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. A 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.

Keywords: Nitrification, denitrification, sequencing batch reactor, aquaculture, *Vibrio harveyi*, *Bacillus*.

1. 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 1930’s when Japanese scientists began raising kuruma shrimp (*Penaeus japonicas*) in hatcheries, but it wasn’t until the early 1980’s 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

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

94
95 While shrimp aquaculture has been successful and grown significantly throughout
96 the developing world, the few shrimp farms in the US continue to face issues while
97 struggling to compete against foreign shrimp farms. In 1991, 1.18 billion pounds of
98 shrimp were imported into the US, representing 85% of the total US shrimp supply and
99 less than 5% of the them were grown in the Western hemisphere. This amounts to a
100 burgeoning trade deficit, which was approximately 3.1 billion dollars in 1998. The
101 United States Department of Agriculture has been aware of this trade deficit and
102 subsequent lack of food security, and in response created the United States Marine
103 Shrimp Farming Program (USMSFP) to increase shrimp production in the US (Boopathy
104 and Lyles 2008).

105
106 Many key areas have been identified by the USMSFP that require new research
107 and development to enable the US shrimp farming to successfully compete with the rest
108 of the world. Traditional farms in the US are built as large ponds close to coastal areas to
109 provide ease of water exchange between the ponds and the marine environment.
110 Commercial coastal land is often difficult to find and typically more expensive than
111 inland property, and ponds exposed to the local seasons limit shrimp production to certain
112 times of the year (Browdy and Moss, 2005). The amount of waste produced by high
113 intensity aquaculture is another issue that must be dealt with. Typically effluents from
114 aquaculture are characterized by increased nitrogen species (ammonia, nitrites, and
115 nitrates), organic carbon, phosphates, suspended solids, and high biological oxygen
116 demand (BOD) and chemical oxygen demand (COD) (Boopathy and Lyles 2008).

117 Significant issues can result in the release of nutrient rich effluents such as these
118 including increased algal blooms, degradation of benthic communities, oxygen depletion,
119 and overall degraded water quality (Boyd 2003). Governmental entities within the U.S.
120 such as the Environmental Protection Agency (EPA) have ruled that in order to protect
121 United States waterways certain standards and limitations must be met before wastewater
122 can be released. These regulations are set under the Clean Water Act, and restrict
123 discharges from aquatic animal production facilities into public waterways in the U.S.
124

125 During the 1970's sequencing batch reactor (SBR) technology and system design
126 were still very much in the beginning phases of research (Irvine and Ketchum, 1989). A
127 SBR consists of a single reactor vessel in which the activated sludge process is adapted to
128 perform nitrification and denitrification in a timed sequence (Boopathy *et al.*, 2005). A
129 typical SBR sequence consists of the following 5 unique stages, loading/fill, react,
130 settling, effluent extraction/decanting, and idle (Boopathy and Lyles, 2008; Marsili-
131 Libelli 2006). The react stage typically contains a fixed schedule of both an aerobic phase
132 and an anaerobic phase, enabling nitrification and denitrification to take place.
133

134 Throughout the years a variety of SBR systems have been adapted for the
135 treatment of many types of waste water such as human waste, animal waste, tannery
136 waste, and slaughterhouse waste (Bernett *et al.*, 2000; Murat *et al.*, 2002;
137 Sirianuntapiboon and Manoonpong, 2001). While standard wastewater treatment
138 systems have shown to be ineffective on large high intensity systems, SBR systems have
139 shown significant success (Boopathy *et al.*, 2005). Many benefits exist of SBR's over
140 other treatment options, such as increased control over the reliability, precision and
141 versatility of the reactor (Stricker and Béland 2006). SBR infrastructure requires less
142 capital investment and significantly less plumbing, and space requirements when
143 compared to other treatments (Boopathy and Lyles, 2008; Irwine and Ketchum 1989).
144 These benefits make SBR technology an ideal candidate for treating high intensity shrimp
145 wastewater.
146

Successful cycling of a 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 ratio has been determined to yield ideal reduction in shrimp wastewater by Fontenot *et al.* (2007). Hydrolyzed molasses can be a cheap and effective external carbon source to correct the C:N ratio of the wastewater (Fontenot *et al.*, 2007; Quan *et al.*, 2004; 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.*, 2007 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*.

2. Materials and Methods

2.1. 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.

2.2. 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 *B. subtilis*, *B. megaterium*, *B. licheniformis*, and *B. cereus* in equal cell numbers. The consortium was maintained in tryptic soy broth (TSB). Each reactor received 1000 L of wastewater at the beginning of the experiment. The reactors were aerated using an aerator. Aeration was turned off for the system to run anaerobically. The reactors were operated aerobically for the first three days and anaerobically for three days followed by one day aerobic operation as shown in Figure 1. SBR process for nitrogen removal may be divided into 2 stages as follows: Aerobic Stage: In this stage the carbon oxidation and nitrification are combined into the single process to achieve nitrification and COD removal and Anaerobic Stage: The second stage is an anaerobic process in which denitrification is accomplished.

2.3 Shrimp Pathogen Removal Experiment

An experiment was conducted to study the removal of a shrimp pathogen, *Vibrio harveyi* in the SBR operation. *Vibrio* species are the most numerous culturable bacteria commonly found in the marine environment, including shrimp farms (Gopal et al. 2005). Pathogenic *Vibrio harveyi* is responsible for vasculitis, enteritis, and eye lesions in marine fish, and is associated with luminous vibriosis in shrimp and lobster (Austin and Zhang, 2006). Virulent strains of *V. harveyi* was identified to cause mass mortality in farmed larval tiger shrimp (*Penaeus monodon*), and both luminescence and antibiotic

resistance were observed with the virulent strains of *V. harveyi* isolated from dead tiger shrimp (Karunasagar et al. 1994). The major bacterial diseases include vibriosis caused by vibrio species and necrotizing hepatopancreatitis (NHP) caused by obligate intracellular rickettsia-like bacteria (Lightner, 2005). The same set of experimental conditions described in section 2.2 were maintained with the addition of 10^{10} *V. harveyi* (ATCC 14126) at the beginning of the experiment. *V. harveyi* in the SBR was monitored by bacterial plate counts using *V. harveyi* medium as described by Adams and Boopathy (2013). *V. harveyi* was obtained from the American type culture collection (ATCC), Manassas, Virginia, USA

2.4. Analyses

Thirty ml wastewater was removed from each reactor and centrifuged at 5000 rpm for 10 minutes and the supernatant was used for the chemical analysis during every sampling event. Nitrite, nitrate, and ammonia were analyzed periodically by colorimetric methods with a Hach water analysis kit (Hach 1999). The chemical oxygen demand (COD) was analyzed using standard methods (APHA, 1998). Total COD was analyzed using the whole sample and soluble COD was analyzed using filtered sample using 0.2 μ m filter paper. The soluble COD excludes carbon from microorganisms in the wastewater and it represents the soluble organic carbon in the wastewater. The dissolved oxygen (DO), salinity, and temperature were measured using an YSI DO and salinity probe (Model No. 85-10FT, Yellow Spring, OH). The pH was measured using a pH probe (Model UB 10, Denver Instruments, Boulder, CO). The quality control was maintained by EPA protocol for quality assurance and quality control (QAQC)

2.5. Statistical Analysis

All data were subjected to an analysis of variance (ANOVA) test ($p \leq 0.05$) followed by a tukey “*post hoc*” analysis when needed (SAS). COD concentrations were analyzed using a paired t-test analysis ($p \leq 0.05$; SAS).

3. Results and Discussion

3.1. Characteristics of Shrimp Wastewater

The initial characteristic of the shrimp wastewater is given in Table 1. The wastewater contained high concentration of carbon (COD), ammonia, nitrate, and nitrite. The pH of the wastewater was 8.1.

3.2. Performance of SBR

The initial carbon in the form of soluble COD was very high in the shrimp wastewater. All three treatments of SBR showed removal of COD. However, the rate of removal of COD was high in the SBR that received *Bacillus* consortium as an inoculum at the beginning of the experiment followed by SBR without *Bacillus* inoculum and the SBR with *Bacillus* that received autoclaved wastewater to kill all bacteria in the waste (Fig. 2). Specifically, 99.75% COD was removed in the SBR that received non-autoclaved wastewater with *Bacillus* consortium as inoculum. This removal was achieved within six days of reactor operation. In the SBR that did not receive *Bacillus*, the COD removal was 96% in eight days. The SBR that was operated with autoclaved waste with *Bacillus* removed 93% COD within eight days. This results showed that the initial one time addition of *Bacillus* consortium to the reactor significantly enhanced the COD removal and the presence of other bacteria in the wastewater really helped in a synergistic way to remove the organic carbon from the shrimp wastewater. Shrimp wastewater contains significant amount of bacteria including nitrifiers and denitrifiers (Boopathy et al. 2005; Fontenot et al. 2007; Boopathy and Lyles, 2008).

Figure 2 shows the total nitrogen including ammonia, nitrite, and nitrate in various SBR reactors. Similar to COD removal, the best nitrogen removal was achieved in the SBR that received *Bacillus* consortium with indigenous bacteria present in the shrimp wastewater. More than 99% of nitrogen was removed within 7 days of reactor operation in this SBR. Nitrogen removal was 95% in the SBR without *Bacillus* addition in 8 days. In the SBR that received the autoclaved wastewater with *Bacillus* consortium performed the least in terms of nitrogen removal with 85% nitrogen removal in 8 days. This is

because of the loss of nitrifiers and denitrifiers that are present in the shrimp wastewater due to autoclaving of the wastewater. This results showed that the addition of *Bacillus* consortium helps the indigenous bacteria present in the wastewater to enhance the nitrogen removal.

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 ratio has been determined to yield ideal reduction in shrimp wastewater by Fontenot *et al.* (2007). Hydrolyzed molasses can be a cheap and effective external carbon source to correct the C:N ratio of the wastewater (Fontenot *et al.*, 2007; Quan *et al.*, 2004; 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.*, 2007 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) using SBR without external inoculum source. In the present study, the waste removal was enhanced by the addition of external inoculum, which helped faster removal of carbon and nitrogen in the wastewater. This will help in better management of recycling of water in the shrimp production system.

The SBR successfully removed carbon and nitrogen from the wastewater of an active shrimp aquaculture system. 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 (Irvine and Ketchum 1989; Masse and Masse 2000; Lo *et al.* 1991). The wastewater problem in shrimp aquaculture is addressed by activated sludge process, foam fractions, use of filter

systems, and sludge management (Browdy and Moss, 2005; Holloway 2002). 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 three days and anaerobic process for three days to remove 99% of carbon and nitrogen in the wastewater. The wastewater contained heterogenic populations of bacteria to carry out nitrification and denitrification 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 wastewater during the aerobic cycle. The denitrifying organisms dominated the system during the anaerobic operation of SBR. This was supported by the fact that the levels of nitrite and nitrate dropped significantly under the anaerobic phase of the reactor operation. The carbon was effectively removed under both aerobic and anaerobic conditions in the SBR. However, the addition of *Bacillus* consortium enhanced both carbon and nitrogen at a faster rate. The application of SBR technology for intensive shrimp production is an attractive alternative to various methods currently used in shrimp aquaculture. The hydraulic retention time can be shortened by three days from 8 to 5 when *Bacillus* consortium was used as an inoculum.

3.3. Pathogen Removal in the SBR

Mass mortality, slow growth, and deformity of shrimp cause major economic losses in shrimp aquaculture. Multiple viral and bacterial pathogens are globally reported to be suspected agents that cause mass mortalities. One of the most common diseases that cause significant damage to shrimp industry is Vibriosis caused by a bacteria called *Vibrio harveyi*. An experiment was conducted to study the effectiveness of the *Bacillus* consortium as a probiotic to control *V. harveyi* in the shrimp wastewater. As described in the method section, three sets of SBR were operated with the addition of *V. harveyi* at 10^{10} cells/ml of the reactor at the beginning of the experiment. The population of *V. harveyi* in the SBR was monitored daily using a special *V. harveyi* medium. Figure 3 shows clearly the probiotic effect of *Bacillus* culture as the SBR that received autoclaved

wastewater with *Bacillus* and *V. harveyi* showed significant decrease in *V. harveyi* counts. This reactor showed a six-log decrease in *V. harveyi* within 8 days. This was followed by the reactor that received *Bacillus* with indigenous bacteria (non-autoclaved wastewater), which showed four-log decrease in the pathogen count. The SBR that did not receive any *Bacillus* cultures showed no loss of *V. harveyi* and its numbers were constant at 10^{10} CFU/ml throughout the study.

In order to show the probiotic properties of the *Bacillus* consortium, a simple study was conducted where *Bacillus* culture was grown as a single streak in the middle of a tryptic soy agar plate and this culture was allowed to grow for 24 hours at 30°C. The following day four different *Vibrio* species, namely, *Vibrio harveyi*, *Vibrio parahaemolyticus*, *Vibrio cholerae*, and *Vibrio vulnificus* (obtained from ATCC) were streaked across the *Bacillus* streak as shown in Fig. 5 and allowed to grow for 24 hours. The results showed the clear inhibition of bacterial growth in *V. harveyi*, *V. cholerae*, and *V. vulnificus* indicated by no growth near *Bacillus* streak. These three *Vibrio* species are sensitive to the presence of *Bacillus* species. The only *Vibrio* that grew in the presence of *Bacillus* was *V. parahaemolyticus*, which showed resistance as there was significant growth near *Bacillus* culture (Fig. 5). *Bacillus* is a known antibiotic producer, which produces antibiotics like bacitracin and polymyxin in nature (Batzing, 2002). In the SBR that received *Bacillus* consortium these antibiotics might have been produced to suppress the *V. harveyi* counts as shown in Fig. 4. This experiment demonstrated the added advantage of using *Bacillus* consortium as an inoculum. It not only enhanced waste digestion, but also inhibited the growth of one of the dangerous shrimp pathogens from the wastewater. This will benefit the shrimp industry in two ways, namely carbon and nitrogen removal and pathogen control and provide better management of shrimp wastewater in treatment as well as reuse of the treated water in shrimp production.

4. Conclusions

This study showed successful removal of carbon in the form of COD, ammonia, nitrate, and nitrite in a high intensity shrimp raceway system by a SBR. The removal efficiencies and rates were improved by the addition of *Bacillus* consortium as an

inoculum. This *Bacillus* consortium has probiotic properties as demonstrated by the suppression of growth of a shrimp pathogen, *V. harveyi*. Use of *Bacillus* inoculum will provide better management of shrimp wastewater and reuse in the shrimp production system. The advantage of using SBR technology is its simplicity. A SBR is a variation of the activated sludge process. This process uses multiple steps in the same reactor and the nitrogen removal was accomplished by the sequential operation of the reactor aerobically followed by anaerobic process. Though there was no start-up period, inoculum addition did enhance carbon and nitrogen removal rates in the wastewater.

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Table 1. Characteristics of the shrimp wastewater*

Parameter	Concentration
Total COD (mg/L)	1996 ± 24
Ammonia (mg/L)	289.1 ± 9.1
Nitrate (mg/L)	101.3 ± 2.4
Nitrite (mg/L)	66 ± 2.7
Total nitrogen (mg/L)	456.4 ± 4.7
Salinity (ppt)	28.6 ± 0.4
pH	8.1 ± 0.1

*Average of 5 analyses with standard deviation

Figure Legends

Figure 1. This diagram shows the sequencing series for wastewater treatment, which consists of the following process stages: fill, react, settle, decant and idle. These process stages occur in a timed sequence in a single reactor basin.

Figure. 2. Soluble COD concentration in all three SBR reactors. The S.D. was less than 5%. The statistical analysis showed significance with a p value of <0.05 .

Figure. 3. Total nitrogen including ammonia, nitrite, and nitrate in all three SBR reactors. The S.D. was less than 5%. The statistical analysis showed significance with a p value of <0.05 .

Figure. 4. Population of *Vibrio harveyi* in the SBR. At the beginning of the experiment 10^{10} Colony Forming Unit (CFU)/ml of the bacteria were inoculated in all three SBR. *V.harveyi* was monitored daily using a *V. harveyi* medium.

Figure. 5. Effect of Bacillus consortium on various Vibrio species. Bacillus consortium was streaked single line in the middle of a Tryptic Soy Agar (TSA) plate and allowed to grow overnight (allow time for probiotic to leach into media). Various ATCC Vibrio cultures were streaked from right to left and incubated at 30° C for 24 hours. No growth near Bacillus streak = sensitivity. Vibrio species: Vh: *Vibrio harveyi*, Vp: *Vibrio parahaemolyticus*, Vch: *Vibrio cholerae*, and Vv: *Vibrio vulnificus*. All Vibrio species were obtained from American Type Culture Collection.

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Fig. 3. Total nitrogen including ammonia, nitrite, and nitrate in all three SBR reactors. The S.D. was less than 5%. The statistical analysis showed significance with a p value of <0.05 .

Figure. 4. Population of *Vibrio harveyi* in the SBR. At the beginning of the experiment 10^{10} Colony Forming Unit (CFU)/ml of the bacteria were inoculated in all three SBR. *V.harveyi* was monitored daily using a *V. harveyi* medium.

Fig. 5. Effect of Bacillus consortium on various Vibrio species. Bacillus consortium was streaked single line in the middle of a Tryptic Soy Agar (TSA) plate and allowed to grow overnight (allow time for probiotic to leach into media). Various ATCC Vibrio cultures were streaked from right to left and incubated at 30° C for 24 hours. No growth near Bacillus streak = sensitivity. Vibrio species: Vh: *Vibrio harveyi*, Vp: *Vibrio parahaemolyticus*, Vch: *Vibrio cholerae*, and Vv: *Vibrio vulnificus*. All Vibrio species were obtained from American Type Culture Collection.