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Biofloc Technology: An Overview and its application in animal food industry

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Abstract

On growing demand of protenacious food, particularly animal protein necessitates animal husbandry and fisheries. Over exploitation of capture fisheries forces aquaculture production intensification. But now a day's aquaculture facing both environmental and economic unsustainability due to the overexploitation of fish meal, oil and environmental pollution through the emission of toxic metabolites. Biofloc Technology (BFT), one of the innovative waste management and nutrient retention technologies offers a solution to solve the problems. In aquaculture ponds Biofloc Technology removes toxic metabolites and through microbial mass protein production helps to retain more nitrogen in the form of fish or shrimp biomass. BFT is a C/N ratio optimization technology and for its optimal practice requires more knowledge about this. Paper deals with the history, benefits and major works in this field and also tried to express factors for aggregation of microbes within the floc and ionic binding by means of extracellular polymeric substances. Various physic- chemical parameters such as mixing intensity, temperature, pH, organic loading rate and organic carbon sources affects the flocculation, size quality and quantity. Microbial flocs also have probiotic effect through the production of poly β- hyroxy butyrate to the surroundings. Through the quorum sensing and co- aggregation of microbial groups determines the floc quality and also helps to remove or suppress the influence of pathogens in the aquaculture system. Biofloc now a day's used in the reproduction of fin fishes and Aquaponics also. Finally describes the over all added value of biofloc technology and future research possibilities. Articles suggests more molecular technologies are necessary for the characterization of functional genes in the BFT system such as FISH, PCR and DGGE.

Keywords: Aquaculture, Biofloc Technology, microbes co-aggregation, Bioflocculation, C/N ratio optimization, sustainable aquaculture

1. Introduction

Increasing trends of human population from 1.5 to 6.4 billion and is predicted to increase to 9 billion by the 2050 necessitates the production diversification to reduce the malnourishment, which has been estimated 840 million[1]. To ensure the health of world population by providing nutritionally balanced especially protein rich food, animal husbandry and fisheries are the two sources of animal protein. Fish and fishery products are the safest group of animal protein, containing all essential amino acids, essential fatty acids of n-3, n-6 series [2,3, 4,5]. But today's production and demand have a huge gap compared to the exponential growth of world population. To solve these problems culture of organisms under controlled conditions in water ie; aquaculture is the only way. But in aquaculture intensification is necessary to meet the food demand.

Issues in aquaculture

The aquaculture industry is growing fast at a rate of 9% per year since 1970's[6], but is going through unsustainability in the meaning of environmentally and economically, haunted by number of environmental and social issues [7].Over usage of antibiotics, disease and exotic fish transfer to wild and omitting of heavy load nutrients are under consideration. Over dependence of fish meal and privatization of public common leads to physical conflicts among fishermen and depletion of natural resources. All these factors searches opportunities for economic and environmental developments permeate in to many facets of the industry[8]. Of the total production expenses 50% represents for feed cost, which is predominantly due to the cost of protein component in commercial diet[9]For the production of 1 kg live weight fish needs 1-3 kg dry wt feed(FCR is about 1-3, [10]. Of the total nitrogen input in culture ponds only 20-30% retain in biomass and the rest becomes a polluting agent of the water by producing high level toxicants such as ammonia and nitrite leads to eutrophication in the surrounding water of the aquaculture farms [11,12,13]. Approximately 36% of feed excreted as in the form of organic waste. Thus tool to make up industry more sustainable pond management should be geared towards improving nutrient retention [14].

Biofloc Technology

Now a days closed aquaculture systems are more safe for biosecurity, having environmental and marketing advantages over conventional extensive and semi intensive systems [15]. Due to the re usage of water reduces introduction of pathogens and exotic to the external environment. Such a type of environment friendly technology for closed aquaculture is Biofloc Technology(BFT). More nutrient retention and recycling has been take place internally without so more cost compared to the conventional waste management technologies such as recirculating aquaculture systems and biofiltration, both needs complex internal filtration[16], expensive, laborious and economically not feasible[17]. BFT is an innovative one, from conventional autotrophic to autotrophic- heterotrophic system. Microbes like bacteria are generally regarded as a disease causing agent in animal and plants. But in controlled conditions dense and active aerobic microbial communities control water qualities through immobilization of toxic metabolites to microbial protein and recycle the feed residue and raise the feed efficiency [18,19,20]. Compared to the conventional photoautotrophic system BFT has more advantages such as diurnal variation of dissolved oxygen and hydrogen ion concentration is limited. Assimilation of inorganic nutrients through bacteria in suspension is an additional protein source than the biosynthesis of algae. Through the decomposition of organic matter by microorganisms leads to the production of new bacterial cell amounting 40-60% of the metabolized organic matter[21]. This aerobic decomposition can be higher in high C/N ratio (through the addition carbohydrate source should be economically feasible) with high oxygenic conditions.

BFT is a green approach in aquaculture knowing in different names such as zero exchange autotrophic- heterotrophic system[22,23,24] active sludge or suspended bacterial based system[25], Single cell protein production system[18], Microbial floc system etc. Now biofloc has been focused internally as an alternative protein for fish meal, mainly produced in the form of microbial meal[26]. Many researchers has been conducted the study of nutritional acceptability of biofloc.

History of Biofloc Technology(BFT)

Historically BFT was first developed in French Research Institute for exploitation of the Sea, Oceanic centre of Pacific[27]. It is become a popular technology in the family of Tilapia, *Penaeus monodon*, Pacific white shrimp *Litopenaeus vannamei*, giant freshwater prawn *Macrobrachium rosenbergii*[28], *Fenneropenaeus merguiensis* and *Litopenaeus stylirostris*[29,30]. To studying more about the BFT Ifremer started a research programme “Ecotron” in 1980. In the same period Israel and USA (Waddel mariculture centre) started R&D in BFT with Tilapia and white shrimp. Commercial level application of BFT started in 1988 by Sopomer farm[31] and Belize aquaculture farm of Central America producing approximately 26tons/ha/cycle using 1.6 ha lived grow out ponds. Green house BFT farms is successfully run in Maryland, USA. Now a days successful implementation expanded in Latin and Central America and small scale in USA, South Korea, Brazil, China, Italy, Indonesia(P.T Central Prtiwi Bahari), Australia[32], and India.. Research institutes of many countries now concentrate to more application of Biofloc technology such as energy kinetics, bacterial identification and economics, low cost processing etc. Diversification to the level of reproductive application in shrimps and fishes are also continuing. In Belize 13.5 mt shrimp/ ha was an achievement at the time. The same model is applied in Indonesia. The technology combined with partial harvest was repeated in Medan, Indonesia with better results during 2008& 09. Now biofloc technology is common in Bali and Java. In Indonesia intensification of shrimp aquaculture is doing in HDPE lined and plastic sheet covered ponds by using this technology. Malaysia is currently initiating a 1000 ha integrated shrimp farming project at Seitiu, Terengganu by Blue Archipelagio. In India this technology is not yet popular. But researches and onfarm trials were conducted in Cochin University of Science and Technology, Kerala in 2006 [33,14]. It was proved that its application is possible in the larval culture of *Macrobrachium rosenbergii* by[34]. Nursery rearing of the same species with the BFT gave an opportunity for improving the larval stocking density and animal heath welfare compared to normal conventional systems[28]. Sensitivity of floc at different hydrogen ion concentrations was conducted by [35] and optimized 7.5 is optimum for good flocculation in shrimp aquaculture. [36] stated the C/N ratio in aquaculture sludge is maximum 3and to reach the optimal range of 10-15 addition of extra carbon source is necessary. BFT is an insitu production system increase the nitrogen retention as body mass in culturing organisms. Since BFT was introduced, several research groups elaborated this technology. More application of this innovative waste management technology is an ongoing research.

Benefits of BFT

BFT has several benefits compared to the semi intensive ponds and Recirculating Aquaculture Systems(RAS) such as it can improve biosecurity, feed conversion ratio, water use efficiency, land use efficiency, water quality and have a reduced sensitivity to light fluctuations. But some disadvantages also, it required high energy for mixing and aeration, reduced response time because water respiration rates are elevated, needs alkalinity supplementation and an increment of nitrate in the system[37].Table1 describes the major beneficiary works in the field.

Table 1 Some researches based on BFT

Species	Results	References
Oreochromis Niloticus	Use of 30% protein feed -55ton/ha in 51 days use of 20%protein feed: 76ton/ha in 51 days - 31%reduction in feed cost	[38]
Penaeus monodon	Use of 40% protein feed:448 kg/ha in 94 days, use of 25% protein feed+ tapioca flour:644kg/ha in94 days-increase in nitrogen retention from20% to 45%.	[14]
Litopenaeus vannamei	Supplementation of 30% protein feed with molasses: no effect on growth	[39]
Macrobrachium rosenbergii	C/N 10: 445 kg/ha- 120 days C/N 20:(by addition of tapioca starch): 583 kg/ha-120 days	[40]
Oreochromis niloticus	35% protein feed:21ton/ha in 84 days. 24%protein feed+ wheat flour: 31 ton/ha in 84 days. 35% protein feed+ wheat flour:30 ton/ha in 84 days.	[16]
Oreochromis niloticus	Addition of starch and labeled 15N- 25% of feed protein could be replaced with Biofloc protein	[41]
Litopenaeus vannamei	Normal feed- 3.1 ton/ha in 35 days. Biofloc incorporationinfeedat7.8%-4.7ton/hain35daysand15.6%incorporation-4.6ton/ha in 35 days.	[26]

Penaeus monodon	Increase of C/N ratio by carbohydrate addition and augmentation of shrimp production- nitrogen retention from added feed by 13%.	[33]
Artemia franiscana	Glycerol grown Biofloc controlled quorum sensing regulated bioluminescence of *Vibrio harveyei*-(showed the probiotic effect of Biofloc)	[43]
Penaeus monodon	Pl-15 grown for 45 days in BFT systems with and without artificial substrate at higher stocking density, higher weight was observed in substrate added treatments (.40gm) without affecting the water quality.	[44]
Litopenaeus *vannamei*	Inclusion of whole shrimp floc and floc fraction to a formulated diet- improved shrimp growth rate without affecting survival	[45]
Poecilia reticulata	Ammonia reduction by improving C/N ratio by addition of tapioca flour- higher growth rate at C/N ratio of 10&20.The C/N ratio of 20&30 worked more effectively.	[46]
Macrobrachium rosenbergii	Effectiveness of BFT with different fin fishes. Combination with Tilapia gave good yield. Maize was used as the carbohydrate source.	[47]
Macrobrachium rosenberggi post larvae	Bioflocs developed with different carbohydrate source and used as feed(glycerol, acetate and glucose). The glycerol bioflocs have higher protein, higher prawn survival) study concluded that selection of carbohydrate source determines the floc quality.	[48]
Macrobrachium rosenbergii(nursery system)	BFT application with different stocking density.200BFT give highest survival rate of 60%(conditional index same in all BFT tanks(18/20). Lowest RNA/DNA ratio was reported at 200BFT	[28]
Macrobrachium rosenbergii	Influence of BFT on prawn growth at 32%,28% and 24%protein levels. Higher growth rate was observed at 24%protein.	[28]
Macrobrachium rosenbergii	Application of BFT in mixed culture with major carps showed higher prawn growth rate with 100% Catla added treatments.	[28]
Penaeus monodon	Optimization of bioflocculation at different hydrogen ion concentrations-7.5 give good flocculation both qualitatively and quantitatively.	[35]
Fenneropenaeus paulensis	BFT increases 50% weight and 80% biomass in the early post larval stages compared to conventional clear water system.	[49]
Litopenaeus	Application in nursery rearing system improved	[50,51]

vannamei	survival rate ranging from 55.9-100%, 97% and 100% respectively.	
Fenneropenaeus brasiliences	BFT system with and without pelletized feed addition gave good results compared to conventional clear water system	[52]
Litopenaeus vannamei	Juveniles fed with35% CP pelletized feed grew significantly better in BFT conditions compared to clear water system	[24]
Litopenaeus vannamei	BFT helped to decrease FCR and feed cost reduction	[23]
Litopenaeus vannamei	Heterotrophic bacterial based system showed no influence on the growth and FCR value with 30% and 45% crude protein and 39% and 43% CP in diets.	[53]
Penaeus monodon	Evaluated the performance of various locally available carbohydrate source as bioflocculating agent and tapioca flour was selected as a good one.	[54]
Macrobrachium rosenbergii larvae	Addition of tapioca flour increased the survival rate of larvae	[34]

Aggregation of microbes in the floc and factors affecting floc formation

Floc is actually an aggregation of microbes. [55] said that microbial flocs consist of a heterogeneous mixture of floc forming and filamentous bacteria, particles, colloids, organic polymers, cations and dead cells and they may become the size of 1000µm[fig:2&3]. Aggregation of bacterial cells to the floc helps them to overcome the physical constraints for acquisition of food, problem facing if they live independently. During aggregation microbes counter the nutrient diffusion problem [56]. Individual cells with in the floc do not have a higher substrate uptake compared to dispersed planktonic cells[57]. The additional energy they getting from the nutritious advantage may invest in order to sustain floc formation[58]. The floc permeability allows advective flow to pass through the pores and water follow the path of least resistance, then higher nutrient supplementation can be possible. The relative uptake factor determines the advantage of floc. It is actually is the function of power input the water for aeration and mixing. Optimal power input for floc formation is 0.1-10Wm^3[59]· Aggregation have another benefits such as cells may become less susceptible to predation majorly by protozoan grazing[60]. Microbial floc formation is a complex process, which involved many physical chemical and biological phenomena. Extracellular polymeric substances(EPS, produced by bacteria), which is made up of polysaccharides, protein, humic compounds, nucleic acids and lipids form a matrix and encapsulates(produced under various nutritional conditions particularly in case of limitation by nutrients like nitrogen) the microbial cells and play a major role in binding the floc components together[56]. The negatively charged EPS determines the zeta-potential [61].Positively charged counter ions tightly fixed to the surface and forms a stern layer. An ion cloudy layer outside of this is known as diffusive layer, which is electrically neutral. When such a particle moving through the water some of the charges will be lost and form a shear plane at zeta- potential. Due to equal surface charges, particles are repelled from each other and are kept in dispersion, but which is countered by Van der Waals forces, which is developed through the polarization of molecules in to dipoles and induce an attractive power between the particles. So the zeta- potential and Van der Waals forces determines the floc formation[62, Fig.1]. Several other factors may also behind the floc formation such as, proton- translocation dehydration[63], cation bridging, and steric interactions etc. [56] reported that Quorum sensing act as a regulatory factor of flocculation. It is the regulation of gene expression programmes[64]. Depending on the cell density it is the way of cell to cell communication between the bacteria[65]. As in biofilms it can reasonably be expected to be active in bioflocs[66], which has mainly been shown to result in a differentiation of existing aggregated structures. So the micro colony formation or aggregation of some bacteria improves the quorum sensing and the related colony association increased the activity of the genes encoding for the production of polypeptide signaling molecules known to induce extracellular polymeric substance production forms a well differentiated qualitative floc. For example co cultivation of *Thermotoga maritime* and *Methanococcus jannaschii* induced increased flocculation compared to a *Thermotoga* monoculture[67]. The molecular and biochemical mechanisms behind the quorum sensing remains far from known. In the study of [56] recommended the seedling of quorum sensing species with in the ponds may allow them to integrate in the flocs and thus improve the floc formation and this is one of the interesting application prospective and one of the existing research direction in this field. Floc formation is actually the end product of many physical, chemical and biological processes and all these factors may affect floc formation and its quality. Size and quality variation improves its accessibility in throughout the life stages of an organism. Mixing intensity, dissolved oxygen, hydrogen ion concentration, organic carbon source, organic loading rate and temperature are the factors mainly affects the floc formation (floc volume index, floc size, communal representatives, nutritional aspects etc). Of all the dependary factors, mixing intensity have much more priority. Intensive turbulent mixing is essential for floc formation and kept in suspension. The unsuspended settled microbial flocs creates an anaerobic zone in the water and produce toxic metabolites such as hydrogen sulphide, methane, Ammonia etc [37]. Increasing of the microbial concentrations in BFT systems demands high oxygen because of that the unavailability of the oxygen leads to the failure of the system and death of the culturing organism too. Response time in the event of a system failure is often less than 1 hour[37]and also biofloc systems are not good choice in areas where power supplies are unreliable or electricity is expensive. [37] also said that other sensitive factor for determining the transitional stages of biofloc from algae to bacteria is the feeding rate. The bacterial domination in Biofloc is directly related to the increasing feeding rate.The degree of beneficiary effects contributed by flocculation depends greatly on the community structure of the floc, which evolves over the coarse of culture and which is affected by the culture conditions [68,69]. Now a days no research has been conducted on the relationship between the operational parameters and functioning of BFT [56]. It opens a new way of research. Fig 2 and 3 gave a basic idea about the community structure of biofloc. Selection of proper candidate species is the success of BFT, it is the basic factor in designing a Biofloc system. Biofloc system work best with species that are able to derive some nutritional benefit from the direct consumption of floc. For example the physiological adaptations of Tilapia and shrimp is able to consume biofloc properly and have the capacity to digest this microbial protein. But some fishes such as channel cat fish and hybrid striped bass are not good candidate for Biofloc systems. The species should have the ability to survive very high solid concentrations. Fishes having omnivorous feeding habits are advisable.

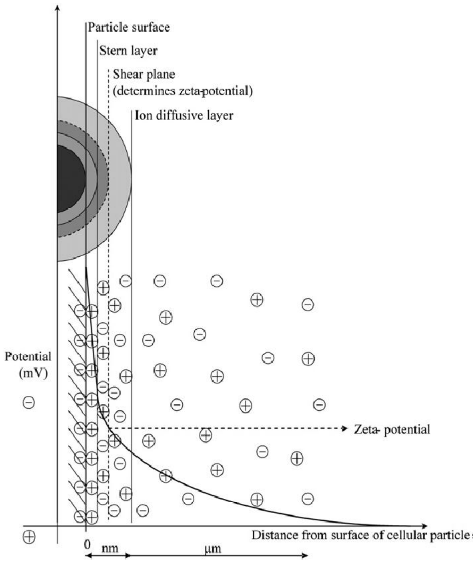

Fig:1 Schematic view of a charged cellular particle with its counter charges and the potential in the area of a particle surface -source:[70].

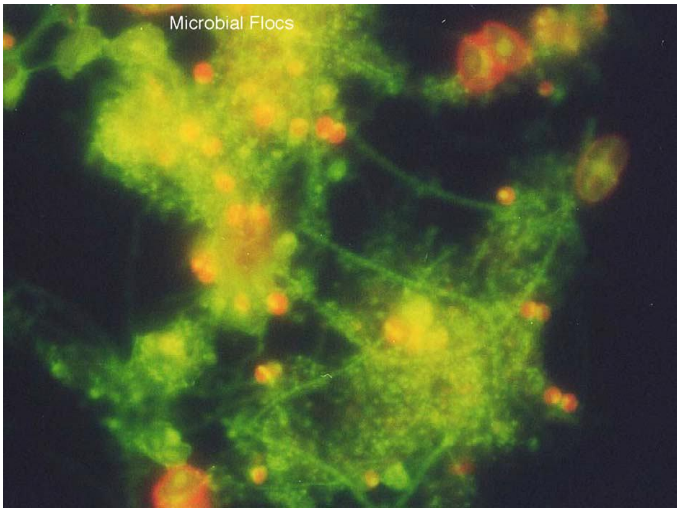

Fig:2 Epifluorescence microscope photo of floc from a shrimp ASP, stained with acridine orange. Courtesy of Michele Burford

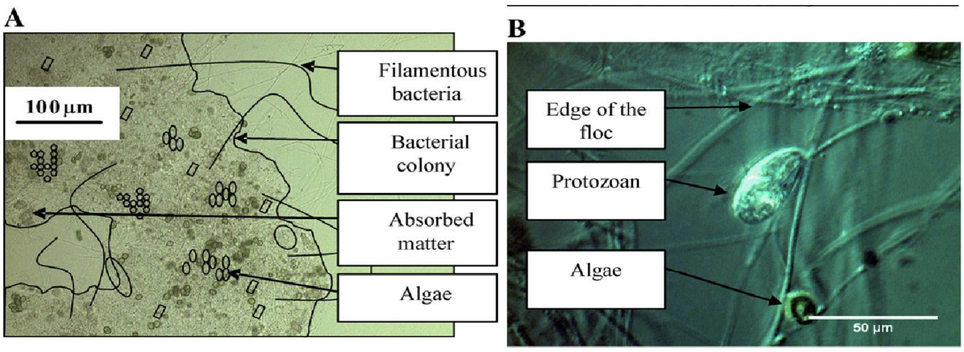

Fig. 3. A. Image of a floc structure within a BFT-system and its composition; B: a protozoan that is grazing at the edge of a floc removes the cells that tend to leave the floc. Source[70]

Nutritional benefits and water quality maintenance.

Macroaggregates plays an important role in nutrient recycling through the assimilation of toxic nitrogenous metabolites[71,33,14], and retention of nitrogen from added feed up to 13% [33, 72]. BFT is an active suspension technology overcomes the limitations of light dependent conventional phytoplankton based system[16]. Regarding to the maintenance of water quality control of heterotrophic bacterial aggregation over the autotrophic bacteria is achieved through the balancing of C/N ratio [38] by the addition of extra carbohydrate source or the application of low protein contained feed. The amount of carbohydrate needed for improving the C/N ratio to 10 or 15 was worked by[21]. Many practical and processed materials have been used as carbon sources in Biofloc systems, including grain pellets, molasses, sugarcane bagasse, and chopped hay among the others. The system will be attain more economically sustainable one through the selection of low cost carbohydrate source. High amount of carbon in water supersede the carbon assimilatory capacity of the algae and increases the heterotrophic bacterial production. They are single cell proteins need nitrogen for their development that increases the assimilatory activity of the bacteria reduces the toxic ammonia from the culture system effectively [38]. One of the feature of low exchange Biofloc system is the accumulation of high concentration of nitrate. The best method to avoid this condition is the periodical removal of the solids [37]. It has the capacity to improve water quality through the elimination of toxic materials such as ammonia and nitrite[69,73]. Farmers spend 50% of the production cost as for producing complete diets comprising 18-50% protein, 10-25% lipid, 15-20% carbohydrate and trace amounts of vitamins and minerals [74]. For sustainability in aquaculture industry needs to investigate alternative protein sources to replace costly fish and soy like ingredients. But the selected source should have good digestibility, palatability energy content and a well balanced amino acid profile [75]. Biofloc(diversed microbiota) can be produced directly in the aquaculture system by the addition of carbohydrate source otherwise through bioreactors.[37] reported that the dry weight protein content of biofloc ranged from 25-50% with most estimates between 30 and 45%and essential fatty acids, pigments such as carotenoids promote the growth of the culturing organisms.

Microbial particles can provide important nutrients such as protein[16,49], lipids[24,70,], amino acids[22] and fatty acids[76,77].Biochemical composition of different bioflocs from various researches outlined in Table2.The most important factors determining the feasibility of Biofloc as a feed in aquaculture is the high protein, Polyunsaturated fatty acids(PUFA) and lipid content[56]. Lot of studies reported the presence of floc has been confer beneficial effects on aquaculture. Floc enhanced animal growth from its nutrients and micronutrients [78]. Floc improved animal product quality, such as color and sensory properties from pigments such as astaxanthin and flour materials (eg:bromophenols)[79, 80]. [45] reported that on the basis of EAA and EAAI protein fractions of the floc, if it is algae dominated or bacteria dominated were comparable to those of fish or soybean meal. Due to this comparability Biofloc is now used as a replacement ingredient for meal and it was applied in nursery, grow out and in latest the breeding system of shrimp and fish. In the study of [81] found it can be possible to reduce 10% protein content of the pelletized feed in the nursery rearing system of *Fenneropenaeus paulensis* with BFT. A low protein Biofloc meal based pellet(25%CP) was evaluated as a replacement conventional high protein fish meal diet (40% CP) for *Litopenaeus vannamei* in a relatively low temperature under Biofloc conditions[27]. Many studies conducted recently have been demonstrated the replacement possibilities of fishmeal by other protein sources under BFT conditions directly or by the incorporation of Biofloc meal in feed.[82] done such a type replacement of fish meal in the level of 40 and 100 % by soya meal and viscera meal in diets of *Litopenaeus vannamei* reared under BFT system. The result concluded 40% replacement is possible. Now a days the developed Biofloc from one culture system can be used as the feed of other system of aquaculture.(ex: floc generated from Tilapia effluent was incorporated in the feed of shrimp by[26]. [83] detected fish meal can be completely replaced with soy protein concentrate and Biofloc meal.[8]evaluated the effect of two types of bioflocs derived from biological treatment of fish effluent as feed ingredients for Pacific white shrimp *Litopenaeus vannamei* and found that all shrimp receiving diets that included bioflocs exhibited faster growth rates from 3.5to 15% faster, mean of 10%, compared to the control diet. Microbial floc meal based diets significantly outperformed control fishmeal based diet in terms of weight gain per week with no difference in survival. Other applications of Biofloc meal in animal industry should be evaluated mainly considering the nutritional profile and relatively low costs as compared to other protein sources. Knowledge of the community structure of the floc and its nutritional value especially amino acid composition help to develop cost effective shrimp feed formulations[84].

Table:2 Proximate analysis of Biofloc particles in different studies

Crude protein%	Carbohydrate%	Lipids%	Crude fiber%	Ash%	Reference
43.00	_	12.5	_	26.5	[85]
31.2	_	2.6	_	28.2	[73]
12.0-42.0	_	2.0-8.0	_	22.0-46.0	[86]
31.1	23.6	0.5	_	44.8	[24]

26.0-41.9	_	1.2-2.3	_	18.3-40.7	[87]
30.4	_	1.9	12.4	38.9	[45]
49.0	36.4	1.13	12.6	13.4	[26]
38.8	25.3	<0.1	16.2	24.7	[8]
28.8-43.1	_	2.1-3.6	8.7-10.4	22.1-42.9	[88]
30.4	29.1	0.5	0.8	39.2	[89]
18.2-29.3	22.8-29.9	0.4-0.7	1.5-3.5	43.7-51.8	[90]
18.4-26.3	20.2-35.7	0.3-0.7	2.1-3.4	34.5-41.5	[91]
28.0-30.4	18.1-22.7	0.5-0.6	3.1-3.2	35.8-39.6	[92]

Source:[93]

Biofloc- As a bio control agent in aquaculture

High C/N ratio is the basic principle behind the heterotrophic growth in BFT systems. The frequent supplementation of feed and/or additional carbon in the system results in alternating periods of carbon excess and limitations for the microorganisms in the water[56], that can trigger the accumulation of poly β- hydroxybutyrate(PHB), a bacterial storage compound in the family of polyhydroxyalkonates. [94] reported that in situation of limited essential nutrients like nitrogen with the presence of excess carbon source PHB will be produced and degraded in the gut of culturing organism, which have an antibacterial activity similar to short chain fatty acids, act as a preventive curator against *vibrios*[95]. Preliminary studies reported the presence of PHB in the mixed cultures of BFT. This intracellular biodegradable polymer involved in bacterial carbon and energy storage. In the case of cell death or lyses degradation of PHB is performed by the activity of extracellular PHB depolymerised enzymes of bacteria and fungi results 3-hydroxybutyrate in to the surrounding environment(Trainer and Charles,2006,96) and offer a probiotic advantage to the culturing organism. PHB accumulating bacteria may abate pathogenic bacteria in aquaculture[97,98]. Antibacterial activity remain needs further study. In the study of [99] suggested bioflocs containing microbes down regulate virulence factor expression and positively influence the gut health of animals.[100] found that in Tilapia fingerling BFT system showed less ectoparasites in gills compared to conventional clear water system. [43] investigated the biocontrol efficiency of glycerol grown biofloc against opportunistic pathogen *Vibrio harveyi* in the gnotobiotic brine shrimp(*Artemia franciscana* larvae)production and stated that biofloc and biofloc supernatants decreased quorum sensing regulated luminescence of *Vibrio harveyi* and indicate in addition to water quality control and insitu feed production biofloc technology could help in controlling bacterial infections with in the aquaculture ponds. The mechanism behind the probiotic effect of biofloc is actually the competition between microorganisms in the floc and pathogen for space and some essential nutrients, and prevent pathogens multiplication.

Bioflocs on reproduction

[93] described the possibility of BFT in brood stock rearing tanks considering its insitu nutritive capability ie., 24 hours per day. He says that bioflocs could be helped to first stages of gonad formation and ovary development. It avoids the unstability of

water quality parameters, related to the conventional brooders rearing system. This opinion is coincides with the study of [101]. They applied BFT in the breeding ponds of *Litopenaeus stylirostris* and *Farfantepenaeus duorarum* respectively and obtained high number of eggs per spawn and higher spawning activity. [93] also suggested that in breeding system should control Total suspended solids(biofloc) such as15 ml/l.But now a days it applied only in shrimps, not in fish breeding systems. It might open a new research gate. Biofloc contained high amount of ARA (16), fatty acid crucial for reproduction and as hormone precursor, is suggestive for its application in breeding.

BFT in Aquaponics

Aquaponics is a sustainable production system combines traditional aquaculture with hydroponics. Now a days aquaponics is common in western countries. The waste excreted from aquaculture species and uneaten feed is converted in to beneficiary nutrients in water. These aquaculture waste water is directly pumped to raceways of hydroponics vegetables. Typical plants raised in aquaponics include lettuce, chard, tomato and fruits such as passion fruit, strawberry, watermelon etc. Now a days BFT have been successfully applied in Aquaponics. The presence of rich microbiota and variety of micro and macronutrients especially Nitrate(In BFT systems have high amount of nitrate compared to other systems). A well known BFT and aquaponics interaction was developed by United Virgin Islands(UVI). But this BFT aquaponics system is questionable and needs more investigation. It require solid management and excess adhesion of microbes in the roots of plants is problematic because of reduced oxygen and growth.

Conclusion

Although researches with the forerunners to biofloc systems has been underway since the early 1990s and commercial application have been in place since early2000s, But some key issues of biofloc system function are still poorly understood [37]. As per researches biofloc technology is the best for closed system management and providing biosecurity in aquaculture. One of the major risky factor of these technology is the periodic increment of total suspended solids, cause clogging in shrimp and fish gills and demands more energy to meet the oxygen demand.[70] described the parameters of importance for the characterization of flocs. He mentioned the physical and chemical characteristics of the flocs and suggested the optimum. Based on this the suspended solids(SS) and Floc Volume Index(FVI)should be 0.2 to 1.0gL^{-1} and>200mlg^{-1} respectively. System stability is necessary for good results. Any failure occurs that will lead to the filamentous bulking(out break of filamentous bacteria), makes slow settlement of flocs and make it difficult to control solid concentration [37]. Ignorance of farmers about this technology is another problem. Promotion programmes must be conducted. Another defect of this technological application is that more concentrating to the Shrimps and Tilapia that’s why general criteria development for the system configuration is difficult. Species diversification is necessary for the successful elaboration of this technology. Bioflocs probiotic effect should be investigated. It has the capacity to control *Vibrios,* particularly in shrimp culture systems. But some studies reported that *Vibrios* are also present in bioflocs but a switch on off systems works there that determines the capacity to encourage or discourage the possibility of disease outbreak. In the opinion of [37] the major thing determines this capability of floc is the total solid concentrations. The co culture of aquaculture species and heterotrophic bacterial biomass by the addition of carbohydrate source is the common way of BFT systems. But the multiplication of the heterotrophic bacteria causes excessive turbidity in the system which may have negative effects on sensitive fish species, not all are adaptable to growing in turbid water [102]. So most of the scientists suggest the compartmental design of BFT. In such applications, the culturing of fish and microbial production are in separate compartments, which allows a better management possibilities. A good understanding of the microscopic mechanisms (microbes especially bacteria) that are involved involved in bioflocculation is necessary for future BFT practice. Advanced biological biofloc monitoring technologies are necessary for the closed examination of microbes[70], such as FISH procedure[103] and Real time Polymerised Chain Reaction(PCR)and Denaturing Gradient Gel Electrophoresis(DGGE). A quantitative way allows for the simultaneous quantification of functional genes involved in the technique. Advective flow and quorum sensing will argument our capability to steer the microbial aggregation and there by optimization of good floc as in terms of size and distribution[56]. He also suggested that more studies are concentrating to the water quality management aspects of bioflocs not so much on the compositional aspects, the later can represent a major added value for aquaculture. The effects of physico- chemical parameters on flocculation needs depth investigation. Focusing on the shifting of microbial population is an another interesting area of research. In microbial flocs filamentous and flocculating bacteria are in a balance. Overperformance of filamentous causes floc bulking and thereby leading to system unstability and incomplete nitrogen removal and pin point flocs or immediately settling flocs. [48] suggested the addition of chemoattractants to the pond or incorporation to the bioflocs can be an interesting field of study that further aims at increasing the nutrient intake by the cultured animal through BFT. Anyway this ecofriendly, probiotic, stable (as in terms of water quality),insitu nutrients giving and recycling, non risky technology needs future research for knowing the complicated things behind it.

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