Potential of Bioremediation in Treating Aquaculture Sludge: Review Article

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Abstract

Rapid development in shrimp farming has raised major concerns on the pond effluents that could negatively impacts the surrounding ecosystem triggered by the increased of the nutrients input. Sludge is formed due to large quantities of unwanted organic material mainly derived from excess feed and organic degradation. Due to its harmful effect, sludge need to be discharged from the culture ponds frequently. Appropriate treatment is needed before sludge could be discharged to the environment. One of the options that have gain interest of many researchers is through bioremediation process, which has been considered as an environmental friendly method in treating organic waste that does not involve any chemical usage. In this review, toxic components in aquaculture waste is discussed together with the potential of beneficial microbes in bioremediating aquaculture sludge.

Introduction

Aquaculture has been the fastest growing sector over the last few decades with the average annual growth rate of more than 8 % (Mirzoyan et al., 2012). The rapid development of aquaculture has results in intensification of culture practice to obtain high yields in order to meet the world's demands for seafood. However, this preferred type of culture practice has raise concerns on its impacts to the environment as the waste produce from this activity is a major contributor to organic waste and toxic compounds (Gondwe et al., 2012; Lananan et al., 2014).

Sludge is a solid type of aquaculture waste which contains nitrogenous compound, phosphorus and other dissolved organic carbon that could affects the environment negatively when the concentration present is higher than usual. Sludge is formed due to large quantities of excessive feed and organic degradation matters. Among the main components of sludge are uneaten feed, phytoplankton, other decaying plant materials, animal wastes, mineral sediment, airborne debris, protozoa, bacteria, fungi and residues of prophylactic and therapeutic input (Mirzoyan et al., 2010). Sludge will affect the habitat availability of cultured animals, produce toxic matters that can endanger the lives of aquatic animals. Due to its harmful effect, sludge need to be removed from the culture ponds frequently and treated properly before discharged to the receiving water bodies (Amirkolaie, 2008; Hopkins & Villalon, 1992). There are strict policy regarding solid waste management and handling prior to disposal. For examples in Malaysia, National Policy on Environment under Ministry of Energy, Science, Technology, Environment and Climate Change of Malaysia emphasize on the idea of achieving clean environment, safe, healthy for present and future generation (DOE, 2002). Hence, the Environmental Act 1974 plays important role in realizing the idea by controlling, regulating and solid waste managements (Mohammad, 2011).

In recent years, there have been a tremendous interest in using beneficial bacteria as potential bioremediators in aquaculture. However, bioremediation is often used to remediate culture water and wastewater instead of sludge. Hence, this paper intends to review the characteristics of aquaculture sludge as well as the potential of bioremediation in managing and treating the sludge for a safe disposal.

Aquaculture Sludge

Waste produces from aquaculture can be categorized into four forms; gases, liquids, semi-solid and solid. Waste that settled on the pond bottom is considered as semi –solid and solid wastes (Latt, 2002). Past researchers refer the materials deposited at the bottom of pond with several names which are “pond bottom soil”, “sediment”, “mud”, “ooze” and “sludge” (Ting, 2002).

Solid wastes or sludge is further divided into two categories; suspended solids and settled solids. Suspended solids are fine particles which remain suspended in culture water except when sedimentation method is used. This type of sludge is very hard to discharge from culture water (Cripps & Bergheim, 2000). On the other hand, settled solids are larger particles that settled in short period of time and is very easy to be removed (Ebeling & Timmons, 2012).

Nevertheless, both types of solid wastes are dangerous in culture system and need to be removed as soon as it accumulates in culture water in order to maintain a good water quality (Latt, 2002). This is because sludge contains high volume of total solids and total solid dissolved with high concentration of nitrogenous compounds. This will result in the increases of aerobic bacterial activity which eventually will reduce the oxygen amount in the culture water (Akinwole et al., 2016).

Nitrogenous compound

Water quality of receiving water bodies deteriorate when accumulated with nitrogenous compound (ammonia, nitrite and nitrate) in sludge discharged from ponds. These compounds when present in high concentration is highly toxic to aquatic plants and animals (Anthony & Philip, 2002). In general, excess amount of nitrogenous compounds can affect the aquatic systems, cause eutrophication which leads to mortality of aquatic flora and fauna (Lananan et al., 2014).

Ammonia in sludge is closely related with type and amount of organic waste such as feed, fertilizers, metabolic wastes and decaying matter in culture system. Nevertheless, the main contributors to the production of ammonia is from the activity of protein metabolisms by the cultured animals. Ammonia is the final product of protein metabolisms (Romano & Zeng, 2013). Fish and shrimp requires feed with high number of protein but not many retained in their body (Dauda et al., 2018). Most of the residues are transferred into culture water and become wastes (Piedrahita, 2003). Ammonia overload results in decreased rate of excretion of aquatic animals which lead to increase in ammonia level in their blood and tissues, decrease oxygen consumption by tissue and effect metabolic enzyme activity. All these changes will cause stress to the animals making them more susceptible to disease infections (Yusoff et al., 2011).

On the other hand, nitrite is a transitional forms of ammonia conversion to nitrate. It is very unstable which makes nitrite more toxic when reach above level of 0.5mg/L (Ajani et al., 2011). High concentration of nitrite will cause problems in receiving waters as it will infer with the oxygen carrying capacity of aquatic animals leading to anemic condition and eventually mortality (Alcaraz & Espina, 1995).

Nitrate is the last component of nitrification process. It is not as toxic compared to ammonia and nitrite. The concentration can go as high as 200mg/L and still will not disturb the water quality and aquatic plants or animals (Dauda & Akinwole, 2015). However, when concentration become higher than the safe level, it will defect the growth and survival of aquatic animals in culture system or in receiving water bodies.

Phosphorus

Phosphorus is an important metabolite product of aquaculture feeds beside nitrogen. Excess phosphorus is due to uneaten feed and undigested phosphorus in feces. However, the value of excess phosphorus varies depending on the culture system, species of cultured animals, type and ratio of ingredients used in feed formulation. Phosphorus requirement depends on the grade of tissue growth and structure of digestive tract of culture organisms (Jahan et al., 2003). These factors will affect the absorption and digestibility of phosphorus (Herath & Satoh, 2015).

There are three classes of phosphorus presents in wastewater; particulate phosphorus (settle at the bottom), suspended phosphorus (less dense than particulate phosphorus) and soluble phosphorus (completely dissolved in water) (Sugiura, 2018). Fish excrete unwanted and undigested phosphorus in feces as particulate phosphorus (Coloso et al., 2003). Prior wastewater discharged, some particulate phosphorus is able to be collected in settling ponds but soluble phosphorus will remain and eventually discharged to the environment (Sugiura, 2018).

Kawasaki et al. (2016) reported on the estimated phosphorus released with wastewater per freshwater pond (0.5 ha) annually for Selangor River was at 10 to 15 kg. On the other hand, for every 1kg breeding of grass carp, 0.033 kg of phosphorus were discharged in water (Bian et al., 2012). High concentration of phosphorus in water bodies will cause eutrophication that will disrupt the water ecosystem.

Hydrogen Sulfide

Sulfur is an important element for plants, animals and bacteria which exist naturally in natural water or in aquaculture ponds mainly as sulfate ion (Boyd, 2014). In aerobic conditions of suspended sediments, sulfur decomposes to sulphide and oxidized into sulphate. When heavy feeding is applied to the culture system, it promotes the accumulation of organic detritus causing severe and extended anoxia conditions in bottom sediments (Musyoka, 2016). Without sufficient oxygen, certain anaerobes bacteria will use the oxygen molecules in sulphate to metabolize producing hydrogen sulfide (Anthony & Philip, 2006).

High concentration of hydrogen sulfide will interrupt with aquatic animal's respiration, cause stress and making them susceptible to diseases (Boyd, 2014). This is the main reason why solid waste need to be discharged from the pond frequently, however, releasing the waste without appropriate treatments will later affect the receiving water bodies.

Management of Aquaculture Sludge

Solid waste or sludge is the most deleterious waste in culture system (Dauda, 2019). Currently, different sludge treatment practices have been suggested for industrial sludge. The preferred method depends on sludge type and characteristics, as well as economic, social, and climatic conditions onsite. In developed countries, critical factors for sludge-treatment choice are efficiency and reliability of the operation, sludge disposal aspects, and land requirements. However, construction and operational costs, sustainability and simplicity of the sludge treatment operation may be the main decisive factors (Mirzoyan et al., 2012).

To date, most of the research related to sludge management prioritize on management of aquaculture feed to optimize feed consumption and digestibility, hence reducing the production of sludge (Turcios & Papembrock, 2014). However, this method depends on the type and size of cultured species, potential of overfeeding and feed characteristics (Westers, 1995).

The other management method focuses on improving the water recycling in Recirculation Aquaculture System (RAS) and aquaponics (Monsees et al., 2017). The aim is mainly to reduce the amount of sludge in the culture system but not on treatment method or management of the sludge. Waste discharged to the receiving water bodies without proper treatment could results in harmful algae bloom and new emerging diseases (Rubert, 2008). There are various other options to reduce the nutrient loading from aquaculture. However, some of these methodologies only transform the nutrients into less toxic forms and do not really reduce the "output" of nutrients to the environment (Matos et al, 2006). Several methods of biological treatment of effluent have been tested although no single treatment method always works (Brown et al, 1999). Nutrient removal can be a cost-effective treatment method, and recently, various microbes have been proposed to be able to remove nitrogen and phosphorus (Sonune & Ghate, 2004). Several researches have been studied on the use of bioremediation method in treating wastewater in aquaculture (Table 1).

Table1: *Previous researches in bioremediation of wastewater in aquaculture*

No	**Species**	**Effects**	**References**
1	*Marichromatium gracile* YL28	Removed 99.96% nitrite in aquaculture pond water in 7 days.	Zhu et al., 2019
2	*Bacillus pumilus* and *Lactobacillus delbrueckii*	Reduced total ammonia nitrogen after 7th week in common carp culture system	Dash et al., 2018
3	*Nitrobacter*, yeast, *Bacillus subtilis*	Removed 99.74% total nitrogen and 62.78% total phosphorus in brackish aquaculture wastewater	Mohamad et al., 2017
4	*Bacillus* sp.	Reduce total ammonia nitrogen level in common carp culture water	Naderi Samani et al., 2016
5	*Bacillus vietnamensis* and *Gordonia bronchialis*	Reduce total ammonia nitrogen and nitrite in *in vitro* assay	Muthukrishnan et al., 2015
6	Mixture of *Bacillus* sp.	Reduced level of total ammonia nitrogen, nitrite and nitrate in *Litopenaeus vannamei* culture water	Zokaeifar et al., 2014
7	*Bacillus amyloquefaciens*	Removed 93% of total ammonia nitrogen within 24 hours	Yu et al., 2012
8	*Bacillus* sp.	Reduced ammonia, nitrite and pH in culture water of *Macrobrachium rosenbergii* after 60 days cultivation	Mujeeb Rahiman et al., 2010

9	*Bacillus subtilis*, *Bacillus mycoides* and *Bacillus licheniformis*	Reduced ammonia, nitrite, nitrate and phosphate ions in recirculation tanks	Lalloo et al., 2007
10	*Bacillus subtilis* and *Bacillus megaterium*	Reduced total ammonia nitrogen, chemical oxygen demand and transparency in red parrot fish recirculation tanks	Chen & Chen, 2001

Concept of Bioremediation

Bioremediation is a known process where beneficial microbiological agents are used to treat contaminated water or waste. Some refers bioremediation as removing, reducing and converting contaminated compounds by inducing its biological process (Divya et al., 2015). This method of treatment relies on hydrogeological conditions, the content and toxicity of contaminants, ecology of microbial and other spatial and temporal factors.

Microbial bioremediation is among the most preferred type of bioremediation as it is cost effective and able to destroy or immobilize contaminants efficiently (Gadd, 2000). These microbes used contaminants as their energy sources. For instance, microbes utilized nitrogen and phosphorus as their nutrients source. It is not necessary to use the natural existing microbes in the waste. It is possible to use exogenous species, or introduced genetically engineered microbes as a mean of bioremediation (Hassan et al., 2003).

Microorganism in bioremediation is differentiate based on the source of carbon they need. Autotrophs is able to synthesis their own food by utilizing the inorganic substances presence as in fixing the inorganic carbon (CO_2) (Musyoka, 2016). Autotrophs are further categorized into photolitotrophs, which need solar as source of energy meanwhile chemiolitotrophs use cellular transferring of electrons as its source of energy. Ammonia-oxidizing bacteria and nitrite-oxidizing bacteria which are essential in nitrification and denitrification process falls under autotrophs bacteria. Autotrophic bacteria are capable in adsorbing and transforming soluble biologically available phosphorus and nitrogen in order to proliferate. This ability is the main highlight in becoming a good bioremediators (Merchant and Helmann, 2012).

Meanwhile heterotrophic bacteria, destroy or immobilize non-living organic matter to produce carbon to build their own cells. These cells in return act as electron donor in catalyzing oxidation of these chemical. Unlike autotrophic bacteria, heterotrophic bacteria are not a major contributor in nitrification and denitrification process, but they can transfer ammonia nitrogen into non-harmful products known as microbial mass (Ebeling et al., 2006). They could break down organic waste (uneaten feed, feces and dead matter) and use it as nutrient sources for growth purpose. Biomass formed can be consumed by aquatic animals as their source of nutrients (Das, 2014). These biomasses are commonly known as biofloc.

According to Bratvold et al. (1997) to achieve a successful bioremediation, the optimum nitrification rates should able to keep the ammonia concentration low, optimization of denitrification rates in order to eliminate excess nitrogen from ponds as nitrogen gas, maximize sulphide oxidation to decrease the accumulation of hydrogen sulfide and maximize carbon mineralization to CO_2 for minimum sludge production.

Application of Bioremediation in Wastewater Aquaculture

Bioremediation of nitrogenous compound

Bacteriological denitrification and denitrification is the most practical method in removing toxic nitrogenous compound in aquaculture (Amin et al., 2013). Accumulation of ammonia occur due to excess feed, mineralization of organic matter and metabolic excretion. Ammonia-oxidizing bacteria play an important role in oxidizing ammonia to nitrite. Autotrophic and heterotrophic bacteria from genera *Nitrosomonas*, *Nitrosovibrio*, *Nitrosococcus* and *Nitrospira* are common nitrifiers used in aquaculture (Anthony & Philip, 2006).

Since nitrite is also consider toxic, it needs to be further removed or converted to nitrate, a safer form of nitrogen. Commonly known nitrite-oxidizing bacteria belongs to genera *Nitrobacter, Nitrococcus* and *Nitrospira.* There are also some heterotrophic nitrifiers that produce only low levels of nitrite and nitrate and often use organic source of nitrogen rather than ammonia or nitrite (Ming Yu Li et al., 2011). Denitrification is the final phase in nitrogen cycle. At the end of denitrification process, nitrogen gas, the safest form of nitrogen will be released to the environment. At least 14 genera of denitrifiers have been identified; *Pseudomonas, Bacillus, Rheinheimera, Pannonibacter, Rhizobium, Gordonia, Stenotrophomonas, Brevundimonas, Paracoccus, Rhodococcus, Pseudochrobactrum, Arthrobacter, Gemmobacter* and *Alcaligenes.*

Incorporation of nitrifying bacteria in reducing the ammonia and nitrite level has started as early as 1991. Used of *Bacillus* sp. has been proved to be able to reduce ammonia in culture water (Porubcan, 1991). Studies from Mayer et al. (2012) showed the ability of strain *Paracoccus pantorophus* 768 in reducing toxic waste compounds leading to a better sediment and water quality in ponds. In addition, *Bacillus amyloliquefaciens* was able to reduce ammonia up to 93%

after 24 h of inoculations with initial concentration of ammonia 200mg/l (Yu et al., 2012). Studies by Abraham et al. (2004) disclosed that a combination of *Nitrosomonas* sp. and *Bacillus* sp. was the most effective in reducing 96% total ammonia.

In another study, Ghosh et al. (2007) found that *Bacillus subtilis* isolated from the intestine of *Cirrhinus mrigala* (Hamilton) after introduced in the rearing water of ornamental fishes significantly lowered total ammonia concentration. *Bacillus amyloliquefaciens* HN exhibited high tolerance towards 80 mg/l of nitrite and ammonia. It could effectively remove 20 mg /l of nitrite (Xie et al., 2013).

Bioremediation of phosphorus

Phosphorous is generated from organic compound as PO_4 by certain bacteria that produce enzymes such as phosphotases and phytases. The solubility of inorganic phosphotases is primarily a function of pH. Bacteria are capable of liberating PO_4 from these compounds through the production of organic and mineral acids.

There are less studies have been conducted in remediating phosphorus in aquaculture. Recently, Lananan et al. (2014) reported on the potential of symbiotic bioremediation of phosphorus using effective microorganisms (EM) and microalgae. High percentage removal of phosphorus has been recorded which is 99.15% equivalent to 0.524mg/l removal per day. In another research, it is shown that mix *Bacillus* sp. were able to reduce phosphorus level in ponds by 81% (Reddy et al., 2018).

Bioremediation of hydrogen sulfide (H_2S)

The photosynthetic benthic bacteria which can break down H_2S at the bottom of ponds have been broadly applied in aquaculture to sustain a favorable environment. It comprises of bacteria chlorophyll that can absorbs light (blue to infrared spectrum, depending on type of bacterio-chlorophyll) and undergo photosynthesis during anaerobic conditions. There are two types of photosynthetic bacteria; purple and green sulphur bacteria which can grow at an anaerobic area of the sediment. Photosynthetic purple nonsulphur bacteria are capable in decomposing organic matter, H_2S, NO_2 and other toxic components.

Chromatiaceae and *Chlorobiaceae* are the two families of photosynthetic sulphur bacteria that preferred anaerobic conditions with solar energy and sulphide to grow. *Chromatiaceae* has sulphur particles in its cells but *Chlorobiaceae* precipitate them out. The family *Rhodospirillaceae* is not of any use for H_2S removal as they mainly utilize organic material, such as lower fatty acid, as source of hydrogen. But they can be used as efficient mineralizes 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 H_2S 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 applied at the pond bottom to reduce H_2S toxicity.

Potential of Bioremediation in Treating Aquaculture Sludge

Over the years, the application of biological degradation using microbes in aquaculture sludge centralized on anaerobic digestion method. Anaerobic digestion has long been used for the stabilization and reduction of wastewater sludge (although not in aquaculture), mainly because of the simplicity of the operation, reduced sludge generation, production of biogas and possible high loading rates (Appels et al., 2008; Cakir and Stenstrom, 2005; Krzystek et al., 2001; Marchaim, 1992). The main purpose of sludge digestion is to produce biogas for agriculture purpose as well as reducing the organic matter in the sludge through composting. However, this practice requires high cost as big scale digestion needs bigger batch reactor.

Early studies on bioremediation of aquaculture sludge was done by Gomez et al. (2019) where fish sludge was incubated with polychaetes *Abarenicola pussila* for 45 days. At the end of the experiment, level of organic matter, nitrogen and carbon was significantly reduced. However, this is only on pilot scale and have not been tested in full scale.

Since many studies on bioremediation focus only on wastewater and culture water, the ability of bioremediation on solid waste (sludge) has still not been documented. Theoretically, there is a promising potential in degrading contaminants of sludge using beneficial bacteria as it has almost the same concept with remediating wastewater and culture water. However, this is highly dependent on the bacteria species, contaminants concentration, environmental condition and other significant factors.

Challenges in Bioremediation

As good as the idea of bio remediate contaminants in aquaculture sludge, there are several challenges that need to be considered in order to come out with a success bioremediation tools. Bioremediation is limited to compounds that can be degraded biologically. In addition, there are some products of biodegradation that are more toxic than the original form (Adnan Amin et al., 2013).

Biological process is highly specific. Many factors affect the effectiveness of bioremediation process. Successful bioremediation required some suitable environmental conditions; pH level, temperature, dissolve oxygen, alkalinity and salinity. Also, appropriate levels of contaminants do effect the bioremediation process as some bacteria cannot withstand high level of contaminants (Ebeling et al., 2006). Some waste generated from aquaculture may not be suitable for the preferred bacteria for degradation (Das, 2014).

In developing bioremediators, it is a challenge to move from bench and pilot scale to full scale trials. Research is needed to develop and engineer bioremediation technologies that are appropriate for sites with complex mixtures of contaminants that are not evenly dispersed in the environment. Contaminants may be present as solids, liquids, and gases (Bhatnagar & Kumari, 2013).

Conclusion

There have been less awareness and interest in developing technologies to reduce environmental problem. Bioremediation has been considered to be among the best practical method in treating contaminants in waste by enhancing degradation process as one of its modus operation. Better understanding on the relationship between microbial communities and contaminants and how microbes responds in the presence of certain level of contaminants may lead to an ultimate breakthrough in waste management research studies. In aquaculture, bioremediation is more focus on maintaining water quality in culture ponds but not on managing waste especially solid waste (sludge). Managing sludge is one of the biggest concern as the conventional method are time consuming and highly cost. Hence, more researches need to be done to explore the potential of bioremediation in reducing and eliminating highly toxic contaminants in aquaculture sludge for a better future ahead.

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