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Use of Bacillus consortium in waste digestion and pathogen control in shrimp aquaculture

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ABSTRACT

The wastewater from shrimp aquaculture industry contains high concentration of nitrogen and carbon. The use of microorganisms to remove nitrogen and carbon from shrimp aquaculture wastewater was investigated. A consortium of Bacillus species was used to enhance the performance of a sequencing batch reactor (SBR) for the treatment of shrimp aquaculture wastewater. An SBR is a variation of the activated sludge biological treatment process. This process accomplishes equalization, aeration, and clarification in a timed sequence in a single reactor basin to take the place of multiple tanks in conventional treatment systems. This is achieved through sequencing stages, which includes fill, react, settle, decant, and idle. The shrimp wastewater initially contained a high concentration of carbon and nitrogen. By operating the reactor sequentially, viz, aerobic, anaerobic, and aerobic modes, nitrification and denitrification were achieved as well as removal of carbon. Specifically, the initial chemical oxygen demand concentration of 1996 mg/L was reduced to 4 mg/L within eight days of reactor operation. Ammonia in the wastewater was nitrified within three days. The denitrification of nitrate was achieved by the anaerobic process and more than 99% removal of nitrogen was observed. The addition of Bacillus consortium also controlled the growth of shrimp pathogen, *Vibrio harveyi* in the wastewater.

Introduction

Humans have capitalized on the abundance of the ocean for a large portion of their sustenance for almost 2 million years. In recent times the human population has been increasing exponentially alongside our demand for seafood. As wild fishery stocks were noticeably decreasing due to overfishing and other causes it became economically and environmentally necessary to adapt the agrarian lifestyles into the “field” of seafood. The farming of aquatic organisms, hereinto referred to as aquaculture, has occurred for at least 1500 years (Costa-Pierce, 1987). Traditionally aquaculture was done on a very small scale using the natural resources at hand. Modern day aquaculture annual production has risen over the past 58 years from 1 million metric tons in 1950 to 52.55 million metric tons in 2008 (FAO, 2010). This places aquaculture as the source of 38% of the worlds seafood supply in 2009 (FAO, 2010). As wild fisheries continue to be exploited and therein decline, and human demand continues to grow, aquaculture will no doubt expand to provide higher proportions of the world's seafood. With this growth comes the need to mitigate the environmental impacts that aquaculture produces, while at the same time increasing its economic viability.

Shrimp have become a huge seafood staple, particularly in the US where the average citizen consumes 1.6 kg a year. Subsequently making shrimp the largest seafood commodity in value terms at approximately 15% of all globally traded seafood (FAO, 2010). This has led to declining shrimp populations and has resulted in a subsequent increase of bycatch. Shrimp trawl bycatch was most recently reported at a worldwide estimate of 11,207,761 metric tons. An average of 8.14 kg of bycatch is landed for every 1 kg of shrimp caught (Alverson et al., 1994). These environmental factors and as well as the state of our economy have given rise to increase focus on the aquaculture of shrimp.

Modern shrimp aquaculture began in the 1930s when Japanese scientists began raising kuruma shrimp (*Penaeus japonicas*) in hatcheries, but it wasn't until the early 1980s that commercial production of farm-raised shrimp began to occur (Weidner and Rosenberry, 1992). Most shrimp grown today use either an extensive, semi-intensive or intensive designs. Extensive farms focus on low investment and result in low yield and occur in low-lying

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impoundments alongside bodies of water. Semi-intensive farms increase shrimp density by stocking juveniles, supplementing feed, and are typically built above high tide line, requiring moderate investments, and resulting in increased production. Intensive farms require significant investments such as building a facility, feeding, waste removal, aeration, and round-the-clock management. Commercial scale super intensive raceways farms often suffer from the same issues, but some success has been made in recent years with the use of biofloc technology (Weidner and Rosenberry, 1992; Krummenaur et al., 2011).

While shrimp aquaculture has been successful and grown significantly throughout the developing world, the few shrimp farms in the US continue to face issues while struggling to compete against foreign shrimp farms. In 1991, 1.18 billion pounds of shrimp were imported into the US, representing 85% of the total US shrimp supply and less than 5% of them were grown in the Western hemisphere. This amounts to a burgeoning trade deficit, which was approximately 3.1 billion dollars in 1998. The United States Department of Agriculture has been aware of this trade deficit and subsequent lack of food security, and in response created the United States Marine Shrimp Farming Program (USMSFP) to increase shrimp production in the US (Boopathy and Lyles, 2008).

Many key areas have been identified by the USMSFP that require new research and development to enable the US shrimp farming to successfully compete with the rest of the world. Traditional farms in the US are built as large ponds close to coastal areas to provide ease of water exchange between the ponds and the marine environment. Commercial coastal land is often difficult to find and typically more expensive than inland property, and ponds exposed to the local seasons limit shrimp production to certain times of the year (Browdy and Moss, 2005). The amount of waste produced by high intensity aquaculture is another issue that must be dealt with. Typically effluents from aquaculture are characterized by increased nitrogen species (ammonia, nitrites, and nitrates), organic carbon, phosphates, suspended solids, and high biological oxygen demand (BOD) and chemical oxygen demand (COD) (Boopathy and Lyles, 2008). Significant issues can result in the release of nutrient rich effluents such as these including increased algal blooms, degradation of benthic communities, oxygen depletion, and overall degraded water quality (Boyd, 2003). Governmental entities within the U.S. such as the Environmental Protection Agency (EPA) have ruled that in order to protect United States waterways certain standards and limitations must be met before wastewater can be released. These regulations are set under the Clean Water Act, and restrict discharges from aquatic animal production facilities into public waterways in the U.S.

During the 1970s sequencing batch reactor (SBR) technology and system design were still very much in the beginning phases of research (Irwine and Ketchum, 1989). An SBR consists of a single reactor vessel in which the activated sludge process is adapted to perform nitrification and denitrification in a timed sequence (Boopathy et al., 2005). A typical SBR sequence consists of the following 5 unique stages, loading/fill, react, settling, effluent extraction/decanting, and idle (Marsili-Libelli, 2006; Boopathy and Lyles, 2008). The react stage typically contains a fixed schedule of both an aerobic phase and an anaerobic phase, enabling nitrification and denitrification to take place.

Throughout the years a variety of SBR systems have been adapted for the treatment of many types of waste water such as human waste, animal waste, tannery waste, and slaughterhouse waste (Bernet et al., 2000; Sirianuntapiboon and Manoonpong, 2001; Murat et al., 2002). While standard wastewater treatment systems have shown to be ineffective on large high intensity systems, SBR systems have shown significant success (Boopathy et al., 2005). Many benefits exist of SBRs over other treatment options, such as increased control over the reliability, precision and versatility of the reactor (Stricker and Béland, 2006). SBR infrastructure requires less capital investment and significantly less plumbing, and space requirements when compared to other treatments (Irwine and Ketchum, 1989; Boopathy and Lyles, 2008). These benefits make SBR technology an ideal candidate for treating high intensity shrimp wastewater.

Successful cycling of an SBR requires monitoring of the conditions present to ensure the ideal characteristics for the metabolism of the specialized bacteria (Marsili-Libelli, 2006). The proper carbon/nitrogen ratio is important for the ideal growth of the bacteria and ideal nutrient reduction. A ratio of 10:1 C:N has been determined to yield ideal reduction in shrimp wastewater by Fontenot et al. (2006). Hydrolyzed molasses can be a cheap and effective external carbon source to correct the C:N ratio of the wastewater (Quan et al., 2005; Fontenot et al., 2006; Roy et al., 2010). Adequate aeration is also vital for proper aerobic metabolism during the aeration phase (Burt et al., 1990). Venturi tubes have been used to provide sufficient aeration to waste water and have many benefits over other technologies including increase efficiency, simplification, and aeration (Baylar et al., 2007). Oxidation reduction potential (ORP), dissolved oxygen (DO), and pH have shown to be valuable indicators as to the current state of the bacteria in the reactor and can be used to automate the cycling of the reactor to shorten the cycle times and yield idealized processing (Marsili-Libelli, 2006). Fontenot et al. (2006) found that temperature ranges of 22–37 °C showed significant nutrient reduction capabilities over higher temperatures. In the past, our laboratory has shown successful treatment of shrimp production wastewater (Boopathy et al., 2005; Boopathy and Lyles, 2008). In the present study, we enhanced the performance of SBR by the use of Bacillus consortium as an inoculum treating the wastewater from an intensive shrimp raceway aquaculture system. The hypothesis is the addition of Bacillus will not only help in organic carbon and nitrogen reduction in the SBR, but also will reduce shrimp pathogens as Bacillus is known to be a probiotic microbe. The results from this operation indeed showed successful removal of nitrogen and carbon in the wastewater along with the removal of shrimp pathogen, *Vibrio harveyi*.

Materials and methods

Shrimp wastewater

Shrimp wastewater from an intensive raceway system was collected from a sediment settling tank at the Gulf Coast Research Laboratory (GCRL), Ocean Springs, Mississippi, USA. The sample was analyzed for various characteristics including chemical oxygen demand (COD), ammonia, nitrate, nitrite, and pH. This wastewater was used to run the sequencing batch reactor.

Sequencing batch reactor (SBR)

Three sets of identical SBRs were operated with shrimp wastewater and in each set triplicate SBRs were maintained with a total of nine SBRs. One set of reactor was operated without additional inoculum (indigenous bacteria in the waste served as a bacterial source for waste digestion). The second set of reactor received an inoculum of Bacillus consortium at 10^6 cells/mL along with the indigenous bacteria in the waste. In the third set, the waste was autoclaved to kill the indigenous bacteria and 10^6 cells/mL of Bacillus consortium was used as inoculum for waste digestion. The Bacillus consortium was developed from our earlier work (Boopathy and Lyles, 2008) and the consortium contains *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus licheniformis*, and *Bacillus*