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Bioremediation - A potential tool for management of aquatic pollution

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Abstract

Aquaculture aimed at high production through intense aquaculture practices which produce large amounts of organic wastes that often leads not only to the alteration in water quality but also to severe disease problems. Development of aquaculture sector can be achieved by adopting eco-friendly aquaculture practices by minimizing impact on the surrounding environment. Oxidation of these waste compounds depletes the level of dissolved oxygen in aquatic environment and encourages the formation of toxic metabolites such as ammonia (NH_3), nitrite (NO_2^-) and hydrogen sulfide (H_2S). Such conditions increase the mortality rates in aquaculture farming. Hence, NH_4^+ or NO_3^- removal processes (nitrification and denitrification) become essential for the pond water quality. This can be carried out by nitrifying or denitrifying bacteria such as *Nitrobacter*, *Thiobacillus* and *Paracoccus*. *Thiobacillus* also allows the removal of two contaminants (such as H_2S and NO_3^-) in a single step, converting them into environmentally favorable compounds (SO_4^{2-} and N_2). Dimethyl sulfide (DMS) and oil spill are also play major role in aquatic pollution. Bioremediation are inexpensive, effective, environment friendly and safe technology that improving water quality and maintaining the health and stability of aquaculture systems offers innovative way to clean up hazards waste mineralized to carbon dioxide, maximising primary productivity, nitrification and denitrification. It is biodegradable as well as recalcitrant.

Keywords: Bioremediation, Eco-friendly technology, N_2 -cycle, Probiotic and Aquatic management.

1. Introduction

Bioremediation is a beneficial microbiological agents, mainly e.g. yeast, fungi or bacteria to clean up contaminated soil and water. It is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes. All waters bodies (such as Lakes, rivers, wetlands etc.) are affected by either point or non-point source of pollution. Point sources of pollution occur when the polluting substance is emitted directly into the waterway. The common point sources of pollution are municipal and industrial wastewater effluents; run-off and leachate from solid disposal sites; run-off from industrial sites; run-off and drainage from industrial sites; discharge from vessels. The non-point source includes flow of water from agricultural fields and orchards, urban run-off from unsewered areas, etc. The effects of water pollution are not only devastating to the aquatic animal but also to the terrestrial animals and birds. More seriously, contaminated water destroys aquatic life and reduces its reproductive ability. Ultimately, it becomes a hazard to human health. Waste disposal has an environment cost and a financial cost also. It can reduce by using of bioremediating agent. Increased production is being achieved by the expansion of land and water under culture and the use of more intensive and modern farming technologies that involve higher usage of inputs such as water, feeds, fertilizers and chemicals. As a result, aquaculture is now considered as a potential polluter of the aquatic environment and a cause of degradation of wetland areas (Pillay, 1992).

India endowed with potential aquatic resources including Coast line: 8118km, EEZ: 2.02million km^2 , Continental shelf: 0.53 million km^2 , Estuarine: Area of brackish water: 1.44 million ha, Rivers: 29000 km, Reservoirs: 3.15 million ha, Tanks and ponds: 2.25 million ha, Flood plains, wetlands and oxbow lakes: 3.54 lakh ha (Ayyappan.S, 2011).

2. Bioremediation – Concept

It is an advance technology that use to improve water quality in aquaculture is the application of probiotics and/or enzymes to the ponds. This type of biotechnology is known as “*bioremediation*”, which involves manipulation of microorganisms in ponds to enhance mineralization of organic matter and get rid of undesirable waste compounds.

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2.1 Principle of Bioremediation

- Bioremediation is as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities.
- As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation (enzymatically attack the pollutants) to proceed at a faster rate and convert them to harmless products.
- The use of competitive exclusion for improving a specific ecology is called “probiotics” (Parker, 1974). The probiotic treatments may be considered as methods of biological control, the so called “Biocontrol” that termed the limitation or the elimination of pests by the introduction of adverse organisms, like parasites or specific pathogens.

2.2. Source of Waste Production in Aquaculture

The wastes in aquaculture farms can be categorized as: (1) Residual food and faecal matter; (2) Metabolic by-products; (3) Residues of biocides and biostats; (4) Fertilizer derived wastes; (5) Waste produced during moulting; and (6) Collapsing algal blooms (Sharma and Scheeno, 1999). The new ecofriendly technology for improving water quality in aquaculture is the application of microbes/enzymes to the ponds, known as ‘bioremediation’. When macro and microorganisms and/or their products are used as additives to improve water quality, they are referred to as bioremediators or bioremediating agents (Moriarty, 1998).

2.3 Bioremediation in Domestic Sewage

Nitrifying bacteria (*Nitrosomonas spp.* and *Nitrobacter spp.*) thrive in lakes and streams with high input of sewage and waste water because of high ammonia content. 16s rDNA clone libraries shows that *Klebsiella sp.* and *Citrobacter sp.* - dominant within the initial biofilm thickness of 0-250 μ . Overall results infer that coliform bacteria participated in the Nitrate and Phosphorus removal from domestic waste water. Sulphur oxidising bacteria such as *Beggiatoa* and *Thiothrix sp.* present in biofilm thickness over 250 μ . Cis 2 – decenoic acid secreted by *Pseudomonas aeruginosa* and the Yeast *Candida albicans*: Inducing Dispersion and inhibiting growth of biofilm colonies.

2.4 Microbial Precipitation of Metal Phosphates

Citrobacter sp. bacteria immobilised in a polyacrylamide gel or colonised on glass helices and supplied with citrate and glycerol 2- phosphate remove more than 90% of the lead and uranium. Mechanism of Precipitation is the liberation of HPO_4^{2-} from suitable organic phosphate substances by means of a surface located metal resistant enzymes which precipitates metal ion.

2.5 Bioremediation in Paper and Pulp Industry

Dimethyl sulfide (DMS) is one of the sulfurous pollutants present in the waste generated from the pulp and paper industry (B.S.Giri, 2012). The potential culture for degradation of DMS was identified as *Bacillus sphaericus* by 16s rRNA molecular analysis. Utilization of pulp, paper, and cardboard industry sludge and waste water for the isolation and screening of polyhydroxyalkanoates (PHAs)

accumulating bacteria *Enterococcus sp.* NAP11 and *Brevundimonas sp.* NAC1 can be considered as good candidates for industrial production of PHB from cardboard industry waste water (Anish Kumari Bhuwal *et al.*, 2013). PHAs (biocompatible bioplastics) can be completely degraded within a year by variety of microorganisms into CO_2 and water.

Predominant bacteria in paper mill effluent for evaluating the degradation efficiency of combination of isolates to treat the released effluent. Effective floc formation and degradation was attained in *Pseudomonas (alkaligenes + Enterobacter spp.)* combination which enhance clearing and settling process in the treatment plants.

2.6 Bioremediation in Oil Spill

Bacteria (*Pseudomonas sp.*, HC1 and *Raulstonia*) can break down oil to carbon dioxide and water which is used by primary producer.

2.7. Bioremediators as Disease Controlling Agents

Beneficial microbes, such as non-pathogenic isolates of *Vibrio alginolyticus*, can be inoculated into shrimp culture systems to suppress the pathogenic vibrios like *Vibrio harveyi*, *Vibrio parahaemolyticus* and *Vibrio splendens* and reduce the opportunistic invasion of these pathogens in shrimps (Jameson, 2003). Most probiotics proposed as biological control agents in aquaculture belong to the Lactic Acid Bacteria (*Lactobacillus*, *Carnobacterium* etc.), *Vibrio (Vibrio alginolyticus)*, *Bacillus*, and *Pseudomonas* (Singh *et al.*, 2001). Abraham *et al.* (2001) studied *in-vitro* antagonistic activity of penaeid shrimp larvae associated bacterium, *Alteromonas*, against several opportunistic crustacean pathogens and found that the *Alteromonas* species suppressed the activity of *Vibrio harveyi* and improved the survival of *Penaeus indicus* larvae *in-vivo*. Larvae are highly exposed to gastrointestinal microbiota-associated disorders, because they start feeding even through the digestive tract is not yet fully developed, though the immune system is still incomplete. Thus, probiotic treatments are particularly desirable during the larval stages.

3. Probiotics

The concept of biological disease control, particularly using microbiological modulator for disease prevention, has received widespread attention. A bacterial supplement of a single or mixed culture of selected non-pathogenic bacterial strains was termed probiotics.

3.1 Beneficial Effects of Probiotics Are

- Neutralization of toxin
- Suppression of viable count
- Production of antibacterial compounds
- Competition for adhesion sites
- Alternation of microbial metabolism and Production of enzymes
- Stimulation of immunity in the host
- Accelerate the sediment decomposition by producing organic acids
- Production of hydrogen peroxide

Table 1: Types of Probiotics

Non-viable probiotics	These are dead.
Freeze-dried probiotics	These will die rapidly upon leaving refrigeration.
Fermentation probiotics	These are produced through fermentation.
Viable probiotics	This is live with guaranteed shelf life, guaranteed number of organisms, have a protocol for counting and to be very stable and efficacious.

Table 2: Function of probiotics/beneficial organisms

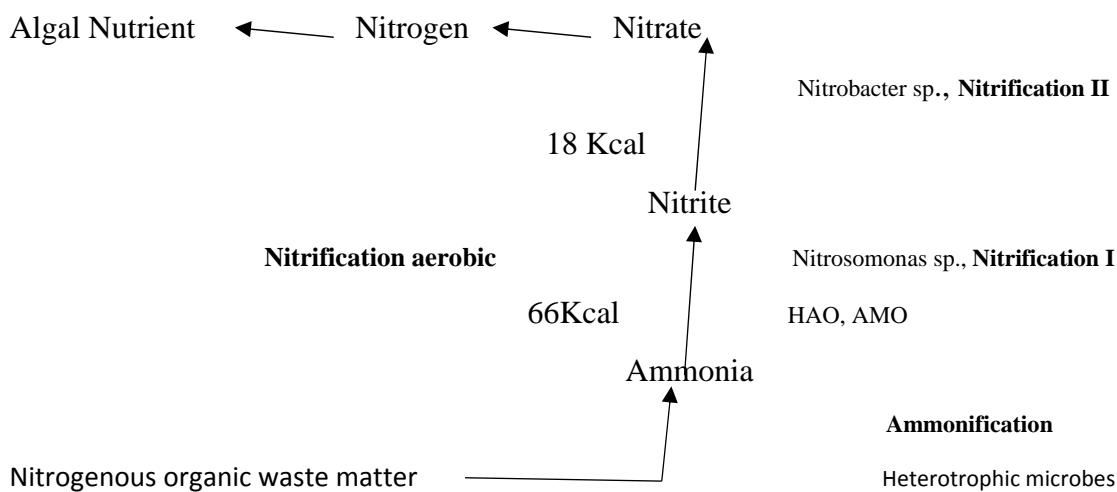
Probiotics	Role/ function
<i>Bacillus</i> sp.	Mineralization and Breakage of proteins
<i>Nitrosomonas</i> sp.	Oxidation of ammonia
<i>Nitrobacter</i> sp.	Oxidation of nitrites
<i>Aerobacter</i> sp.	Reduction of organic matter
<i>Cellulomonas</i> sp.	Breakage of plant material

4. Bioremediation of Organic Detritus

Beneficial bacteria produce a variety of enzymes that break down proteins and starch to small molecules, which are then taken up as energy sources by other organisms. The selection of indigenous bacteria able to degrade a wide range of natural organic compounds (e.g., lipids, proteins and carbohydrates) constitutes a new approach to study the potential of bioaugmentation in eutrophicated environment. A good bioremediator must contain microbes that are capable of effectively clearing carbonaceous wastes from water. Members of the genus *Bacillus*, like *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus cereus*, *Bacillus coagulans*, and of the genus *Paenibacillus*, like *Paenibacillus polymyxa*, are good examples of bacteria suitable for bioremediation of organic detritus. *Lactobacillus* is also used along with *Bacillus* to break down the organic detritus. The removal of large organic compounds reduces water turbidity (Haung, 2003).

However, these bacteria are not normally present in the

5. Nitrification Process



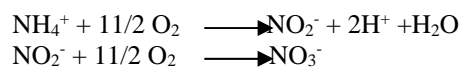
(Unconsumed feed, faecal matter, moults, dead algae, excretory waste etc.)

required amounts in the water column, their natural habitat being the sediment. They compete with the bacterial flora naturally present for the available organic matter, like leached or excess feed and shrimp faeces (Sharma, 1999).

6. Bioremediation of Nitrogenous Compounds

The principal sources of ammonia are fish excretion and sediment flux derived from the mineralization of organic matter and molecular diffusion from reduced sediment, although cyanobacterial nitrogen fixation and atmospheric deposition are occasionally important (Ayyappan and Mishra, 2003). For the removal of ammonia from closed aquaculture systems, bacteriological nitrification is the most practical method and it is commonly achieved by setting of sand and gravel bio-filter through which water is allowed to circulate. The ammonia oxidizers are placed under five genera, *Nitrosomonas*, *Nitrosovibrio*, *Nitrosococcus*, *Nitrolobus* and *Nitrospira*, and nitrite oxidizers under three genera *Nitrobacter*, *Nitrococcus* and *Nitrospira* (S.P. Antony and R. Philip, 2006).

Applications of Nitrogen in pond assimilatory capacity can lead to deterioration of water quality through the accumulation of nitrogenous compounds (e.g. Ammonia and nitrite) with toxicity to fish and shrimp. Nitrification proceeds as follows



Nitrification not only produces nitrate but also alters the pH slightly towards the acidic range, facilitating the availability of soluble materials (Ayyappan and Mishra, 2003). The vast majority of aquaculture ponds accumulate nitrate, as they do not contain a denitrifying filter. Denitrifying filters helps to convert nitrate to nitrogen. It creates an anaerobic region where anaerobic bacteria can grow and reduce nitrate to nitrogen gas (Rao, 2002). Among these, *Pseudomonas*, *Bacillus* and *Alcaligenes* are the most prominent numerically. Nitrate is the end product of nitrification.

7. Nitrogen Cycle

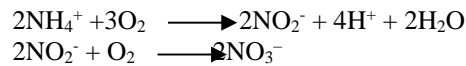
The principal excretory product of most aquatic organisms is ammonia. It is toxic, acutely and chronically, to fish and invertebrates thus it is a critical water quality factor. Fish, prawn and shrimp excrete ammonia as waste from their gills, kidneys and normal respiration. Ammonia also develops from unconsumed feeds, shell moults of prawn and shrimp, dead algae, zooplankton etc. by the microbial activity.

But inputs of ammonia cannot be eliminated from the water body. Ammonia should be maintained below 0.1 mg/L (total ammonia). The most efficient way to do this is by the establishment of a biological filter. A biological filter is a collection of naturally occurring bacteria, which oxidize ammonia to nitrite, and other bacteria, which then convert nitrite to nitrate. Nitrite is formed either by the oxidation of ammonia (nitrification) or the reduction of nitrate (denitrification). Nitrite is toxic to fish and some invertebrates and should be maintained below 0.1 mg/L. It is also a critical water quality factor.

In general, nitrate should be maintained below 50 mg/L (measured as NO₃-N) but it is not a critical water quality factor. The most common ways to reduce nitrate are water changes and growing live plants. A denitrifying filter creates an anaerobic region where anaerobic bacteria can grow and reduce nitrate to nitrogen gas. A poorly run denitrifying filter does not convert nitrate all the way to nitrogen gas but instead produces nitrite.

The nitrogen 'cycle' is the oxidation of ammonia to nitrite by bacteria of the genus *Nitrosomonas* and the subsequent oxidation of the nitrite to nitrate by bacteria belonging to the genus *Nitrobacter*. A species of bacteria called

"*Nitrosomonas*" converts this ammonia into nitrite. A second species of bacteria called "*Nitrobacterium*" converts this nitrite into nitrate.



Algae and aquatic plants utilize nitrate to produce chlorophyll, which are in turn consumed by zooplankton and then by fish, prawn and shrimp. Then the cycle repeats. These bacteria are important to aqua farmers because without them it is difficult to maintain healthy environmental conditions in the aquaculture ponds.

8. Bioremediation in Municipal Solid Waste

From Municipal Solid Waste numbers of the genera *Pseudomonas*, *Achromobacter* and *Bacillus* are found in most aerobic soils; where conditions are anaerobic *Clostridium* will occur. Bacteria such as *Thiobacillus thiooxidans* and *Acetobacter* sp. are capable of growing at the very low pH values between 0 and 2. Some *Bacillus* sp. can grow at pH 11. *Vibrio*, *Streptococcus faecalis* and *Escherichia coli* can tolerate an alkaline reaction (pH 8-9). This have increased the scope of finding industrially important bacteria from municipal waste dump sites and these isolates could be vital source for the discovery of industrially useful enzymes like Protease in the case/molecules.

9. Commercial Products

Bioremediations commercially available in the market mainly include Nitrifiers, Sulphur bacteria, *Bacillus* sp. and *Pseudomonas* sp.

List of commercially available bioremediators for aquaculture applications.

Product	Microbial content	Company / firm
ABIL nitrifying package	Nitrifiers	Tropical marine centre, London
Alken clear-flo 1002	<i>Bacillus</i> sp.	Alken Murray Corp., New York
Alken clear-flo 1100	Nitrifying bacteria	Alken Murray Corp., New York
Alken clear-flo 1400	3 species of <i>Bacillus</i> + 2 species of Nitrifying bacteria	Alken Murray Corp., New York
Ammonix	Nitrifying bacteria	Prowins Bio- Tech Pvt. Ltd., India
Bactaclean	Nitrifiers	Enviro-Comp. Services, Inc., Dover, USA.
Biogreen	<i>Bacillus subtilis</i>	Activa Biogreen Inc., Wood Date, USA
Biostart	<i>Bacillus</i> sp.	Bio-CAT, Inc., Verginia, USA
BRF-13A	Nitrobacter, Nitrosomonas	Enviro-reps., Ventura, CA, USA.
BRF-1A	Nitrifying bacteria	Enviro-reps., Ventura, CA, USA.
BRF-4	Nitrobacter, Nitrosomonas	Enviro-reps., Ventura, CA, USA.
BRF-4	Nitrifying bacteria	Enviro-reps., Ventura, CA, USA.
BZT Aquaculture	Nitrifiers	United-Tech, Inc., Indiana, USA
Detrodigest	<i>Bacillus</i> sp.	NCAAH, CUSAT, India
Eutroclear	Nitrifying bacteria	Bioremediate. Com.LLC, Atlanta.
Nitroclear	Nitrobacter, Nitrosomonas	Bioremediate. Com.LLC, Atlanta.
PBL-44	<i>Bacillus</i> sp. / Nitrifying bacteria	Enviro-reps., Ventura, CA, USA.
Probac BC	<i>Bacillus</i> sp.	Synergy Biotechnologies, India
Pronto	<i>Bacillus</i> sp.	Hort-Max Id., New Zealand
Ps-1	<i>Pseudomonas</i> sp.	NCAAH, CUSAT, India
Remus	Nitrifying bacteria	Avecom, Belgium
Super PS	Sulphur bacteria	CP aquaculture Pvt. Ltd., India.

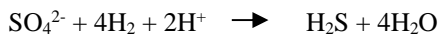
(S.P. Antony and R. Philip, 2006)

10. Bioremediation of Hydrogen Sulphide

Organic loading can stimulate H₂S production and reduction in the diversity of benthic fauna. H₂S is soluble in water and is suggested as the cause of gill damage and other ailments in fish. Unionized H₂S is extremely toxic to fish at concentrations that may occur in natural waters as well as in aquaculture farms.

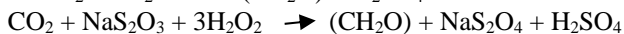
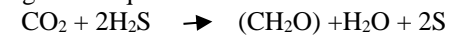
In aerobic conditions, organic sulphur decomposes to sulphide which in turn gets oxidized to sulphate. Sulphate is highly soluble in water and so gradually disperses from sediments. Sulphide oxidation is mediated by microorganisms in the sediment, though it can occur by purely chemical processes. Under anaerobic conditions,

sulphate may be used in place of oxygen in microbial metabolism. This process leads to the production of hydrogen sulphide gas. The H₂S is produced by a series of microbial mediated reductions.



The photosynthetic benthic bacteria that break H₂S at pond bottom have been widely used in aquaculture to maintain a favorable environment. These bacteria contain bacteria-chlorophyll that absorbs light (blue to infrared spectrum, depending on type of bacterio-chlorophyll) and performs photosynthesis under anaerobic conditions. They are purple and green sulphur bacteria that grow at the anaerobic portion of the sediment-water interface. Photosynthetic purple non-sulphur bacteria can decompose organic matter, H₂S, NO₂ and harmful wastes of ponds. The green and purple sulphur bacteria split H₂S to utilize the wavelength of light not absorbed by the overlying phytoplankton. The purple and green sulphur bacteria obtain reducing electrons from H₂S at a lower energy cost than H₂O splitting photo autotrophy and thus require lower light intensities for carrying out photosynthesis.

The general equation of this reaction is as follows:



Chromatiaceae and Chlorobiaceae are the two families of photosynthetic sulphur bacteria that favour anaerobic conditions for growth while utilizing solar energy and sulphide. Chromatiaceae contain sulphur particles in cells but Chlorobiaceae precipitate them out. The family Rhodospirillaceae is not of any use for H₂S removal as they mainly utilize organic material, such as lower fatty acid, as source of hydrogen. But they can be used as efficient mineralizers at pond bottom as they grow in both aerobic and anaerobic conditions as heterotrophic bacteria even in the dark without utilizing solar energy.

For bioremediation of H₂S toxicity, the bacterium that belongs to Chromatiaceae and Chlorobiaceae can be mass cultured and can be applied as pond probiotic. Being autotrophic and photosynthetic, mass culture is less expensive and the cultured organisms can be adsorbed on to the sand grains and applied so that they may reach the pond bottom to enrich the hypolimnion and ameliorate H₂S toxicity.

11. Microbes as Bioremediators

Microorganisms both Gram positive and Gram negative have been tested for their efficacy as bioremediations in aquaculture. *Bacillus* is the most commonly used organism followed by *Aeromonas* and *Pseudomonas*.

12. Role of Biotechnology

Genetically modified microorganisms have shown potential for bioremediation applications in soil, ground water, and activated sludge environments, exhibiting enhanced derivative capabilities encompassing a wide range of chemical contaminants. The recombinant DNA and molecular biological techniques have (i) enabled amplification, disruption and modification of the targeted genes that encodes the enzymes of metabolic pathways, (ii) minimized bottlenecks pathway (iii) enhanced redox and energy generation, and (iv) play important role in recruiting

heterogenous genes to give new characteristics. It is possible that this process, known as bio-augmentation, will open a new range of possibilities for future process of bioremediation. Transgenic of plants has also become a powerful tool for enhancing the efficiency of phytoremediation of organic-polluted soil.

13. Limitations

Bioremediation is limited to only those compounds that are biodegradable and not all compounds are susceptible to rapid and complete degradation. There are various factors affecting the process of bioremediation such as depletion of preferential substrates, lack of nutrients, toxicity and solubility of contaminants, oxidation or reduction potential and microbial interaction. The outcome of each degradation process depends on microbes (biomass concentration, population diversity and enzyme activities), substrate (physicochemical characteristics, molecular structure and concentration), and a range of environmental factors (pH, temperature, moisture content, Eh, availability of electron acceptors, and carbon and energy sources). The type of microbial transformation depends on whether the compound serves as a primary, secondary or co-metabolic substrate.

14. Conclusion

Management of pond microbial ecology is an area where applied research can lead to important findings for improving the productivity and environmental "friendliness" of the shrimp farming industry worldwide, particularly in view of recent negative environmental impacts of shrimp farms. It seems likely that the use of bioremediations will gradually increase and the success of aquaculture in future may be synonymous with the success of bioremediations that, if validated through rigorous scientific investigation and used wisely, may prove to be a boon for the aquaculture industry. The physical or chemical remediation process might be replaced or supplemented with this biological process, which are cost effective and eco-friendly for aquatic management system. A successful bioremediation involves: optimising nitrification rates to keep low ammonia concentration; optimising denitrification rates to eliminate excess nitrogen from ponds as nitrogen gas; maximising sulphide oxidation to reduce accumulation of hydrogen sulphide; maximising carbon mineralization to carbon dioxide to minimize sludge accumulation; maximising primary productivity that stimulates shrimp production and also secondary crops; and maintaining a diverse and stable pond community where undesirable species do not become dominant (Bratvold *et al.*, 1997). The application of beneficial bacteria, probiotics and biodegrading microorganisms, to the pond water and soil (bioremediation) is a sustainable approach to minimize the environmental impact of aquaculture.

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