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SHORT COMMUNICATION

Effect of carbon to nitrogen (C:N) ratio on nitrogen removal from shrimp production waste water using sequencing batch reactor

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Abstract The United States Marine Shrimp Farming Program (USMSFP) introduced a new technology for shrimp farming called recirculating raceway system. This is a zero-water exchange system capable of producing high-density shrimp yields. However, this system produces wastewater characterized by high levels of ammonia, nitrite, and nitrate due to 40% protein diet for the shrimp at a high density of 1,000 shrimp per square meter. The high concentrations of nitrate and nitrite (greater than 25 ppm) are toxic to shrimp and cause high mortality. So treatment of this wastewater is imperative in order to make shrimp farming viable. One simple method of treating high-nitrogen wastewater is the use of a sequencing batch reactor (SBR). An SBR is a variation of the activated sludge process, which accomplishes many treatment events in a single reactor. Removal of ammonia and nitrate involved nitrification and denitrification reactions by operating the SBR aerobically and anaerobically in sequence. Initial SBR operation successfully removed ammonia, but nitrate concentrations were too high because of carbon limitation in the shrimp production wastewater. An optimization study revealed the optimum carbon to nitrogen (C:N) ratio of 10:1 for successful removal of all nitrogen species from the wastewater. The SBR operated with a C:N ratio of 10:1 with the addition of molasses as carbon source successfully removed 99% of ammonia, nitrate, and nitrite from the shrimp aquaculture wastewater within 9 days of operation.

Keywords Nitrification · Denitrification · Biofilter · Sequencing batch reactor · Shrimp wastewater

Introduction

Aquaculture is the farming of aquatic organisms including fish, mollusks, crustaceans, and aquatic plants and it constitutes the fastest growing global farming system in the world with an annual increase in production of over 9% per year since 1985 [9–11]. The aquaculture industry produces a substantial amount of the fish and shellfish consumed around the globe by using ponds and raceways far from the seas and oceans. The consistent increase in global aquaculture production is staggering and this increase in production is being driven by consumers' increasing global demand for seafood [9, 10]. To meet global seafood demands, improvements in sustainability, biosecurity, and environmental impacts must be incorporated into existing and future aquaculture practices [6, 25].

Shrimp aquaculture production is of particular interest because it represents one of the primary global seafood exports for human consumption [9]. Shrimp aquaculture processes can be highly concentrated to maximize production and yield, and these concentrated processes can produce poor water quality because of the shear density of shrimp of greater than 1,000 shrimp per square meter (m^2) in the system and also because of the nutrient characteristics of the feed used in the aquaculture operations.

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Conventional systems are effective in treating intensive aquaculture process waters [7, 30]; however, the systems can be costly to operate and require the solids that are produced as a component of the conventional treatment processes to be disposed of offsite [7].

The solids typically collected as “backwash or “filter cake” from an aquaculture facility’s biological filters can contain high concentrations of organic carbon and ammonia-nitrogen and NaCl [3]. This backwash can be expensive for aquaculture operators to transport and dispose of according to Clean Water Act (CWA) requirements. Process water and NaCl can be lost through backwash processes and solids containing high levels of NaCl must be disposed of in a manner that will not allow high concentrations of NaCl to accumulate in soil, which can limit the use of land farming as a means of disposal. These considerations can increase the cost of conventional treatment. Because many inland aquaculture operations must purchase process water and NaCl to culture marine species, conventional wastewater treatment can increase production costs if losses of process water and NaCl are substantial.

An onsite biological wastewater treatment solution to reduce the carbon and nitrogen concentration of filter backwash from aquaculture operations to acceptable effluent discharge levels may provide a cost-effective alternative to offsite transport and disposal of backwash solids commonly referred to as “sludge”. This would allow operators to simultaneously reduce facility operation costs while preventing nutrient loading of receiving waters near an aquaculture facility. This would also reduce the total volume of solids from biological wastewater treatment processes being disposed of in non-hazardous sanitary landfills.

The sequencing batch reactor activated sludge (SBR) process was proposed as a potential solution to reduce the nutrient concentration of filter backwash sludge at aquaculture facilities. The selection of a particular treatment process is based on many considerations, including operational objectives, wastewater characteristics, effluent quality, and operating costs. The SBR process is novel because unlike conventional activated sludge processes that use multiple tanks or vessels to achieve the environmental requirements to perform continuous treatment of wastewater [1, 14, 22, 31], the SBR process uses a single reactor vessel that is “sequenced” through a series of stages that alter the environment of the reactor [15, 22]. SBRs can be operated to perform aeration, carbon oxidation, clarification, nitrification, denitrification, and settling in the same vessel [22]. Most SBR treatment processes follow the common sequence of fill, react, settle, draw, and idle [20, 22]. The operation of the SBR can be modified, based on the characteristics of the influent stream, to achieve the desired effluent concentrations [3, 22].

Previous work conducted by Boopathy et al. [3] modeled the microbial kinetics of nitrification, and denitrification to determine operational and environmental variables required to treat aquaculture sludge using suspended growth activated sludge processes. One of the problems in the operation of SBR is the carbon limitation in the system to complete the denitrification process. If enough carbon is not available, an incomplete denitrification occurs resulting in high nitrate and nitrite concentration in the shrimp wastewater [23]. The purpose of this research was to optimize C:N ratio for successful removal of nitrate in the shrimp waste using SBR.

Materials and methods

Shrimp waste

Solids (sludge) that accumulates on the industrial biological bead filters (biofilters) that filter water from the recirculating shrimp aquaculture raceways at the Waddell Mariculture Center (WMC), S. Carolina, is periodically backwashed from the filters and stored in a settling tank at the facility. This sludge was collected in 3-L plastic containers, stored, and shipped at 4°C to Nicholls State University for experimentation. The characteristics of the sludge are given in Table 1. The sludge contained high concentration of ammonia, nitrite, and nitrate. The sludge also had high bacterial load of 10^{-10}/ml total heterotrophic bacteria. Bacteria in the sludge include *Nitrosomonas, Nitrobacter*, and *Pseudomonas* spp. [5], and this sludge provided microorganisms for nitrification and denitrification reactions in the SBR.

Initial SBR operation

The SBR was made of Plexiglass with 4-L working volume. The reactor was equipped with an aeration port, controller, mixing device, feeding, and decanting system. The reactor received 4-L of shrimp sludge at the beginning of the experiment. The reactors were aerated by using air stones,

Table 1 Characteristics of the shrimp wastewater

Parameter	Concentration
Organic carbon (mg/l)	793 ± 36
Ammonia (mg/l)	103.7 ± 6.1
Nitrate (mg/l)	168.3 ± 1.4
Nitrite (mg/l)	176 ± 22.7
Salinity (ppt)	28.6 ± 0.4
pH	8.1 ± 0.1

Average of 4 analyses

and the wastewater was mixed during aerobic operation at a rate of 100 rpm using a stirring motor. Aeration and mixing were turned off for the system to run anaerobically. The reactors were operated aerobically and anaerobically in sequence (Fig. 1) and these modes of operation depend on ammonia or nitrate concentration in the sludge. During the aerobic phase, ammonia in the shrimp sludge is converted to nitrate by nitrifying bacteria, and during the anaerobic stage denitrification is accomplished where all the nitrates are converted to nitrogen gas by denitrifying bacteria. Triplicate reactors were operated and the data presented in the results section are the average of these three reactors. The experiment was run twice to see the reproducibility of the system. The reactor was operated at ambient temperature (22°C).

C:N ratio study

To study the effect of the carbon to nitrogen ratio on the operation of the SBR in treating sludge collected from the WMC, a laboratory study was conducted in a smaller scale using laboratory flasks. Thus, 500 ml of sludge was placed in 1-L flasks that served as the SBR vessels. Air stones, plastic tubing, and a small air pump were used for aeration and mixing. The reactors were configured with four different C:N ratios of 10:1, 20:1, 30:1, and 40:1 through the addition of molasses to add carbon or ammonium salts to add nitrogen. The reactors were operated at room temperature (22°C). Three reactors per C:N ratio treatment ($N = 3$) were used. The reactors were sequenced based on initial work of Boopathy et al. [3] in the following manner: aerobic 3 days, followed by 6 days of anaerobic phase.

Operation of SBR with optimum C:N ratio

After the C:N ratio optimization study as explained above, the optimum C:N ratio for successful removal of nitrogen was found to be 10:1. A triplicate batch of SBR was run very similarly to earlier SBR runs except this time the initial C:N ratio of the shrimp sludge was adjusted to 10:1 with the addition of molasses and ammonium salt. Samples were periodically taken for the analysis of ammonia, nitrate, and nitrite. The SBR was operated in triplicate.

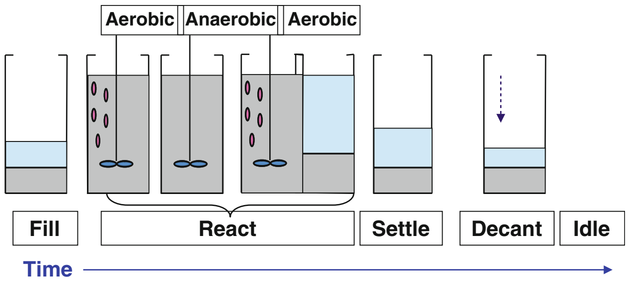

Fig. 1 Schematic of sequencing batch reactor (SBR)

Analyses

Samples were periodically taken from the SBR and C:N ratio experimental flasks for various analyses. Nitrate, nitrite, ammonia, and organic carbon were analyzed by colorimetric methods as per standard methods [16]. The pH was measured by using a pH probe (model UB 10, Denver Instruments, Boulder, CO). The salinity, temperature, and dissolved oxygen concentration were measured by using a YSI multi-meter (YSI, Inc., Yellow Spring, Ohio: 45387 USA, Model No. 85-10 FT; SN 02E0656). Statistical analysis of data was done by using SAS [28].

Results and discussion

Performance of initial SBR

The shrimp sludge used in this study contained high concentration of ammonia, nitrate, and nitrite (Table 1). The performance of SBR in removing nitrogen in the shrimp sludge was studied. During the aerobic phase of the reactor operation all the ammonia is converted to nitrate and there was 98% removal of ammonia within the first week of reactor operation (data not shown). However, the concentration of nitrite and nitrate remained high for 2 weeks during the anaerobic phase of SBR (Fig. 2). On day 28 of the experiment, additional carbon was added to all reactors in the form of molasses (1% v/v). After the addition of molasses, both nitrite and nitrate levels dropped significantly within 38 days in both runs of SBR. This study showed carbon limitation in the system and this lack of carbon did not support denitrifying bacteria to convert all the nitrate and nitrite to nitrogen gas and as a result partial denitrification occurred in the system.

C:N ratio study

The small-scale C:N ratio experiment showed the optimum C:N ratio for maximum removal of nitrogen in the shrimp sludge. Table 2 shows the results from this study. Among the various C:N ratio studied, maximum removal of ammonia (100%), nitrite (91%), and nitrate (99%) was observed with a C:N ratio of 10:1 followed by 5:1, 20:1, and 30:1. This result showed that the C:N ratio of 10:1 is better than higher C:N ratios. By simply doubling the C:N ratio from 5:1 to 10:1 via the addition of an inexpensive molasses the performance of SBR could be improved significantly. Previous literature suggests a C:N ratio of 20:1 and 30:1 for various wastewater treatments such as

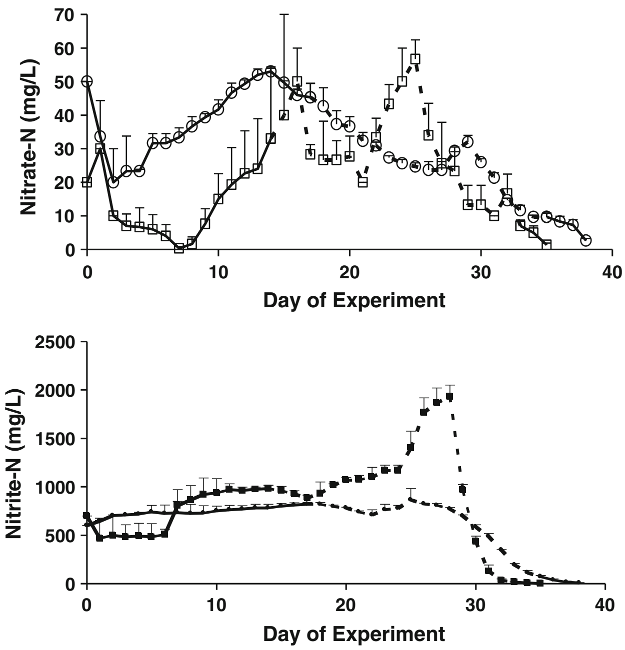

Fig. 2 Mean (±SD; $n = 3$) of nitrite and nitrate-N for trial 1 (*squares*) and trial 2 (*circles*) for each day of the experiment. The *solid lines* represent times the SBR was operated aerobically and the *dashed lines* represent when the SBR was operated anaerobically. On day 28 molasses (1% v/v) was added to increase the carbon in the SBR. *Error bar* indicates standard deviation with three replications

poultry waste, cow manure, and coffee waste [2, 8, 29]. Successful shrimp aquaculture requires maintenance of water quality conducive for the growth of shrimp. Common water quality concerns for shrimp aquaculture include suspended solids, organic carbon, dissolved oxygen (DO), and nitrogen [4, 7, 13, 26, 27]. Low-water exchange aquatic animal culture systems rely on technological filtration systems to biologically and mechanically treat wastewater to reduce carbon and nitrogen [7, 30]. A major drawback with this type of system is the accumulation of sludge, which must be concentrated, collected, and then physically removed from the aquaculture facility [7, 30]. The present study showed that the nitrogen in the shrimp sludge could be removed by using SBR and the optimum C:N ratio for successful operation is 10:1.

SBR operated with 10:1 C:N ratio

The C:N ratio study demonstrated the optimum C:N ratio of 10:1 and to prove that use of this ratio will successfully remove nitrate and nitrite from the shrimp sludge, an experiment was conduced with a 4-L SBR and the initial C:N ratio was adjusted to 10:1 as described in the "Materials and methods" section. The results indicated successful removal (greater than 99%) of all three nitrogen species in the shrimp sludge within 9 days of reactor operation (Fig. 3). Biological treatment of organic waste using activated sludge is a proven technology used in municipal sewage treatment facilities. Conventional anaerobic treatment processes have been used to reduce the organic carbon concentration of liquid, but these processes have not been successful in reducing both carbon and nitrogen at a reasonable cost. However, the sequencing batch reactor (SBR) minimizes the capital costs by incorporating both aerobic and anaerobic processes in a single reactor [21].

The successful operation of the reactor showed that the wastewater contained the nitrifying and denitrifying organisms such as *Nitrosomonas, Nitrobacter*, and *Pseudomonas* spp., to carry out the metabolism of nitrogen in the wastewater. There was no need to add specific microbes for the metabolism of carbon and nitrogen as these were present in the shrimp wastewater and these microorganisms were not affected by the change of conditions from aerobic to anoxic modes of operation and vice versa.

We believe that backwash from biological filters currently used in shrimp aquaculture can be directed to the

Table 2 Mean (±SD; $N = 3$) initial concentration (mg/l), final concentration, and percent reduction of nitrate-N, nitrite-N, and total ammonia-N of shrimp sludge treated with a sequencing batch reactor for different C:N ratios

Parameter	C:N ratio	Initial concentration	Final concentration	% Reduction
Ammonia-N	5:1	69 ± 3	0 ± 1^{B}	100
	10:1	69 ± 3	0 ± 1^{B}	100
	20:1	69 ± 3	11 ± 3^{A}	84
	30:1	69 ± 3	13 ± 5^{A}	81
Nitrate-N	5:1	128 ± 3	43 ± 4^{C}	66
	10:1	128 ± 3	1 ± 1^{D}	99
	20:1	128 ± 3	66 ± 14^{B}	48
	30:1	128 ± 3	91 ± 6^{A}	29
Nitrite-N	5:1	105 ± 49	64 ± 3^{B}	39
	10:1	105 ± 49	9 ± 15^{C}	91
	20:1	105 ± 49	56 ± 8^{B}	47
	30:1	105 ± 49	105 ± 6^{A}	0

Final concentrations within variable groups that share a common letter are not significantly different ($\alpha = 0.05$)

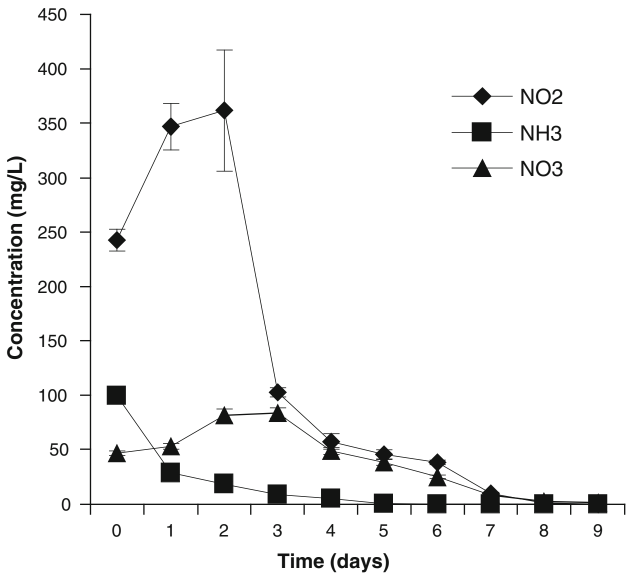

Fig. 3 The concentration of ammonia, nitrate, and nitrite in the laboratory-scale SBR operated with C:N ratio of 10:1. The results are average with SD for three reactors. Reactors were operated aerobically for the first 3 days followed by anaerobic operation for 6 days

SBR. The SBR will then digest the carbon and nitrogen associated with the backwash. Once the carbon and nitrogen are digested, water can be decanted from the SBR and returned to the culture system, so water loss will be negligible. The reactor design is simple and very easy to operate. The SBR system has been successfully used for various wastewaters including slaughterhouse wastewater, swine manure, dairy wastewater, and sewage [12, 21, 22, 24, 32]. In the literature, it is shown that the wastewater problem in shrimp aquaculture is addressed by activated sludge process, foam fractions, use of filter systems, and sludge management [17–19]. These systems are costly and expensive to operate. The SBR system is very simple in design and this process uses multiple steps in the same tank to take the place of multiple tanks in a conventional treatment system.

In this study, it has been shown that the SBR could be used to treat shrimp wastewater produced from intensive shrimp raceway production system. The operation mode is simple which includes aerobic process for first 3 days and anaerobic process for 6 days to remove 99% of nitrogen in the shrimp sludge. The sludge contained heterogenic populations of bacteria to carry out nitrification and denitrifiation reactions as well as carbon metabolism. The nitrifying organisms dominated the system during the aerobic operation of the reactor. This was evidenced by the data on removal of ammonia in the sludge (Fig. 3). The denitrifying organisms dominated the system during the anoxic operation of SBR. This was supported by the fact that the levels of nitrite and nitrate dropped significantly under the anaerobic phase and eventually reached less than 1 mg/l (Fig. 3). Similar results were demonstrated earlier by Boopathy et al. [4] in an SBR treating low-salinity shrimp aquaculture wastewater. At the end of the operation the sludge can be dewatered and the water can be recycled back into shrimp production. The application of SBR technology to intensive shrimp production is an attractive alternative to various methods currently used in shrimp aquaculture. The only concern was initial low C:N ratio in the sludge and this can be easily overcome by adjusting the C:N ratio to the optimum level 10:1 by adding low concentration (1%) of molasses as demonstrated in this study. Molasses is an inexpensive carbon source, which makes the treatment process more economical.

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