

Review Article

Aggregates in aquatic ecosystems and implications for aquacultures

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ABSTRACT

Agglomerations of suspended particulate matter serve various roles in aquatic ecosystems. They participate in nutrient and energy fluxes and are involved in important food web processes. While comprehensive studies on aggregates are available from natural freshwater and marine ecosystems, little is known about the roles of aggregates in aquacultures, particularly in shrimp pond farming. As particle-rich systems, shrimp ponds and marine aquaculture (mariculture) areas constitute interesting objects for aggregate studies, particularly as a source of natural feed, particle fluxes, microbial communities, including pathogenic bacteria, and possible vector of disease widespread. The aims of this review are i) to compile the current knowledge on the role of aggregates in aquatic ecosystems, particularly in aquaculture areas covering advantages and negative side effects of aggregates in aquacultures, ii) to explore the role of aggregates in disease ecology, and iii) perspective of aquaculture management in the context of aggregate utilization and management. Since Southeast Asia, especially Indonesia, is among the most important regions for aquaculture activities, this review focuses on Indonesian aquacultures. Although aquacultures produce important amounts of aggregates, including its associated microbial communities, they are rarely investigated in Indonesian aquacultures, particularly in shrimp pond farming. In contrast, most of the studies focused on bacterial cultivation and utilization of isolates for aquacultures. Thus, understanding the ecological roles of aggregates in aquacultures may support the improvement of aquaculture management and yields.

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1. Introduction

Aggregates formation is a common phenomenon in the aquatic ecosystem, in lakes (fresh-water), estuaries (brackish water), and in marine ecosystems (seawater). The aggregate formation is influenced by physical, chemical, and biological factors, which often affect the size and type of aggregates (Allredge & Silver, 1988). An increase in aggregate density causes an increase in the sinking sedimentation rate. Therefore, the aggregates play

a major role in vertical material transport and biogeochemical processes in aquatic ecosystems (Jackson & Burd, 1998). In addition, the presence of aggregates in natural ecosystems represents a food source for benthic organisms such as mussels, oysters, and clams via the capture of organic particles (Ward & Kach, 2009). As a result, they supply natural feed for the animals (Crab *et al.*, 2007). In aquatic ecosystems, aggregates can be found from surface layers to the deep sea waters (Passow *et al.*, 2012; Simon *et al.*, 2002).

As organic-rich particles, aggregates provide a suitable habitat for microorganisms. They exploit aggregates not only for nutrient supply but also for protection from microbial predators such as zooplankton and protozoa, as

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well as from destructing physical factors such as pH, UV light, and salinity (Kramer *et al.*, 2013; Lyons *et al.*, 2010). Aggregates have a larger bacterial abundance and higher diversity than the adjacent water column (Alldredge & Silver, 1988). Kramer *et al.* (2013) mentioned that bacterial colonization and extinction rates depend on bacterial density and diversity as well as aggregate size. They proved that the colonization rates increased linearly with the bacterial background and the aggregate size.

Aggregates represent microbial hotspots and are often densely colonized by bacteria. They may even become an effective agent for the survival and spread of pathogens (Froelich *et al.*, 2013; Lyons *et al.*, 2005). They may thus serve as a specific target for pathogen monitoring. Despite their role in disease ecology, aggregates may also be used to improve aquaculture practices. They influence inorganic nutrient cycles, reduce toxic substances, enhance immune systems, and improve breeding periods of cultured animals (Hargreaves, 2013).

This review compiles and carefully discusses the roles of aggregates in aquacultures. It consists of four sections: i) an overview about aggregates and their ecological roles, ii) roles of aggregates in aquacultures methods for bacterial community identification in aggregates, iii) current status of microbial ecology study focusing on bacterial ecology in aquacultures, and iv) future research perspective relating to aggregates in Indonesian maricultures.

2. Overview of the Aggregates

2.1 Formation and life span of aggregates

Aggregates are the conglomeration of living and non-living particles, which occurs in the water column of fresh, brackish, and seawater. The term aggregates cover marine snow, micro- and macro-sinking particles, detritus (including amorphous detritus and phyto-detritus), flocs, and mucilage (Alldredge & Silver, 1988; Danovaro *et al.*, 2009; Grossart *et al.*, 2006; Kjørboe *et al.*, 2003). These aggregates typically consist of inorganic particles, detrital organic particles, and biological particles (e.g., mucus feeding webs, molts, transparent exopolymer particles (TEP), polysaccharides, lignin, humic materials), phyto- and zooplankton, and microbes (Alldredge *et al.*, 1998; Alldredge & Silver, 1988; Danovaro *et al.*, 2009; Grossart *et al.*, 2006).

The formation of aggregates is influenced by a number of physical, chemical, and biological interactions, which often impact the size of aggregates (Biddanda, 1988; Kjørboe, 2001). Physical coagulation by turbulent shear causes dissolved particles to collide and to attach (Alldredge & Silver, 1988). Floating zooplankton feces or chitinous shells collide, and individual or several species of phytoplankton cells adhere (Jackson & Burd, 1998; Kjørboe, 2001). Specific interactions between phytoplankton such as *Skeletonema* and *Thalassiosira* with bacteria as for example, *Marinobacter* sp. support aggregate formation (Grossart *et al.*, 2007; Gärdes *et al.*, 2011).

In the ocean, marine snow varies in size from millimeters to centimeters (Alldredge & Silver, 1988). They episodically exist in a large formation called "mucilage" (i.e., large mucus-rich marine snow known, e.g., from the Adriatic and the Tyrrhenian Sea), which can

reach up to 3 meters in diameter (Precali *et al.*, 2005). Specific physical conditions (i.e., water column stratification and low mixing) and biological patterns in the water column, including nutrient dynamics, production of TEP by microbes and phytoplankton, and other extracellular polymeric substance (exopolysaccharide/EPS), can lead to such huge marine snow formation, which was observed in the Mediterranean sea (Precali *et al.*, 2005). Interactions among bacteria, phytoplankton, and zooplankton initiate the formation of smaller aggregates (Alldredge and Silver, 1988; Simon *et al.*, 2002).

The life span of aggregates depends on their sizes and environmental (water column) conditions. Marine mucilage floating at the surface or within the water column can have a life span of up to three months, while smaller aggregates (up to 4 mm spherical diameter) may have a life span of around one week (Danovaro *et al.*, 2009; Kramer *et al.*, 2013). High density may accelerate the sinking of aggregates resulting in a decrease in the numbers of aggregates in the water column. Once aggregates settle on the seafloor, they coat the sediments. Depending on the amount of export, subsequent bacterial processes involved in the degradation of the organic matter may cause hypoxic or even anoxic conditions. Moreover, pathogens, which may be thriving in aggregates, tend to persist, reside or even proliferate on the seafloor (Kramer *et al.*, 2013).

2.2 Types of aggregates in aquatic ecosystems

Aggregates can be categorized based on component materials that build them up. These materials can be living or dead cells or organic matter in the form of detritus (Alldredge & Silver, 1988). Several publications reported different major components of aggregates, which are phytoplankton-bacteria based aggregates (Gärdes *et al.*, 2011, Grossart *et al.*, 2006), dinoflagellates based aggregates (Alldredge *et al.*, 1998), diatoms-fecal pellet based aggregates (Kjørboe, 2001), and heterotrophic bacteria with oil droplet aggregates (Passow *et al.*, 2012).

2.2.1 Phytoplankton-bacteria based aggregates

Aggregation of phytoplankton and bacterial cells is an important process in marine ecosystems leading to the sinking of particulate organic matter in the form of marine snow (Grossart *et al.*, 2007; Grossart & Simon, 1998). The phytoplankton-bacteria aggregation is facilitated by phytoplankton exudates. Transparent exopolymer particles (TEPs) act as glue for particle aggregation. Heterotrophic bacteria may attach to phytoplankton cells. *Skeletonema costatum*, *Thalassiosira weissflogii*, and *Chaetoceros debilis* form phytoplankton-bacteria aggregates with different colonization rates (Grossart *et al.*, 2007). Gärdes *et al.* (2011) proposed a bilateral model system for an interaction of the diatom *T. weissflogii* with the bacterial strain *Marinobacter adhaerens*. The aggregation mechanism appeared to be highly dependent on the bacteria, meaning without *M. adhaerens*, the aggregation of *T. weissflogii* never occurred. Photosynthetically active status of *T. weissflogii* seemed to be required to produce large amounts of TEP precursor material (Gärdes *et al.*, 2011). Thus, marine bacteria which actively interact with the algae may influence TEP production and increase particle aggregations (Gärdes *et al.*, 2011). In addition, Amin *et al.*

(2012) described that some bacteria consistently associate with growing diatoms, while other bacteria colonize sinking diatom particles and decompose organic matter therein. Hence, heterotrophic bacteria increase the aggregation of microalgae and other particles (Decho, 2000). However, striking differences in aggregation dynamics and TEP abundances were observed when diatom cultures were inoculated with either diatom-attaching or free-living bacteria. Free-living bacteria might not influence aggregation, whereas bacteria attaching to diatom cells may increase aggregate formation (Gärdes *et al.*, 2011).

2.2.2 Dinoflagellates based aggregates

The aggregates of dinoflagellates are considered very large (centimeters long), brown, sticky, and unusually strong and cohesive. They are held together with thick, viscous, mucus-like material and do not readily break apart when disturbed by a diver underwater (Alldredge *et al.*, 1998). The thecate dinoflagellates, including *Gonyaulax polyedra*, *G. polygramma*, *G. grindleyi*, and *G. koeffoidi* make up over 90% of aggregates. However, other organisms are usually available on this type of aggregates, for example, diatoms, *Ceratium*, *Dynophysis*, *Prorocentrum*, *Protoperdinium*, empty frustules of dinoflagellates, and some zooplankton fecal matters and detritus (Alldredge *et al.*, 1998). Dinoflagellate marine snow differs from other types of marine snow in three major ways. First, it contains conspicuous abundances of large thecate dinoflagellates. Second, it yields more particulate organic carbons (POC), particulate organic nitrogen (PON), and chlorophyll per unit volume than other types of marine snow. Third, it is held together by copious and viscous mucus (Alldredge *et al.*, 1998). Even though it has high mucus content, the transparent exopolymers (TEP) concentration in dinoflagellate types of aggregates is considered very low. Alldredge *et al.* (1998) analyzed the TEP content of *G. polyedra* in a laboratory experiment and found almost no TEP. They proposed that the mucus-like material in dinoflagellate aggregates is probably not TEP (acidic polysaccharides containing sulfated half-ester groups) but some other type of mucus. This mucus appears to be of considerably higher cohesiveness, density, and stickiness than TEP. Hence, they might attach component particles together more tightly and generate more compact particles with higher mass content per unit size of aggregates.

2.2.3 Aggregates derived from larvacean houses or fecal pellet

Zooplankton such as pteropods, larvaceans, and salps produce gelatinous feeding nets and houses which, together with their fecal pellets, also occasionally form aggregates (Alldredge & Silver, 1988). These spherical or elliptical houses consist of an outer mucopolysaccharide wall and fine mucus nets. Then, various particles from surrounding seawater (i.e., phytoplankton cells, bacteria, flagellates, ciliates, fecal pellets, and mineral grains) were attached to the houses (Hansen *et al.*, 1996). Due to the accumulation of living organisms, these houses may become sites of elevated microbial activity (Davoll & Silver, 1986), and their high particle content makes them suitable as food for zooplankton (Steinberg *et al.*, 1998). Hansen *et al.* (1996) reported that the aggregates from larvacean houses sized larger than 0.8 equivalent

spherical diameters (ESD) or larger than 0.3 μm and were composed by one abandoned house of the larvacean *Oikopleura dioica* with numerous diatoms, fecal pellets, ciliates and amorphous detritus.

Figure 1 shows different types of aggregates. a) organic marine aggregates formed in the laboratory (photo by M. M. Lyons in Lyons *et al.*, 2005), b) Micrograph of disrupted aggregates (photo by B. Froelich in Froelich *et al.*, 2013), c) Synechococcus-based aggregates (Photo by B. A. Biddanda in Biddanda, 1988), d) Scanning electron microscopy of a *T. weissflogii* cell with four cell of HP15 strain (Photo by A. Gärdes in Gärdes *et al.*, 2011), In situ photograph of marine snow of mixed composition (e), diatom *Chaetoceros* sp (f), diatom aggregates (g) (Photo e, f, g by A. Alldredge, in Kjørboe, 2001), h) photo of quahog pathogen unknown (in dot-circle, photo by M.M. Lyons in Lyons *et al.*, 2005), i) and j) schema of bio-flocs (by P. De Schryver in De Schryver *et al.*, 2008).

2.3 Roles of aggregates in aquatic ecosystems

Aggregates serve various processes in aquatic ecosystems. For example, they are responsible for the majority of the downward transport of organic materials as well as microorganisms by gravitational settling in aquatic ecosystems (Alldredge *et al.*, 1998; Alldredge & Silver, 1988; Grossart *et al.*, 2006). The aggregates provide a suitable substrate for the growth of a diverse range of prokaryotic microbial communities, whose attachments and detachments influence the microbial communities in the water column (Kjørboe, 2001). In the water column, they can also represent a major food source for meso- and macrozooplankton grazers (Kjørboe, 2001) and increase uptake of nutrients in the form of particulate matter for sessile-benthic organisms, such as oysters and mussels (Ward & Kach, 2009).

During algal blooms, the congregation of algae cells, either among algae cells only or between particles such as detritus or clay with algae cells, may decrease the algae cell density in surface waters (Anderson, 2009). This may increase light penetration and avoid oxygen depletion in surface water layers. For several invertebrate larvae, aggregates serve as a sinking vehicle, so they can settle and then undergo metamorphosis (Shanks & Del Carmen, 1997). Lastly, aggregates remove pollutants from aqueous solutions through assimilation, adsorption, biodegradation, and other conjoint processes (Wu *et al.*, 2012).

3. Role of Aggregates in Aquaculture: Are They Curse or Blessing?

An intense aquaculture setting coincides with considerable amounts of particulate and soluble wastes due to an excess of organic materials and nutrients from excreted metabolites (i.e., feces and urine) and left-over feed. These materials may cause an acute toxic effect and long term environmental risks (Piedrahita, 2003), not only for the aquaculture areas but also for the ecosystems nearby aquaculture activities (Herbeck *et al.*, 2013). These include, for example, algal blooms or the mass mortality of cultured or wild organisms due to the depletion of dissolved oxygen (Alonso-Rodríguez & Paez-Osuna, 2003; Sidabutar, 2016). Thereby, aggregates can be involved in processes, which have negative effects on ecosystems and reared biota.

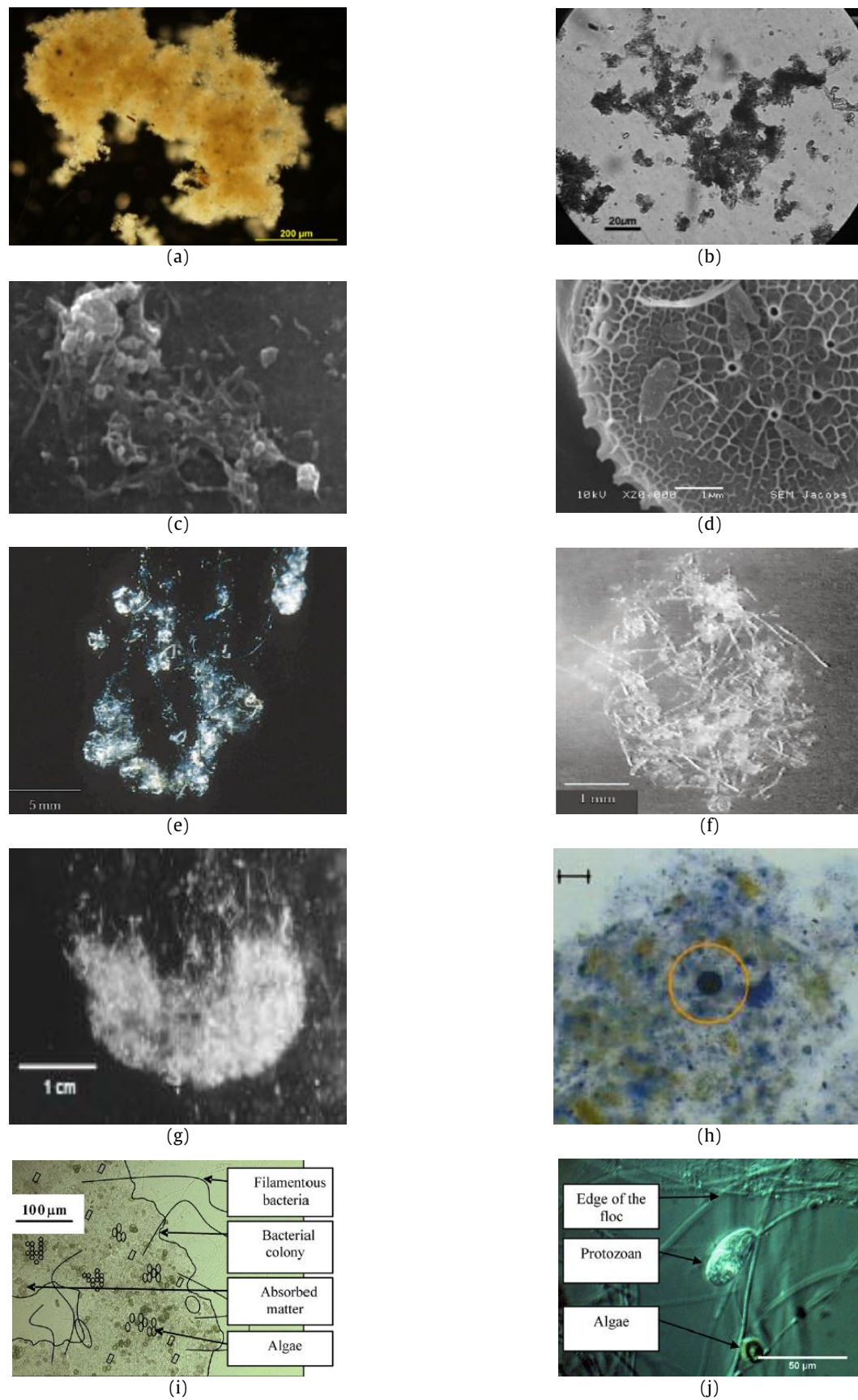


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In contrast, the aggregates have also proven to be part of the solution for aquacultures. For instance, shrimp and tilapia aquaculture practices use aggregates, commonly known as “bio-flocs”, during the rearing (Avnimelech, 2015). Therefore, this section will carefully elucidate how aggregates can influence aquaculture processes.

3.1 Aggregates as islands for microorganisms

Microorganisms, such as bacteria, in aquatic environments can be distinguished into free-living (FL), i.e., floating in the water column, and particle-associated (PA), i.e., associated with a substrate, bacteria. Colonization of aggregates by the so-called PA-bacteria is mainly determined by bacterial attachment and detachment probabilities (Kiorboe *et al.*, 2002). The adhesion of bacteria to both biotic (e.g., phytoplankton, macrophytes, zooplankton, benthic invertebrates, pelagic vertebrates) and abiotic particles (e.g., clay and sediment grains) surfaces are considered a protective mechanism to survive (Liu *et al.*, 2017; Davies *et al.*, 1995). Thereby, the bacterial attachment may reduce the effects of environmental stressors (e.g., sunlight, changes in temperature, salinity, pH, competition, and lack of nutrients) and predation, which may suppress bacterial growth.

The abundance of bacteria associated with an aggregate depends on the size of the aggregate and the abundance and diversity of microbes in the ambient water (Kramer *et al.*, 2013). The concentration of heterotrophic bacteria on phytoplankton-based aggregates is about 10^5 to 10^7 cells per aggregate (Kiorboe, 2001). In addition, Lyons *et al.* (2005) reported that the concentration of total heterotrophic bacteria (THB) in aggregates is also influenced by seasonal change, with concentration ranges of 10^3 - 10^7 cells mL⁻¹, while THB concentration in the water column is about 10^3 - 10^4 cells mL⁻¹. Once aggregates formed, free-living bacteria will attach and colonize the aggregate surface.

Aggregates are often considered to represent microscale islands in a sea of potential colonists, which can be understood with reference to the theory of island biogeography (MacArthur and Wilson, 1967 in Lyons *et al.* 2010). The theory suggests that bacterial colonization and extinction rates largely depend on the size of the aggregate and its distance from the source of dispersing organisms/ water (Lyons *et al.*, 2010). Originally, the island biogeography theory was developed to explain the composition of biological communities found on oceanic islands. The theory predicts a dynamic equilibrium between colonization of new species and extinction of resident species in which the total number of species (i.e., species richness) is an increasing function of island size and a decreasing function of the distance to a source of potential colonizers (e.g., continental mainland) (Lyons *et al.*, 2010). They observed bacterial populations in some individual organic aggregates in size from 1.9 to 3.7 mm with surface areas from 2.6 to 8.6 mm² and volume of 0.024 to 0.137 ml. They found that a dynamic equilibrium within the aggregates-associated microbial community was reached within one day and was maintained for at least one week. During this period, the species composition in an aggregate might change, but the bacterial numbers remained constant.

3.2 Aggregates facilitate organic matter degradation

Heterotrophic aggregate-associated bacteria are mainly involved in the degradations of organic matter in aggregates (Grossart *et al.*, 2007; Mecozzi *et al.*, 2005). These complex processes can lead to major draw-downs of oxygen in the water column and on the sediments (Holmer *et al.*, 2003). This can result in the generation of anaerobic conditions, which are often observed in sediments below farming structures. In pond farming, the anaerobic conditions may occur due to the outgassing of hydrogen sulfide, ammonia, and methane (Avnimelech & Ritvo, 2003). Hence the over-fertilization of aquaculture systems with organic matter and subsequent aggregate formation can have detrimental effects on the environment and aquaculture process. For instance, water deteriorations and disease outbreaks are often linked to high rates of sedimentation of organic matter (Martin *et al.*, 1998; Holmer *et al.*, 2003; Hsieh *et al.*, 2007).

3.3 Aggregates influence water quality

Organic matter and microbes in aggregates may change water quality. Increase loads of aggregates in water column affect turbidity and light penetration, which will affect other interconnecting biological, chemical, and physical processes such as photosynthesis, organic matter degradation, and change of pH, alkalinity, hardness, and oxygen saturation (Avnimelech, 2015; Boyd and Tucker, 2014; Emerenciano *et al.*, 2013). In addition, several publications reported that living microorganisms in aggregates might uptake inorganic nutrients from the water column for their growths (Ray *et al.*, 2010; Crab *et al.*, 2012; Leyva-Flores *et al.*, 2018), resulting in potential microbial proliferation, including pathogenic ones. Later, it may lead the aquaculturists or scientists to consider aggregates for disease surveillance tools.

3.4 Aggregates as a hotspot for surveillances of pathogenic microorganisms

The risk of disease for cultured animals in aquaculture often increases with low water quality, intense culture, and stocking density, and particularly when polyculture is replaced by monoculture (Kautsky *et al.*, 2000). Furthermore, the presence of potentially pathogenic bacteria, as well as antibiotic-resistant bacteria, raises particular concern in aquaculture, where a large use of antibiotics has been common in recent years (Seyfried *et al.* 2010). Aquaculture is believed to contribute to the spread and persistence of antibiotic resistant bacteria in the environment, and indeed, antibiotic-resistant bacteria have frequently been detected at aquaculture sites (Tamminen *et al.*, 2011, Di Cesare *et al.*, 2013). Even though no antibiotic treatment was applied in aquaculture, a potential threat of antibiotic-resistant bacteria in aquaculture, especially coastal aquaculture, remains viable (Di Cesare *et al.*, 2013). Antibiotic-resistant bacteria can reach aquaculture sites also via agricultural and urban wastewaters, which contain intestinal flora and pathogens of animals and humans, which are usually resistant to antibiotics (Cabello, 2006). These emerging contaminants may accumulate in the underlying sediments of aquaculture. Even in the absence of a continuous antimicrobial application, antibiotic-resistant microorganisms, as well as pathogenic bacteria, can

persist in protected reservoirs such as sediments or cultured animal gut. Thus, certain groups of microbes may potentially be pathogens or provoke diseases in cultured animals (Reichardt *et al.*, 2013; Zhang *et al.*, 2014).

Pathogenic or parasite bacteria have been found on aggregates from aquatic ecosystems and shellfish aquaculture. Quahog hard clam (*Mercenaria mercenaria*) unknown parasites (QPX), mesophilic pathogenic bacteria (*Vibrio cholera*, *V. vulnificus*, *V. alginolyticus*, *Aeromonas hydrophyla* and *Pseudomonas aeruginosa*), and *E. coli* were found on the aggregates from aquaculture of hard clams and oysters (Lyons *et al.*, 2005, Lyons *et al.*, 2010). Other studies reported that human pathogenic bacteria such as *V. parahaemolyticus* and *V. cholera* were detected on aggregates from a Bangladesh river (Colwell *et al.*, 2003; Reichardt *et al.*, 2013). Therefore, it is essential to analyze the presence of potentially pathogenic bacteria on aggregates. The presence of pathogen-laden aggregates in areas subjected to disease outbreaks would suggest a vector for the spread and survival of pathogens between epidemics. Aggregates may then provide a specific target for environmental monitoring of those pathogens.

3.5 Aggregates and its associated bacteria as nutritious feeds for cultured biota

Aggregates provide two critical services for aquaculture in terms of nutrient supply. Firstly, they provide nutrition for the cultured organisms, and secondly, they can treat wastes from left-over pellets (Hargreaves, 2013). Once bio-flocs are formed, the supply of food sources in an aquaculture system naturally increases, which may provide additional sources of proteins (around 25 to 50%), fats (around 0.5 to 15 %), vitamins, and minerals, and especially phosphorus (Hargreaves, 2013). An addition of extra carbons (i.e., an external carbon source or elevated carbon content of the feed) may enhance bio-floc formation (Crab *et al.*, 2012). Amongst those carbon sources, molasses, glucose, and starch give the best bio-floc formation (Avnimelech, 2015).

The addition of molasses in zero water exchange aquaculture of shrimp *Penaeus monodon* can ameliorate shrimp culture by reducing the ammonium and nitrate concentrations, increasing the heterotrophic bacterial numbers, and improving the shrimp survival rates, the percentage of weight gain, and the feed conversion ratio at optimum levels (Panjaitan, 2010). Furthermore, the application of bio-flocs in aquacultures can reduce water exchange rates in pond aquacultures. In fact, subsequent rearing can be undertaken using the same water. Therefore, this system is applied in areas where water is scarce (Crab *et al.*, 2012).

In shrimp aquaculture, bio-flocs can be a novel strategy for disease management in opposite to conventional approaches such as antibiotic, antifungal, probiotic and prebiotic applications. The natural probiotic effect in bio-flocs could act internally and externally against, i.e., *Vibrio* sp., and ectoparasites, respectively. Sinha *et al.* (2008) reported that bacteria like *Bacillus* sp., *Alcaligenes* sp., and *Pseudomonas* sp. may synthesize and accumulate polyhydroxyalkanoates (PHA). These polymers may comprise 16% of the bio-flocs dry weight, and when they are degraded in an animal gut, they will provide antibacterial activity similar to short chain fatty acids (Liu *et al.*, 2010).

Studies in shrimp farming *Litopenaeus stylirostris* and *Farfantepenaeus duorarum* showed that bacteria in bio-flocs could enhance spawning performance which might be caused by better control of water quality parameters and continuous availability of food in the form of fatty acids rather than in conventional systems (Emerenciano *et al.*, 2013). Furthermore, Crab *et al.* (2012) inoculated bio-flocs with a probiotic *Bacillus* mixture in an attempt to produce probiotic bio-flocs for improving shrimp growth performance, survival, immunity, and disease resistance. They showed that the water of shrimp tanks fed bio-flocs inoculated with *Bacillus* had an on average five times lower *Vibrio* population when compared to the shrimp tanks fed an artificial feed. It is possible that bacterial conglomeration mechanisms in bio-flocs involve cell-to-cell interactions (quorum sensing). However, the understanding of this mechanism is far from complete. Consequently, more studies of the microbial dynamics in the bio-flocs are required (De Schryver *et al.*, 2008).

4. Bacterial Communities in Indonesian Marine Aquacultures (Maricultures)

Despite diverse mariculture areas in Indonesia, studies on microbial ecology, including bacterial community compositions in Indonesian aquaculture systems, are still limited. Most of the published studies related to aquaculture were focused on the isolation, identification, and utilization of strain levels. Screening of bacteria through the cultivation method was done to determine pathogenic bacteria as well as to isolate bacteria, which may inhibit the pathogenic ones (Azizah *et al.*, 2017; Hatmanti *et al.*, 2008; Herfiani *et al.*, 2010). Furthermore, some studies attempted to use bacterial isolates to improve water quality, specifically to reduce the ammonium concentration (Suantika *et al.*, 2013; Azizah *et al.*, 2017).

Identification of the microbial symbiont composition of fish and shrimp via 16S rRNA amplicon sequencing has been recently reported. Hennersdorf *et al.* (2016) and Oetama *et al.* (2016) analyzed the microbial symbionts in cultured and wild fish *Epinephelus fuscoguttatus* and shrimp *Penaeus monodon* to compare the microbial symbionts in both types of organisms, respectively. Hennersdorf *et al.* (2016) reported that feces samples from cultured fish *E. fuscoguttatus* revealed a highly stable distribution of several orders of bacteria such as *Vibrionales*, *Pseudomonales*, *Rhizobiales*, and non-classifiable *Alphaproteobacteria*, while Oetama *et al.* (2016) described that wild *P. monodon* contained more pathogenic bacteria such as *Pseudoalteromonadales* and *Vibrionales*, e.g., *Vibrio alginolyticus* and *Photobacterium damselae*, as well as viruses, which provoke white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), and yellow head virus (YHV).

Recently, Alfiansah *et al.* (2018) put into consideration the investigation of aggregate-associated bacterial fraction. They reported that potential pathogenic bacteria, such as *Vibrio parahaemolyticus*, *Alteromonas*, *Pseudoalteromonas*, and *Photobacterium* were found in aggregate samples from shrimp *P. vannamei* aquacultures (Alfiansah *et al.*, 2020). Thus, more research on aggregates and their microbial communities is still required.

5. Future Research Perspective Relating to Aggregates in Indonesian Maricultures

Active marine and pond aquaculture areas in Indonesia are about 200.000 and 650.000 hectares, respectively, with seaweed, shrimp, and fish as the main commodities amounting to a harvest of 6.5, 0.5, and 0.6 million tons, respectively (Kementerian PPN/Bappenas, 2014). Marine and pond aquaculture have to deal with ecological aspects such as degradation of environmental quality and disease outbreaks (KKP, 2015). Even though water monitoring, as well as preventive and curative disease procedures, have been established, water deterioration and diseases, especially viral and bacterial diseases, have frequently occurred in Indonesian aquaculture (Kementerian PPN/Bappenas, 2014). Therefore, a comprehensive study of pathogenic microbial ecology, etiology, and widespread modes are fundamental to avoid aquaculture failures. In this context, aggregates can be one of the proposed objects for water quality monitoring.

Aggregates might also be an interesting object in a new aquaculture system called "integrated multi-trophic aquaculture (IMTA)." In this system, several species of different trophic levels are cultivated together to optimize the use of nutrients (Buschmann *et al.*, 2009). The idea of the system is to re-use the resources, especially with regard to space and feed, to get more than one harvest. To achieve the objective, farmers have to cultivate the main biota such as fish or shrimp, detritivore such as sea cucumbers, and nutrient absorbers such as seaweed or mussels. This promising aquaculture system was tested in an IMTA in Nusa Tenggara Barat, Indonesia, which included oyster (*Crassostrea* sp.), seaweed (*Gracilaria* sp.), and black tiger shrimps (*Penaeus monodon*) (Astriana, 2012). Unfortunately, microbial parameters have not been observed yet. Research on the aggregates can be performed in this aquaculture system, for example, in the topics of the particle fluxes, aggregate captures and sedimentation rates, bio-fouling, as well as beneficial and pathogenic microorganisms which may be available in the water column.

Aquaculture management, which includes a microbial perspective, is needed to sustain Indonesian aquaculture. The assessment of pathogenic bacteria via cultivation-based method as well as single bacterial utilization in outdoor facilities seems to be ineffective to improve rearing practices. Besides the need for rapid and precise assessments of the causative diseases, other investigations which involve microbial monitoring, particle fluxes in the water column as well as carrying capacity for aquaculture activities are highly necessary to be taken. In addition, regular records of water quality parameters over rearing periods are still needed to improve rearing processes (Alfiansah & Gärdes, 2019). It is expected that having holistic information about the bacterial community compositions in the water column as well as biogeochemical parameters of aquaculture systems with particular emphasis on shrimp pond aquaculture will minimize rearing failures and improve both quality and quantity of production.

6. Conclusions and Outlook

Aquatic ecosystems, particularly aquaculture areas, contain aggregates that are loaded with microorganisms. They play vital roles in ecosystems, especially on the vertical transports, biogeochemical cycles, and nutrient supplies. It is evident that certain pathogenic microorganisms, including bacteria, live in aggregates. Their presence may endanger aquaculture. Therefore, identification of the microbial community compositions, especially pathogenic bacteria on aggregates, is necessary to gain better knowledge on the abundance and diversity of potentially pathogenic microorganisms. As an integral part of aquatic ecosystems, particularly in the aquaculture ecosystem, aggregates may play significant roles in the sustainability of shrimp aquaculture and mariculture, including IMTA. Regular observation of aggregates in terms of their quantity and quality, sinking rates, and microbial communities with an emphasis on pathogenic bacteria surveillance may improve aquaculture and harvest yield via enhancement of cultured animal performance and reduction of operational costs and avoid rearing failure due to disease, eutrophication and water quality deterioration.

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