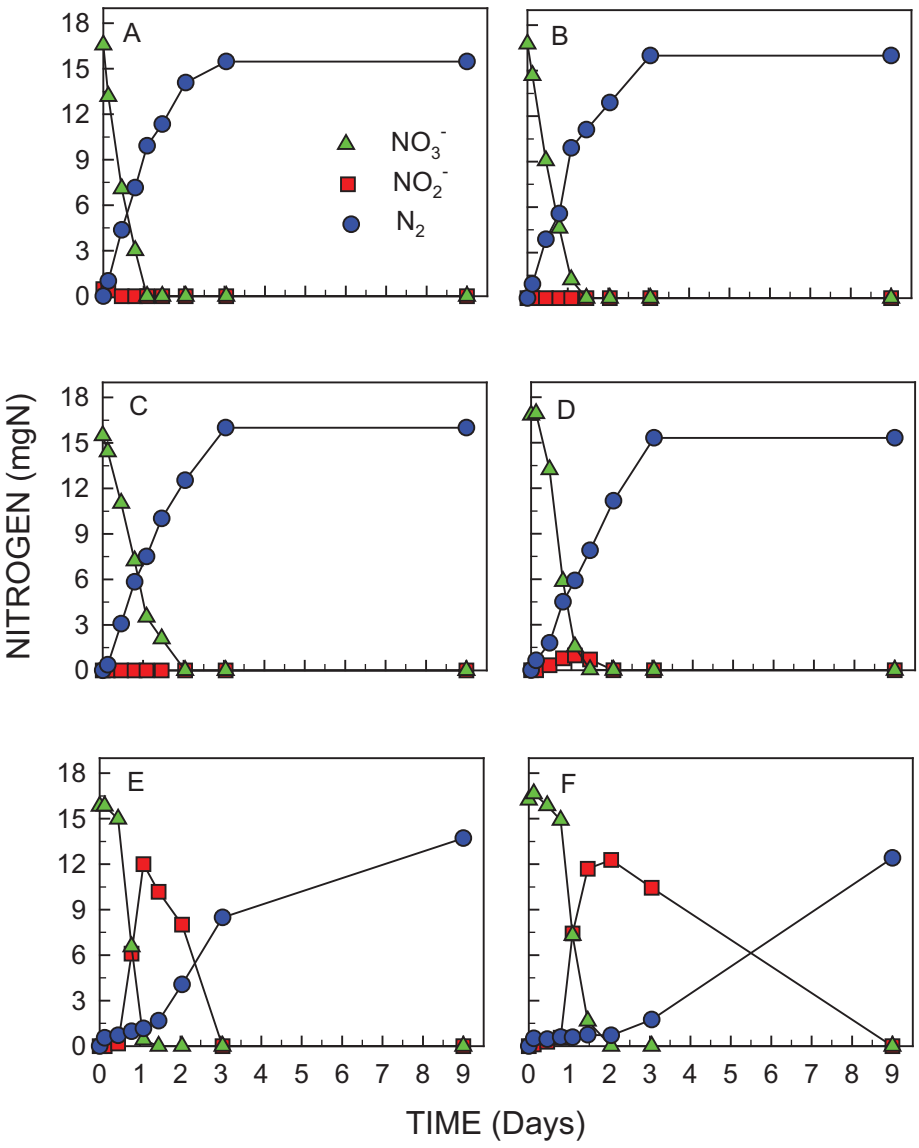

Figure 4.11. Time course of nitrogen species during the batch denitrification assay conducted with BAC-unexposed mixed denitrifying culture and initial BAC concentrations of 0 (A), 10 (B), 25 (C), 50 (D), 75 (E) and 100 mg/L (F).

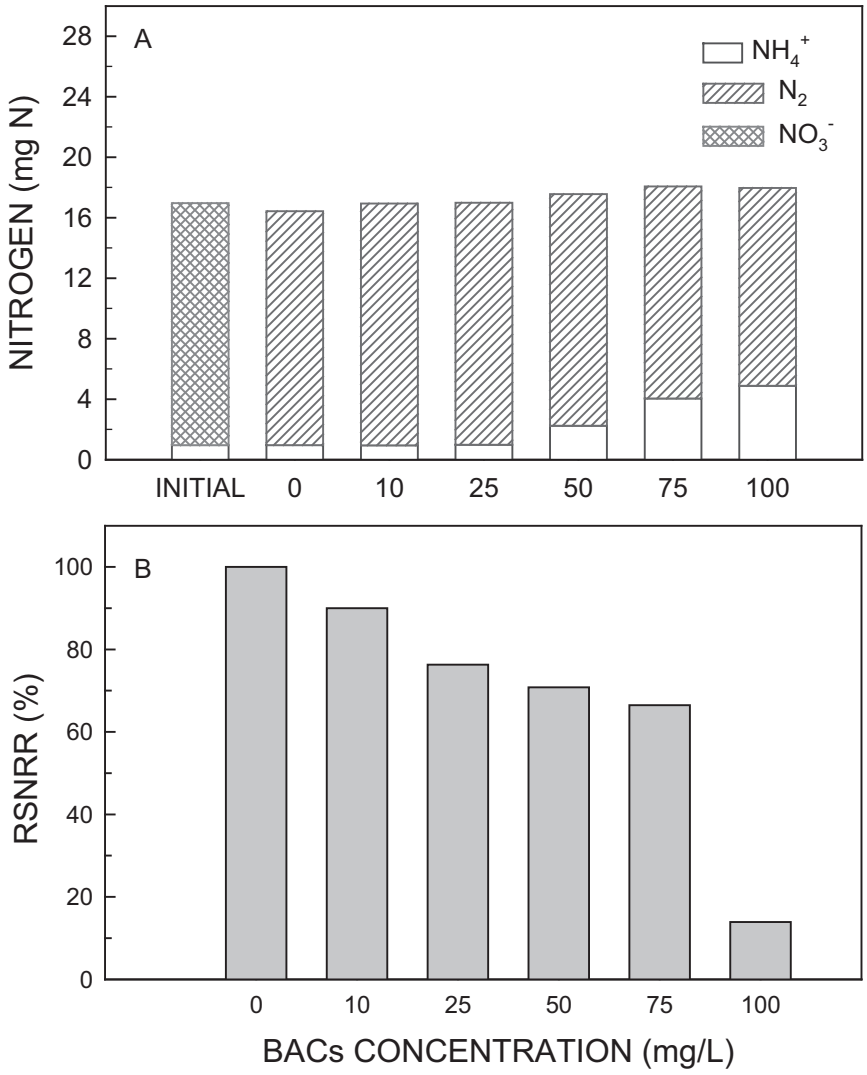

Figure 4.12. Distribution of the nitrogen species at the end of the incubation period (A) and relative specific nitrate removal rate (RSNRR) (B) in the batch denitrification assay conducted with BAC-unexposed mixed denitrifying culture.

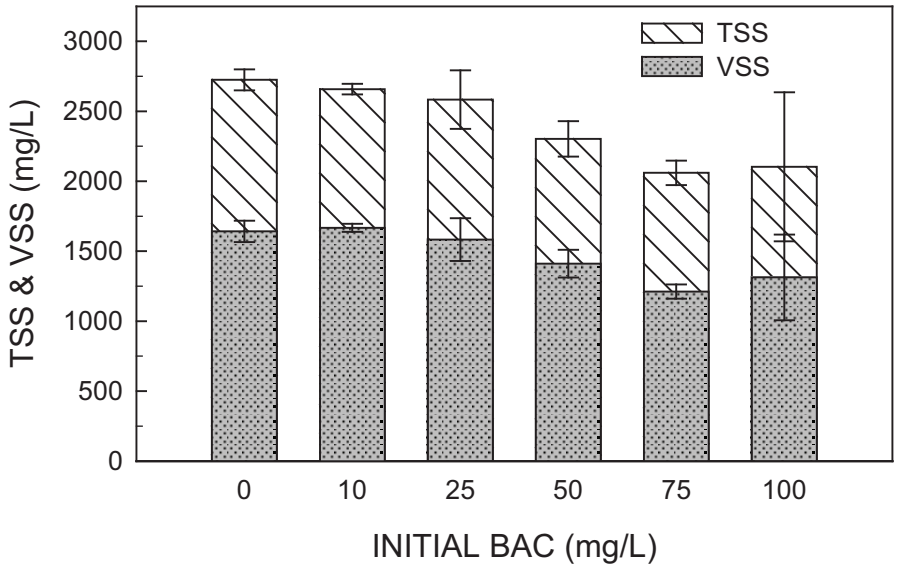

Figure 4.13. Total and volatile suspended solids (TSS and VSS) at the end of the incubation period in the batch denitrification assay conducted with BAC-unexposed mixed denitrifying culture. Error bars represent one standard deviation of the means (n = 3).

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 4.14 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).

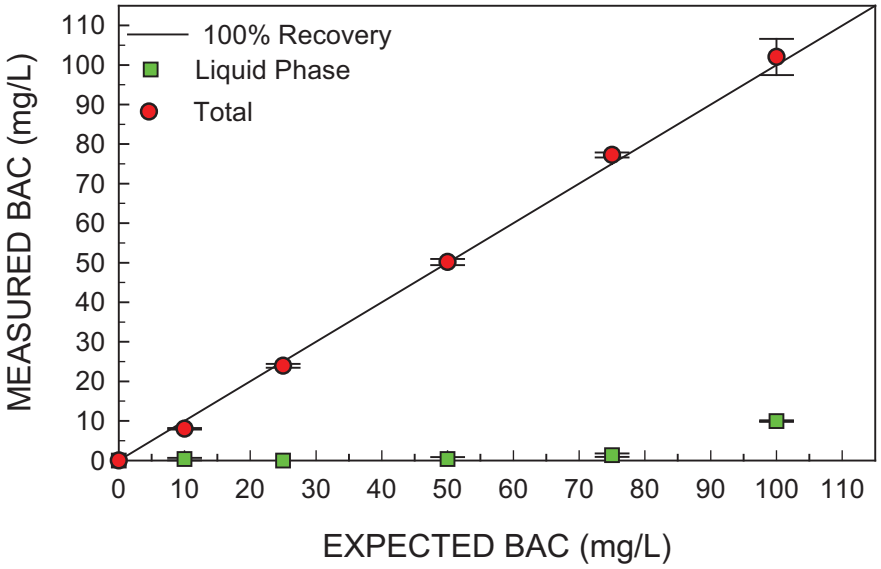

Figure 4.14. BAC recovery at the end of the incubation period in the batch denitrification assay conducted with BAC-unexposed mixed denitrifying culture. Error bars represent one standard deviation of the means (n = 3).

In the denitrification assay of the present study 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).

4.3.4.3 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. The validity of this conclusion was further investigated and results are reported in Chapter 5, below.

4.4 Summary

A laboratory-scale, continuous-flow, multi-stage BNR system treating poultry processing DAF underflow wastewater was constructed and tested. The system achieved 100 and 75% ammonia and total nitrogen removal efficiency, respectively, during 30 days of continuous operation, treating real poultry processing wastewater that was

comprised mainly of protein, lipids, and carbohydrates. Nitrifiers accounted for 5.1% of the aerobic reactor population, with 2.8% AOB and 2.2% NOB.

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 removal 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.

BAC led to a significant decrease in the nitrate reduction rate, but did not affect the extent of nitrate removal, where nitrate was mostly and completely reduced to dinitrogen in all BAC-amended culture series in the denitrification assay. On the other hand, BAC inhibited denitrification at a concentration equal to and higher than 50 mg/L. 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.

Overall, BAC was detrimental to the BNR process, affecting nitrification and denitrification at a varying degree of severity. Nitrogen removal efficiency will

deteriorate while treating BAC-bearing poultry processing wastewater as a result of long-lasting inhibition of the nitrifying population. Moreover, it could be deduced that treating BAC-bearing poultry processing wastewater could irreversibly deteriorate the BNR system performance depending on the extent of BAC exposure (concentration) in the aerobic reactor.

CHAPTER 5

BIOLOGICAL NITROGEN REMOVAL SYSTEM – OPERATION WITH BENZALKONIUM CHLORIDE

5.1 Introduction

As discussed in Chapter 2, the fate and effect of QACs (mainly BAC antimicrobial compounds) on different biological processes utilized in HSWW 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 BAC on multiple reactions under typically encountered conditions (e.g., sequence of nitrification/denitrification). The latter condition is typical in continuous-flow, engineered wastewater treatment systems in which multiple environmental conditions are specifically created to sustain different groups of microorganisms which biologically mediate the overall treatment process.

As for any QAC, the fate and effect of BAC in BNR systems, as well as in natural systems, is determined by three processes: adsorption, inhibition, and biotransformation. The BAC phase distribution in the continuous-flow BNR system will constantly change because of continuous solubilization/aggregation of new particulate matter as a result of

hydrolysis, substrate utilization, and growth/decay of biomass coupled with mixing and dilution effects throughout the system. BAC biotransformation is possible under aerobic conditions (assuming the presence of competent microbes), while it is not possible under either anoxic or anaerobic conditions. An exception is the abiotic, yet biologically initiated, nucleophilic substitution reaction with BAC in a modified Hofmann reaction under very specific conditions regarding nitrite and liquid BAC concentrations, as previously reported by (Tezel and Pavlostathis, 2009). Finally, the two main biological processes in BNR systems (i.e., nitrification and denitrification) are susceptible to BAC, but the degree and extent of BAC inhibition differ between the two processes. Nitrification was completely inhibited at relatively low BAC concentrations, while denitrification was transiently inhibited at relatively higher BAC concentrations (see Chapter 4, above).

From what has been discussed above, the fate and effect of BAC in a continuous-flow BNR system can only be understood by following the three aforementioned processes (i.e., adsorption, biotransformation, and inhibition) throughout the entire system. Therefore, the objective of the research reported in this chapter was to investigate the performance of a continuous-flow BNR system (Chapter 4) 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.

5.2 Materials and Methods

5.2.1 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 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.

5.2.2 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.

The assay conducted during the BNR operation with BAC-bearing poultry processing wastewater used the BAC mixture (Chapter 4) at initial BAC concentrations up to 45 mg/L (0, 5, 15, 20, 25, 30, and 45 mg/L), while the assay conducted after the BNR operation with BAC-bearing poultry processing wastewater used the BAC mixture at initial BAC concentrations of 0, 5, 10, and 15 mg/L. In both 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 the above-mentioned 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.

5.2.3 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, 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.

5.2.4 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). The assay 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 3.2.2, above, were used to follow the concentration of BAC and four possible BAC metabolites: benzyl trimethyl amine (BTMA), dimethyl amine (BDMA), benzyl methyl amine (BMA), and benzyl amine (BA).

5.2.5 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^{o}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:

$$q_e = K_F C_e^{n} \qquad \text{(Equation 5.1)}$$

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).

5.3 Results and Discussion

5.3.1 BNR Operation with BAC-bearing poultry processing wastewater

5.3.1.1 Initial BAC exposure

After establishing stable BNR system operation regarding nitrogen removal (see Chapter 4), 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 discussed in Chapter 4, 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 5.1 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 5.1). 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.

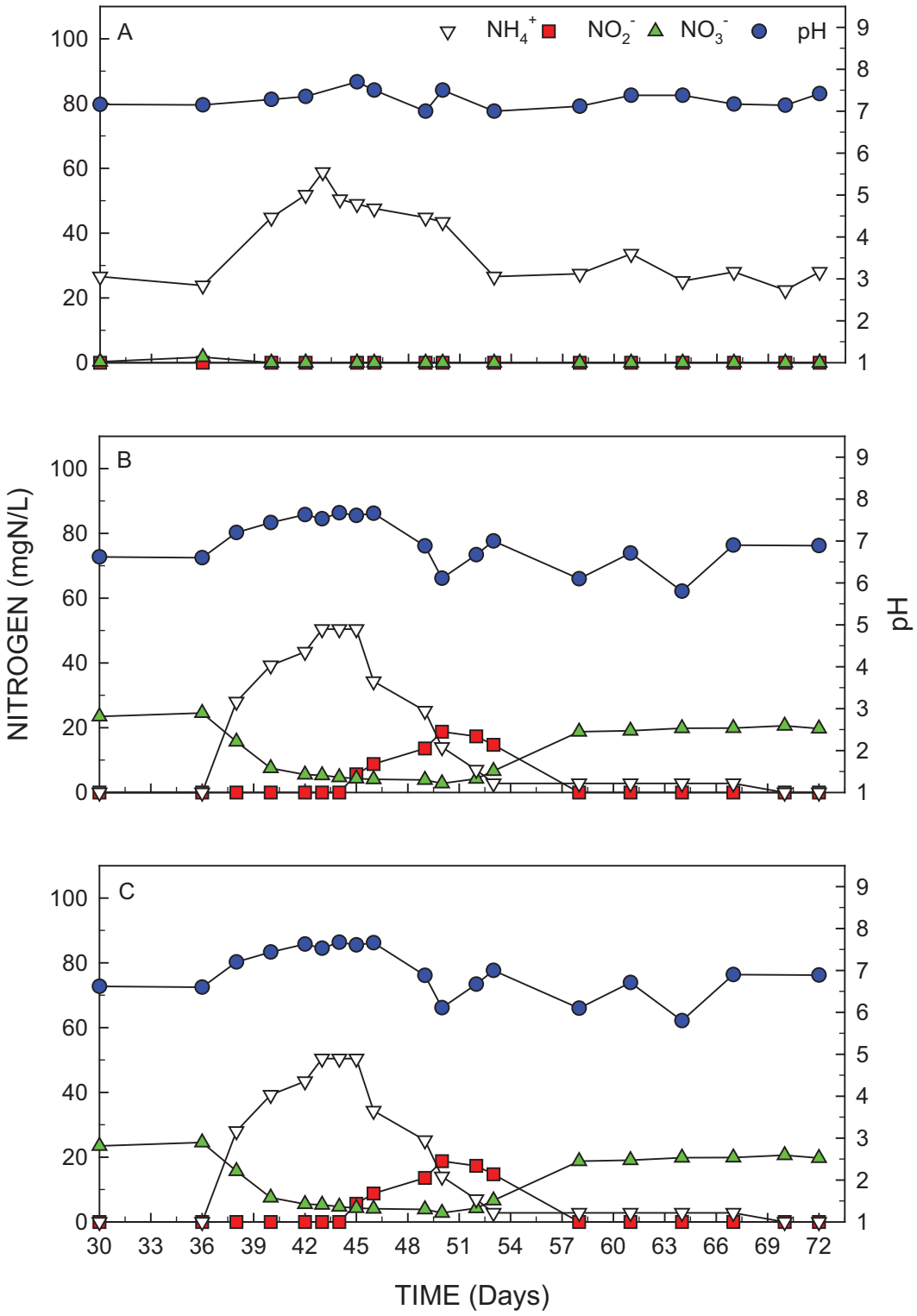

Figure 5.1. Nitrogen species and pH in the BNR system while treating the BAC-bearing poultry processing wastewater at a feed BAC concentration of 5 mg/L (A, R_2; B, R_3; and C, Effluent).

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 Chapter 4, 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.

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

After the complete biotransformation of BAC, the ammonia concentration in R_3 gradually decreased (Figure 5.1). 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 5.1). OUR analysis of the aerobic reactor mixed liquor performed at the 100th day of operation resulted in OUR_1, OUR_2, and OUR_3 values of 16.6 ± 0.7, 15.3 ± 0.8, and 14.2 ±0.2 mg O_2/h, respectively. The three OUR values were marginally less than those measured during the BAC-free operation, which is attributed to a low BAC inhibitory effect on the respiratory enzymes of the aerobic reactor microbial population (Zhang et al., 2011). The above reported OUR values corresponded to AOB and NOB biomass fractions of 2.0 and 3.1%, respectively, in the aerobic reactor population.

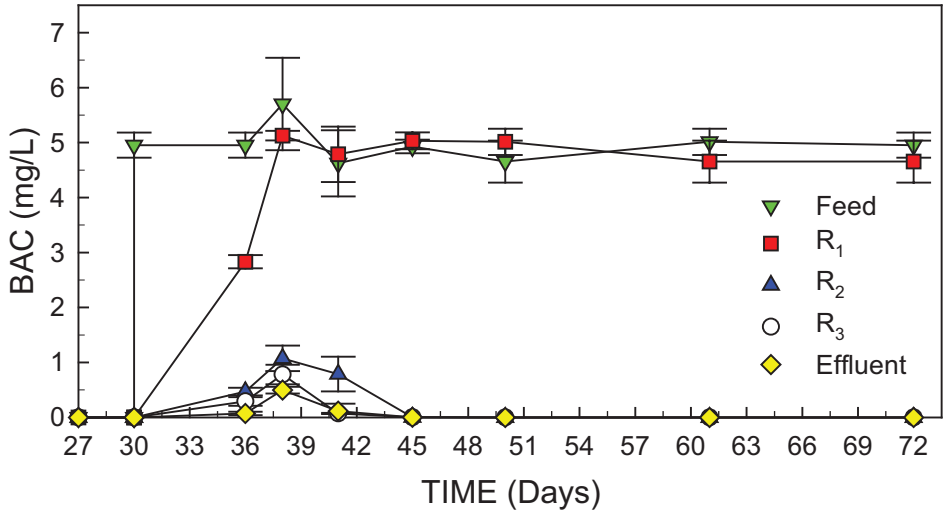

Figure 5.2. Total BAC concentration in the BNR system while treating the BAC-bearing poultry processing wastewater at a feed BAC concentration of 5 mg/L.

These AOB and NOB biomass fractions are similar to the values obtained during the BAC-free operation period.

Table 5.1 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 Chapter 4. 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 detected in the anaerobic reactor effluent while the BNR system feed was maintained at 5 mg/L. As discussed in Chapter 2, the surface active properties of BACs favor their adsorption to organic particulates 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).

5.3.1.2 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 5.3 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 5.2 summarizes the BNR system performance during the same period.

Table 5.1. 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;

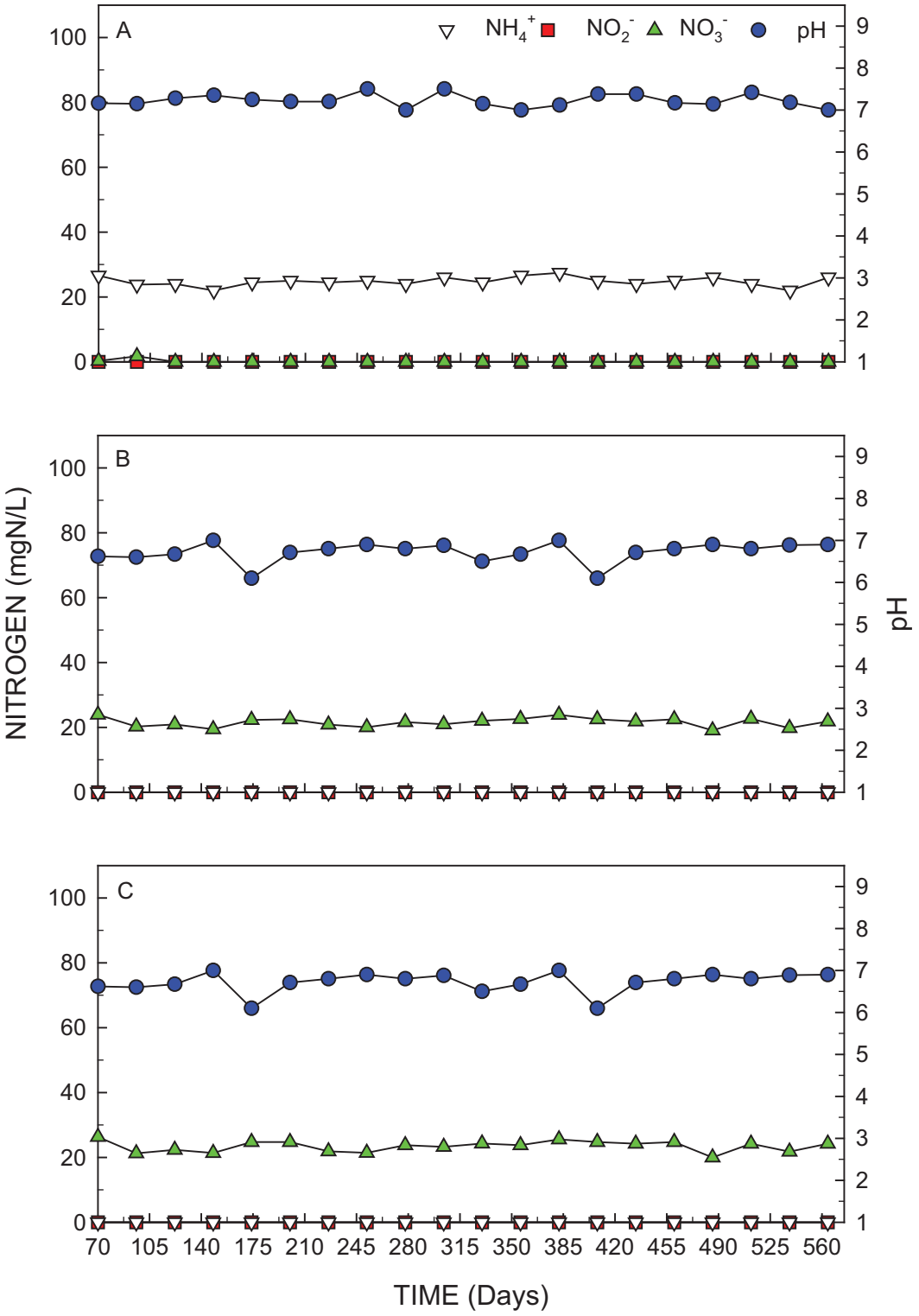

Figure 5.3. Nitrogen species and pH in the BNR system while treating the BAC-bearing poultry processing wastewater at stepwise increased feed BAC concentrations from 5 to 60 mg/L (A, R_2; B, R_3; and C, Effluent).

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 5.3). 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 5.3 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 5.4 shows the BAC concentration throughout the BNR system during the same period. As seen in Table 5.3, 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 5.4.2.3 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 5.2. 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 5.3. 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, $n \geq 8$

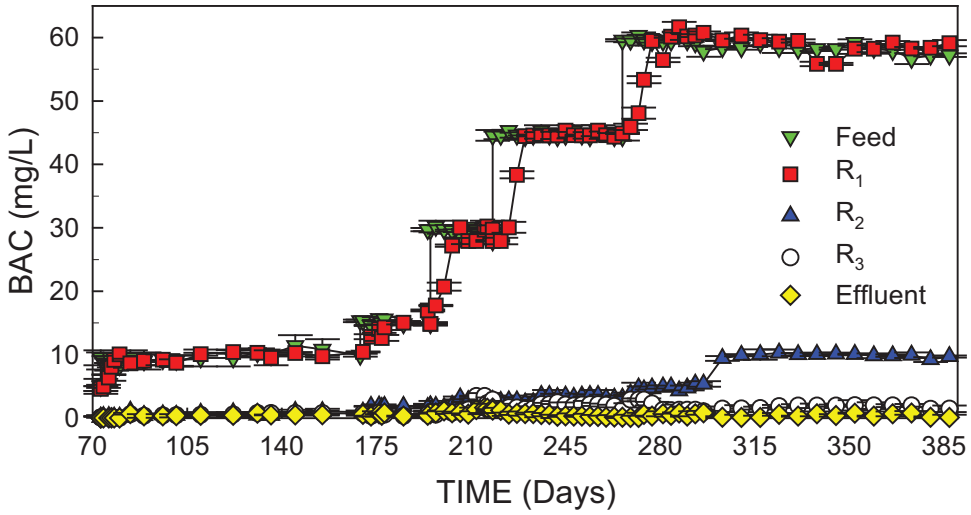

Figure 5.4. Total BAC concentration in the BNR system while treating the BAC-bearing poultry processing wastewater at stepwise increased feed BAC concentrations from 5 to 60 mg/L.

5.3.1.3 BNR system resiliency 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 5.5 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 $\geq$ 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.

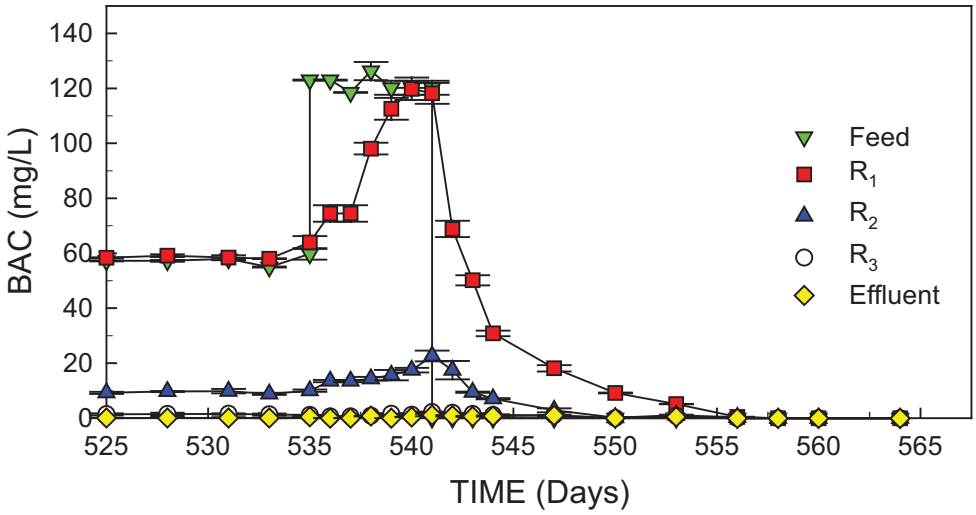

Figure 5.5. Total BAC concentration in the BNR system while treating the BAC-bearing poultry processing wastewater at a stepwise increase of the feed BAC concentrations from 60 to 120 mg/L.

5.3.2 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.

5.3.2.1 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). Figures 5.6 and 5.7 show the time course of sCOD and VFAs production and ammonia release (corrected for the seed culture series), respectively. 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.

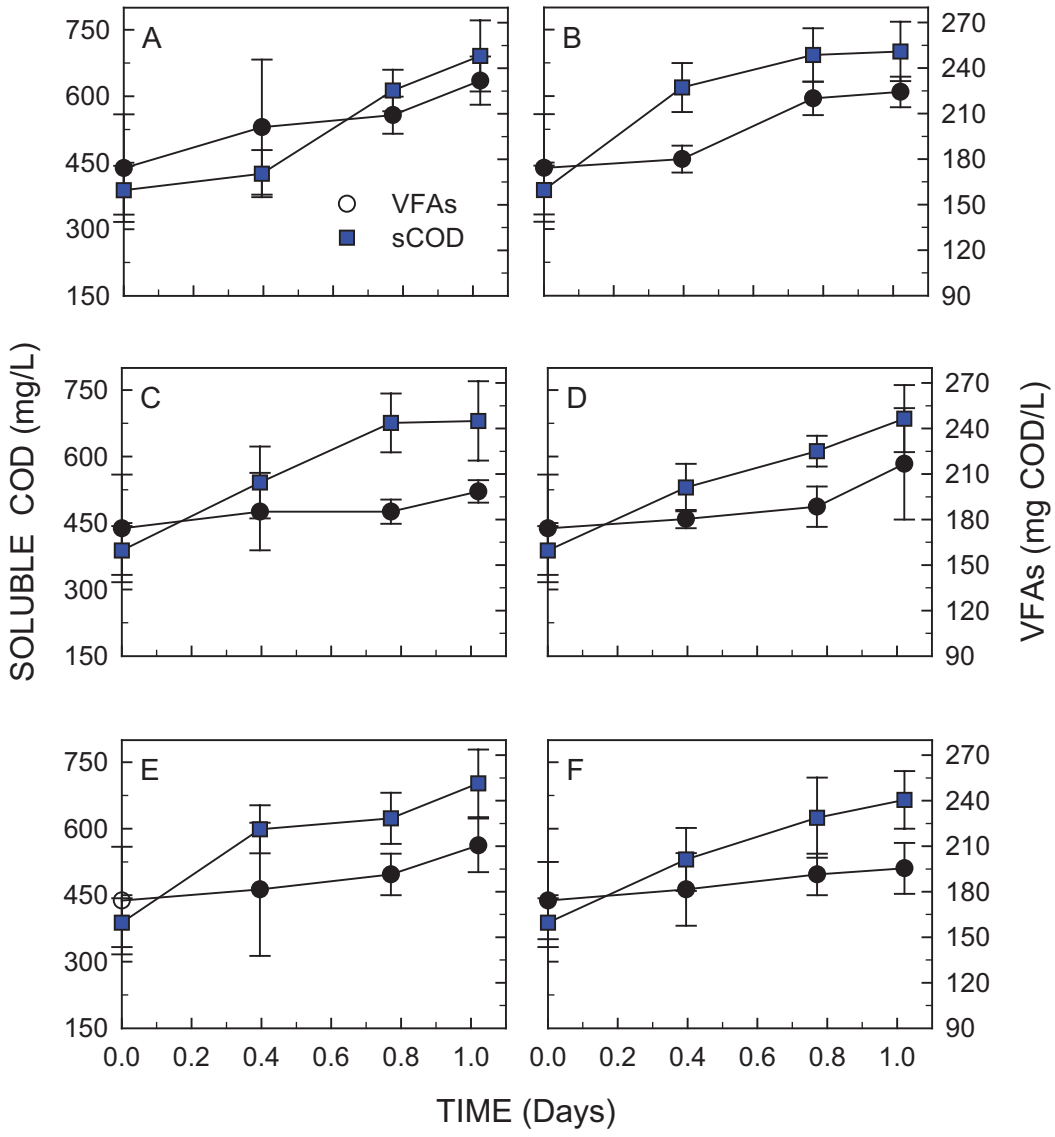

Figure 5.6. Time course of sCOD and VFAs concentrations during the anaerobic batch assay conducted with the anaerobic reactor mixed liquor and poultry processing wastewater at initial BAC concentration of 0 (A), 5 (B), 15 (C), 30 (D), 45 (E), and 60 (F) mg/L.

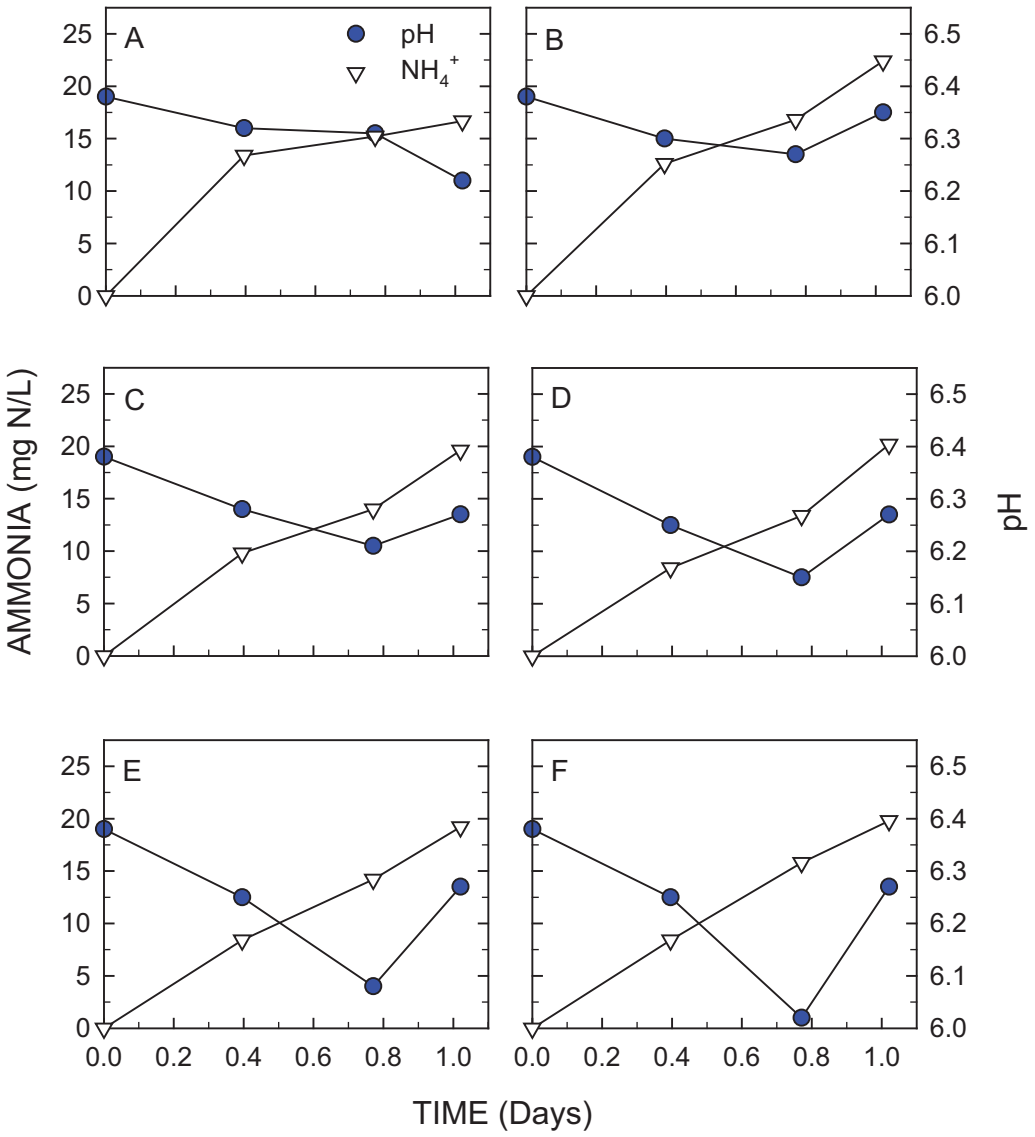

Figure 5.7. Time course of ammonia concentration and pH during the anaerobic batch assay conducted with the anaerobic reactor mixed liquor and poultry processing wastewater at initial BAC concentration of 0 (A), 5 (B), 15 (C), 30 (D), 45 (E), and 60 (F) mg/L.

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 5.8. 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).

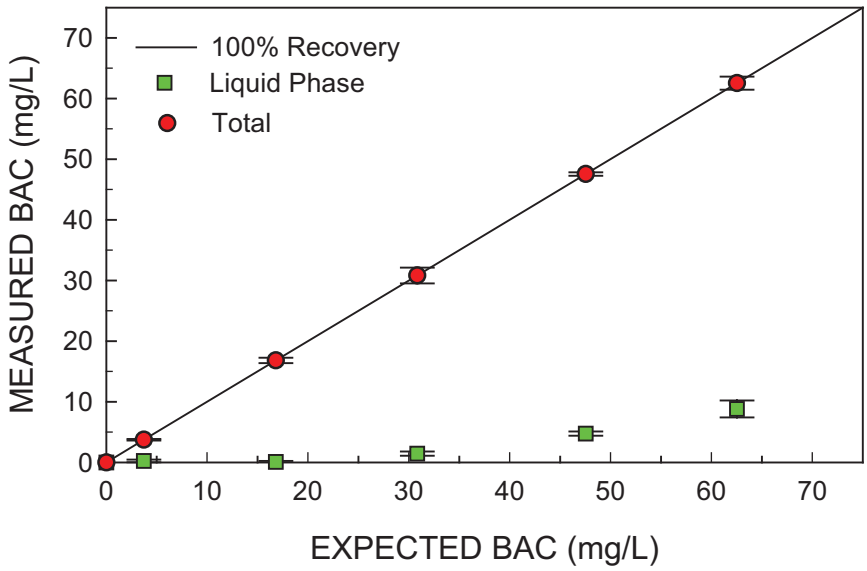

Figure 5.8. BAC phase distribution at the end of the anaerobic batch assay.

5.3.2.2 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. Figure 5.9 shows the time course of the nitrogen species and pH variation during the batch incubation period for all seven culture series. Similarly to the previous nitrification assay (see Chapter 4), 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 (Figure 5.10) brought about by the BAC effect as an antimicrobial agent (Cross and Singer, 1994).

Figure 5.11A 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 5.11B 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.

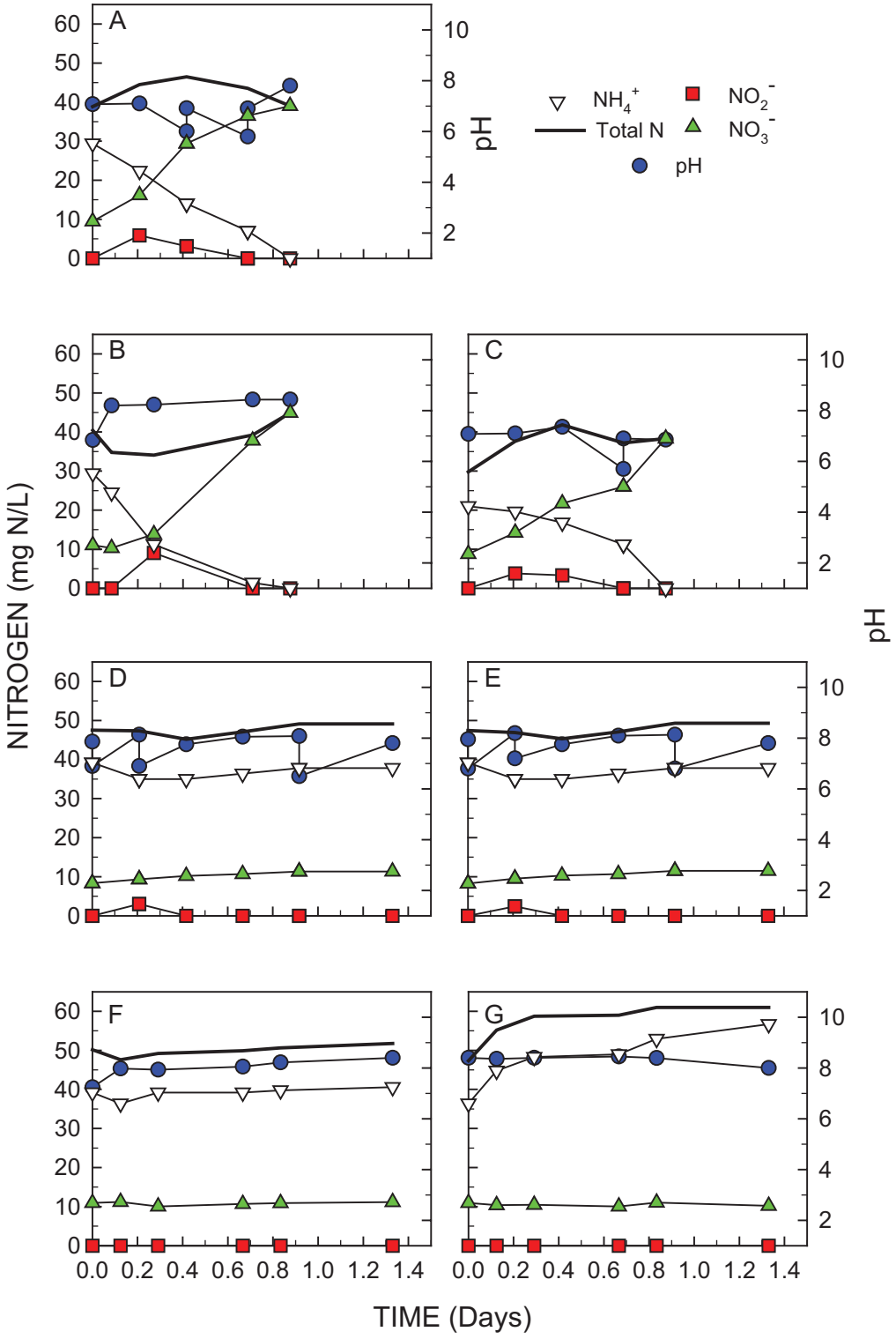

Figure 5.9. Time course of nitrogen species and pH variation during the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 370 and initial BAC concentrations of 0 (A), 5 (B), 15 (C), 20 (D), 25 (E), 30 (F), and 45 (G) mg/L.

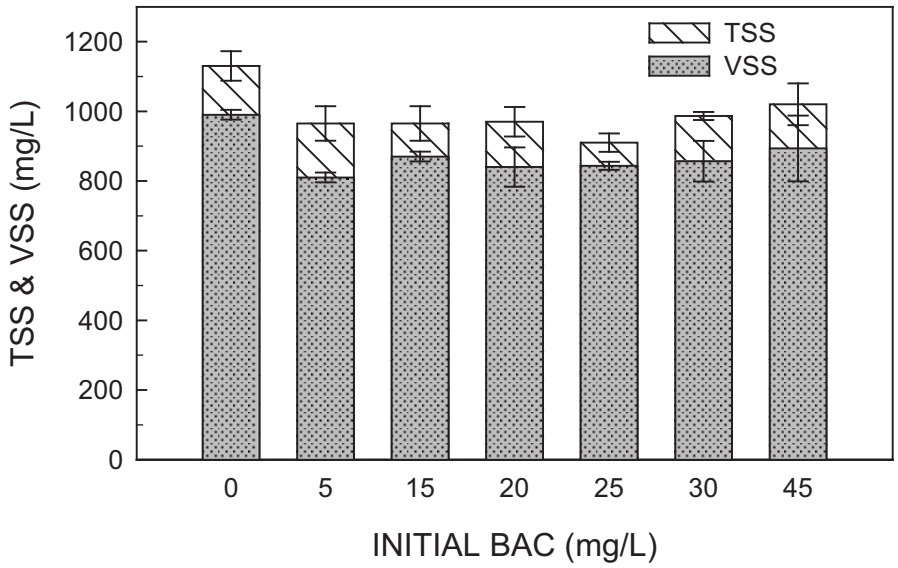

Figure 5.10. Total and volatile suspended solids (TSS and VSS) in the culture series at the end of the incubation period of the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 370 and a range of initial BAC concentration 0 – 45 mg/L. Error bars represent one standard deviation of the means (n = 3).

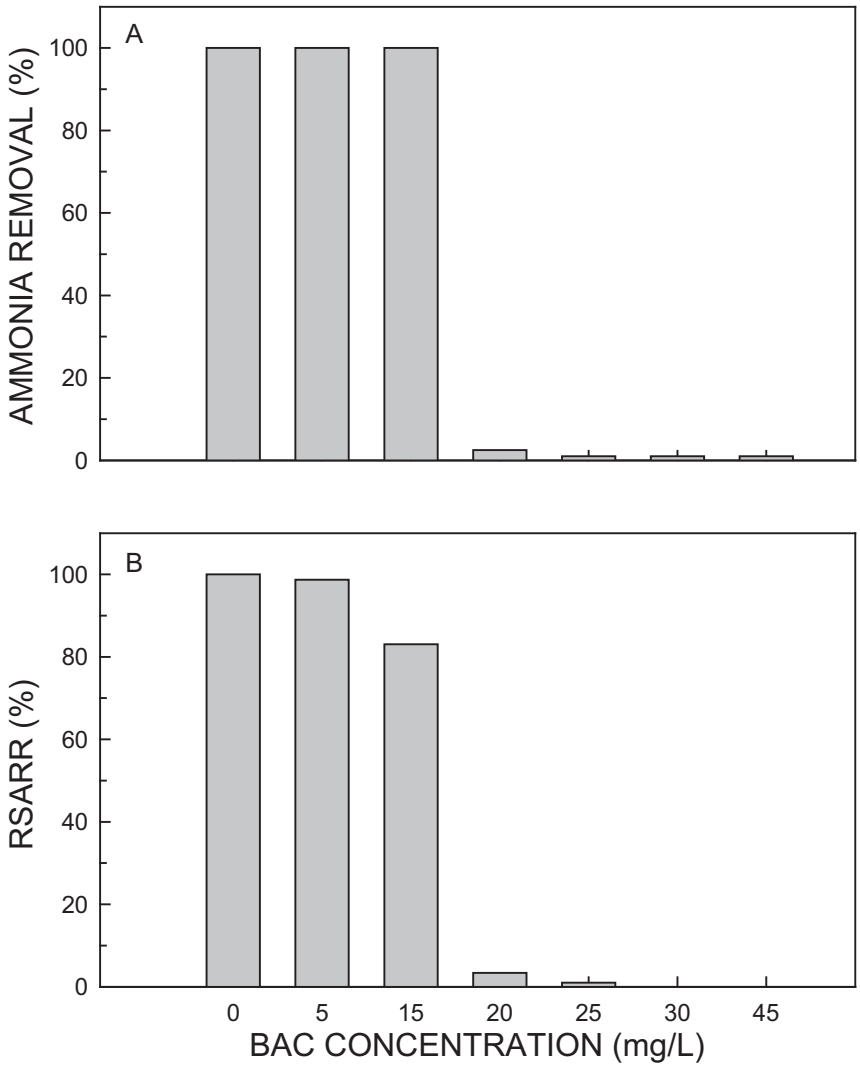

Figure 5.11. Extent of ammonia removal (A) and relative specific ammonia removal rate (RSARR) (B) for the culture series in the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 370 and a range of initial BAC concentration 0 – 45 mg/L.

Figure 5.12 shows the time course of sCOD concentration during the batch incubation period for all seven culture series. 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 5.4.2.1, above), the increase in the sCOD is attributed to BAC-induced inhibition of cell growth and cell lysis. Figure 5.13 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).

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 5.14 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.

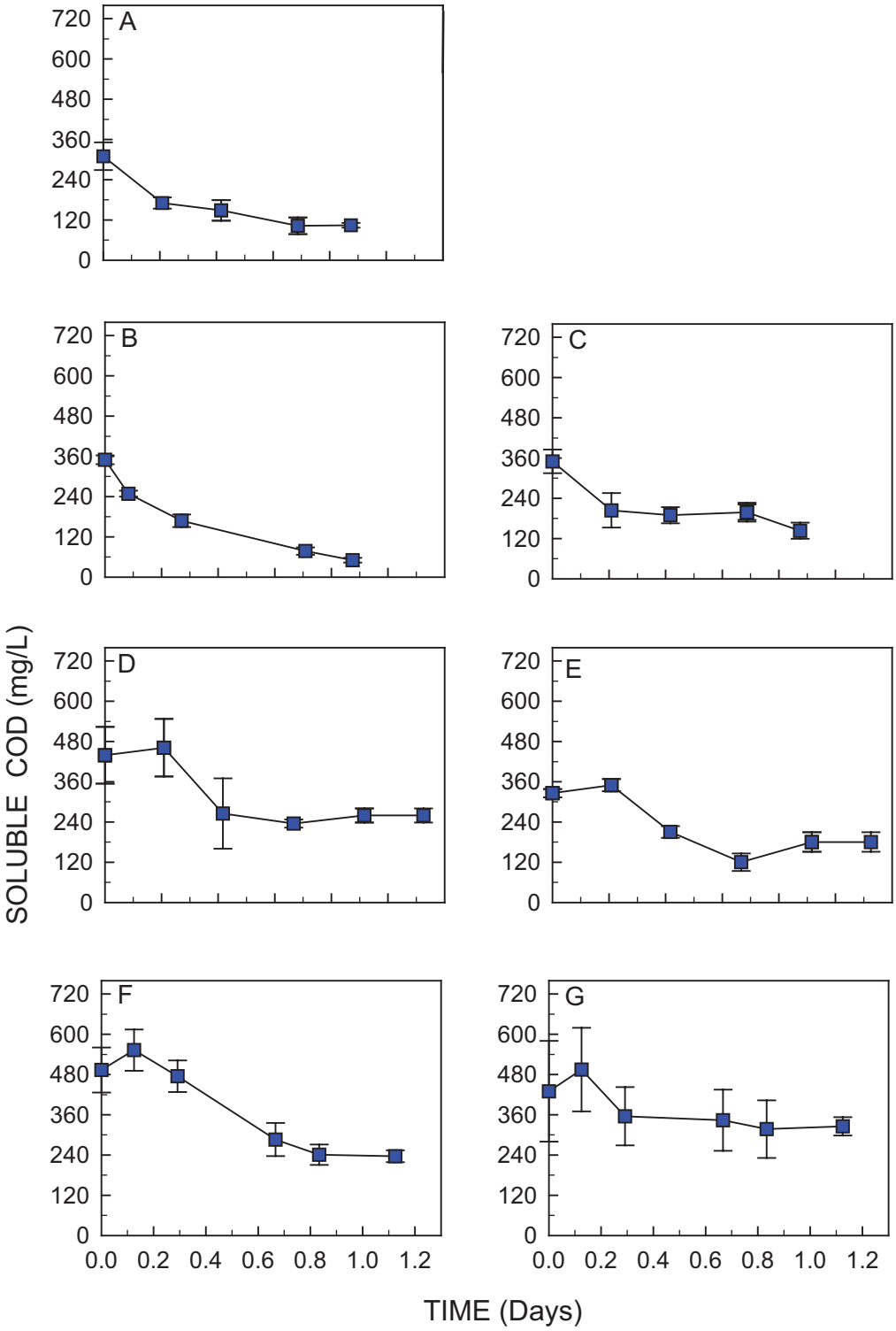

Figure 5.12. Time course of sCOD concentration during the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 370 and initial BAC concentrations of 0 (A), 5 (B), 15 (C), 20 (D), 25 (E), 30 (F), and 45 (G) mg/L. Error bars represent one standard deviation of the means (n = 3).

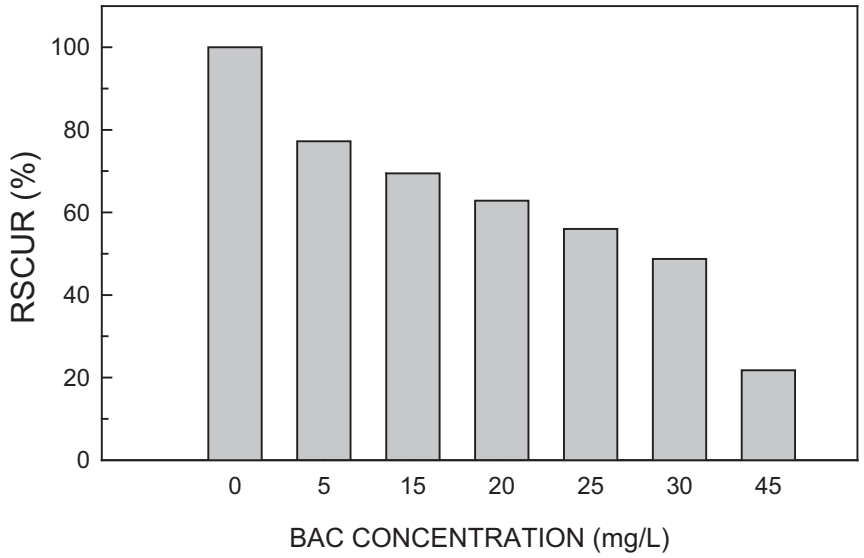

Figure 5.13. Relative specific sCOD utilization rate (RSCUR) for the seven culture series used in the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 370 and a range of initial BAC concentration 0 – 45 mg/L.

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. However, the higher BAC removal rates did not prevent nitrification inhibition at initial BAC concentrations ≥ 20 mg/L.

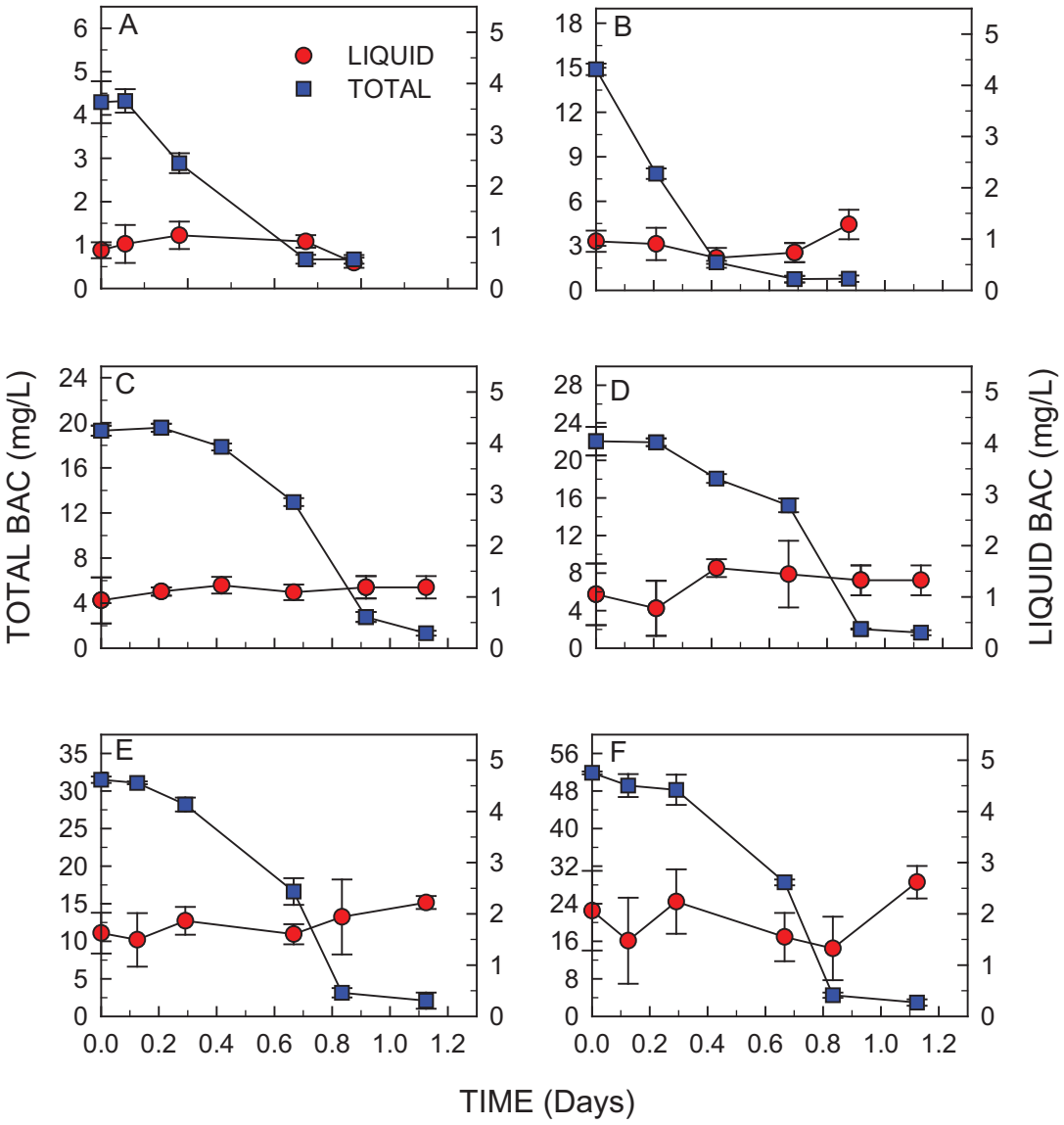

Figure 5.14. Time course of total and liquid-phase BAC concentration during the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 370 and at initial BAC concentrations of 5 (A), 15 (B), 20 (C), 25 (D), 30 (E), and 45 (F) mg/L. Error bars represent one standard deviation of the means (n = 3).

5.3.2.3 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 5.15 shows the time course of nitrogen species in the BAC-free and BAC-amended cultures series and Figure 5.16A 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.

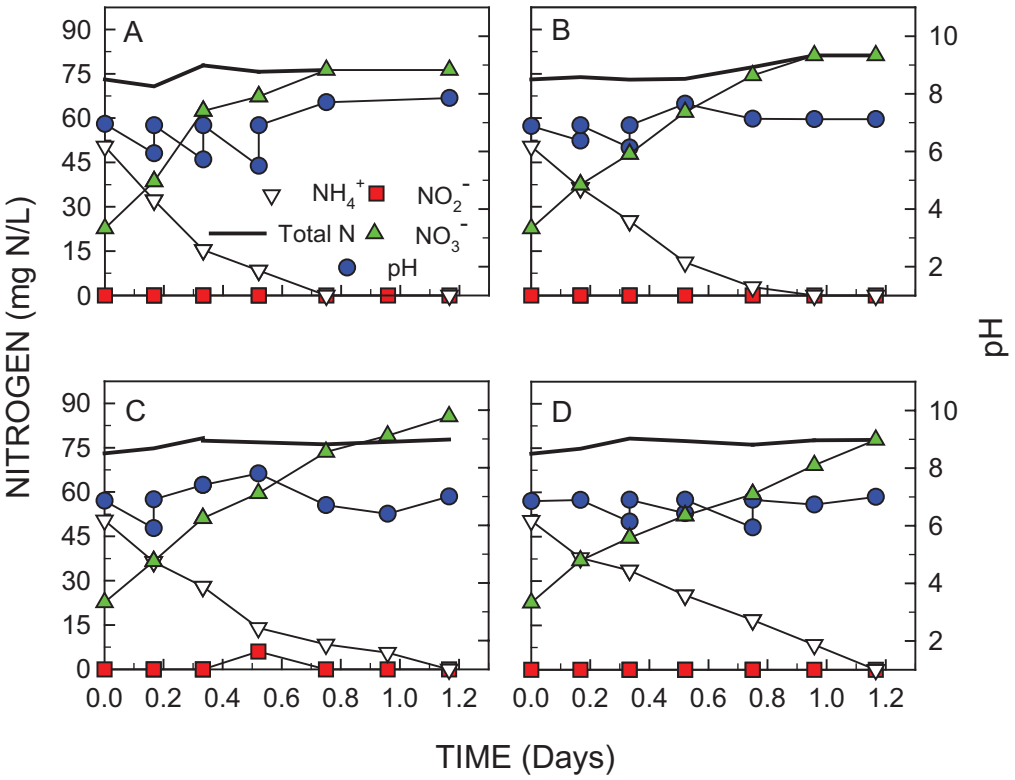

Figure 5.15. Time course of nitrogen species and pH variation during the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 660 and at initial BAC concentrations of 0 (A), 5 (B), 10 (C), and 15 (D) mg/L.

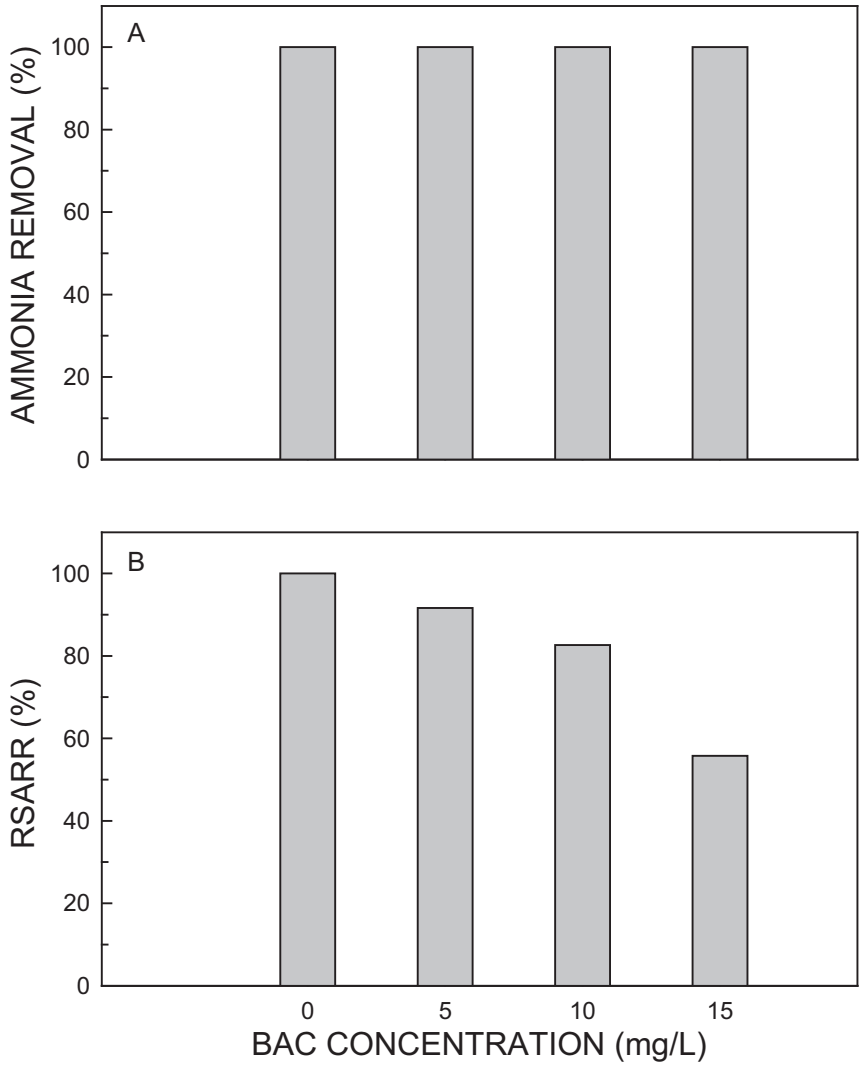

Figure 5.16. Extent of ammonia removal (A) and relative specific ammonia removal rate (RSARR) (B) for the culture series in the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 660 and a range of initial BAC concentration 0 – 15 mg/L.

Figure 5.16B 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 5.17 and was comparable to the initial BAC biotransformation rates obtained with the BAC-unexposed aerobic reactor mixed liquor (see Chapter 4). 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 assay (see Chapter 4) achieved only 43 and 28% ammonia removal, respectively, in spite the fact that BAC was completely removed in these culture series.

This last observation indicates 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.

5.3.2.4 Denitrification batch assay

The assay testing the effect of BAC on denitrification lasted for 1.8 days (42 hours). Figure 5.18 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 5.19A 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 5.20).

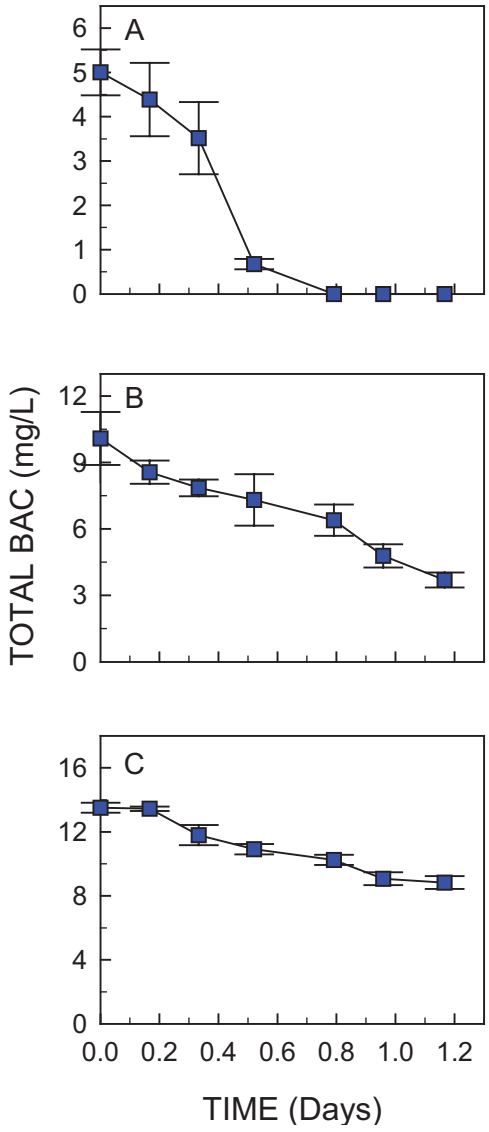

Figure 5.17. Time course of total BAC concentration during the batch nitrification assay conducted with the aerobic reactor (R_3) mixed liquor collected at day 600 and at initial BAC concentrations of 5 (A), 10 (B), and 15 (C) mg/L. Error bars represent one standard deviation of the means (n = 3).

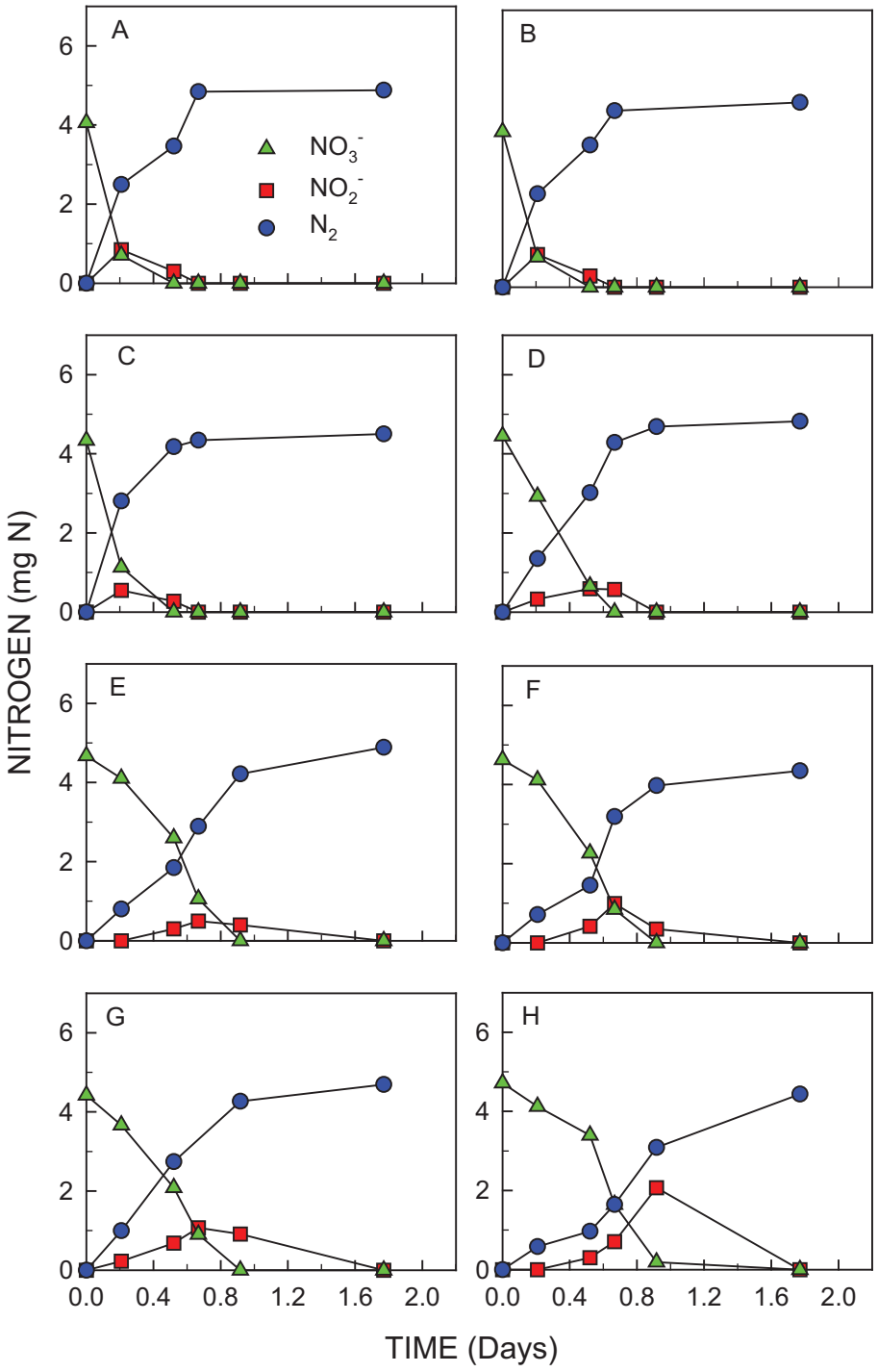

Figure 5.18. Time course of nitrogen species mass per bottle during the batch denitrification assay conducted with the anoxic reactor (R_2) mixed liquor collected at day 400 and at initial BAC concentrations of 0 (A), 5 (B), 10 (C), 15 (D), 20 (E), 25 (F), 30 (G), and 45 (H) mg/L.

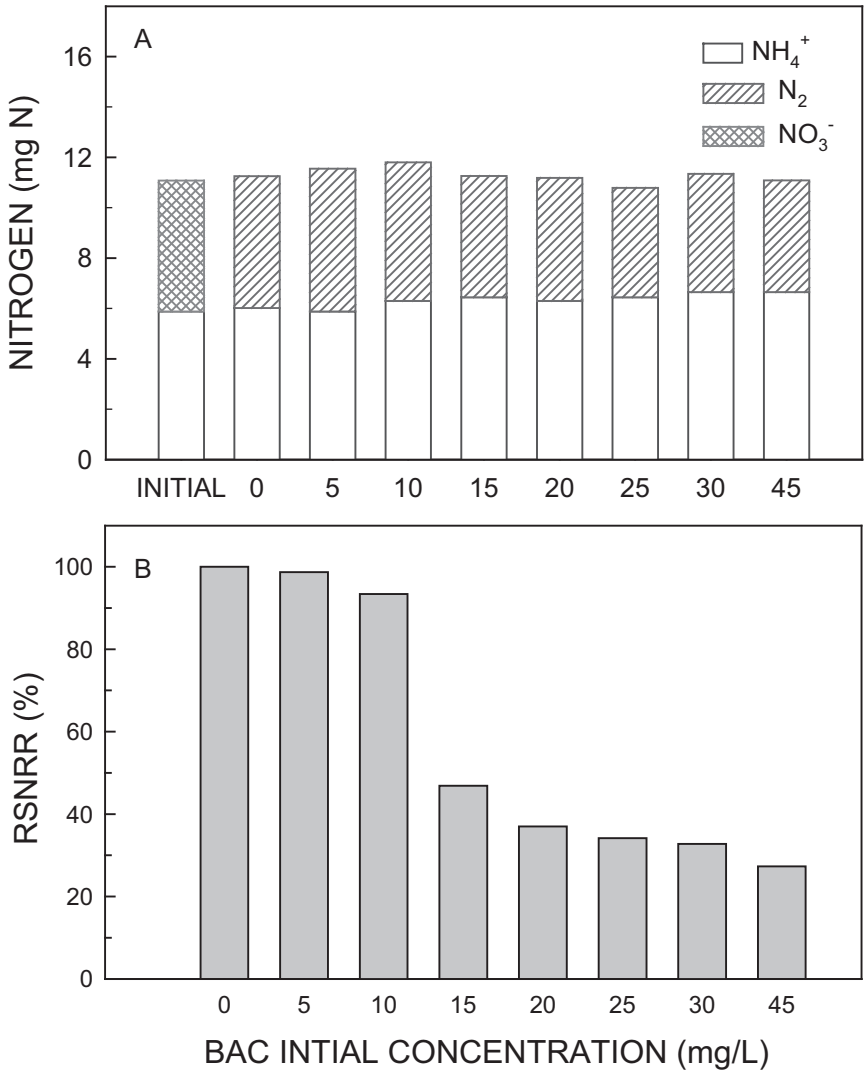

Figure 5.19. Nitrogen species distribution at the end of the incubation period (A) and relative specific nitrate removal rate (RSNRR) (B) in the batch denitrification assay conducted with anoxic reactor (R_2) mixed liquor collected at day 400 and a range of initial BAC concentration 0 – 45 mg/L.

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 5.19B 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 5.19B). Similarly to the initial denitrification assay (i.e., before exposure to BAC; see Chapter 4), 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 culture (discussed in Chapter 4) 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.

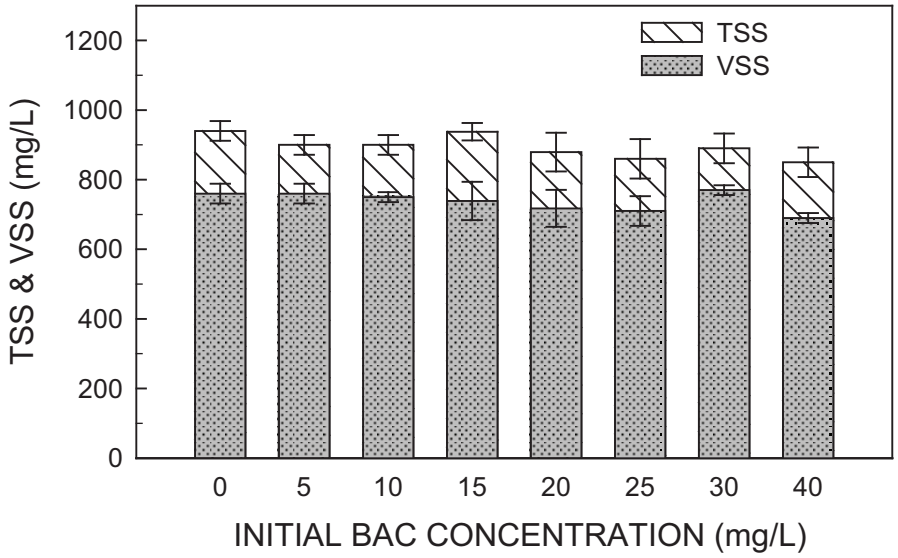

Figure 5.20. Total and volatile suspended solids (TSS and VSS) at the end of the incubation period in the batch denitrification assay conducted with anoxic reactor (R_2) mixed liquor collected at day 400. Error bars represent one standard deviation of the means (n = 3).

5.3.2.5 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 5.21 shows the time course of BAC and detected BAC metabolites during the course of the assay.

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) (see Chapter 2 above), 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 5.22A shows the proposed pathways for BAC in the BNR system aerobic reactor, while Figure 5.22B shows the other reported pathway for BAC (Tezel, 2009).

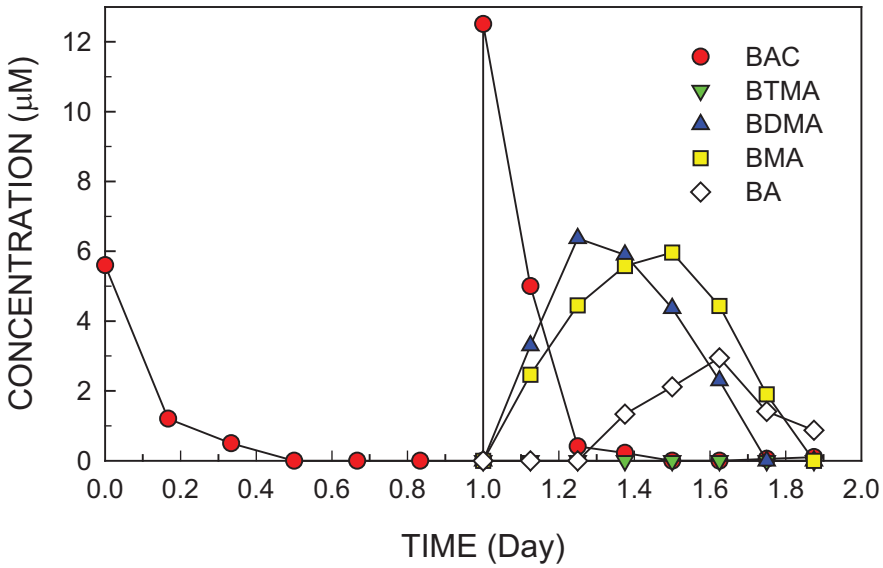

Figure 5.21. Time course of BAC and detected BAC metabolites during the BAC biotransformation assay conducted with aerobic reactor (R_3) mixed liquor collected at day 373.

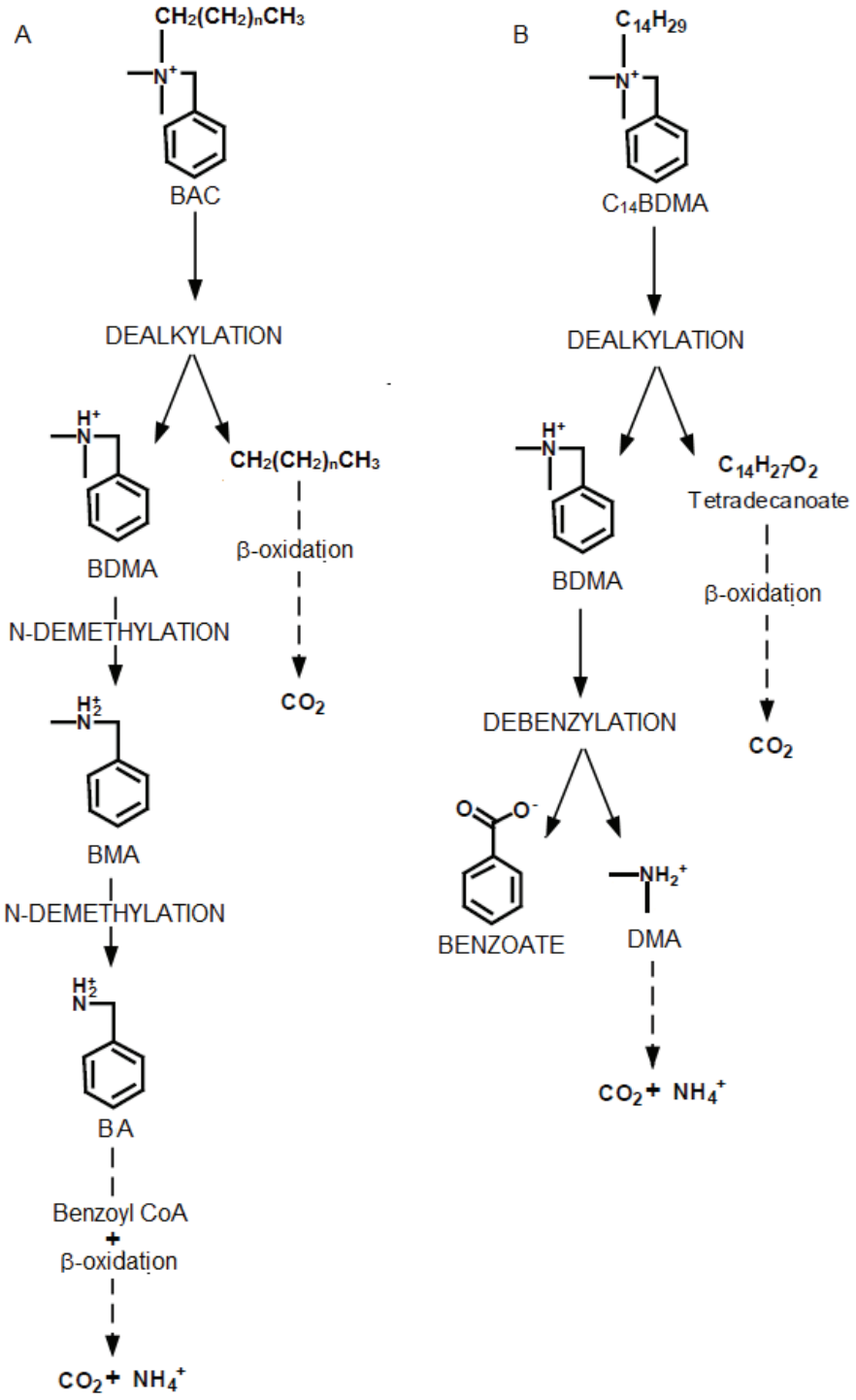

Figure 5.22. Proposed BAC biotransformation pathways as observed in the BNR system aerobic reactor (A) and reported by Tezel (2009) for a highly enriched BAC-degrading culture (B).

5.4.2.6 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 5.23. 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. 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 5.4, while Figure 5.24 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 Chapter 4).

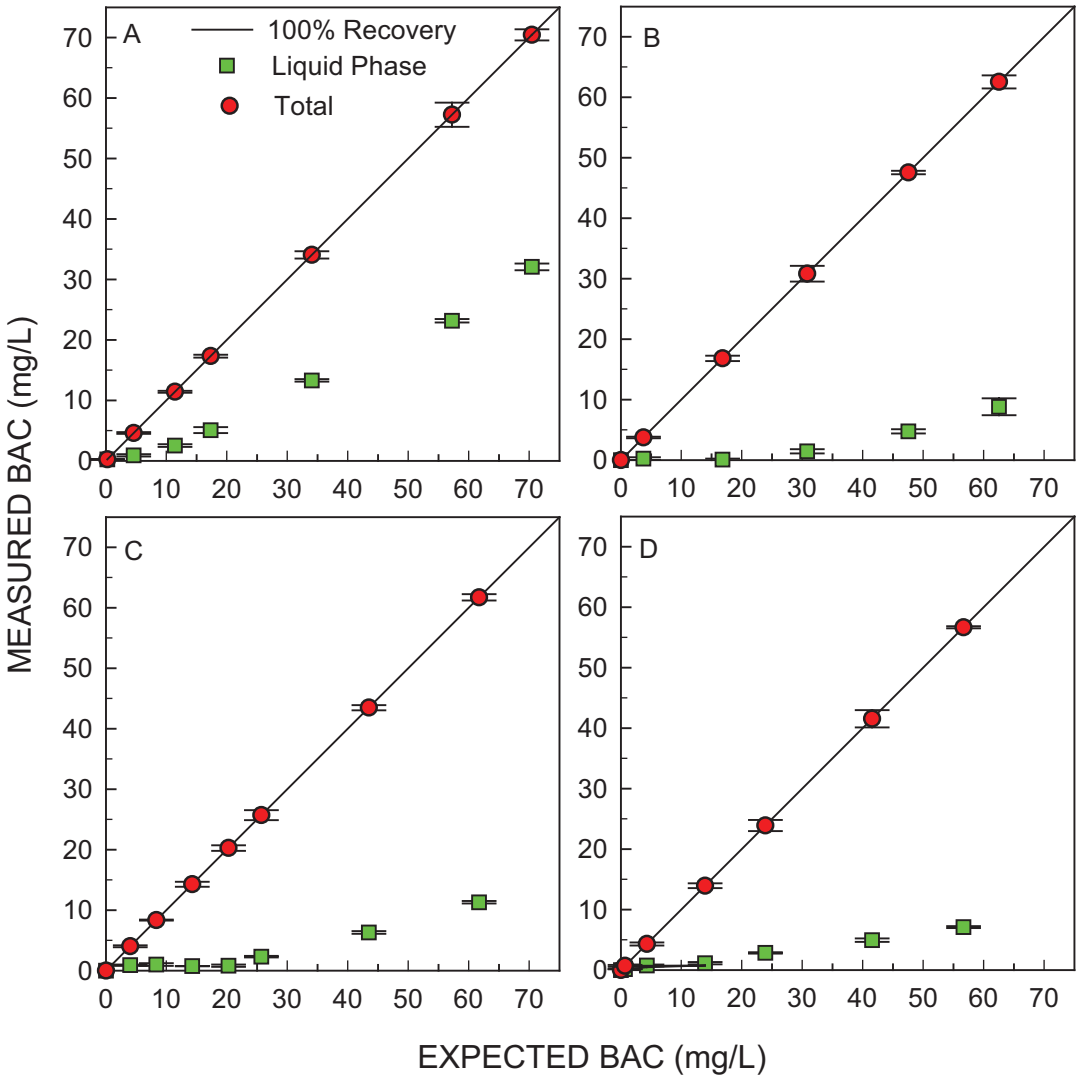

Figure 5.23 BAC phase distribution in the poultry processing wastewater feed (A), and the anaerobic reactor (B), anoxic reactor (C), and aerobic reactor (D) mixed liquors at VSS concentrations of 92 ± 20, 406 ± 17, 938 ± 71, and 1073 ± 94 mg/L (mean ± standard deviation, $n \geq 6$) after 24-hours equilibration. Error bars represent one standard deviation of the means ($n = 3$).

Table 5.4. 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.

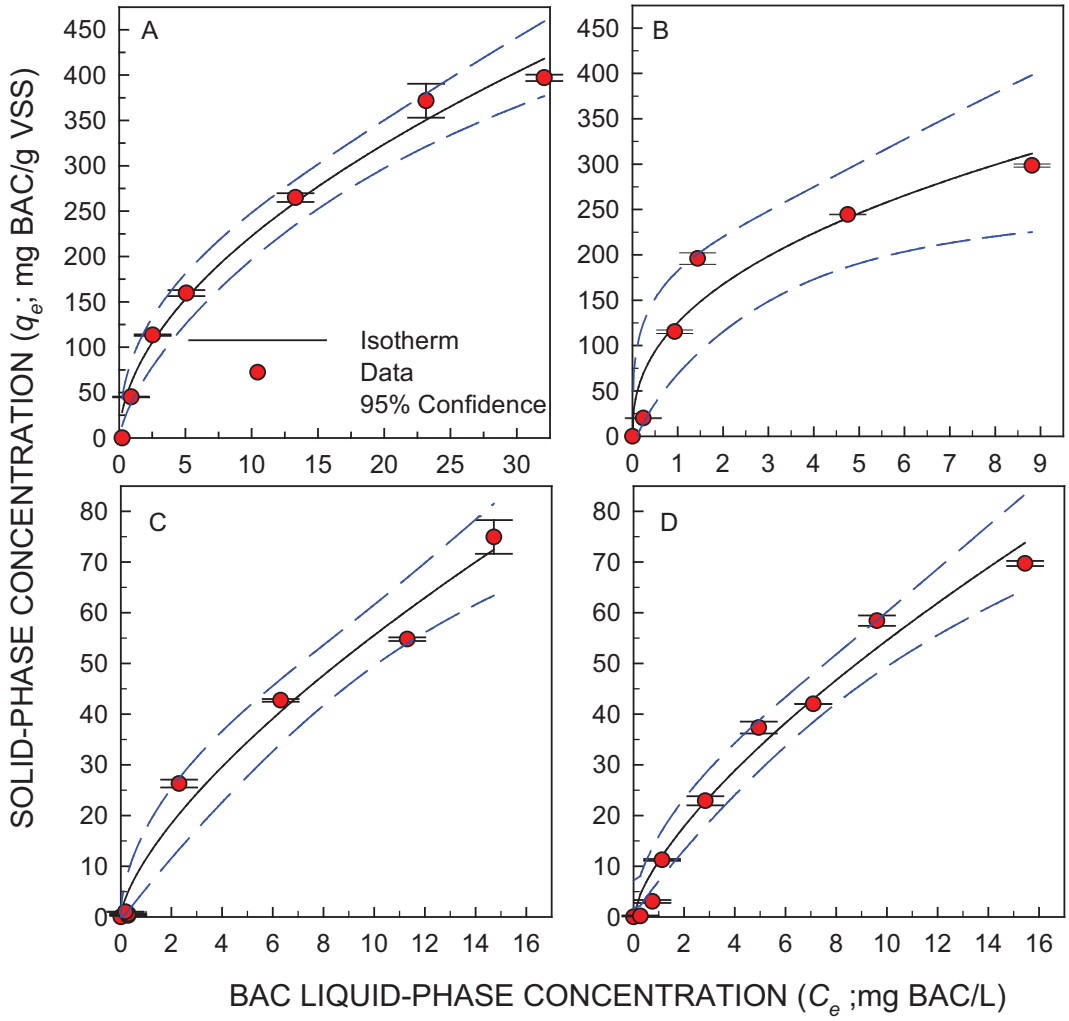

Figure 5.24 Solid- and liquid-phase BAC concentration in the poultry processing wastewater feed (A), and the anaerobic reactor (B), anoxic reactor (C), and aerobic reactor (D) mixed liquors after 24-hours equilibration. Error bars represent one standard deviation of the means (n = 3). Broken lines represent 95% confidence intervals.

The adsorption behavior of the BAC mixture is expected to be different than that of individual BAC homologs because of interactions between the three homologs.

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.

While not evident at this point, 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. Further analysis is required to clearly define the relationship between adsorption and inhibition by BAC, an area that is explored in Chapter 6.

5.4 Summary

The nitrogen removal efficiency of the BNR system was examined while treating BAC-bearing poultry processing wastewater at a range of BAC concentrations. The laboratory-scale, multi-stage BNR system was continuously fed with real poultry processing wastewater amended with a mixture of three benzalkonium chlorides. The nitrogen removal efficiency initially deteriorated at a poultry processing wastewater feed BAC concentration of 5 mg/L due to complete 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.

Batch assays performed using the 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 SARR and complete nitrification at higher BAC concentrations and acquired resistance to BAC, while denitrifiers achieved higher SNRR with lower

transient nitrite accumulation. BAC was also found to be inhibitory to sCOD 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 BDMA, which in turn underwent successive N-demethylations to yield BMA and BA. Time course data of the three intermediates indicated that these biotransformation reactions took place simultaneously.

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.

CHAPTER 6

MODELING THE FATE AND EFFECT OF BENZALKONIUM CHLORIDES IN A BNR SYSTEM

6.1 Introduction

In contrast to available information regarding the fate and effect of BAC on nitrification and denitrification (Chapter 2), little or no information exists regarding BAC nitrification and denitrification inhibition kinetics. Results obtained in both Chapters 4 and 5 showed that BAC has an adverse effect on the BNR system, specifically on the two biological processes responsible for nitrogen removal, i.e., nitrification and denitrification. Three processes contribute to the overall fate of BAC: adsorption, inhibition, and biotransformation. The extent of BAC effect on nitrification and denitrification was influenced by its biotransformation and inhibition. The BAC equilibrium phase distribution (i.e., adsorption) differed in the various components of the BNR system (i.e., feed, anaerobic, anoxic, and aerobic reactors), thus altering the degree and extent of BAC exposure throughout the system. Kinetic modeling of the biological processes in the BNR system reactors (anaerobic, anoxic, and aerobic) with and without BAC exposure will contribute to a better understanding of the interactions between the three system components relative to BAC fate and effect in terms of adsorption, inhibition, and biotransformation. In addition, the incorporation of BAC nitrification and denitrification inhibition kinetics in a comprehensive BNR model will allow the simulation of BAC fate and effect on a BNR system under different operational

conditions, which in turn can guide both the design and operation of BNR systems treating BAC-bearing poultry processing wastewater.

The objectives of the research reported here were to: (a) develop kinetic sub-models for the biological processes occurring in the three reactors in the BNR system; (b) develop BAC inhibition kinetics for nitrification and denitrification; and (c) develop and validate a comprehensive BNR system dynamic model that combines the kinetic sub-models and BAC inhibition kinetics.

6.2 Model Development

6.2.1 Anaerobic Reactor sub-Models

The main processes taking place in the anaerobic reactor are hydrolysis and fermentation (i.e., VFAs production) of particulate organic matter, and the resulting ammonia release (ammonification). Assuming first-order kinetics and no inhibition, the three reactions in the anaerobic reactor are described by three ordinary differential equations (ODEs) as follows:

$$\frac{dXs}{dt} = -k_{hd} X_s \qquad \text{Equation (6.1)}$$

$$\frac{dS_{vfa}}{dt} = f_{vfa} k_{hd} X_s \qquad \text{Equation (6.2)}$$

$$\frac{dS_{nh3}}{dt} = f k_{hd} X_s \qquad \text{Equation (6.3)}$$

where X_S, S_S, S_{vfa} are particulate organic matter, readily degradable organics, and VFAs concentration (mg COD/L), respectively; t is time (days); k_{hd} is the first-order hydrolysis rate constant (day^{-1}); S_{nh3} is ammonia concentration (mg N/L); f_{vfa} and f are the

fractional VFAs (mg COD/mg COD) and ammonia stoichiometric factors (mg N/mg COD), respectively.

6.2.2 Anoxic Reactor sub-Models

The main biological processes taking place in the anoxic reactor are denitrification, utilization of readily degradable organics, and related anoxic microbial heterotrophic growth and decay. Nitrate is assumed to be reduced in a two-step reaction, yielding nitrite as an intermediate and dinitrogen as a final product. Reduction of nitric and nitrous oxides is assumed to be non growth-limiting, and faster than nitrate and nitrite reduction. Readily degradable organics (S_S; mg COD/L) are the source of carbon and electron donor for denitrification. The nitrate and nitrite reduction follows Monod, mixed- or double-substrate kinetics (Rittmann and McCarty, 2001; Sin et al., 2008), where the reduction reactions are further limited by the heterotrophic utilization of the readably degradable organics. Assuming a two-step nitrate reduction rate, and no pH, oxygen, or nitric acid inhibition, the following ODEs describe nitrate reduction in the anoxic reactor:

$$\frac{dS_{no3}}{dt} = -\left(\frac{k_{no3}S_{no3}}{K_{sno3}+S_{no3}}\frac{\eta K_{ss}}{K_{ss}+S_s}\right)X_h \qquad \text{Equation (6.4)}$$

$$\frac{dS_{no2}}{dt} = \left[\left(\frac{k_{no3}S_{no3}}{K_{sno3}+S_{no3}}\frac{\eta S_s}{K_{ss}+S_s}\right)-\left(\frac{k_{no2}S_{no2}}{K_{sno2}+S_{no2}}\frac{\eta K_{ss}}{K_{ss}+S_s}\right)\right]X_h \qquad \text{Equation (6.5)}$$

$$\frac{dS_{n2}}{dt} = \left(\frac{k_{no2} S_{no2}}{K_{sno2} + S_{no2}} \frac{\eta S_s}{K_{ss} + S_s} \right) X_h$$

Equation (6.6)

where S_{no3} , S_{no2}, and S_{n2} are nitrate, nitrite, and dinitrogen concentration (mg N/L) normalized to the reactor liquid volume, respectively; k_{no3} and k_{no2} are the maximum specific reduction rate for nitrate and nitrite (mg N/mg VSS·day); K_{sno3} , K_{sno2} , and K_{ss} are the half-saturation constants for nitrate and nitrite reduction (mg N/L) and readily degradable substrate utilization (mg COD/L), respectively; η is the dimensionless anoxic microbial growth correction factor; and X_h is the VSS concentration of heterotrophic denitrifiers (mg VSS/L). Heterotrophic anoxic microbial growth is described using Monod kinetics, and assumes to be limited by nitrate, nitrite, and readily degradable organics utilization as follows:

$$\frac{dS_s}{dt} = k_{hd} X_S - \left(\frac{k_s S_s}{K_{ss} + S_s} \right) \eta X \qquad \text{Equation (6.7)}$$

$$\frac{dX_h}{dt} = \left[\left(\frac{\eta Y_h k_s S_s}{K_{ss} + S_s} \right) + \left(\frac{Y_{no3} k_{no3} S_{no3}}{K_{sno3} + S_{no3}} \right) + \left(\frac{Y_{no2} k_{no2} S_{no2}}{K_{sno2} + S_{no2}} \right) \right] X_h - b_h X_h \qquad \text{Equation (6.8)}$$

where k_s is the maximum specific readily degradable organics utilization rate (mg COD/mg VSS·day); Y_h, Y_{no3}, and Y_{no2}, are the heterotrophic yield coefficients for readily degradable substrate (mg VSS/mg COD), nitrate and nitrite reduction (mg VSS/mg N), and b_h is the microbial decay coefficient (day^{-1}). In addition, 80% (f_p) of decayed biomass is assumed to be degradable, yielding additional readily degradable organics and

ammonia. Finally, ammonia is assumed to be the nitrogen source for microbial growth in the anoxic reactor, utilized by a factor (*iX*) which equals the nitrogen fraction in biomass:

$$\frac{dX_S}{dt} = -k_{hd} X_S + f_p b_h X_h \qquad \text{Equation (6.9)}$$

$$\frac{dS_{nh3}}{dt} = k_{hd} f X_S - iX \cdot \left(\frac{dX_h}{dt} \right)_{anoxic-growth} \qquad \text{Equation (6.10)}$$

6.2.3 Aerobic Reactor sub-Models

The main biological processes taking place in the aerobic reactor are nitrification, aerobic utilization of readily degradable organics linked to heterotrophic microbial growth, and decay of both nitrifiers and heterotrophs. Nitrification is assumed to be a two-step reaction yielding nitrite as an intermediate, and nitrate as a final product. Autotrophic nitrifiers utilize ammonia and nitrate as the only limiting substrates for microbial growth, and Monod kinetics are used to describe the substrate utilization. Similarly to the anoxic reactor, ammonia is the nitrogen source for microbial growth in the aerobic reactor. Assuming no oxygen limitations, no ammonia inhibition, and constant neutral pH, the following ODEs are used to describe nitrification in the aerobic reactor:

$$\frac{dS_{nh3}}{dt} = -\left(\frac{k_{nh3} S_{nh3}}{K_{snh3} + S_{nh3}} \right) X_a - iX \cdot \left(\frac{dX_a}{dt} + \frac{dX_h}{dt} \right)_{growth} \qquad \text{Equation (6.11)}$$

$$\frac{dS_{no2}}{dt} = \left[\left(\frac{k_{nh3} S_{nh3}}{K_{snh3} + S_{nh3}} \right) - \left(\frac{k^a_{no2} S_{no2}}{K^a_{sno2} + S_{no2}} \right) \right] X_a \qquad \text{Equation (6.12)}$$

$$\frac{dS_{no3}}{dt} = \left(\frac{k^a_{no2} S_{no2}}{K^a_{sno2} + S_{no2}} \right) X_a \qquad \text{Equation (6.13)}$$

where k_{nh3} and k^a_{no2} are the maximum specific oxidation rate for ammonia and nitrite (mg N/mg VSS·day); K_{snh3} and K^a_{sno2} are the half-saturation constants for ammonia and nitrate oxidation (mg N/L); and X_a is the VSS concentration of active autotrophic nitrifiers in the aerobic reactor (mg VSS/L). The growth of heterotrophs and autotrophs in the aerobic reactor is also limited by the readily degradable organics and ammonia/nitrite utilization, respectively:

$$\frac{dS_s}{dt} = k_{hd} X_S - \left(\frac{k_s S_s}{K_{ss} + S_s} \right) X_h \qquad \text{Equation (6.14)}$$

$$\frac{dX_h}{dt} = \left[\frac{Y_h k_s S_s}{K_{ss} + S_s} \right] X_h - b_h X_h \qquad \text{Equation (6.15)}$$

$$\frac{dX_a}{dt} = \left[\left(\frac{Y_{nh3} k_{nh3} S_{nh3}}{K_{snh3} + S_{nh3}} \right) + \left(\frac{Y^a_{no2} k^a_{no2} S_{no2}}{K^a_{sno2} + S_{no2}} \right) \right] X_a - b_a X_a \qquad \text{Equation (6.16)}$$

where Y_{no3} and Y^a_{no2} are the autotrophic yield coefficients for ammonia and nitrate (mg VSS/mg N), and b is the microbial decay coefficient (day^{-1}). Similarly to the anoxic reactor, 80% of decayed biomass is assumed to be degradable (Equation 6.9).

6.2.4 BAC Fate and Effect sub-Models

6.2.4.1 BAC in the Feed Poultry Processing Wastewater and Anaerobic Reactor

Experimental results discussed in Chapter 5 and literature data show that BAC biotransformation is not possible under the conditions of the anaerobic reactor. Moreover, the effect of BAC on the biological processes in the anaerobic reactor was marginal compared to the anoxic and aerobic reactors. The same argument could be extended to the feed poultry processing wastewater. Therefore, the effect of BAC was assumed to be negligible in both the feed and the anaerobic reactor. Nevertheless, because the solids concentration in the feed poultry processing wastewater and the anaerobic reactor are different, the only change that BAC undergoes between these two compartments is its equilibrium phase distribution. Values of K_F and n (Chapter 5) clearly showed a difference in adsorption extent and affinity between the poultry processing wastewater, the anaerobic reactor, and the anoxic/aerobic reactors. In order to accommodate this difference in BAC phase distribution in the BNR system model, the adsorption behavior of BAC must be included in the sub-models. In addition, the nonlinear Freundlich adsorption isotherm will result in an implicit equation when the liquid and solid phase BAC concentrations are correlated with the total BAC concentration. On the other hand, a linear adsorption relationship will result in an explicit equation, which can be easily incorporated in the simulation of the BNR system model. BAC phase distribution for the poultry processing wastewater feed and the anaerobic reactor was assumed to be linear as shown in the following equation:

$$q_e = K_P C_e \qquad \text{Equation (6.17)}$$

where K_P is the linear partition coefficient ((mg/g VSS)(L/mg)). Figure 6.1 shows the linearized adsorption isotherms and the resulting partition coefficient values. As seen in

Figure 6.1 and Figures 5.26A and B, the linear adsorption model did not affect the predicted liquid- and solid-phase BAC concentration, and successfully represented the adsorption behavior of BAC in the first two BNR components (i.e., feed and anaerobic reactor).

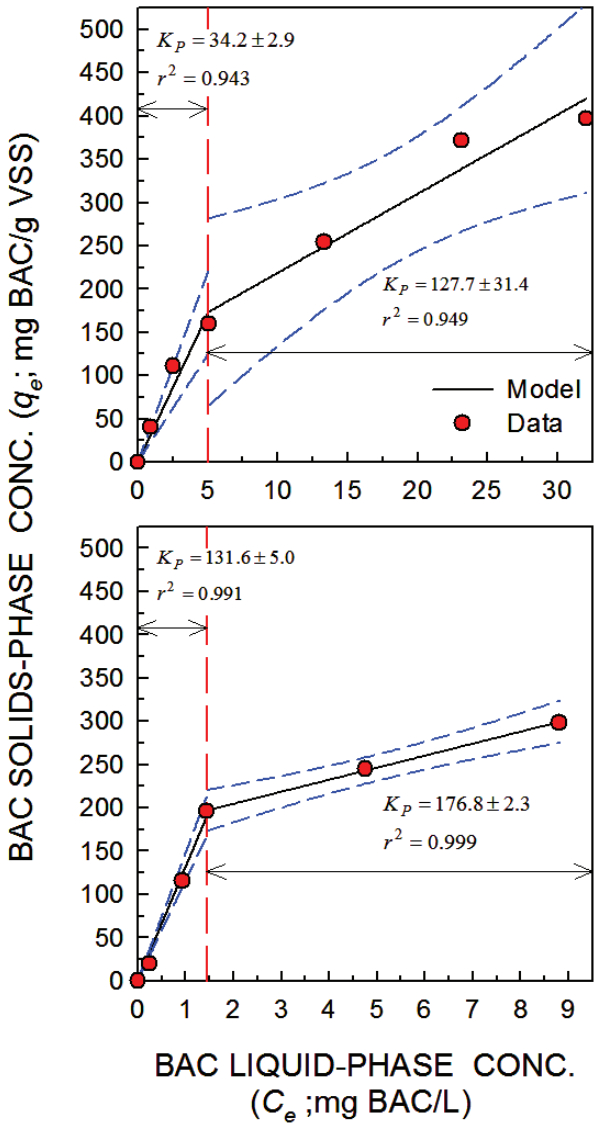

Figure 6.1. BAC linearized adsorption isotherms and partition coefficient values for the feed poultry processing wastewater (A) and the anaerobic reactor mixed liquor (B) (Broken lines are 95% confidence intervals).

6.2.4.2 BAC in the Anoxic Reactor

BAC affected the denitrification rates in the anoxic reactor (Chapters 4 and 5), but similarly to the anaerobic reactor, biotransformation of BAC was not observed. BAC inhibits respiratory enzymes by a mechanism best described by the competitive inhibition model (Zhang et al., 2010), where the inhibitor binds with the enzyme(s) at the active site. In the case of competitive inhibition, the apparent half-saturation constant in Monod-type kinetics increases with increasing inhibitor concentration (Rittmann and McCarty, 2001), as shown in the following Equation:

$$K_s^{'} = K_s(1+\frac{I}{K_I}) \qquad \text{Equation (6.18A)}$$

$$\mu = \frac{k_s}{Y}\frac{S}{K'_s+S} \qquad \text{Equation (6.18B)}$$

where $K_s^{'}$ and K_s are the apparent and un-inhibited half-saturation constants (mg N/L); K_I is the inhibition coefficient (mg BAC/L); I is the inhibitor concentration (mg BAC/L); μ is the specific microbial growth rate (day^{-1}); k_s is the maximum specific utilization rate (mg substrate/ mg VSS · day); S is the rate-limiting substrate concentration (mg/L); and Y is the microbial yield coefficient (mg VSS/mg substrate). Figure 6.2 shows how the competitive inhibition affects the specific growth rate in Monod-type kinetics at different inhibitor concentrations (Equation 6.18B).

6.2.4.3 BAC in the Aerobic Reactor

BAC affected the nitrification rate and extent and the sCOD utilization in the aerobic reactor (Chapters 4 and 5). In addition, BAC was biodegraded under the conditions of the aerobic reactor (Chapter 5).

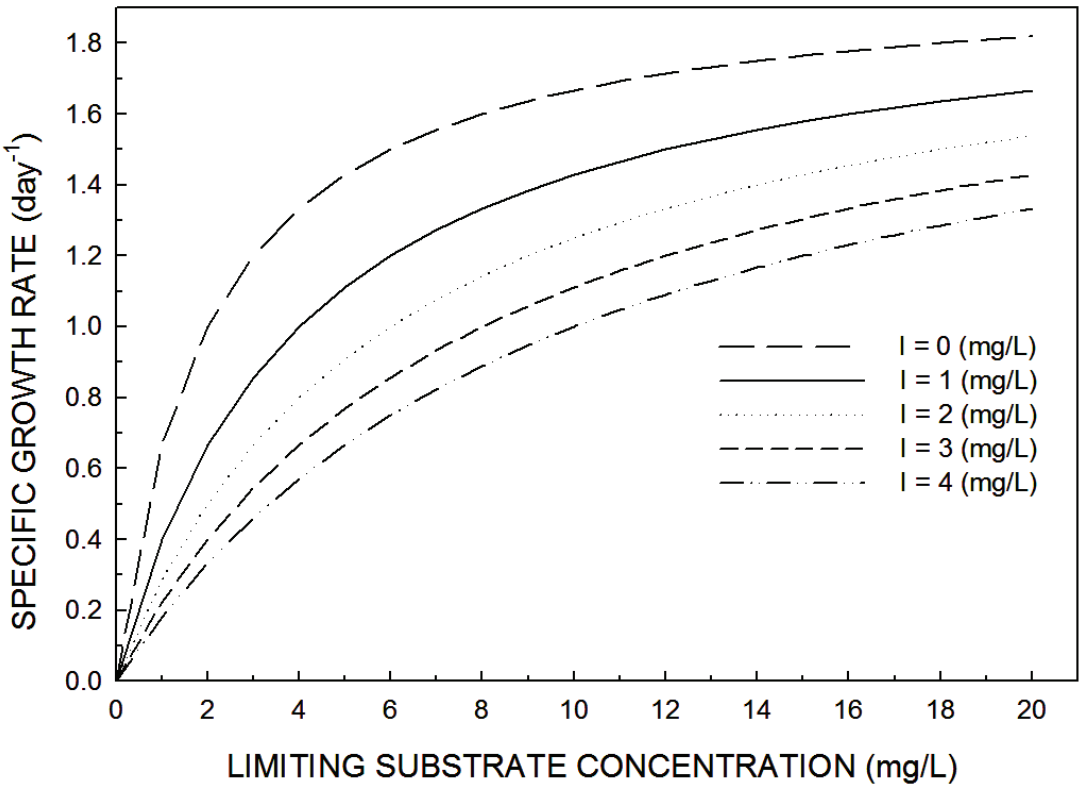

Figure 6.2. Competitive inhibition effects on the specific growth rate (μ) in a Monod-type, kinetic equation at different inhibitor concentrations. k_s = 1 (mg N/mg VSS.day^{-1}); Y = 0.5 (mg VSS/mg N) K_S = 2 (mg N/L); and K_I = 1 mg N/L.

The inhibitory effect of BAC on nitrification is better described by a non-competitive model (Rittmann and McCarty, 2001; Yang, 2007), where the inhibitor binds with the enzyme(s) at a place other than the active site for substrate binding. As a result, the enzyme(s) becomes less reactive towards the substrate(s). In the case of non-competitive inhibition, the apparent maximum specific utilization rate decreases in Monod-type kinetics with increasing inhibitor concentration (Rittmann and McCarty, 2001), as shown in the following Equation:

$$k_s' = \frac{k_s}{(1 + \frac{I}{K_I})} \qquad \text{Equation (6.19A)}$$

$$\mu = \frac{k_s'}{Y} \frac{S}{K_s + S} \qquad \text{Equation (6.19B)}$$

where k'_s and k_s are the apparent and un-inhibited maximum specific utilization rate (mg N/mg VSS· day); K_I is the inhibition coefficient (mg BAC/L); and I is the inhibitor concentration (mg BAC/L). Figure 6.3 shows how the competitive inhibition affects the specific growth rate in Monod-type kinetics at different inhibitor concentrations (Equation 6.19B). As seen in Figure 6.3, a non-competitive inhibitor decreases the growth rate at concentrations far less than a competitive inhibitor; therefore, a non-competitive inhibitor will have a greater effect than a competitive inhibitor at the same concentration. For heterotrophic sCOD utilization, BAC inhibition is best described by the competitive inhibition model, similar to denitrification.

BAC degradation was found to follow mixed-substrate, Monod kinetics, where BAC degradation is competing with readily degradable organic substrate (Zhang et al., 2010), as shown in the following Equation:

$$\frac{dS_{BAC}^{T}}{dt} = \left[\frac{k_{BAC} S_{BAC}^{T}}{S_{BAC}^{T} + K_{SBAC}} \frac{K_S}{S_S + K_S} \right] X_h \qquad \text{Equation (6.20)}$$

where S_{BAC}^{T} is the total BAC concentration in the system (mg/L); k_{BAC} is the total BAC maximum specific utilization rate (mg BAC/mg VSS · day); and K_{SBAC} is the BAC degradation half-saturation constant (mg BAC/L).

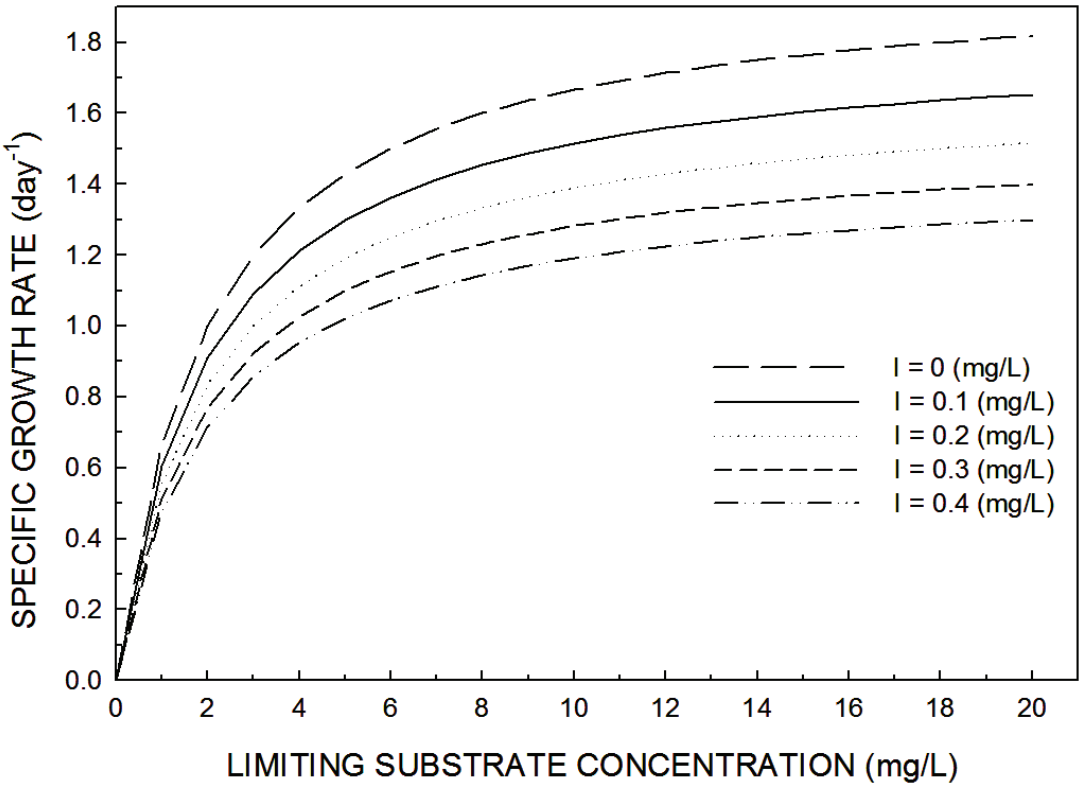

Figure 6.3. Non-competitive inhibition effects on the specific utilization rate (μ) in a Monod-type, kinetic equation at different inhibitor concentrations; k_s = 1 (mg N/mg VSS.day^{-1}); Y = 0.5 (mg VSS/mg N) K_S = 2 (mg N/L); and K_I = 1 mg N/L.

6.2.4.4 BAC Phase Distribution in the Anoxic and Aerobic Reactors

As discussed in Chapter 5, the BAC equilibrium phase distribution was similar in both the anoxic and aerobic reactor. Therefore, the linearization of BAC adsorption behavior (Equation 6.17) was assumed to be the same for both reactors. Figure 6.4 shows the linearized adsorption isotherms and resulting partition coefficient values. Similar to the linear model used for the feed and the anaerobic reactor, the linear BAC adsorption model for the anoxic and aerobic reactors successfully predicted the adsorption behavior of BAC in the latter two BNR components (i.e., anoxic and aerobic reactors) (Figure 5.26C and D).

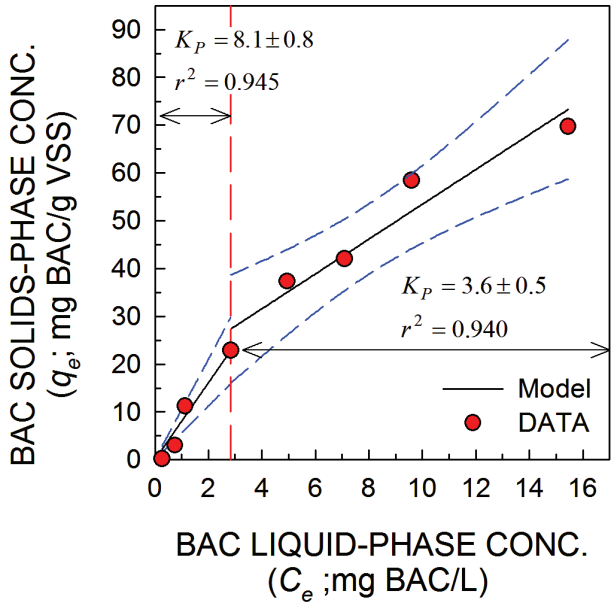

Figure 6.4. Linearized BAC adsorption isotherms and partition coefficient values for the anoxic and aerobic reactors (Broken lines are 95% confidence intervals).

6.2.5. BNR Model

The sub-models discussed above were combined in a comprehensive model, which describes the performance of the BNR system. Figure 6.5 shows an overview of the relationships between the different variables in the BNR model, while Table 6.1 shows the matrix with the BNR model processes and variables.

Mass balance equations on the soluble and particulate variables within the BNR system reactors resulted in the following ODEs:

(A) Anaerobic reactor:

a. Soluble:

$$\frac{dS}{dt} = \frac{Q}{V}(S_{HSWW} - S_{Anaerobic}) \pm rate \qquad \text{Equation (6.21A)}$$

b. Particulate:

$$\frac{dX}{dt} = \frac{Q}{V}(X_{HSWW} - X_{Anaerobic}) \pm rate \qquad \text{Equation (6.21B)}$$

where S and X are the soluble and constituents concentration, respectively (Mass/L); Q is the poultry processing wastewater feed flow rate (L/day); V is the reactor volume (L); and the rate corresponds to the appropriate sub-model in the anaerobic reactor.

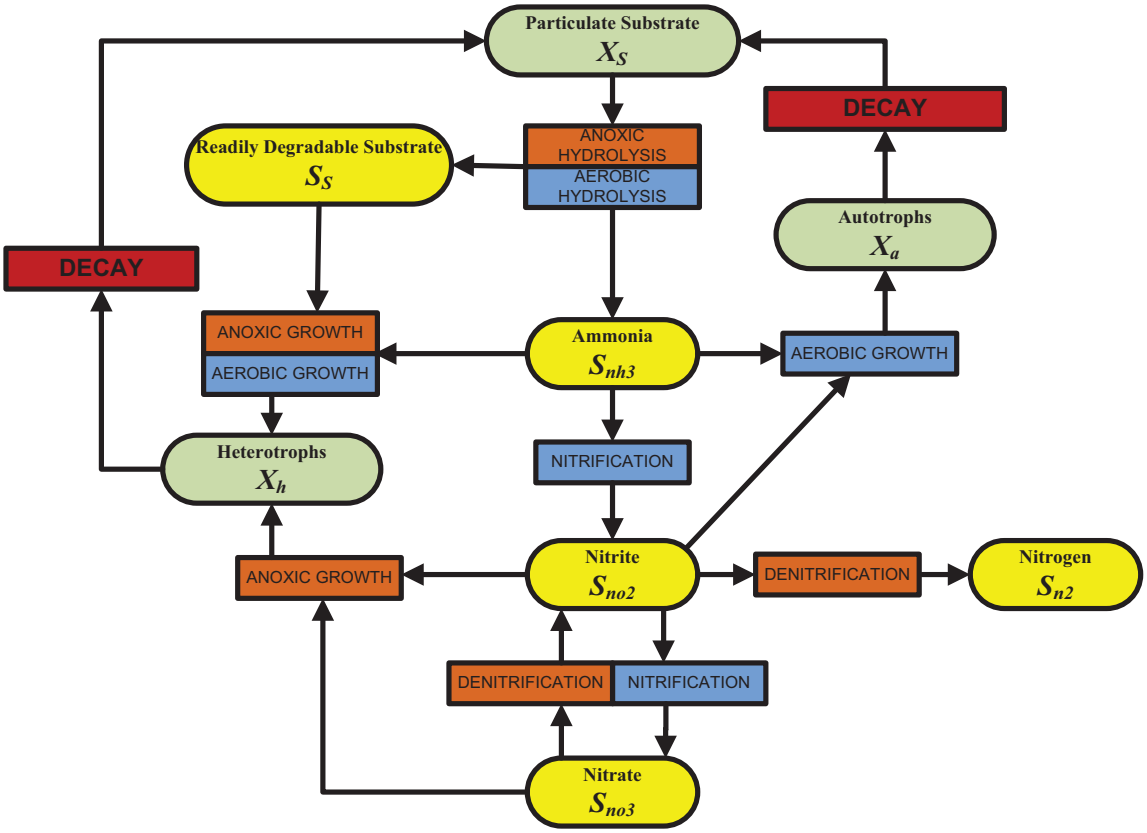

Figure 6.5. An overview of the relationships between the BNR model variables (Yellow, soluble; green, particulate; blue, aerobic; orange, anoxic; red, anoxic and aerobic).

Table 6.1. Matrix of the BNR model processes and variables.

I →	Component→	1	2	3	4	5	6	7	Process Rate
j ↓	Process ↓	S_S	X_S	X_h	X_a	S_{no3}	S_{no2}	S_{nh3}	
1	Ammonification							f	$k_{hd}X_S$
2	Hydrolysis of organics	1	-1						$k_{hd}X_S$
3	Anoxic growth of heterotrophs (Organics)	-1		Y_h				$-i_{XB}Y_h$	$\left[\frac{k_s S_s}{K_{ss}+S_s}\right]\eta X_h$
4	Anoxic growth of heterotrophs (Nitrate)			Y_{no3}		-1	1	$-i_{XB}Y_{no3}$	$\left[\frac{k_{no3}S_{no3}}{K_{sno3}+S_{no3}}\frac{K_{ss}}{K_{ss}+S_s}\right]\eta X_h$
5	Anoxic growth of heterotrophs (Nitrite)			Y_{no2}			-1	$-i_{XB}Y_{no2}$	$\left[\frac{k_{no2}S_{no2}}{K_{sno2}+S_{no2}}\frac{K_{ss}}{K_{ss}+S_s}\right]\eta X_h$
6	Aerobic growth of heterotrophs (Organics)	-1		Y_h				$-i_{XB}Y_h$	$\left[\frac{k_s S_s}{K_{ss}+S_s}\right]X_h$
7	Aerobic growth of autotrophs (Ammonia)				Y_{nh3}		1	$-1-i_{XB}Y_{nh3}$	$\left[\frac{k_{nh3}S_{nh3}}{K_{snh3}+S_{nh3}}\right]X_a$
8	Aerobic growth of autotrophs (Nitrite)				Y^a_{no2}	1	-1	$-i_{XB}Y^a_{no2}$	$\left[\frac{k^a_{no2}S_{no2}}{K^a_{sno2}+S_{no2}}\right]X_a$
9	Decay of heterotrophs		f_P	-1					$b_h X_h$
10	Decay of autotrophs		f_P		-1				$b_a X_h$
		Readily degradable organics (mg COD/L)	Particulate organics (mg COD/L)	Heterotrophs (mg VSS/L)	Autotrophs (mg VSS/L)	Nitrate (mg N/L)	Nitrite (mg N/L)	Ammonia (mg N/L)	

Mass balance equations on the soluble and particulate variables within the BNR system reactors resulted in the following ODEs:

(B) Anoxic reactor:

a. Soluble:

$$\frac{dS}{dt} = \frac{Q(1+r)}{V}(S_{Anaerobic} - S_{Anoxic}) \pm rate \qquad \text{Equation (6.22A)}$$

b. Particulate:

$$\frac{dX}{dt} = \frac{Q(1+r)}{V}(X_{Anaerobic} - X_{Anoxic}) \pm rate \qquad \text{Equation (6.22B)}$$

where r is the mixed liquor recycle ratio, $\frac{\text{Recycle}(L/d)}{Q(L/d)}$, and the rate corresponds to the appropriate sub-model in the anoxic reactor.

(C) Aerobic reactor:

a. Soluble:

$$\frac{dS}{dt} = \frac{Q(1+r)}{V}(S_{Anoxic} - S_{Aerobic}) \pm rate \qquad \text{Equation (6.23A)}$$

b. Particulate:

$$\frac{dX}{dt} = \frac{Q(1+r)}{V}X_{Anoxic} - X_{Aerobic}\frac{Q(f_{eff}+r)}{V} \pm rate \qquad \text{Equation (6.24B)}$$

where f_{eff} is the fraction of solids in the final effluent, i.e., $X_{Effluent}/X_{Aerobic}$. The value of f_{eff} was calculated from the system steady-state operation VSS data to be 7.4%.

Because of the change in BAC phase distribution in the BNR system, the total BAC concentration was followed rather than its liquid- and solid-associated concentrations. Both concentrations are related to the total BAC concentration as follows:

$$S^T{}_{BAC} = C_e + q_e \cdot X \qquad \text{Equation (6.24A)}$$

$$q_e = K_P C_e \qquad \text{Equation (6.24B)}$$

$$C_e = \frac{S^T{}_{BAC}}{K_P X + 1} \qquad \text{Equation (6.24C)}$$

$$q_e = \frac{S^T{}_{BAC}}{X + \frac{1}{K_P}} \qquad \text{Equation (6.24D)}$$

where K_P is the linear partition coefficient in each reactor.

6.3 Methods

6.3.1 Parameter Estimation

The equations in the sub-models discussed above were fitted to time series data obtained from the batch assays presented in Chapters 4 and 5 as follows:

1) The sCOD, VFAs, and ammonia concentration data obtained from the aerobic assays were fitted to Equations 6.1 to 6.3 to estimate the values of k_{hd}, f_{vfa}, and f.

2) The nitrate, nitrite, dinitrogen, and initial/final biomass data obtained from the denitrification assays were fitted to Equations 6.4 to 6.10 to estimate the values of k_{no3}, K_{Sno3}, k_{no2}, K_{Sno2}.

3) The ammonia, nitrate, nitrite, sCOD, and initial/final biomass data obtained from the nitrification assays were fitted to Equations 6.11 to 6.16 to estimate the values of k_{nh3}, K_{Snh3}, k^a_{no2}, K^a_{Sno2}, k_s, and K_{SS}. In addition, the nitrifying population was assumed to comprise 5% of the total biomass in each assay (see Chapter 4 for details).

Values for the remaining parameters where obtained from literature and are summarized in Table 6.2.

Table 6.2**.** Literature values used in the BNR system model.

Parameter	Value (Units)	Reference
Y_{nh3}	0.15 (mg COD/mg N)	Sin et al., 2008
Y_{ano2}	0.04 (mg COD/mg N)	Sin et al., 2008
Y_h	0.67 (mg COD/mg	Henze et al., 2000
Y_{no3}	0.79 (mg COD/mg N)	Sin et al., 2008
Y_{no2}	0.55 (mg COD/mg N)	Sin et al., 2008
f_P	0.8	Henze et al., 2000; Rittmann and McCarty, 2001
iX_B	0.08	Henze et al., 2000; Rittmann and McCarty, 2001
b_h	0.15 (day^{-1})	Rittmann and McCarty, 2001; Sin et al., 2008
b_a	0.11 (day^{-1})	Rittmann and McCarty, 2001; Sin et al., 2008

Two software packages were used for parameter estimation for the aforementioned sub-models. First, Berkeley Madonna Software Version 8.3 (Macey and Oster, 2006) was used and the values of the parameters used in each sub-model were estimated by minimizing the deviation between the model output and the experimental data set. A fixed-step size integration method, Runge-Kutta 4, with a step size of 0.01 day was used in all simulations. The root mean square deviation (RMSD) was used as a measure of the goodness of fit in each simulation. Second, the Fit ODE toolbox in Igor Professional v.5.057 (WaveMetrics, Inc., Lake Oswego, OR) was used to calculate the standard deviation values for the evaluated parameters. Figure 6.6 illustrates the algorithm used for parameter estimation.

6.3.2 Sensitivity and Identifiability Analysis

The sensitivity analysis for the model parameters was performed using the Berkeley Madonna Software Version 8.3 (Macey et al., 2000). Berkeley Madonna provides the sensitivity as the partial derivative of the state variable relative to the parameter (Gujer, 2008):

$$\delta_{y,p} = \frac{\partial y}{\partial p} \cong \frac{y(p+\Delta p) - y(p)}{\Delta p} \qquad \text{Equation (6.21)}$$

where $\delta_{y,p}$ is the local sensitivity parameter of the variable (y) relative to a change (Δ) in the parameter (p). Values of each parameter were changed by 1%. The local sensitivity parameter is plotted as a function of time in order to generate a sensitivity curve for the associated variable (y). In order to obtain an improved visualization of the form of the sensitivity curves, the absolute-relative sensitivity function ($\delta_{y,p}^{a,r}$) was then evaluated by normalizing the local sensitivity by the parameter value as follows:

$$\delta_{y,p}^{a,r} \cong p\frac{y(p+\Delta p)-y(p)}{\Delta p}$$ Equation (6.22)

The identifiability of each estimated parameter was determined by visual inspection of the relative local sensitivity curves. Parameters that have a relative local sensitivity curve with a unique shape are considered uniquely identifiable from the data set used for the parameter estimation. On the other hand, parameters with similar/proportional relative local sensitivity curves are not uniquely identifiable, i.e., the change in one parameter can be compensated by an appropriate adjustment of another parameter value (Gujer, 2008).

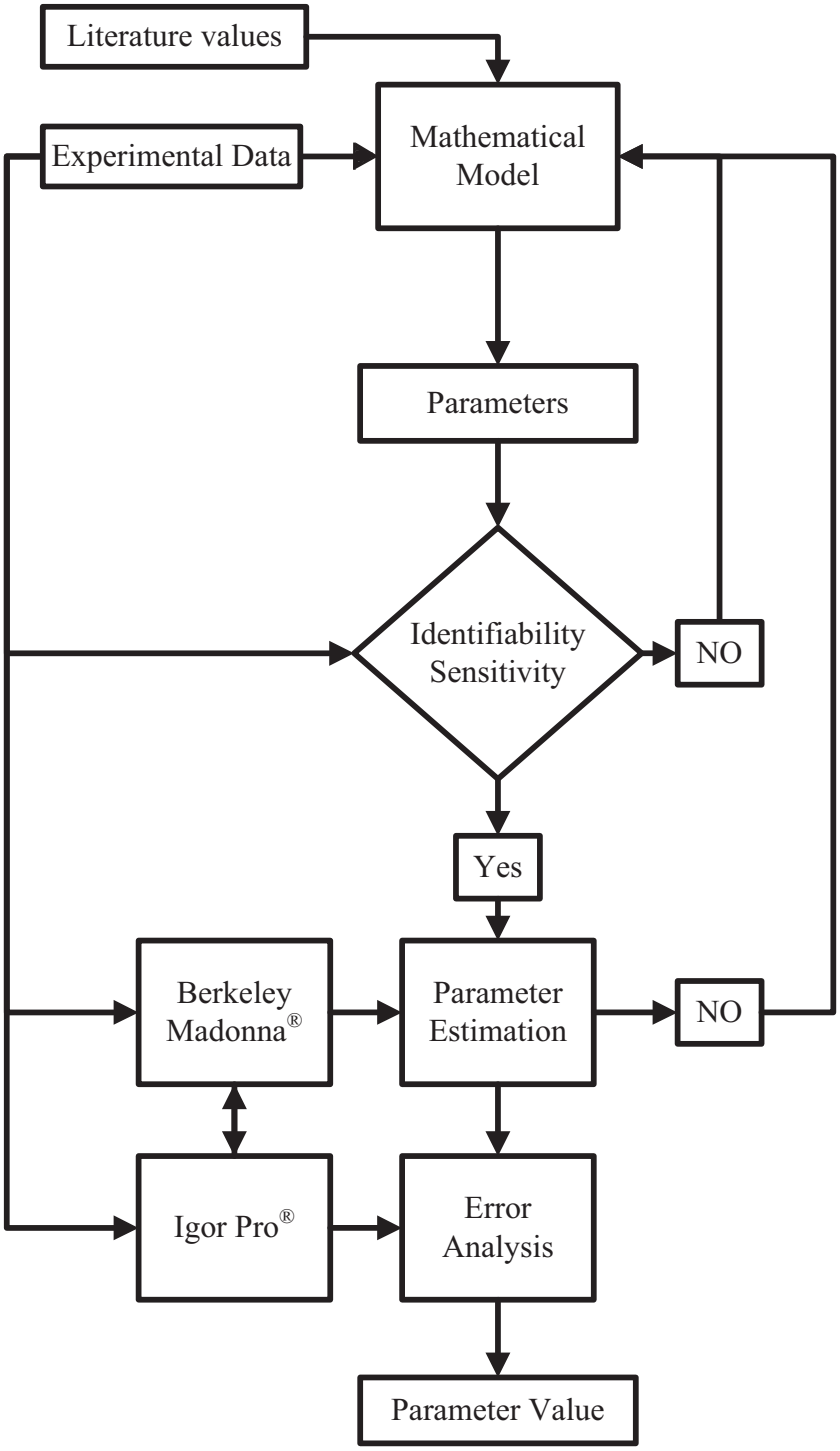

Figure 6.6. An overview of the algorithm used for parameter estimation for the different sub-models.

6.4 Results and Discussion

Results regarding data fitting and parameter estimation are discussed first as they provide more insight regarding the effect of BAC on the BNR system.

6.4.1 Parameter Estimation and Identification

6.4.1.1 Anaerobic Reactor

Table 6.3 summarizes the estimated parameters for hydrolysis, VFAs production, and ammonification, while Figures 6.7, 6.8, and 6.9 show the measured and simulated ammonia, VFAs, and sCOD concentrations in the anaerobic assay. The model simulation for ammonia and VFAs concentrations was better than that for the sCOD concentration. Nevertheless, the estimated hydrolysis rate value (0.45 ± 0.10 mg COD/mg VSS · day) is comparable to literature values. For example, Masse et al. (2003) evaluated the hydrolysis rate for slaughterhouse wastewater and reported a value of 0.63 ± 0.07 (mg COD/mg COD · day).

Figure 6.10 shows the absolute-relative sensitivity function curve for the anaerobic reactor sub-model**.** The ammonia concentration was mostly sensitive to the ammonia stoichiometric factor followed by the hydrolysis rate. The production of sCOD was sensitive only to the hydrolysis rate. The production of VFAs was sensitive to the VFAs stoichiometric factor followed by the hydrolysis rate. By far, the hydrolysis rate is the parameter that has the most sensitivity, and judging by the shape of the absolute-relative sensitivity function, this rate was uniquely identifiable.

Table 6.3**.** Estimated parameter values for hydrolysis, VFAs production, and ammonification in the anaerobic reactor.

Parameter	Value (Units)	RMSD[a]
k_{hd}	0.45 ± 0.10[b] (mg COD/mg VSS · day)	
f_P	0.13 ± 0.01 (mg N/mg COD)	79.9
f_{VFA}	0.19 ± 0.10 (mg COD/mg COD)	

[a] Root mean square deviation $=\sqrt{\sum(\text{measured value} - \text{estimated value})^2}$

[b] Best estimate ± standard deviation

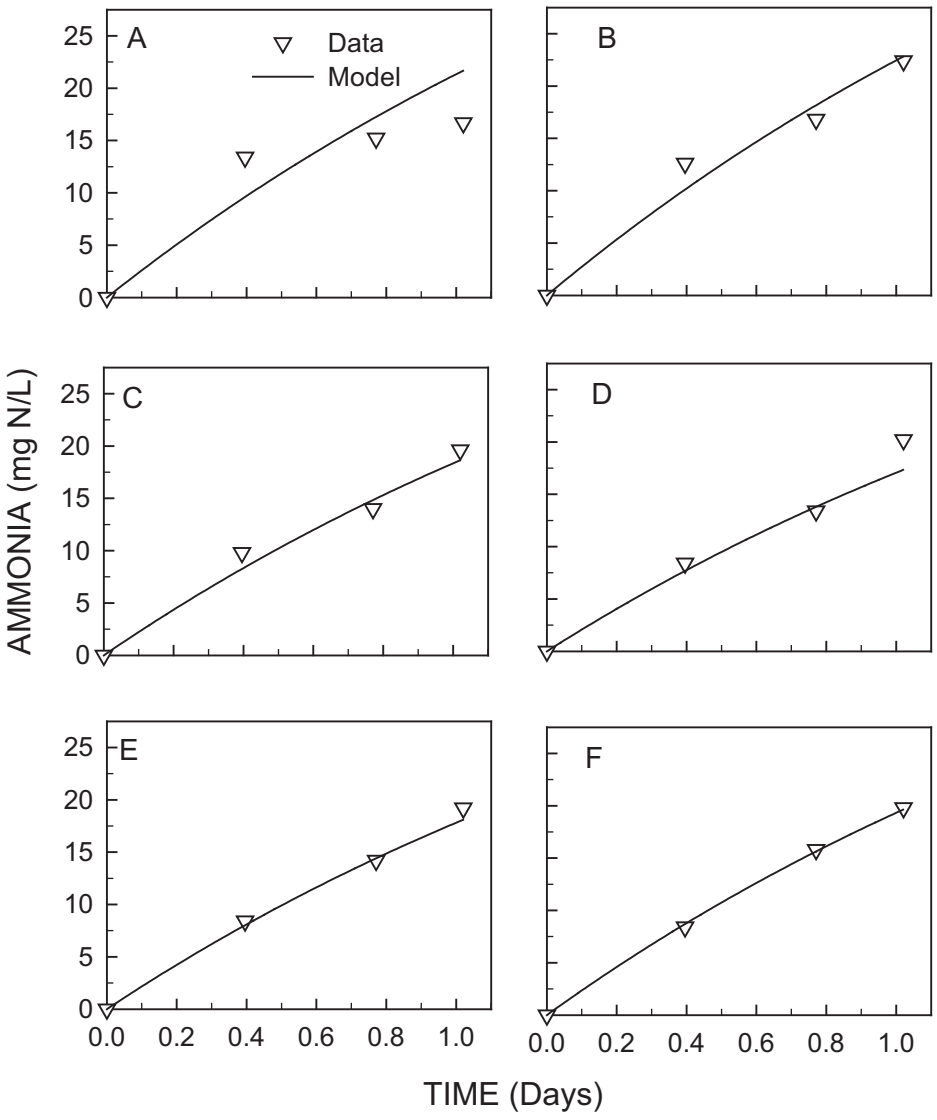

Figure 6.7. Measured (data points) and simulated (lines) ammonia concentration in the anaerobic assay. Culture series at initial BAC concentration of 0 (A), 5 (B), 15 (C), 30(D), 45 (E), and 60 mg/L.

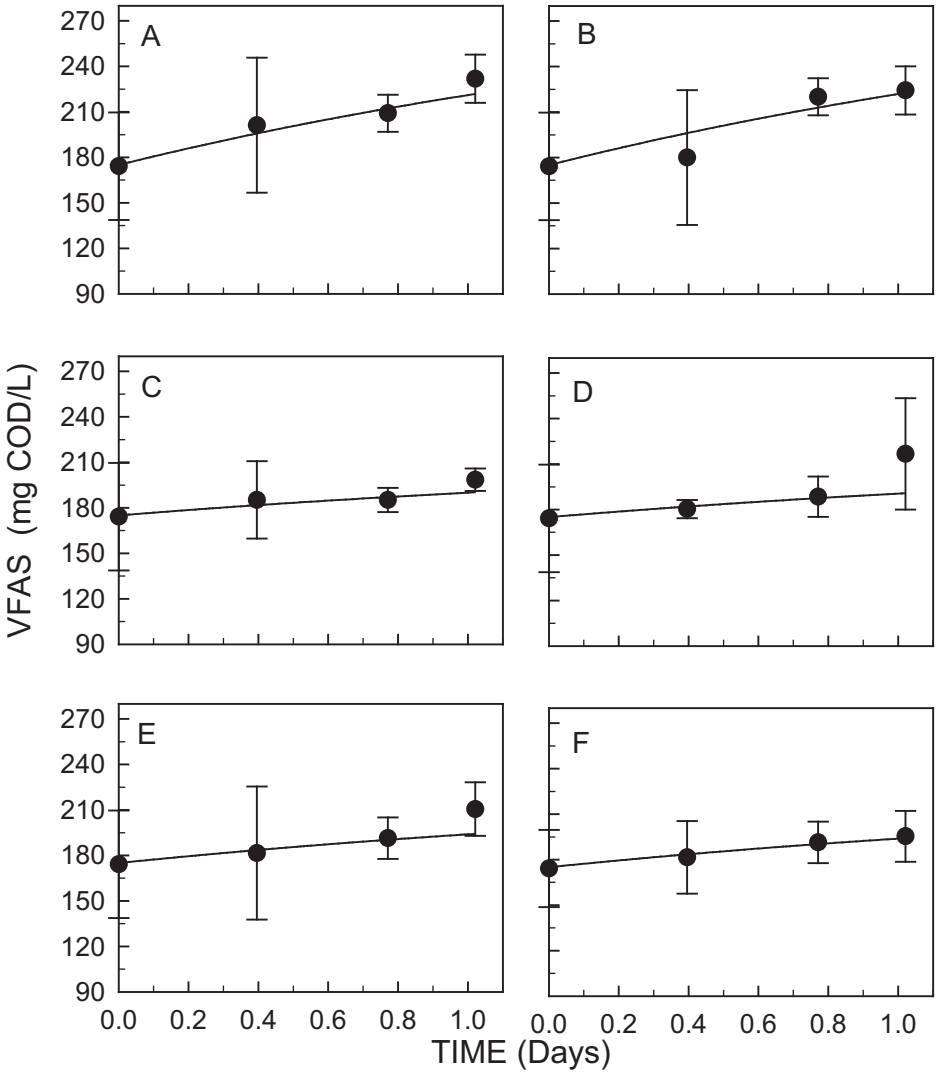

Figure 6.8. Measured (data points) and simulated (lines) VFAs concentration in the anaerobic assay. Culture series at initial BAC concentration of 0 (A), 5 (B), 15 (C), 30(D), 45 (E), and 60 mg/L. Error bars represent one standard deviation from the mean.

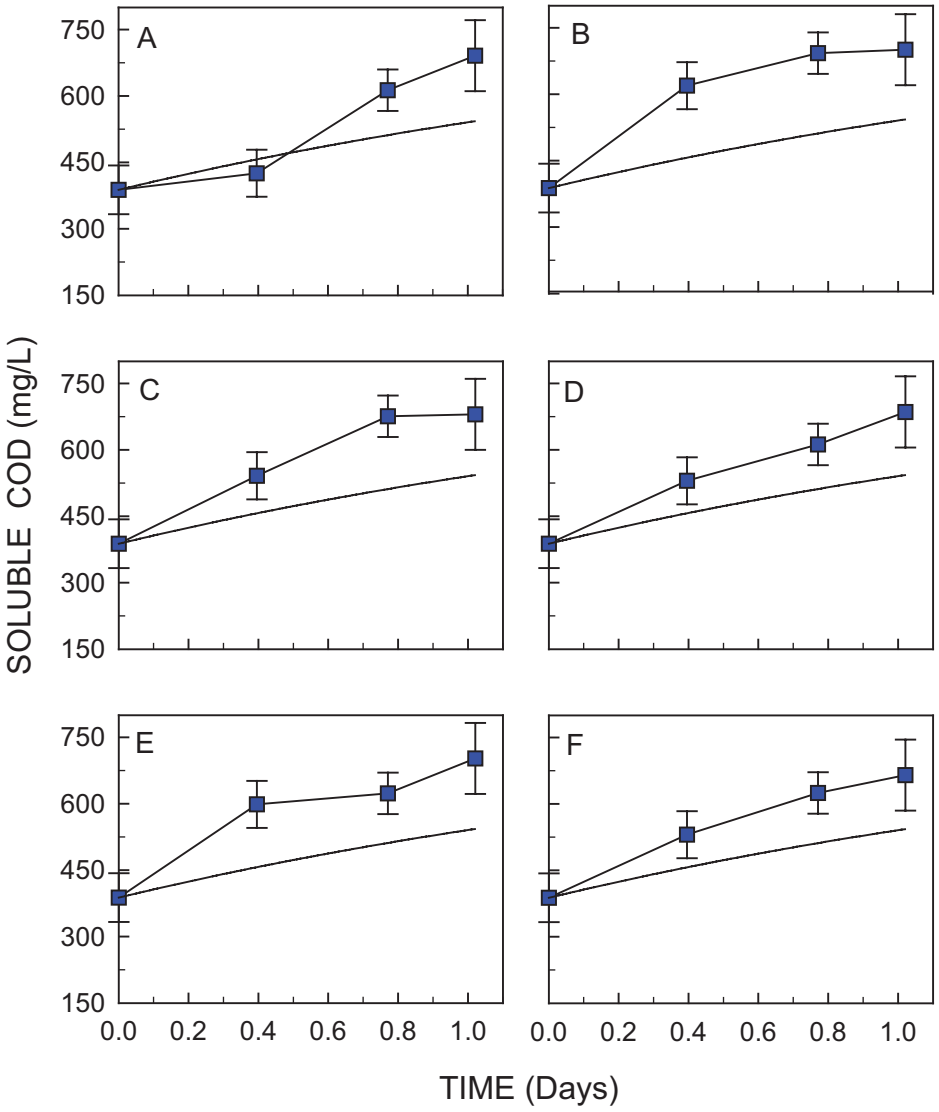

Figure 6.9. Measured (data points) and simulated (lines) sCOD concentration in the anaerobic assay. Culture series at initial BAC concentration of 0 (A), 5 (B), 15 (C), 30(D), 45 (E), and 60 mg/L. Error bars represent one standard deviation from the mean.

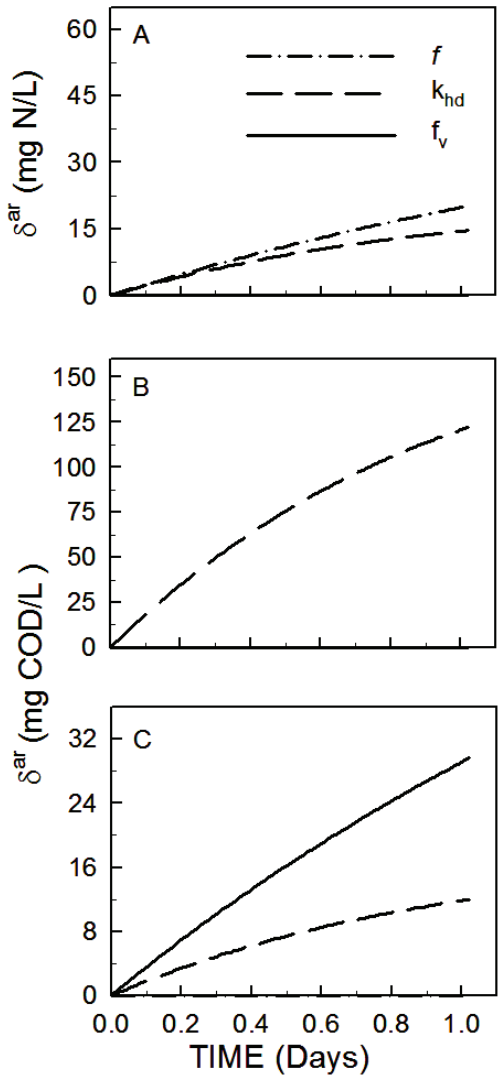

Figure 6.10. Absolute-relative sensitivity curves (δ^{ar}) showing the sensitivity of the anaerobic reactor sub-model parameters to ammonia (A), sCOD (B), and VFAs (C) concentration.

6.4.1.2 Anoxic Reactor

Figures 6.11 and 6.12 show the measured and simulated nitrogen species in denitrification batch assays conducted with the unexposed denitrifying culture and the anoxic reactor mixed liquor. Table 6.4 summarizes the estimated parameters for nitrate and nitrite reduction before and during BAC exposure for the BAC-free culture series.

In both assays, the nitrite reduction rate was faster than the nitrate reduction rate, which agrees with literature reports (Wett and Rauch, 2003; Sin et al., 2008). Moreover, rates in the assay conducted with the denitrifying culture were faster than the rates of the assay conducted with the anoxic reactor mixed liquor. The difference could be attributed to the carbon source used in each assay: glucose in the former and anaerobically processed poultry processing wastewater in the latter. Despite the differences, the effect of BAC on denitrification could be established by comparing the BAC-free culture series in each assay to the BAC-amended culture series in the same assay.

In each of the previous assays, the inhibitory effect of BAC was evaluated by fitting the values of the apparent half-saturation constants for all culture series to the competitive inhibition model, considering the total, liquid phase, and solid-associated BAC concentration in the same culture series as the inhibitor (Figure 6.13). The data was fitted by non-linear regression analysis performed using SigmaPlot.

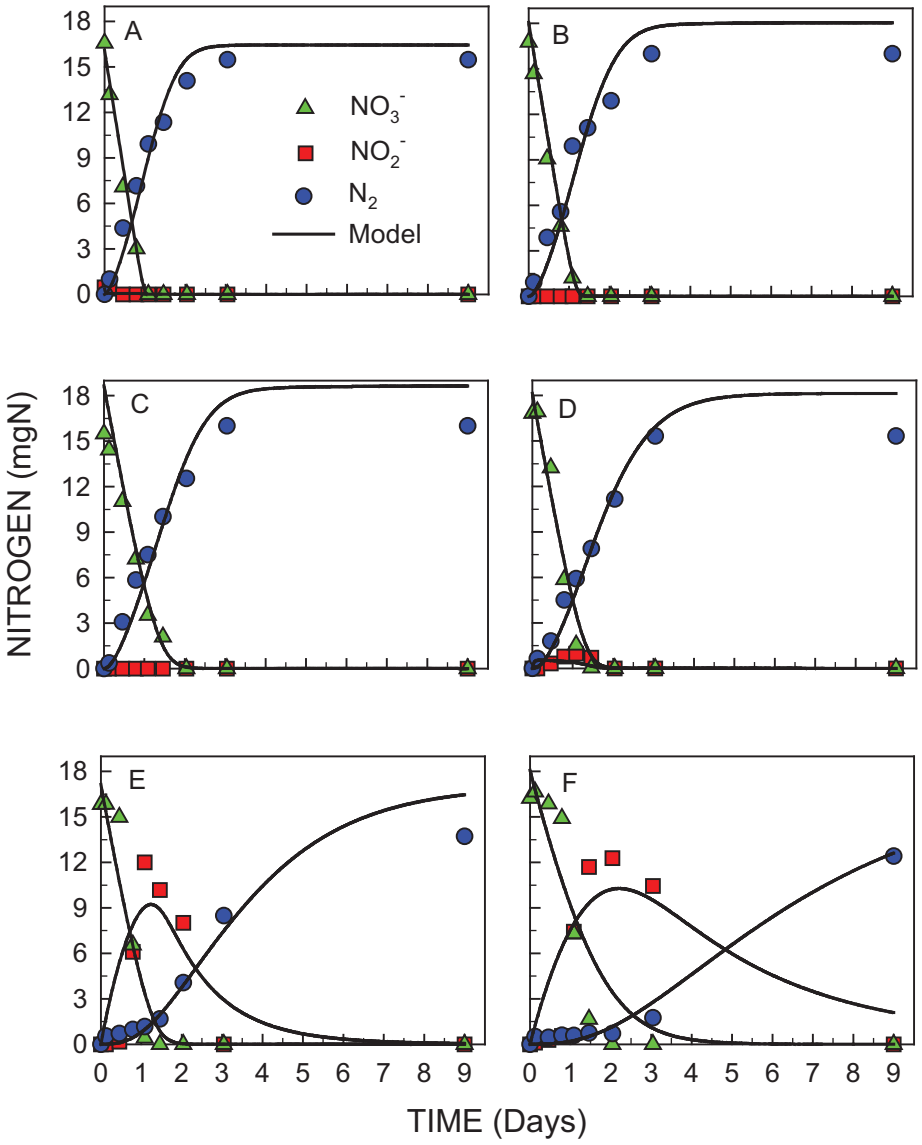

Figure 6.11. Measured (data points) and simulated (lines) nitrogen species in the denitrifying batch assay performed with the BAC-unexposed culture. Culture series at initial BAC concentration of 0 (A), 10 (B), 25 (C), 50 (D), 75 (E), and 100 mg/L.

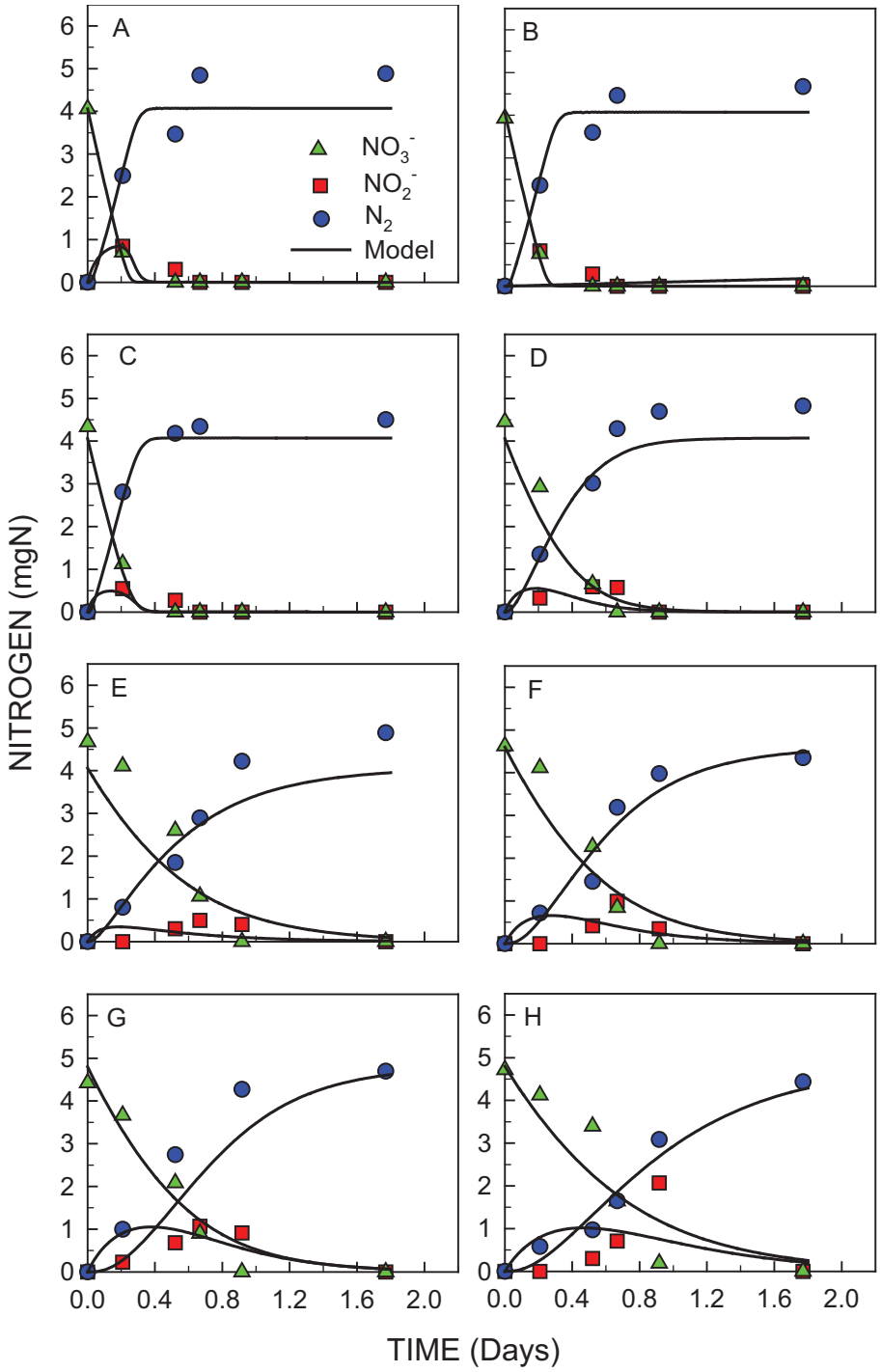

Figure 6.12. Measured (data points) and simulated (lines) nitrogen species in the denitrifying batch assay performed with the BAC-exposed anoxic reactor mixed liquor. Culture series at initial BAC concentration of 0 (A), 5 (B), 10 (C), 15 (D), 20 (E), 25 (F), 30 (G), and 45 mg/L (H).

Table 6.4**.** Estimated parameter values for denitrification before and during BAC exposure.

Parameter		Value (Units)	RMSD[a]
Before BAC Exposure	k_{no3}	0.87 ± 0.08[b] (mg N/mg VSS · day)	17.3
	K_{sno}	4.2 ± 1.2 (mg N/L)	
	k_{no2}	1.32 ± 0.7 (mg N/mg VSS · day)	
	K_{sno}	10.2 ± 1.9 (mg N/L)	
During BAC Exposure	k_{no3}	0.23 ± 0.02 (mg N/mg VSS · day)	12.6
	K_{sno}	2.3 ± 0.7 (mg N/L)	
	k_{no2}	0.34 ± 0.08 (mg N/mg VSS · day)	
	K_{sno}	7.5 ± 0.9 (mg N/L)	

[a] Root mean square deviation $=\sqrt{\sum(\text{measured value} - \text{estimated value})^2}$

[b] Best estimate ± standard deviation

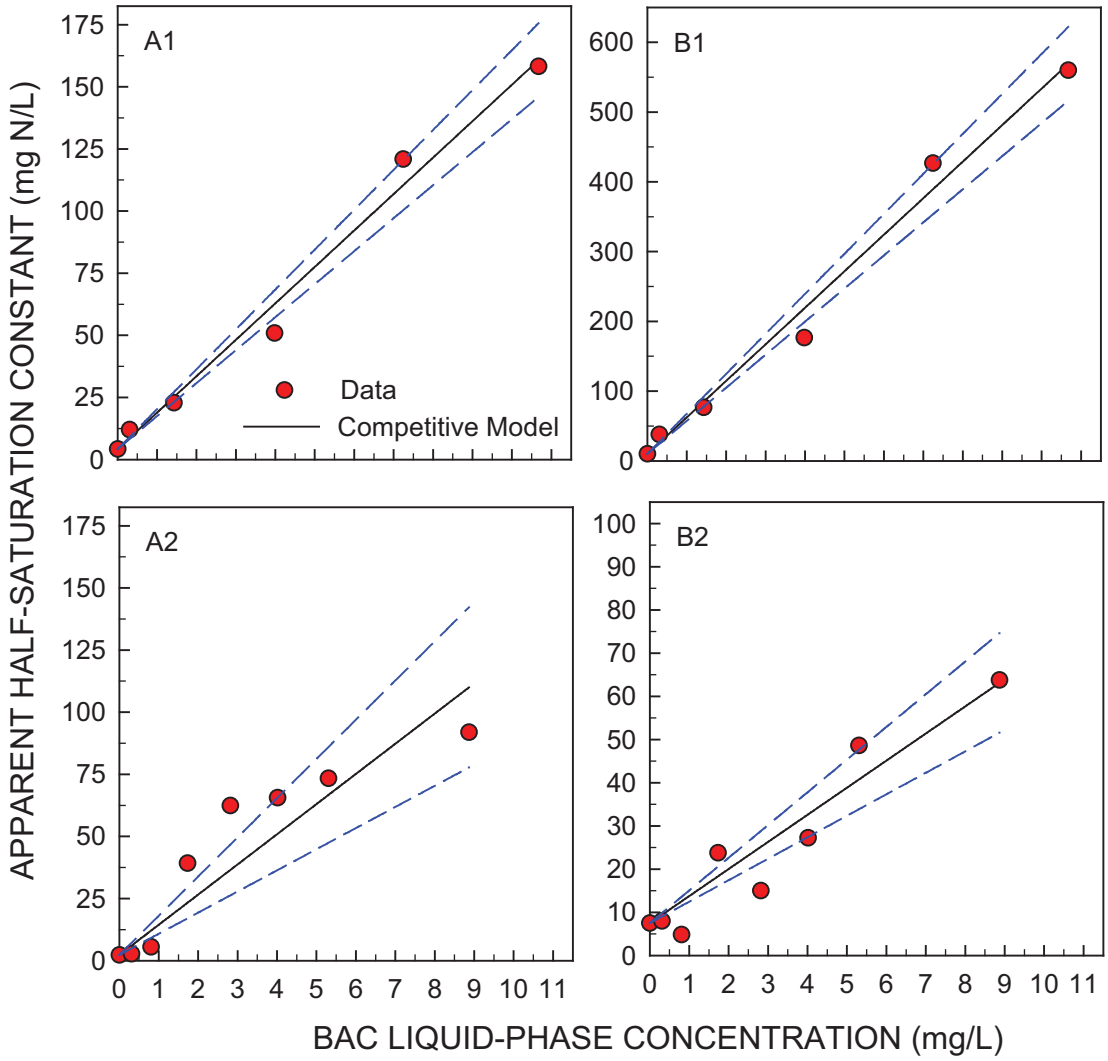

Figure 6.13. Apparent half-saturation constant for nitrate (A) and nitrite (B) reduction in the denitrification assay with the BAC-unexposed denitrifying culture (1) and the BNR anoxic reactor mixed liquor (2) while treating BAC-bearing poultry processing wastewater. [r^2 = 0.987 (A1), 0.987 (B1), 0.867 (A1), and 0.954 (B2)]. (Broken lines are 95% confidence intervals).

Among the three aforementioned BAC concentrations, the liquid-phase BAC concentration had the best fit, i.e., had the highest r^2 value. Zhang et al. (2011) reported that BAC biodegradation by acclimated heterotrophic bacteria is taking place simultaneously in both the liquid-phase and biomass-adsorbed solid-phase BAC, where biotransformation of the solid-phase BAC takes place while it is migrating through the cell membrane. Such a biotransformation mechanism results in the riddance of biomass-associated, solid-phase BAC. Consequently, the liquid-phase BAC fraction is the more bioavailable and inhibits the denitrifying facultative heterotrophs. As a result, it is the liquid-phase BAC concentration which correlates with the observed inhibition of denitrification by BAC (Figure 6.13).

The highest apparent half-saturation constant values were for nitrite reduction evaluated based on the assay conducted before BAC exposure, which had the highest BAC inhibitory effect. In contrast, the apparent half-saturation constant values for the assay conducted during BAC exposure were the lowest, indicating a much lower BAC inhibitory effect. The same observation is extended to nitrate reduction. The competitive inhibition coefficients evaluated based on the assay conducted before BAC exposure were 0.27 ± 0.01 and 0.19 ± 0.01 mg BAC/L for nitrate and nitrite reduction, respectively, and 0.29 ± 0.01 and 0.21 ± 0.10 mg BAC/L for nitrate and nitrite reduction , respectively (estimate ± standard error) for the assay conducted during BAC exposure. Nitrate reduction was less susceptible to BAC compared to nitrite reduction in the assay conducted before BAC exposure. These results explain the nitrite accumulation observed with the initial introduction of BAC. The nitrate reductase is believed to be associated with the inner face of the cell membrane, while most of nitrite reductases is believed to

be in the periplasmic space (Knowles, 1982; Hochstein and Tomlinson, 1988). The spatial arrangement of the reductase enzymes renders nitrite reductase more vulnerable to BAC compared to the nitrate reductases.

Figure 6.14 shows the absolute-relative sensitivity function curve for the anoxic reactor sub-model**.** The nitrate concentration was mostly sensitive to the nitrate maximum specific reduction rate followed by nitrate BAC inhibition coefficient and BAC partition coefficient, while it had a very low sensitivity to the remaining parameters, such as nitrite maximum specific reduction rate, nitrate/nitrite half saturation constant, and nitrite BAC inhibition coefficient. The nitrite concentration was mostly sensitive to nitrate maximum specific rate followed by nitrite maximum specific reduction rate. In addition, the nitrite concentration was sensitive to the nitrite and nitrate reduction BAC inhibition coefficient, as well as to BAC partition coefficient. The dinitrogen concentration was mostly sensitive to nitrate maximum specific reduction rate followed by nitrite maximum specific reduction rate and nitrate reduction BAC inhibition coefficient. Overall, the parameter that has the highest sensitivity level is the nitrate maximum specific reduction rate, which is followed by the nitrite maximum specific reduction rate, nitrate reduction BAC inhibition coefficient, and BAC partition coefficient. Moreover, the shape of the absolute-relative sensitivity function for the two maximum reduction rates indicates that both rates were uniquely identifiable. The same argument could not be extended to the other parameters

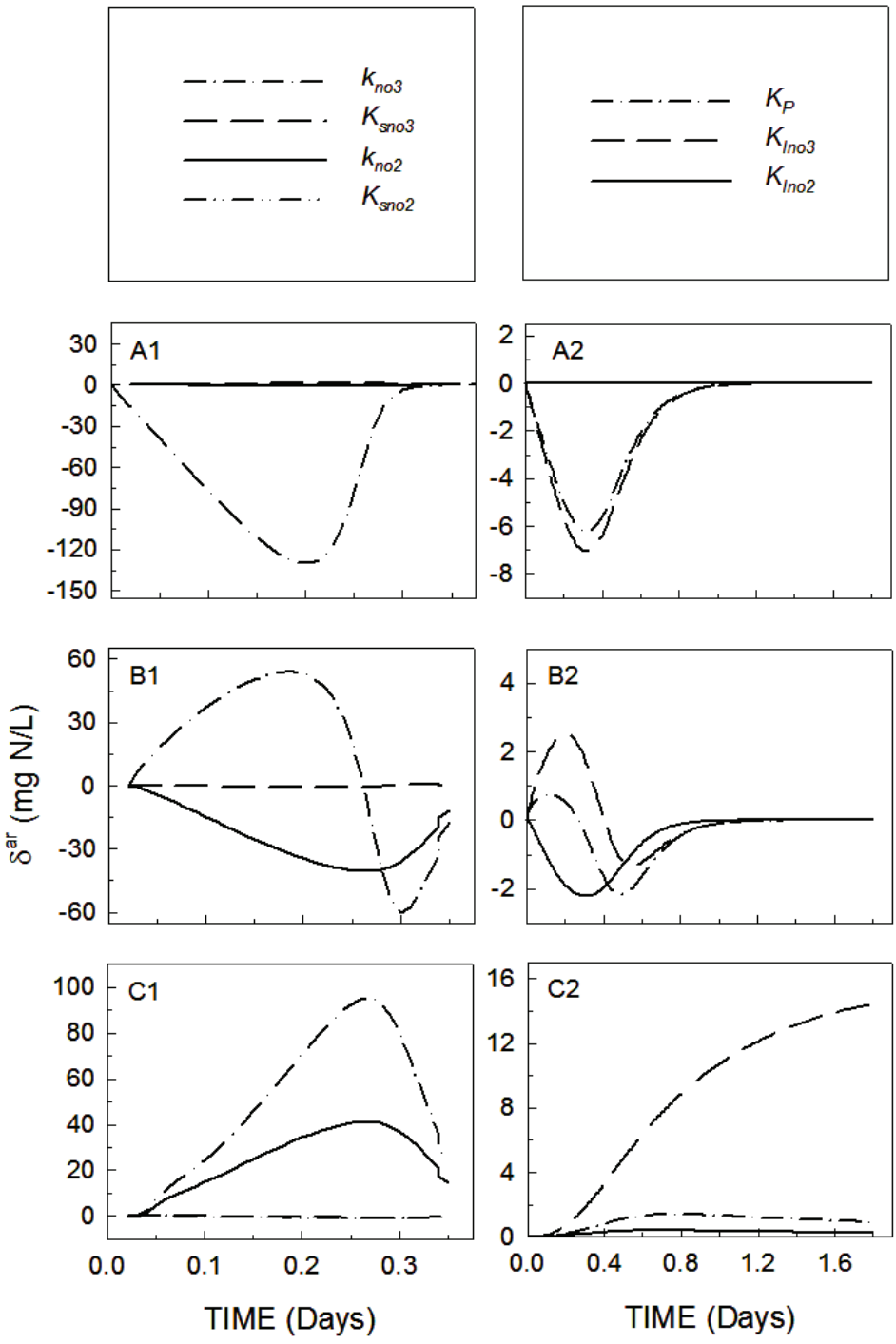

Figure 6.14. Absolute-relative sensitivity curves (δ^{ar}) showing the sensitivity of the anoxic reactor sub-model parameters to nitrate (A), nitrite (B), and dinitrogen (C) concentration; (1) denitrification parameters, and (2) BAC-related parameters.

The BAC phase distribution determined the level of the observed BAC inhibitory effect on denitrification. This observation can be explained by the following two points: 1) Higher liquid-phase BAC concentrations will result in a higher denitrification inhibitory effect; therefore, it could be deduced that decreasing the BAC liquid-phase concentration will reduce its inhibitory effect on denitrification; and 2) For all the nitrogen species, the shape of the absolute-relative sensitivity function curve for the BAC inhibition coefficients and partition coefficient were almost identical, which indicates that the two parameters are related, i.e., the value of one depends on the other. Consequently, the level of BAC inhibition in the anoxic reactor could be controlled by its adsorption behavior, i.e., a different adsorption affinity could result in a different inhibition level.

6.4.1.3 Aerobic Reactor

Figures 6.15, 6.16 and 6.17 show the measured and simulated nitrogen species in the nitrification batch assays conducted with the aerobic reactor mixed liquor before, and during BAC introduction into the BNR system, and after BAC amendment of the system feed was terminated, respectively. Table 6.5 summarizes the estimated parameter values for ammonia and nitrite oxidation.

The BAC-free culture series in the three aforementioned assays was used to evaluate the maximum specific oxidation rates for ammonia and nitrite as well as the half-saturation constants. The half saturation constant values were found to be similar in the three assays. On the other hand, the maximum specific oxidation rates for ammonia and nitrite were marginally different. The highest specific rates were determined in the assay conducted during BAC exposure, followed by those determined in the assay

conducted before BAC exposure, and then those determined in the assay conducted after BAC exposure was terminated. The estimated values for the specific ammonia and nitrite oxidation rates were lower than values reported in the literature (as presented in Chapter 2), but the half-saturation constant values for ammonia and nitrite oxidation were comparable.

Figure 6.18 shows the measured and simulated sCOD concentration in the assay conducted during BAC exposure. The estimated value of the maximum specific readily degradable organics utilization rate was 0.49 ± 0.19 mg COD/mg VSS · day, and the value of the half saturation constant was 11.8 ± 0.2 mg COD/L. Brenner (2000) reported a maximum specific readily degradable organics utilization rate of 6.0 mg COD/mg VSS day, and values for the half saturation constant in the range of 3-15 mg COD/L for a SBR nitrogen removal system treating concentrated municipal wastewater . Zhang et al. (2011) reported a maximum specific glucose utilization rate of 0.4 ± 0.06 mg COD/mg VSS · day, and a value for the half saturation constant of 22 ± 19.8 mg COD/L. Ni and Yu (2008) reported a maximum specific readily degradable organics utilization rate of 1.74 mg COD/mg VSS · day, and a value for the half saturation constant of 2 mg COD/L for an activated sludge system. The reported variation in the values of the maximum specific readily degradable organics utilization rate and the half saturation constant is related to the system design and history as well as the type of treated wastewater.

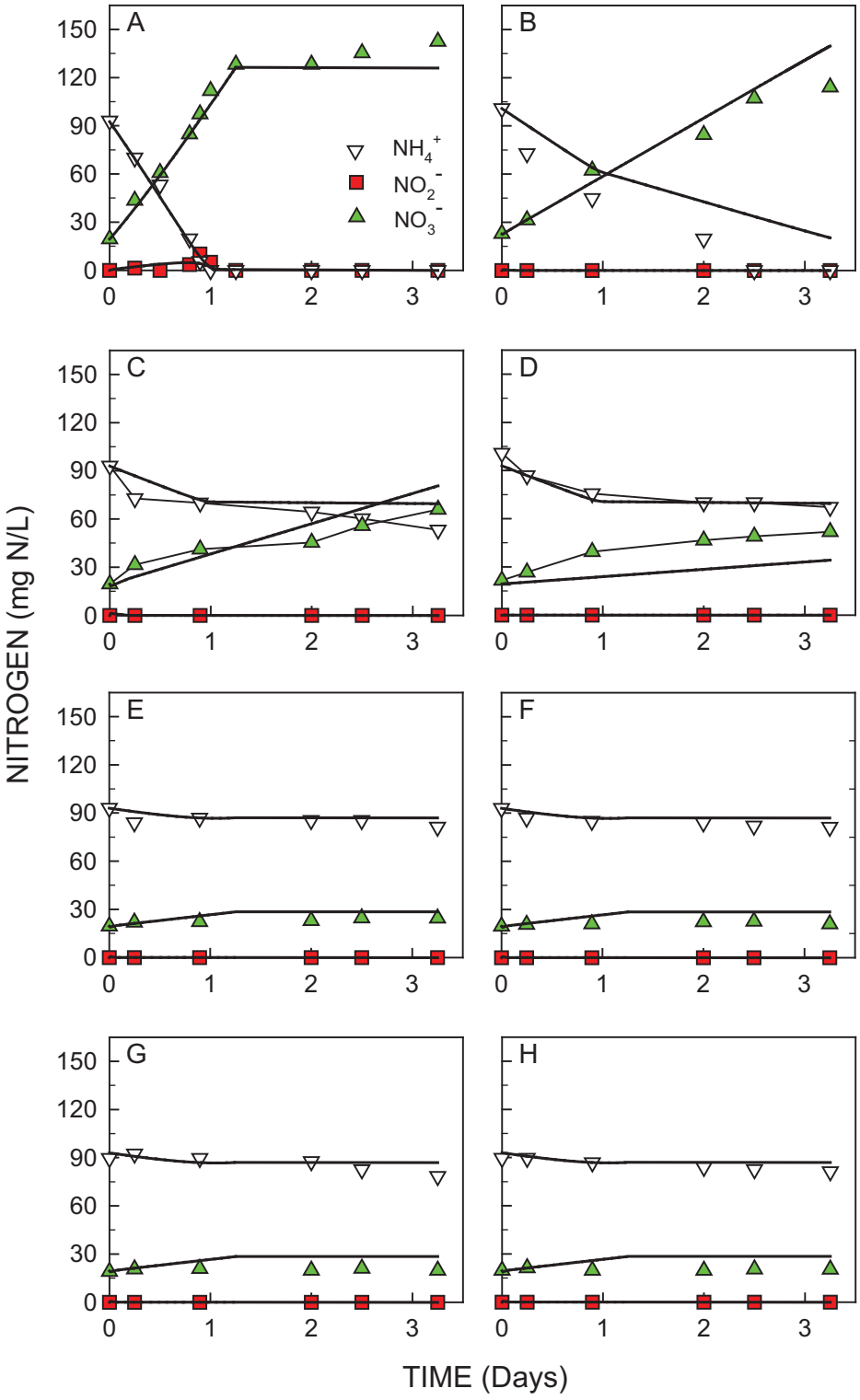

Figure 6.15. Measured (data points) and simulated (lines) nitrogen species in the nitrifying batch assay performed with the aerobic reactor mixed liquor before treating BAC-bearing poultry processing wastewater. Culture series at initial BAC concentration of 0 (A), 5 (B), 10 (C), 15 (D), 25 (E), 50 (F), 75 (G) and 100 mg/L (H).

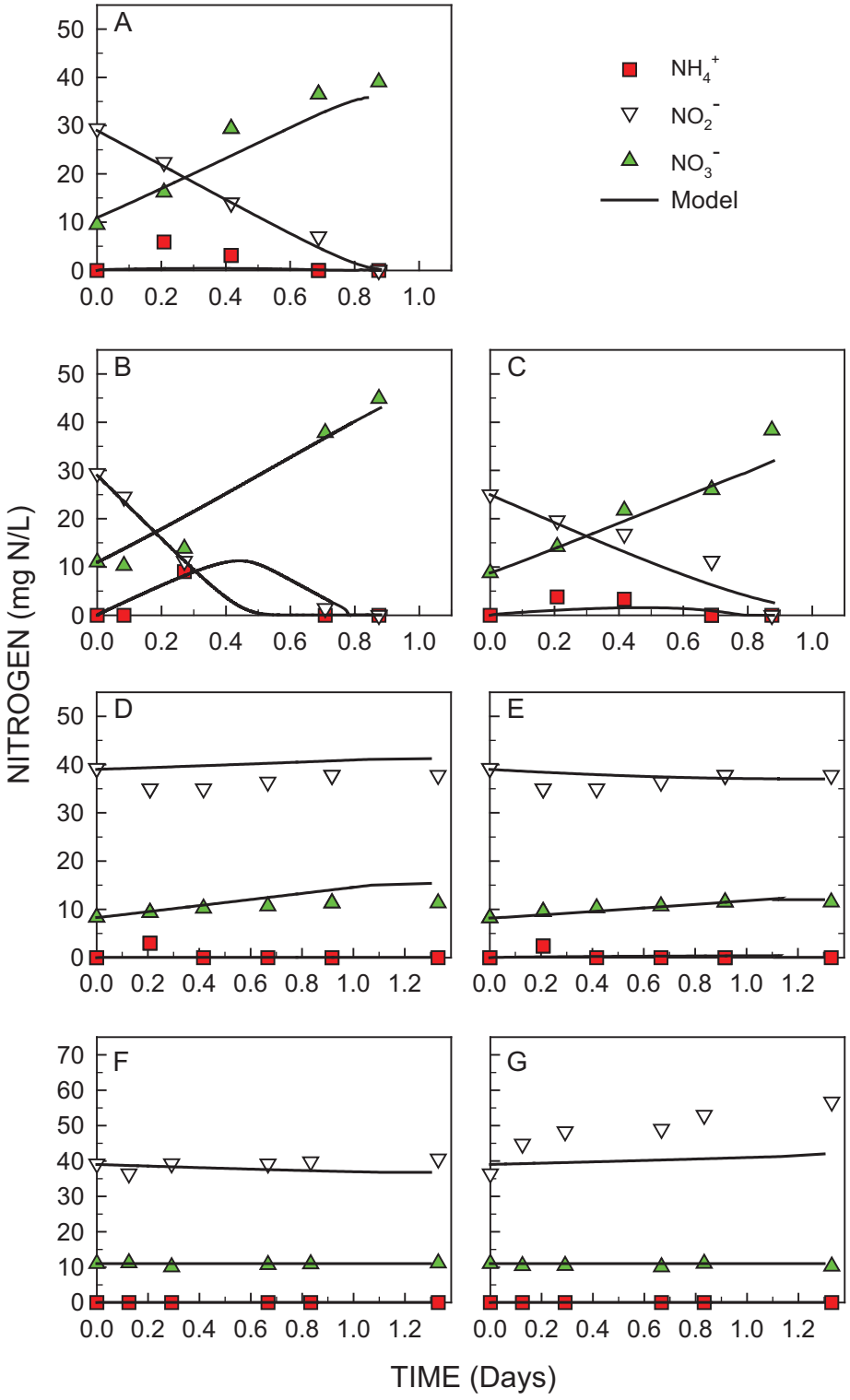

Figure 6.16. Measured (data points) and simulated (lines) nitrogen species in the nitrifying batch assay performed with the aerobic reactor mixed liquor while treating BAC-bearing poultry processing wastewater. Culture series at initial BAC concentration of 0 (A), 5 (B), 15 (C), 20 (D), 25 (E), 30 (F), and 45 mg/L (G).

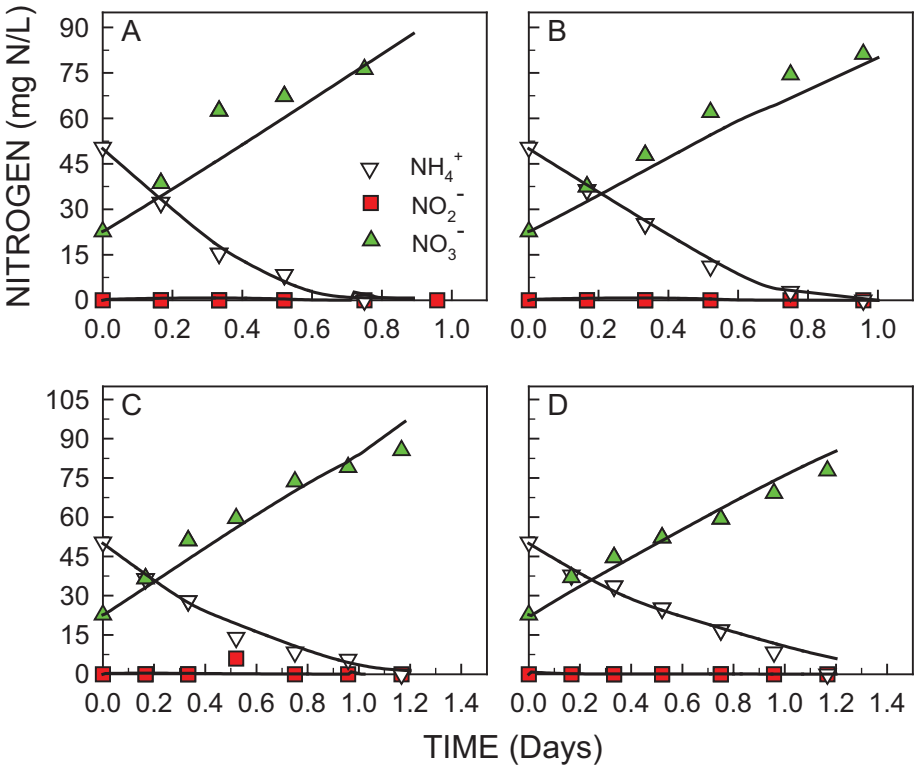

Figure 6.17. Measured (data points) and simulated (lines) nitrogen species in the nitrifying batch assay performed with the aerobic reactor mixed liquor after treating BAC-bearing poultry processing wastewater was terminated for over 100 days. Culture series at initial BAC concentration of 0 (A), 5 (B), 10 (C), and 15 mg/L (D).

Table 6.5**.** Estimated parameter values for nitrification before, during, and after BAC exposure.

Parameter		Value (Unit)	RMSD[a]
Before	k_{nh3}	1.08 ± 0.21[b] (mg N/mg VSS · day)	26.3
	K_{snh3}	2.38 ± 1.5 (mg N/L)	
	k_{ano2}	0.97 ± 0.12 (mg N/mg VSS · day)	
	K_{snao2}	0.019 ± 0.005 (mg N/L)	
During	k_{nh3}	1.56 ± 0.81(mg N/mg VSS · day)	82.4
	K_{snh3}	2.38 ± 1.5 (mg N/L)	
	k_{ano2}	0.75 ± 0.11 (mg N/mg VSS · day)	
	K_{snao2}	0.019 ± 0.005 (mg N/L)	
	k_s	0.49 ± 0.19 (mg COD/mg VSS · day)	
	K_{ss}	11.8 ± 0.2 (mg COD/L)	
After	k_{nh3}	0.88 ± 0.16 (mg N/mg VSS · day)	72.6
	K_{snh3}	2.38 ± 1.5 (mg N/L)	
	k_{ano2}	0.005 ± 0.002 (mg N/mg VSS · day)	
	K_{snao2}	0.63 ± 0.22 (mg N/L)	

[a] Root mean square deviation $=\sqrt{\sum(\text{measured value} - \text{estimated value})^2}$

[b] Best estimate ± standard deviation

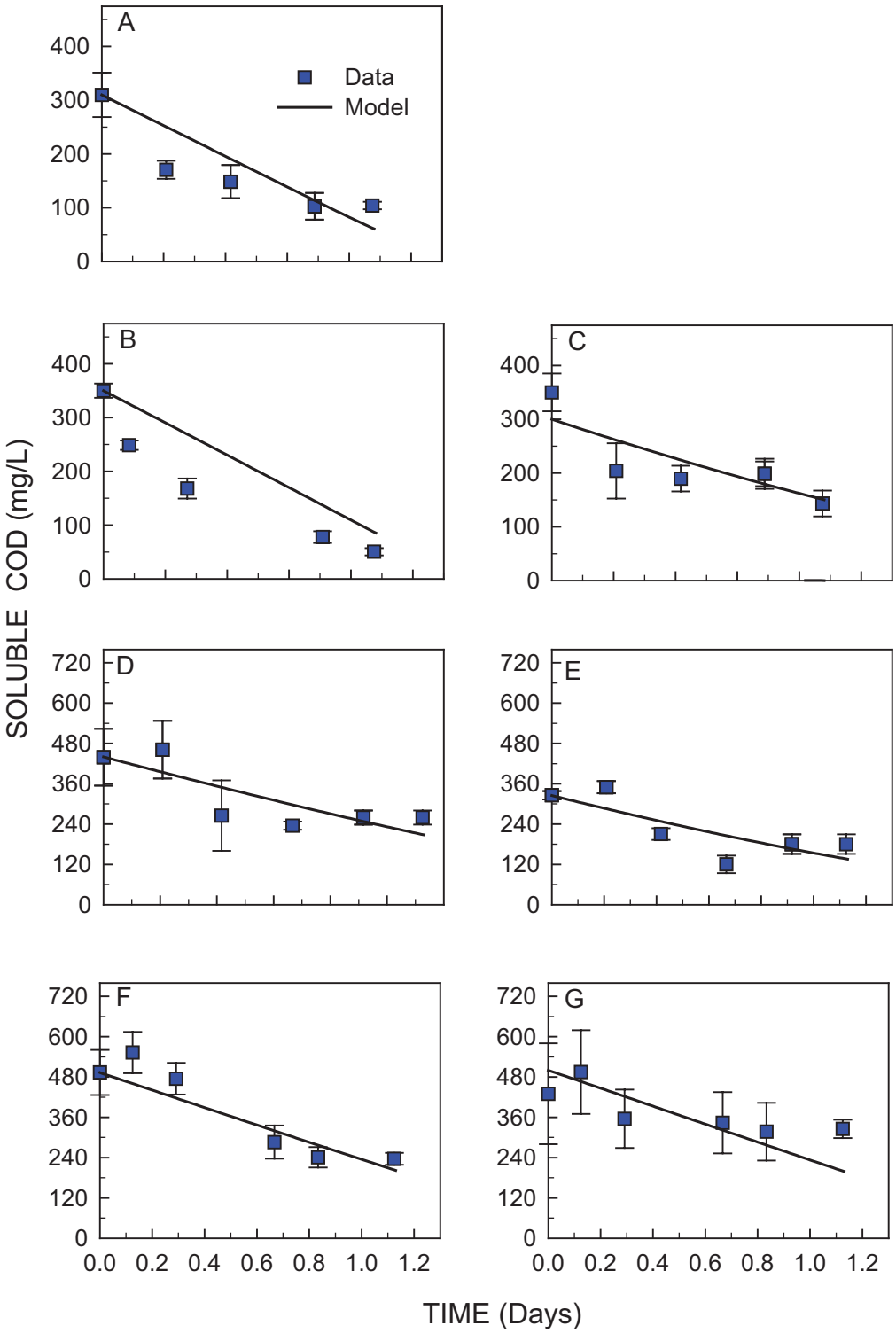

Figure 6.18. Measured (data points) and simulated (lines) sCOD concentration in the nitrifying batch assay performed with the aerobic reactor mixed liquor while treating BAC-bearing poultry processing wastewater. Culture series at initial BAC concentration of 0 (A), 5 (B), 15 (C), 20 (D), 25 (E), 30 (F), and 45 mg/L (G). Error bars represent one standard deviation from the mean.

Figures 6.19 and 6.20 show the measured and simulated total BAC concentration in the batch assays conducted during and after BAC exposure was terminated. As discussed in Chapter 5, it was deduced that the BAC biotransformation rate by the heterotrophic microbial population in the BNR system increased rapidly during the system operation with the BAC-bearing poultry processing wastewater. The estimated values for the total BAC maximum specific utilization rate were 0.49 ± 0.19 and 0.005 ±.002 mg BAC/mg VSS · day during and after BAC exposure was terminated, respectively, while the half-saturation constant was estimated to be 0.63±0.22 mg BAC/L. The BNR system was operated with a BAC-free poultry processing wastewater feed for 100 days prior to the time of the batch assay. As a consequence, it can be deduced that the BAC biotransformation rate in the BNR system (i.e., in the aerobic reactor) was dynamic, with a value which increased with the duration of BAC exposure.

In the previously mentioned nitrification assays, BAC inhibited the extent of ammonia oxidation. Therefore, it was not possible to use all the data obtained from the BAC-amended culture series. Instead, data from culture series which achieved ammonia removal were used to evaluate the inhibitory effect of BAC. The culture series used were those with an initial BAC concentration of 5, 10, 15, and 20 mg/L of the batch assay conducted before BAC exposure, the 5, 15, and 20 mg/L culture series of the batch assay conducted during BAC exposure, and the 5, 10, and 15 mg/L culture series of the batch assay conducted after BAC exposure was terminated.

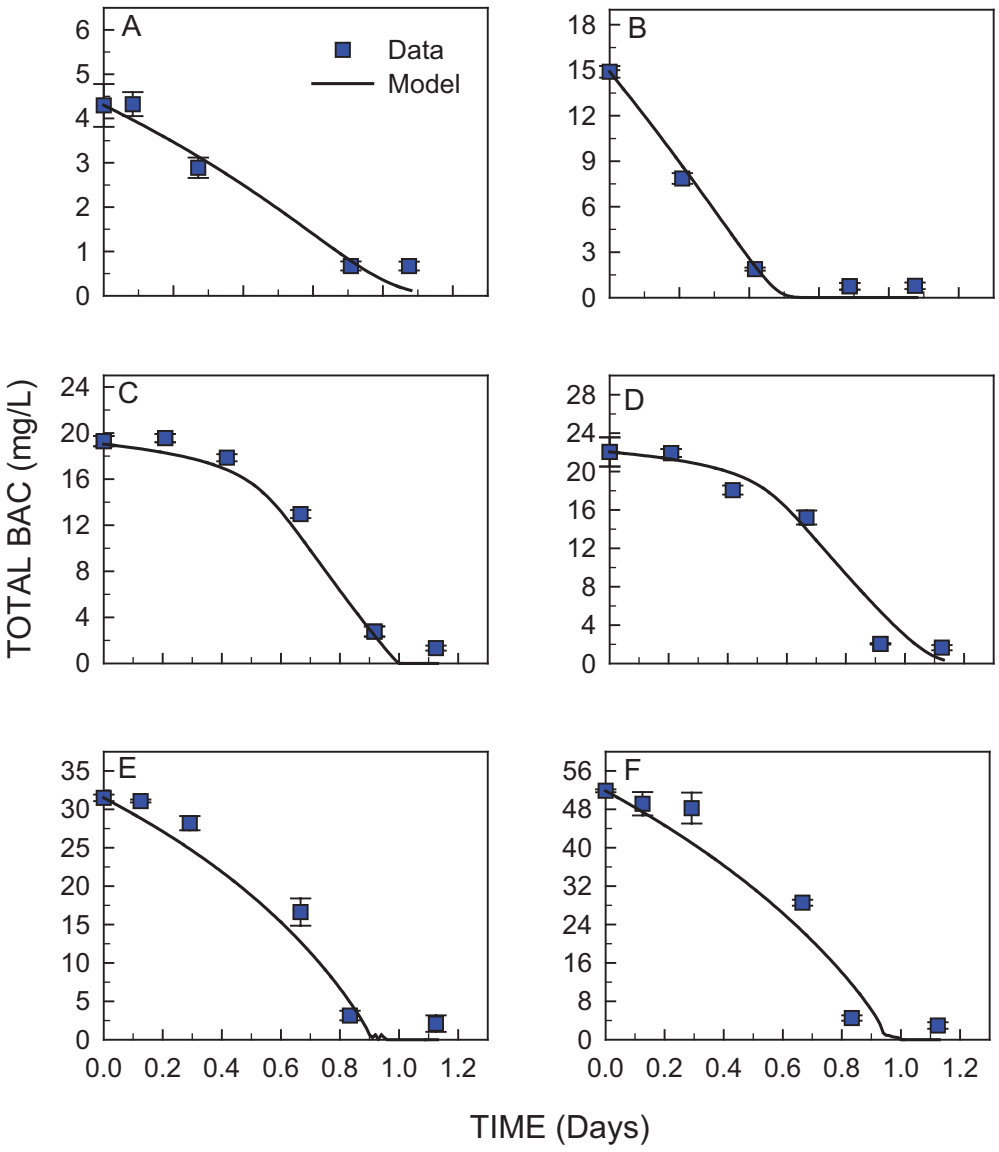

Figure 6.19. Measured (data points) and simulated (lines) total BAC concentration in the nitrifying batch assay performed with the aerobic reactor mixed liquor while treating BAC-bearing poultry processing wastewater. Culture series at initial BAC concentration of 5 (A), 15 (B), 20 (C), 25 (D), 30 (E), and 45 mg/L (F). Error bars represent one standard deviation from the mean.

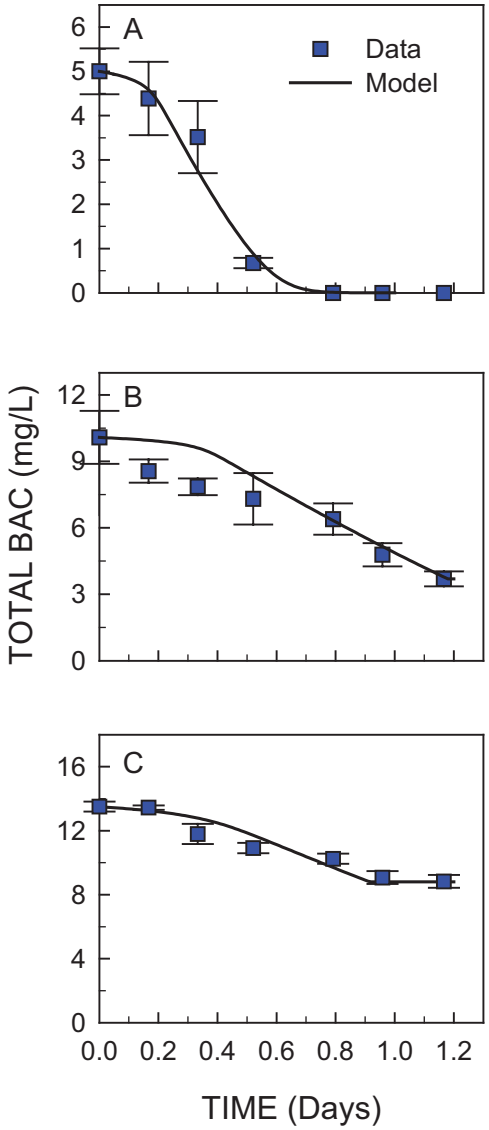

Figure 6.20. Measured (data points) and simulated (lines) total BAC concentration in the nitrifying batch assay performed with the aerobic reactor mixed liquor after treating BAC-bearing poultry processing wastewater was terminated for over 100 days. Culture series at initial BAC concentration of 5 (A), 10 (B), and 15 mg/L (C). Error bars represent one standard deviation from the mean.

The inhibitory effect of BAC was evaluated by fitting the apparent half-saturation constant values for the aforementioned BAC-amended culture series to the non-competitive inhibition model, considering the total, liquid-phase, and autotrophic solid-associated BAC concentration in the same culture series as the inhibitor (Figure 6.21). The autotrophic solid-associated BAC concentration was assumed to be 5% of the solid associated BAC concentration, reflecting that the autotrophic population comprised 5% of the total microbial population in the aerobic reactor mixed liquor. The data was fitted by non-linear regression analysis performed using SigmaPlot. Among the three aforementioned BAC concentrations, regression against the autotrophic solid-associated BAC concentration had the best fit, i.e., had the highest r^2 value. In contrast to the heterotrophic, BAC-degrading population, metabolically, the autotrophic nitrifying microbial population is incapable of BAC biodegradation. Therefore, the biomass-associated, solid-phase BAC fraction is more bioavailable to inhibit the nitrifiers. Consequently, the autotrophic solid-associated BAC concentration had the best correlation with the observed inhibition of nitrification by BAC (Figure 6.21).

The BAC inhibition coefficient values for ammonia and nitrite oxidation differed when the batch assay was performed before, during, and after BAC exposure. The estimated coefficient values were 0.11 ± 0.01 and 0.20 ± 0.01 mg/g VSS for ammonia and nitrite oxidation, respectively, before BAC exposure; 5.41 ± 0.5 and 4.14 ± 0.85 mg/g VSS for ammonia and nitrite oxidation, respectively, during BAC exposure; and 0.55 ± 0.06 and 3.64 ± 1.05 mg/g VSS for ammonia and nitrite oxidation, respectively, after BAC exposure had been terminated for over 100 days.

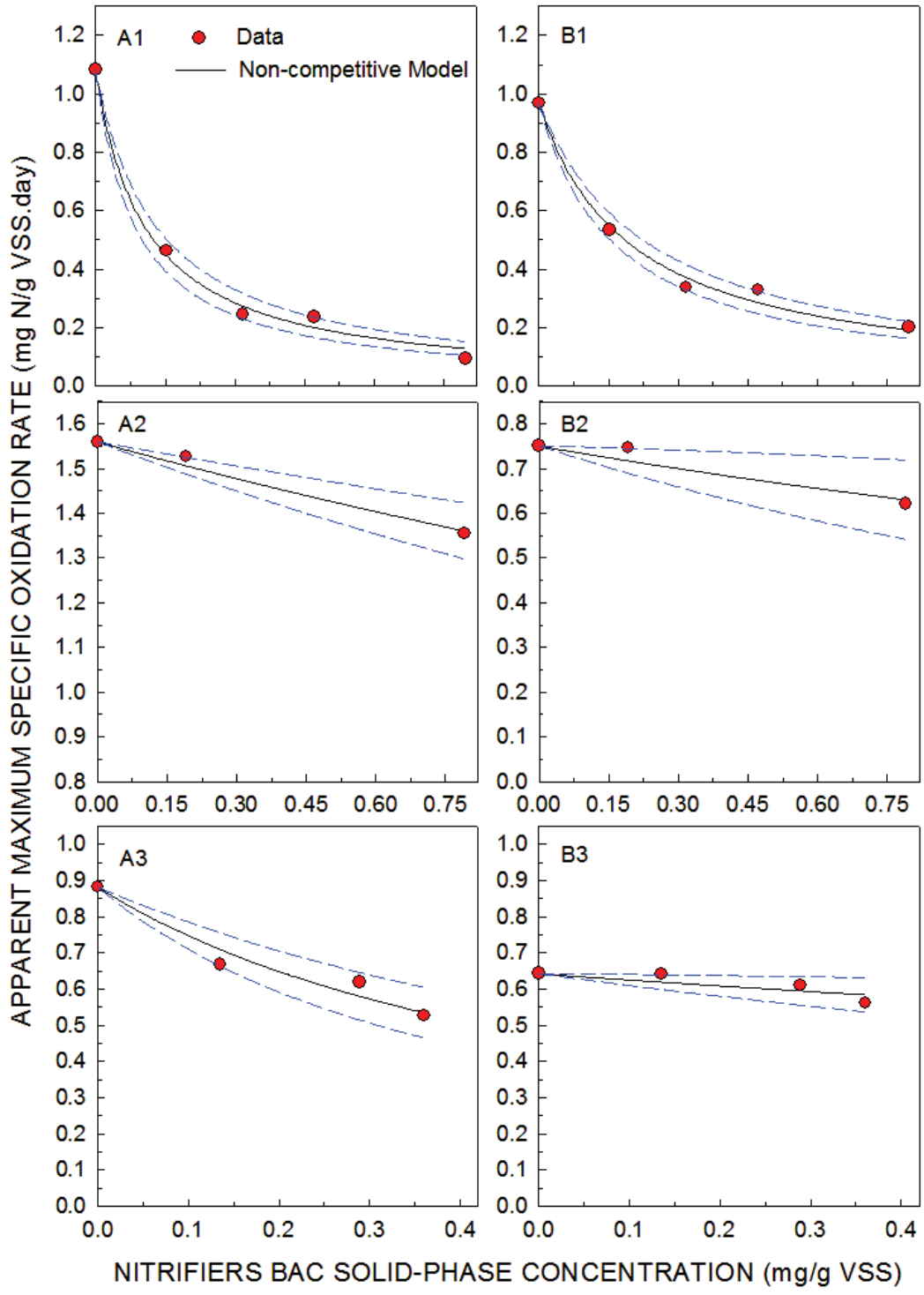

Figure 6.21. Apparent maximum specific oxidation rate for ammonia (A) and nitrite (B) before (1), during (2), and after (3) BAC exposure. [r^2 = 0.994 (A1), 0.991 (B1), 0.981 (A1), 0.914 (B2), 0.951 (A3), and 0.841 (B3)]. (Broken lines are 95% confidence intervals).

The aforementioned values show that both ammonia and nitrite oxidation were less susceptible to BAC during the BNR system operation with BAC-bearing poultry processing wastewater, indicating that the nitrifying population did acquire resistance to BAC. The higher competitive inhibition coefficients obtained after BAC exposure was terminated shows that BAC resistance was retained, albeit to a lesser level. Nitrite oxidizers were more successful in maintaining the acquired resistance to BAC compared to ammonia oxidizers. The discussion above leads to the conclusion that the nitrifiers' resistance to BAC is dynamic, and depends on the duration of the exposure to BAC. The dynamic change in BAC resistance can be modeled as a dynamic change in the non-competitive inhibition coefficient for ammonia and nitrite oxidation.

BAC inhibition of the heterotrophic sCOD utilization was modeled as a competitive inhibition and the half-saturation constants for sCOD utilization for the BAC-amended culture series were correlated with the total BAC concentration. Figure 6.22 shows the effect of the total BAC concentration on the apparent half-saturation constant for sCOD utilization. The competitive inhibition coefficient was evaluated as 14.9 ±1.5 mg BAC/L. BAC biodegradation by the heterotrophic population competes with the utilization of sCOD contributed by organic compounds other than BAC and BAC is more bioavailable at relatively low sCOD concentrations (Zhang et al., 2011). However, sCOD utilization is also inhibited by BAC. This intertwining relation between BAC inhibition and sCOD utilization is more likely the reason behind the observed correlation between sCOD utilization and the total BAC concentration (Figure 6.22).

Figures 6.23 and 6.24 show the absolute-relative sensitivity function curves for the aerobic reactor sub-model**.** The ammonia concentration was by far more sensitive to

the heterotrophic population fraction, followed by the maximum specific ammonia oxidation rate. The nitrite concentration on the other hand was equally sensitive to the heterotrophic population fraction and the maximum specific ammonia oxidation rate, followed by the maximum specific nitrite oxidation rate. The sensitivity of the nitrite concentration was similar to the sensitivity of ammonia concentration, very high to the heterotrophic population fraction, followed by the maximum specific ammonia oxidation rate. Overall, the heterotrophic population fraction and the maximum specific ammonia oxidation rate were the parameters that had the most sensitivity in the aerobic reactor sub-model. For the BAC-related parameters (Figure 6.24), the ammonia and nitrite concentrations were sensitive to the ammonia oxidation BAC inhibition coefficient, but the nitrite concentration was also sensitive to the nitrite oxidation BAC inhibition coefficient as well. Related to BAC, all the nitrogen species concentrations were sensitive to the BAC partition coefficient, albeit at a lesser degree compared to the other parameters. Analyzing the absolute-relative sensitivity function curves for the BAC biotransformation reveals that the BAC biotransformation rate impacted the nitrogen species concentrations to an extent similar to the inhibition coefficients (i.e., resulted in the same sensitivity). The last observation indicates that the BAC inhibition coefficient values are as important as the BAC biotransformation rate. Finally, the shape of the absolute relative sensitivity curves showed that neither of the two maximum specific oxidation rates was uniquely identifiable, but the BAC inhibition coefficients were. The total BAC maximum specific utilization rate and the half-saturation constant were also not uniquely identifiable.

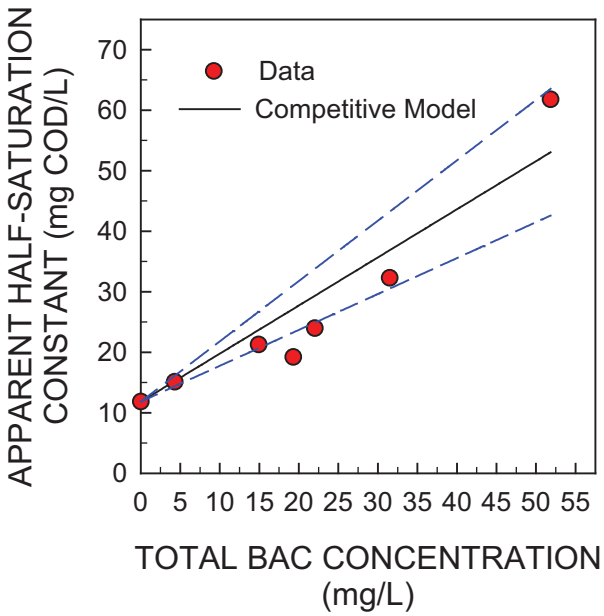

Figure 6.22. Apparent half-saturation constant for heterotrophic sCOD utilization for the assay conducted during BAC exposure (r^2 = 0.994). (Broken lines are 95% confidence intervals).

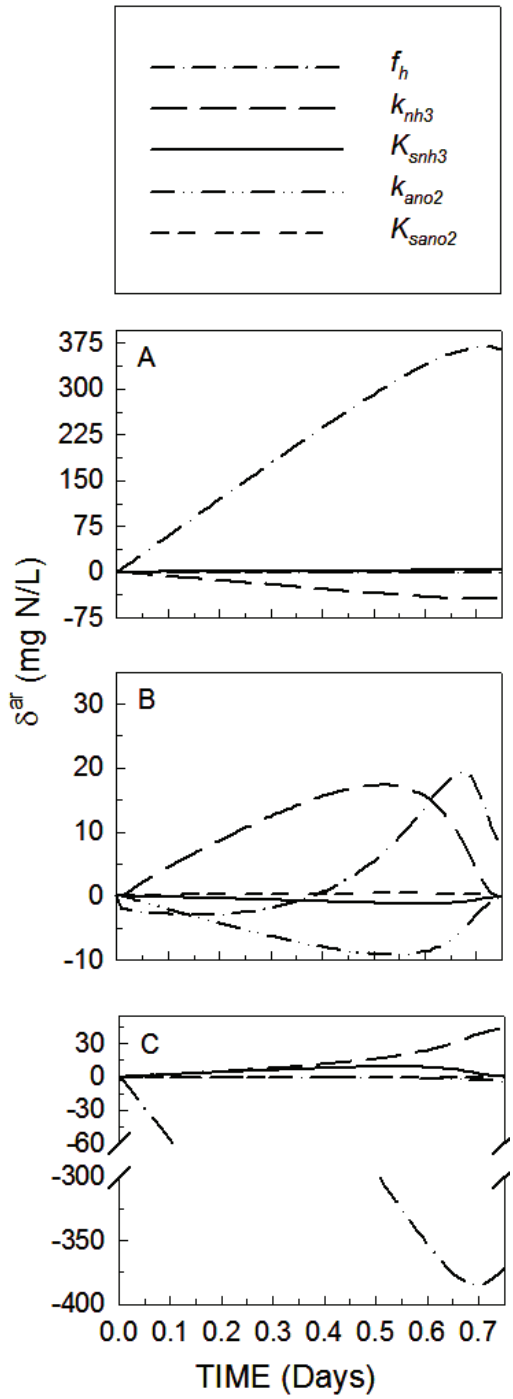

Figure 6.23. Absolute-relative sensitivity curves (δ^{ar}) showing the sensitivity of the aerobic reactor sub-model parameters to ammonia (A), nitrite (B), and nitrate (C) concentration.

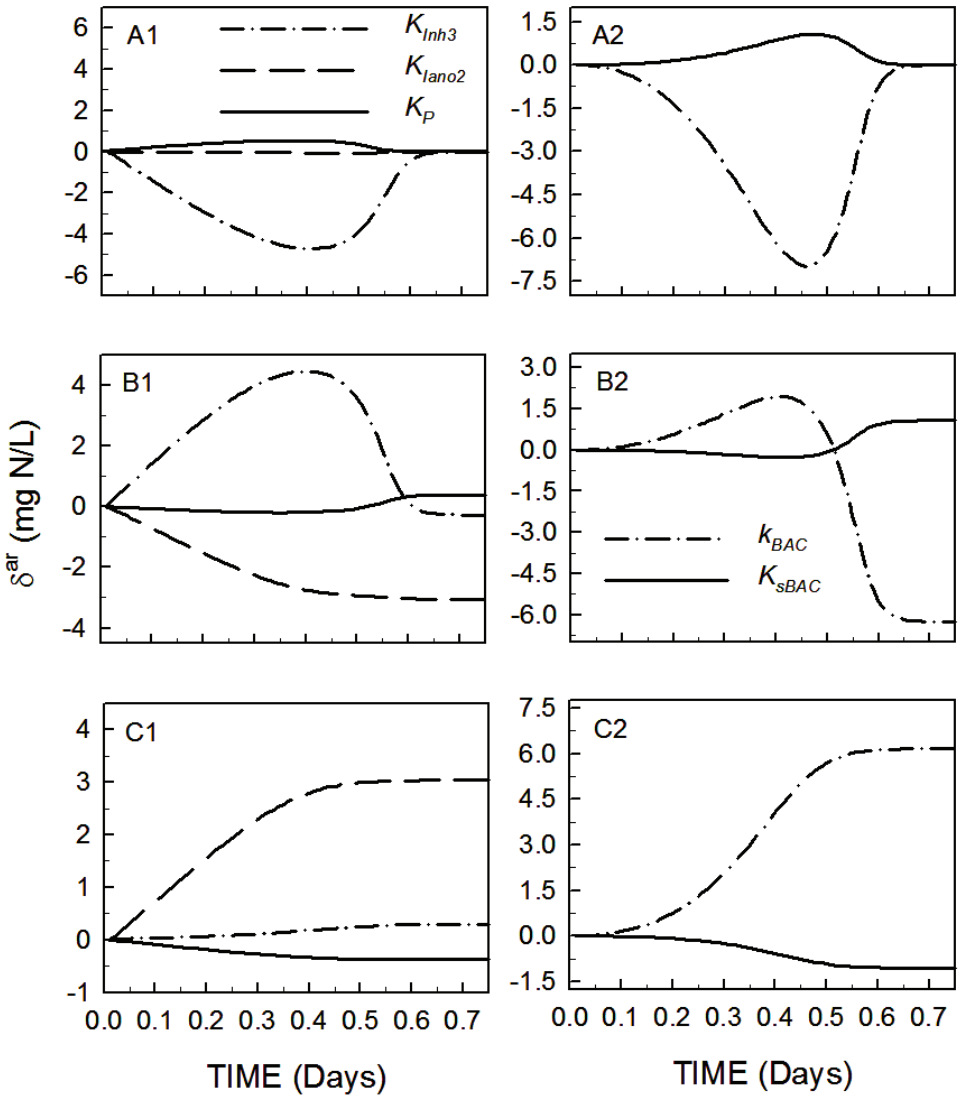

Figure 6.24. Absolute-relative sensitivity curves (δ^{ar}) showing the sensitivity of the aerobic reactor sub-model parameters to ammonia (A), nitrite (B), and nitrate (C) concentration; (1) BAC inhibition parameters , and (2) BAC biotransformation parameters.

6.4.2 BNR System Model

As discussed above, both the BAC biotransformation rate and the nitrification non-competitive inhibition were dynamic, which most probably was brought about by acclimation and enrichment of the heterotrophic and nitrifying populations, respectively, in the BNR system aerobic reactor. For nitrate reduction, the change in the competitive inhibition coefficients over time was small and therefore assumed negligible. The dynamic change in both BAC biotransformation rate and nitrifiers resistance to BAC is also related to the operation of the BNR system. The dynamic step-wise increase in the BAC concentration of the poultry processing wastewater (from 5 to 60 mg/L in the period of 505 days) acted as an increasing selective pressure that resulted in an incremental enrichment of the aerobic reactor microbial community. In contrast, operating the BNR with poultry processing wastewater at a single concentration would have more likely resulted in a static selective pressure in the aerobic reactor, and consequently a fixed level of BAC biotransformation rate and nitrifiers resistance to BAC, i.e., both rates would have reached steady-state values.

To account for that the observed enhancement of the BAC biotransformation, the change in the total BAC maximum specific utilization rate was fitted to the data obtained with the assays conducted during and after BAC exposure, assuming that the rate obtained in the latter assay equals the rate achieved before BAC exposure. The change in the ammonia and the nitrite non-competitive inhibition coefficients was fitted to the data obtained with the assays conducted before and during BAC exposure (i.e., for system run time between 30 and 370 days), corresponding to the time these assays were conducted. The following equations were used:

$$k_{BAC} = k^{i}_{BAC} + M_1 \cdot t \qquad \text{Equation (6.25A)}$$

$$K_{Inh3} = K^{i}_{Inh3} + M_2 \cdot t \qquad \text{Equation (6.25B)}$$

$$K_{Iano2} = K^{i}_{Iano2} + M_3 \cdot t \qquad \text{Equation (6.25C)}$$

where k^{i}_{BAC} is the BAC maximum specific utilization rate (mg BAC/mg VSS · day) evaluated in the assay before BAC exposure; K^{i}_{Inh3} and K^{i}_{Iano2} are ammonia and nitrite non-competitive inhibition coefficients (mg BAC/g VSS), respectively, evaluated in the assay before BAC exposure; M_1, is the acclimation/enrichment coefficient for BAC biotransformation (mg BAC/mg VSS · day^2); M_2 and M_3 are the acclimation/enrichment coefficients for ammonia and nitrite non-competitive inhibition, respectively (mg BAC/g VSS · day). The acclimation/enrichment coefficient values were 0.0013 mg BAC/mg VSS · day^2, 0.015 mg BAC/g VSS · day, and 0.011 mg BAC/g VSS · day for BAC biotransformation and ammonia and nitrite non-competitive inhibition, respectively. Taking into account 505 days operation of the BNR system with the BAC-bearing feed and above-mentioned M_1, M_2, and M_3 values, the BAC maximum specific utilization rate increased by 44.1%, while the ammonia and nitrite non-competitive inhibition coefficients increased by 43.7% and 42.5%, respectively. The nearly identical change in the BAC maximum specific utilization rate and the ammonia and nitrite non-competitive inhibition coefficients indicates that both the BAC-degrading, heterotrophic and the nitrifying microbial populations in the aerobic reactor were enriched to the same degree.

It should be noted that the aforementioned analysis relative to microbial acclimation/enrichment is purely an empirical representation of the behavior of the heterotrophic and nitrifying populations in the aerobic reactor. In reality, changes in the

BAC biotransformation rate and the nitrification non-competitive inhibition resulted from an increase in both BAC-degrading and BAC-resisting microbial species. A more accurate representation of the development of BAC degradation and resistance could be obtained through microbial community analysis of the aerobic reactor's microbial population. Nevertheless, the above presented empirical relationships could serve the goal of simulating the behavior of the heterotrophic and nitrifying populations, albeit with limited applicability to the conditions of BNR system used in this study.

6.4.2.1 Simulation of BAC-free BNR System Operation and Performance

In order to simulate the BNR system operation, the poultry processing wastewater characteristics must be incorporated into the model. Based on COD and sCOD data, the readily degradable organics and particulate organics concentration was considered to be 990 and 300 mg COD/L, respectively. The ammonia concentration was considered to be 45 mg N/L, which was assumed to be the dissolved fraction of the poultry processing wastewater total nitrogen content. The remaining 55 mg N/L were assumed to be part of the particulate organics. The physical characteristics of the BNR system, described in Chapter 4, were used for the simulations discussed in this section.

The BNR system simulation combined the mass balance ODEs discussed in section 6.2.4, above, along with the rate expressions included in the three sub-models. The BNR system BAC-free operation with recycle ratios of 2, 4, and 6Q (where Q is the poultry processing wastewater feed flow rate) were used to benchmark the model output. The BNR performance during the three recycle ratios was obtained from operating a duplicate BNR system, identical to the one which was operated with BAC-amended feed

poultry processing wastewater. The duplicate system was fed with the same poultry processing wastewater, but without any BAC.

Model simulation results along with experimental data are shown in Figure 6.25. The model simulated the performance of the BNR system to a good degree, although some differences in the time trend of nitrogen species were observed.

The steady-state biomass concentration was predicted to be 1023 and 1115 mg VSS/L in the anoxic and aerobic reactors, respectively, compared to the measured values of 1157 and 1251 mg VSS/L. The steady-state model values for the sCOD (mg COD/L), VFAs (mg COD/L), and ammonia (mg N/L) concentrations in the anaerobic reactor were 980, 195, and 93, respectively, compared to 650, 168, and 94, respectively, measured in the BNR system. The model was also able to accurately simulate the trend and magnitude of the change in nitrogen species for the three recycle ratios tested.

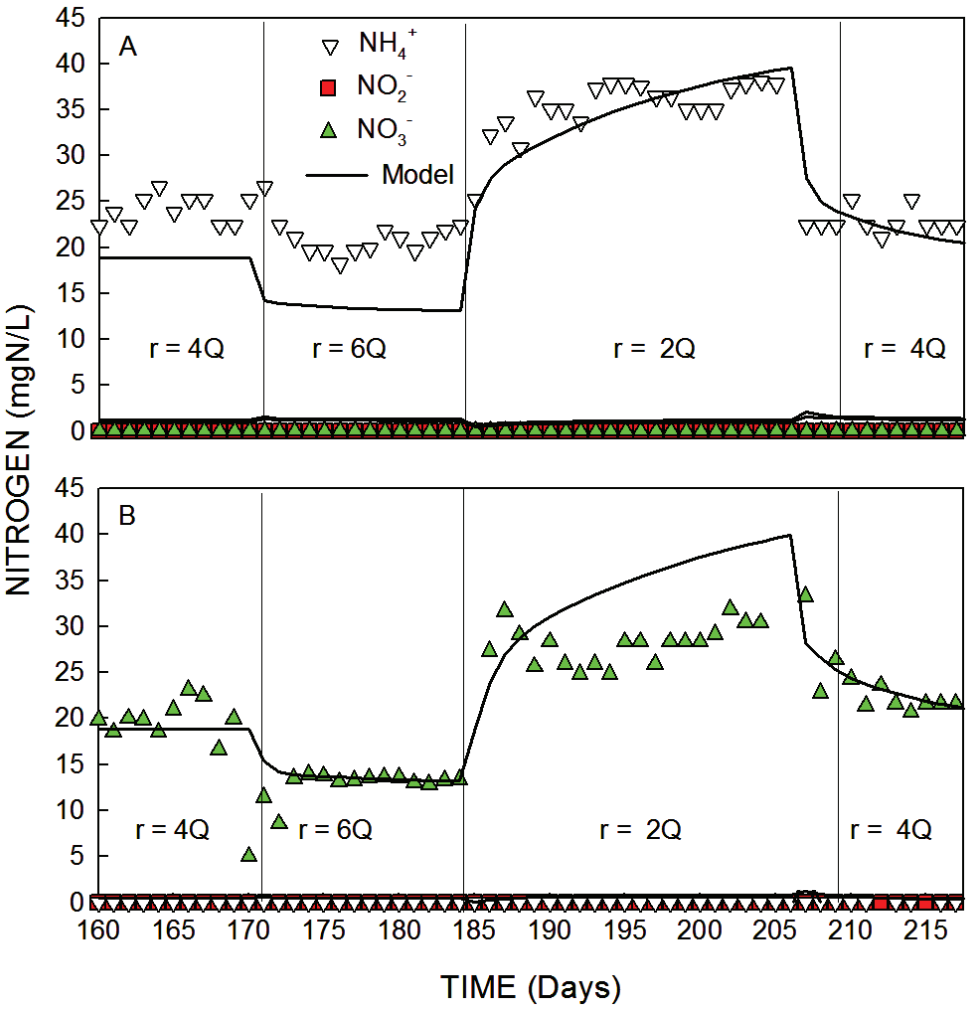

Figure 6.25. Measured (data points) and simulated (lines) nitrogen species in the anoxic (A) and the aerobic (B) reactors of the BNR system during operation with BAC-free poultry processing wastewater feed at different recycle ratios (r) between the aerobic and anoxic reactors.

6.4.2.2 Simulation of the BNR System Operation and Performance with BAC-bearing Poultry Processing Wastewater at 5 mg BAC/L

Figure 6.26 shows the model simulation and experimental data for the BNR system operation period while treating the BAC-bearing poultry processing wastewater at 5 mg BAC/L. The model was not able to predict the exact magnitude of nitrate and ammonia concentrations in the anoxic and aerobic reactors. Nevertheless, the model simulated well the initial response of the BNR system to the BAC-bearing poultry processing wastewater. Figure 6.27 shows the total BAC concentration in the BNR system. The model marginally underestimated the system's ability to degrade BAC and the BAC concentrations were higher in the anoxic and aerobic reactors. Nevertheless, the model was successful in simulating the ensuing rapid BAC degradation. The discrepancy in ammonia, nitrate, and BAC concentrations could be related to the approach used to model the change in BAC biotransformation rate and nitrification inhibition over time. In addition, the reactors' flow regime is assumed to follow that of an ideal continuous-flow stirred tank reactor (CFSTR), but in reality mixing in the system may not have reached ideal levels.

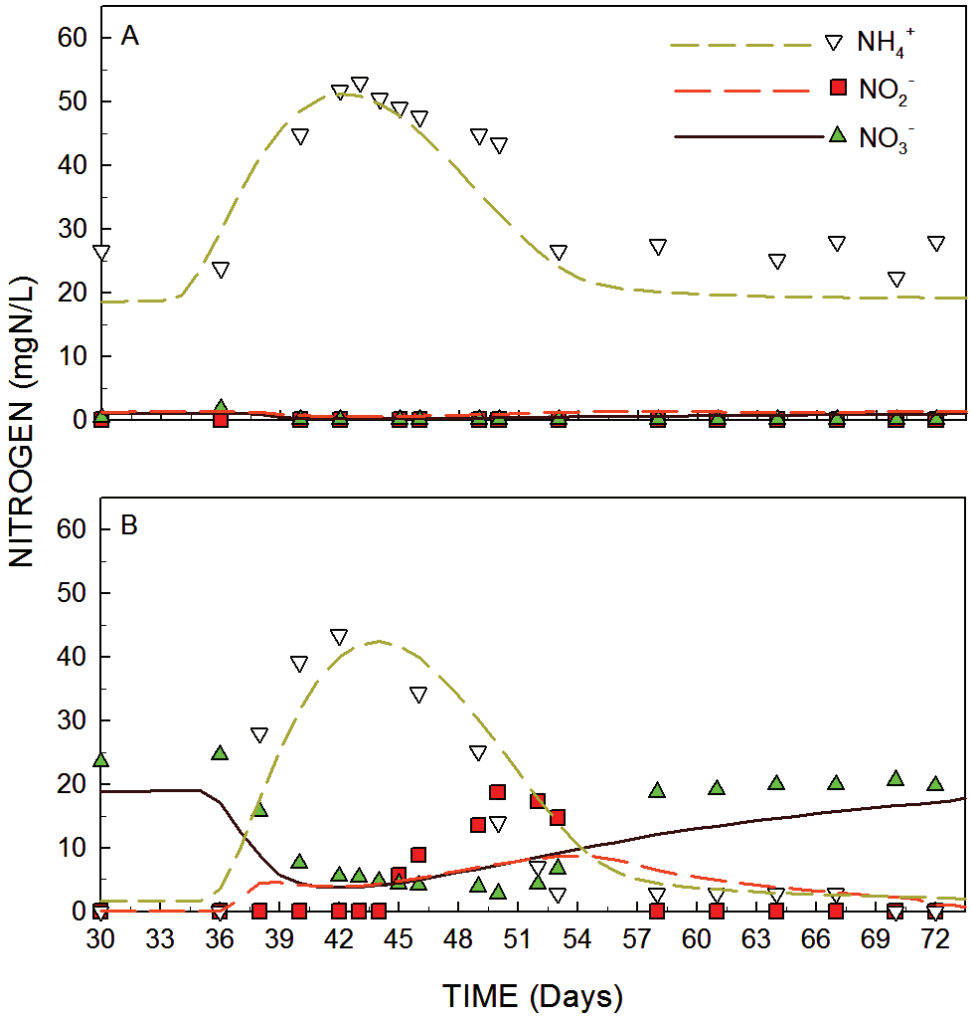

Figure 6.26. Measured (data points) and simulated (lines) nitrogen species in the anoxic (A) and the aerobic (B) reactors of the BNR system during operation with BAC-bearing poultry processing wastewater at 5 mg BAC/L.

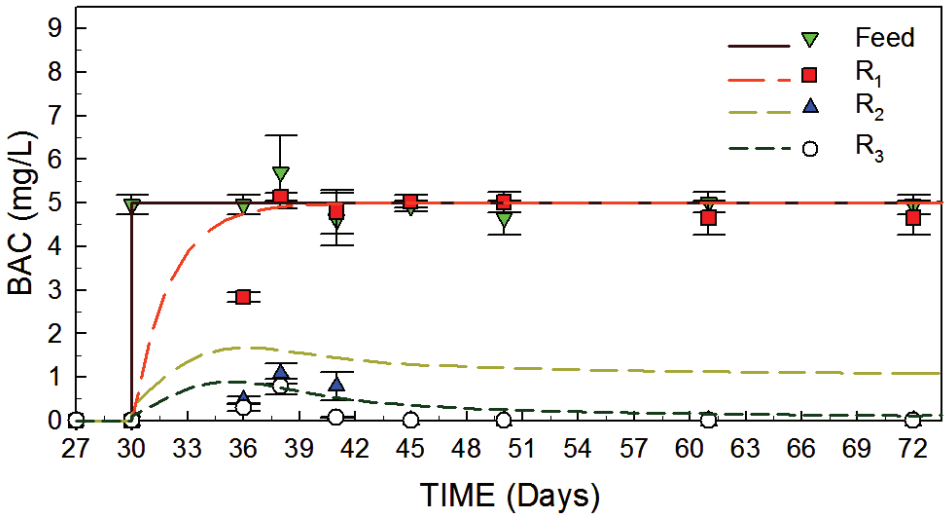

Figure 6.27. Measured (data points) and simulated (lines) total BAC concentrations in the BNR system during operation with BAC-bearing poultry processing wastewater at 5 mg/L

If the model excluded the ensuing increase in BAC biotransformation and decrease in nitrification inhibition, simulation could not match the system's performance while treating the 5 mg/L BAC-bearing poultry processing wastewater. Figure 6.28 shows the simulation results for the BNR system model with BAC biotransformation and nitrification competitive inhibition coefficient values obtained before the system was exposed to BAC and kept constant. The simulation results show that nitrification would never have recovered resulting in a very high ammonia concentration in the aerobic reactor. In addition, the BAC concentration would have reached much higher values in the BNR reactors. Therefore, accounting for the progressive increase in the BAC biotransformation rate and decrease in nitrification inhibition is very important.

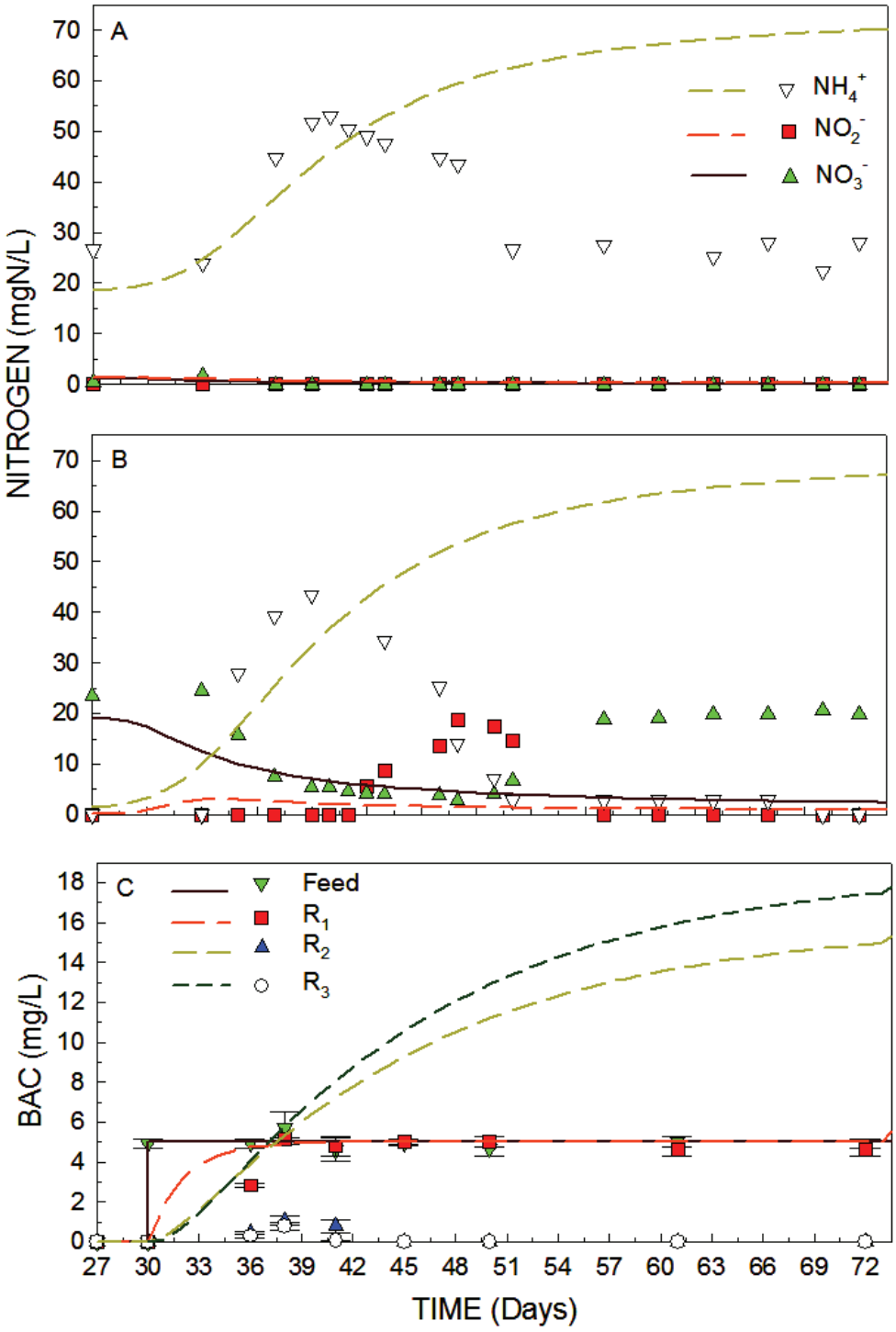

Figure 6.28. Measured (data points) and simulated (lines) nitrogen species in the anoxic (A) and the aerobic (B) reactors and BAC concentration (C) in the BNR system during operation with BAC-bearing poultry processing wastewater at 5 mg BAC/L with parameter values obtained before BAC exposure and system acclimation/enrichment.

6.4.2.3 Simulation of the BNR System Operation and Performance with BAC-bearing poultry processing wastewater at 10 to 120 mg BAC/L

Figures 6.29, 6.30, and 6.31 show the model simulation and experimental data for the BNR system operation period while treating the BAC-bearing poultry processing wastewater at step-increased BAC concentrations from 10 to 120 mg/L. Despite underestimating the ammonia concentration in the anoxic reactor and the nitrate concentration in the aerobic reactor, the model was very successful in simulating the BNR system's performance relative to the nitrogen species.

One consequence that results from BAC high adsorption affinity and its persistence in the anoxic reactor is BAC accumulation in the system. If BAC was not degraded in the aerobic reactor at high rates, the BAC concentration would have reached high levels in both the anoxic and aerobic reactor. In this case, the BAC concentration would have reached levels higher than those found in the poultry processing wastewater feed as shown in Figure 6.32.

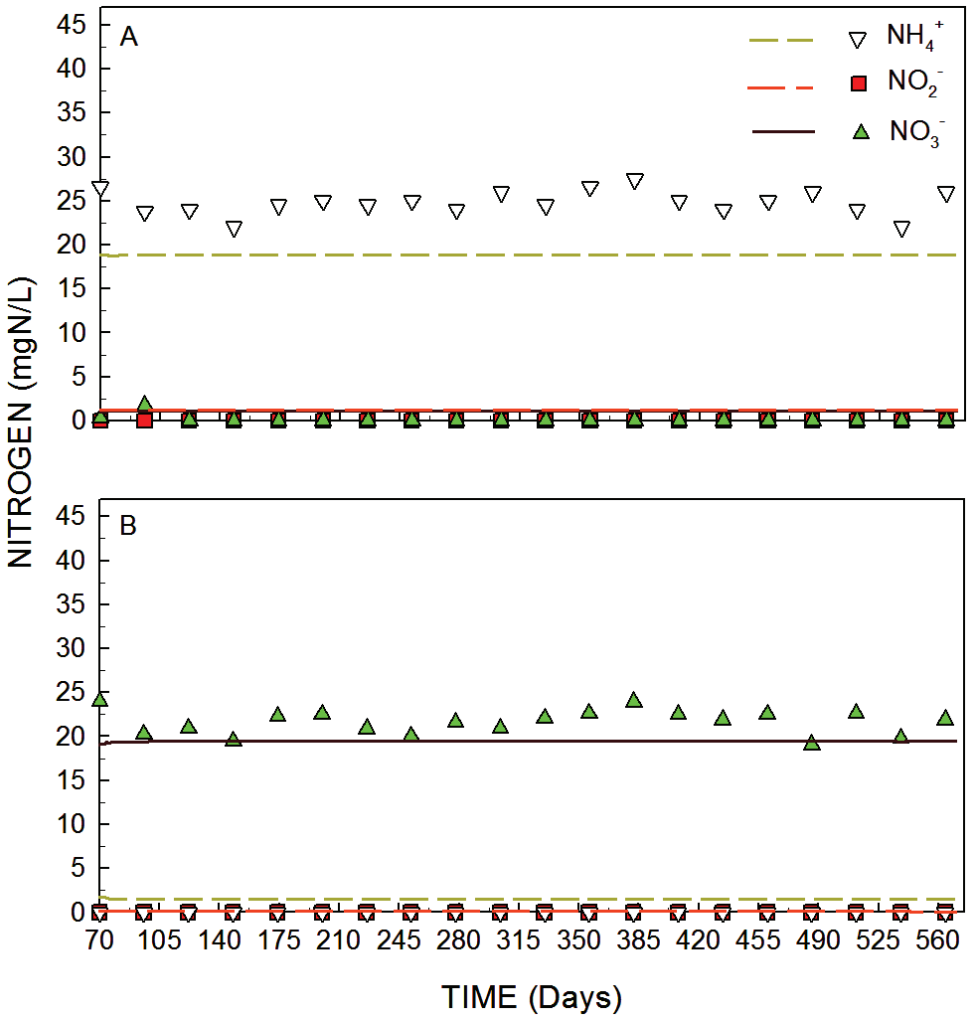

Figure 6.29. Measured (data points) and simulated (lines) nitrogen species in the anoxic (A) and the aerobic (B) reactors of the BNR system during operation with BAC-bearing poultry processing wastewater at step-increased feed BAC concentrations from 10 to 120 mg BAC/L.

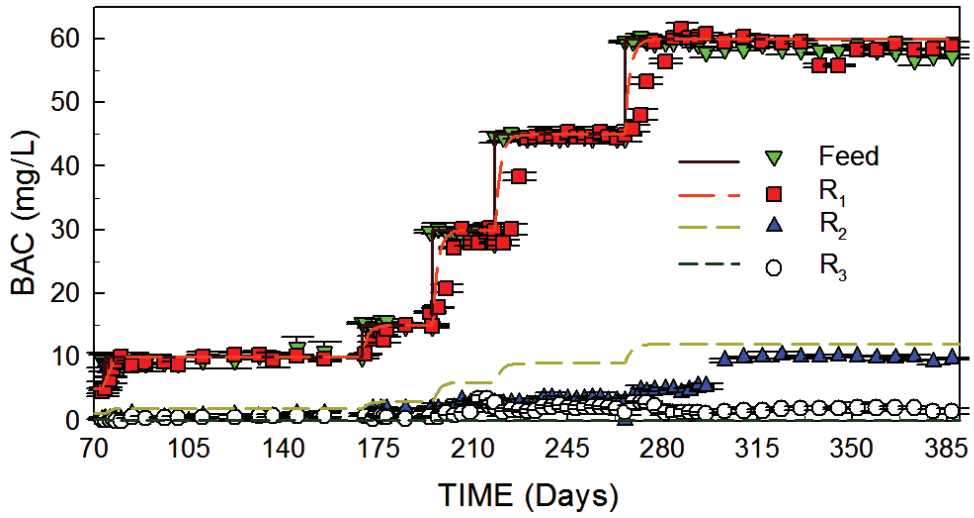

Figure 6.30. Measured (data points) and simulated (lines) total BAC concentrations in the BNR system during operation with BAC-bearing poultry processing wastewater at step-increased feed BAC concentrations from 10 to 60 mg/L.

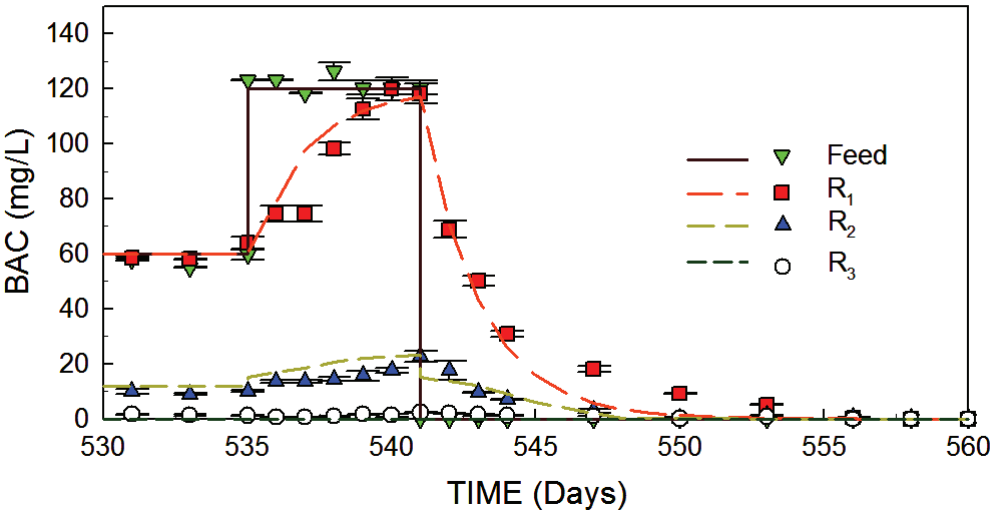

Figure 6.31. Measured (data points) and simulated (lines) total BAC concentrations in the BNR system during operation with BAC-bearing poultry processing wastewater with a step-increase in the feed BAC concentration from 60 to 120 mg/L.

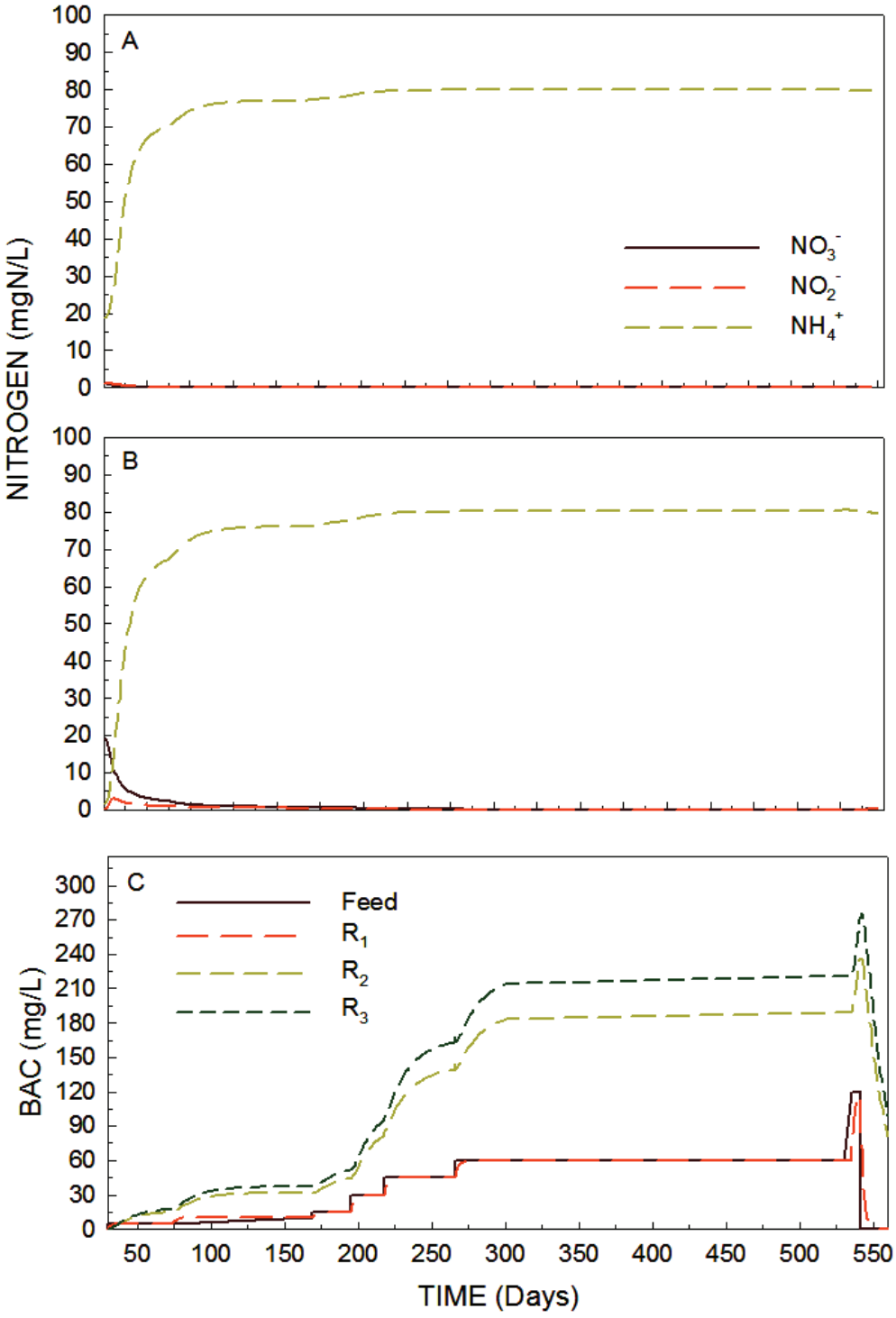

Figure 6.32. Simulated nitrogen species in the anoxic (A) and the aerobic (B) reactors and BAC concentration (C) in the BNR system during operation with BAC-bearing poultry processing wastewater at step-increased feed BAC concentrations of 5, 10, 15, 30, 45, 60, and 120 mg/L without BAC biotransformation and nitrification resistance.

6.5 Summary

Kinetic sub-models for the three reactors of the BNR system were successfully developed. 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 which resulted from independent batch assays discussed in Chapters 4 and 5.

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 parameter that had the most sensitivity in the anaerobic sub-model, while nitrate and nitrite maximum specific reduction rates were the parameters that had the most sensitivity in the anoxic reactor sub-model. The heterotrophic population fraction and the maximum specific ammonia oxidation rate were the parameters that had the most sensitivity in the aerobic reactor sub-model. Kinetic analysis of the 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 BAC exposure of the BNR system.

The three sub-models were combined in a comprehensive ASM1-based model. 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.

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.

CHAPTER 7

CONCLUSIONS AND RECOMMENDATIONS

7.1 Conclusions

The study presented here assessed the fate and effect of benzalkonium chloride, a quaternary ammonium antimicrobial compound, within a laboratory-scale, multi-stage, continuous-flow, high strength wastewater biological nitrogen removal system. Nitrification inhibition and low BAC removal rates resulted in the initial deterioration of the BNR system nitrogen removal efficiency, while treating BAC-bearing HSWW. Enhancement of the BAC removal rate and the acquisition of resistance to BAC by the heterotrophic and nitrifying microbial populations, respectively, ensured stable nitrogen and ammonia removal efficiencies while treating BAC-bearing HSWW with BAC influent concentrations up to 120 mg/L. Batch assays conducted with BNR system reactors mixed liquor showed that the inhibitory effect of BAC varied between the microbially mediated reactions in the system. The extent of BAC inhibition in the BNR system was affected by the phase distribution of BAC between liquid- and solid-phase, which significantly differed between the HSWW feed, the anaerobic, anoxic and aerobic reactors. Three sub-models, which represent the three reactors of the BNR system, along with rate expressions related to the fate and effect of BAC were developed, and their parameters were evaluated using batch assays. The sub-models were then combined in a comprehensive model that was able to simulate the performance of the BNR system fed

with poultry processing wastewater without and with BAC amendment up to 120 mg BAC/L.

The following specific conclusions can be drawn based on the results of this study:

1. The deterioration of nitrogen removal of the BNR system while treating the BAC-bearing HSWW when first introduced at 5 mg BAC/L in the influent was the direct result the BAC inhibitory effect on nitrification. The susceptibility of the microbially-mediated reactions to BAC decreased according to the following series: nitrification > denitrification > readily degradable organics utilization > fermentation.
2. BAC levels in the aerobic reactor exceeding a specific limit (15 mg/L in this study) result in inhibition of nitrification leading to low ammonia removal rate and incomplete nitrification (i.e., accumulation of nitrite). In contrast, complete denitrification takes place even at high BAC levels in the anoxic reactor (up to 100 mg/L in this study).
3. The inhibition of nitrification by BAC was non-competitive and depended on the solid-phase BAC concentration associated with the nitrifying biomass, while the inhibition of the heterotrophic sCOD utilization by BAC was competitive and depended on the total BAC concentration in the aerobic reactor. Similarly, the inhibition of denitrification by BAC was competitive and depended on the liquid-phase BAC concentration in the anoxic reactor.
4. BAC biotransformation took place only under aerobic conditions, and resulted in the simultaneous production and further biodegradation of benzyldimethylamine, benzylmethylamine, and benzylamine.

5. The equilibrium phase distribution of BAC between liquid- and solid-phase in the HSWW feed and the anaerobic, anoxic, and aerobic reactors mixed liquors was accurately described by the Freundlich adsorption isotherm. The BAC sorption capacity of the system decreased according to the following series: anaerobic reactor mixed liquor > HSWW > anoxic/aerobic reactors mixed liquors.
6. Treatment of BAC-bearing HSWW in the BNR system resulted in the development of BAC biodegradation capacity by the heterotrophic microbial population and BAC resistance by the nitrifying population in the aerobic reactor. The BAC biotransformation capacity and the acquisition of BAC resistance were modeled and simulated as dynamic increase of the BAC specific utilization rate and the non-competitive inhibition coefficients for ammonia and nitrite oxidation, respectively. The decrease in the BAC biotransformation capacity of the heterotrophic population and the sustained resistance to BAC by the nitrifying population after BAC was removed from the HSWW feed show that the acquired resistance to BAC by the nitrifying population lasted longer than the enhanced BAC biodegradation capacity by the heterotrophic population.
7. Modeling of the BNR system showed that simulation of a system treating BAC-bearing wastewater is only possible if the dynamic changes in BAC biodegradation capacity by the heterotrophic population as well as acquisition of BAC resistance by the nitrifying population are included in the model.

Overall it is deduced that the fate and effect of BAC in the BNR system can be accurately described if the interactions between BAC adsorption, inhibition, and resistance/biotransformation are considered within the conditions prevailing in each

reactor of the BNR system. Figure 7.1 shows how the aforementioned three interactions affect the fate and effect of BAC within the BNR system. BAC adsorption (phase distribution) determines the level of its inhibitory effect (potency), while BAC biotransformation and resistance define the extent of exposure of the microbial communities to BAC (bioavailability). Finally, the inhibitory effect of BAC is reduced, if not completely removed (i.e., riddance), by the development of BAC resistance and biotransformation capacity.

The integration between the BNR processes in this work showed that the fate and effect of QACs goes beyond their inhibitory effect, which was previously reported based on studies conducted on individual BNR processes and single redox conditions. The difference in BAC phase distribution within the four BNR system components (i.e., feed, anaerobic, anoxic, and aerobic reactors) showed that the BAC exposure levels differ significantly throughout the system, consequently changing the inhibitory effect. Biodegradation of BAC in the aerobic reactor not only resulted in its removal from this reactor, but also succeeded in keeping its concentration very low in the anoxic reactor as a result of mixed liquor recycle. Therefore, BAC did not accumulate in the anoxic reactor to levels that otherwise would have inhibited denitrification. Furthermore, values of the inhibition coefficients indicated that nitrifiers were capable of developing resistance to BAC while denitrifiers did not, causing a shift in the susceptibility to BAC between the two BNR processes. Additionally, the selective pressure of BAC not only resulted in the development of the aforementioned nitrifiers' resistance to BAC but also resulted in the development of enhanced BAC biotransformation rates. After BAC was removed from the BNR system feed poultry processing wastewater, the resistance of nitrifiers to BAC

was retained over a significant time compared to the enhanced BAC biotransformation rate.

The experimental work and modeling approach followed in this work present a framework that allows a better understanding of the fate and effect of BAC in engineered systems. The results of this study 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 food processing facilities, when and where needed, while avoiding any negative impacts on biological treatment systems and the environment.

7.2 Recommendations for Future Work

Most, if not all, BNR systems used by the food processing industry are extensive, open systems, and thus under the direct influence of seasonal temperature variations. Low temperature values during cold seasons will lower microbial growth rate and ultimately the rate of the biotransformation reactions, which might alter the response of such systems to the presence of QACs. Additionally, adsorption of QACs is an exothermic process, which will be affected by temperature. Consequently, the phase distribution of QACs will vary as a function of temperature, thus altering their inhibitory effect. Therefore, assessing the fate and effect of quaternary ammonium antimicrobial compounds in BNR systems at low temperature values is critical and requires further study.

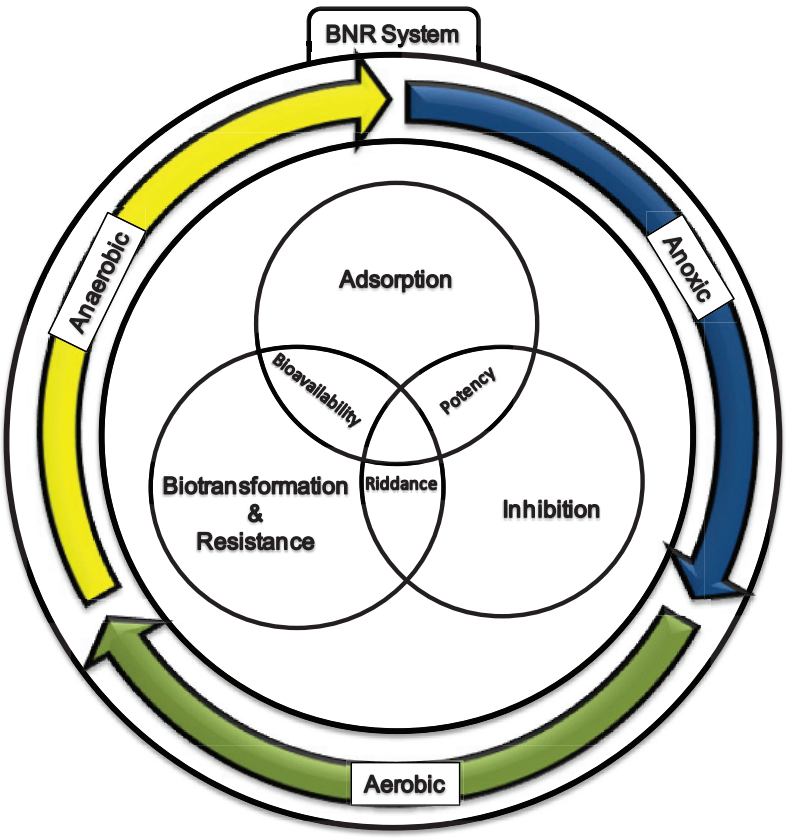

Figure 7.1. Interactions of BAC adsorption, inhibition, and biotransformation/resistance in the BNR system.

The acquisition and retention of resistance to BAC by the nitrifying population played an important role in establishing a stable nitrogen removal performance in the BNR system while treating BAC-bearing HSWW. The conditions and mechanism(s) which lead to the development of such resistance are not clear. Delineating the exact conditions and mechanism(s) which lead to resistance to BAC could help in facilitating the fast recovery of biological treatment systems in facilities which heavily depend on the use of quaternary ammonium antimicrobial compounds in sanitation practices. Recent studies have connected resistance to quaternary ammonium antimicrobial compounds to antibiotic resistance, a consequence of their discharge into the environment. Understanding the acquisition of resistance to QACs could help in the mitigation of antimicrobial resistance problem.

Finally, the mathematical model developed in this study can be used to design as well as optimize the operation of BNR systems treating wastewater with or without QACs, and to identify the best operational conditions by simulation within short periods of time. Such task can be performed by using multi-objective optimization techniques.

7.3 Recommendations for Improved Applications

The inhibitory effect of QACs on nitrification was the main factor that caused the deterioration of the BNR system nitrogen removal, which more likely will be the main concern for real world applications. Additionally, as discussed in section 7.2, above, low temperature values will lower the biological activity in the BNR system, thus exacerbating the inhibitory effect of QACs.

Results presented in this work show that continuous exposure (505 days) to QACs leads to the development of enhanced QACs biotransformation rate and nitrifiers

resistance to QACs. In real world applications, QACs are used intermittently; thus, exposure of the BNR processes to QACs is limited, which in turn limits the enrichment process that leads to the development of the enhanced QACs biotransformation rates and resistance of nitrifiers to QACs. Continuous usage of QACs will insure the development of the aforementioned enrichment and will insure BNR system resiliency towards QACs.

The initial exposure of the BNR system to QACs resulted in deterioration of the BNR system performance, primarily due to inhibition of nitrification, and lasted for 15 days in this work. Such upset to the BNR system could be avoided by manipulating the QACs phase distribution in the BNR system components. Results from this work show that the fate and effect of QACs is closely related to their phase distribution. Modifying the liquid- and solid-phase QACs concentration by the use of effective adsorbents (e.g., granular or powdered activated carbon) is an attractive technique that could limit the inhibitory effect of QACs by either preventing or mitigating such an effect. Additionally, the VSS concentration in the BNR system could be increased by extending the solids residence time, allowing for the development of higher biomass concentration that could act as an effective adsorbent against QACs. Furthermore, higher solids retention times will accelerate the QACs enrichment process within the BNR system, resulting in enhanced microbial resistance and biodegradation rates.

The aforementioned suggested practices could support the continuous use of QACs as antimicrobial agents by the food processing industry when their usage is unavoidable, without any adverse operational or environmental consequences.

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