Heliyon 10 (2024) e25228

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Review article

Swinging between the beneficial and harmful microbial community in biofloc technology: A paradox

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ARTICLE INFO

Keywords:
Heterotrophic bacteria
Amphibolic process
Water quality
Zootechnical variables
Functional characterisation

ABSTRACT

Biofloc Technology (BFT) is proven to be the fulcrum of sustainable recirculating aquaculture system especially under zero water discharge condition. The efficiency of BFT system is reinforced by an unswerving microbial community in the system. Several researchers have made copious reports on the microorganisms in BFT and identified heterotrophic bacteria predominant in the microbial composition. A summary of these researches considers these microorganisms playing the role of chemo-photosynthetic autotrophs, organic detoxifiers, probiotic, decomposers/bio-flocculants, bio-leachers and pathogens. Although these functional roles are well identified, the reports have failed to sufficiently illustrate the borderline at which these microbial communities fail to serve their beneficial roles in BFT system. This review paper firstly presents a snapshot of some indispensable water quality conditions and zootechnical variables aided by the microbial community in floc as well as the amphibolic process that synthesizes nutrient from the organic deposit in BFT. Furthermore, information on the microbial community in BFT is evaluated to have *Bacillus* sp., *Lecane* sp. and *Pseudomonas* sp. serving all-encompassing role in BFT while *Vibrio* sp. and *Enterobacter* sp. are pathogenic under unsuitable water quality conditions. Functional characterisation of the commonly reported microorganisms in BFT categorised 21.95 % as most critical, whose abundance indicates an efficient BFT.

1. Introduction

The advent of biofloc technology (BFT) has resolved the unavoidable challenge of toxic waste accumulation in closed aquaculture systems by the introduction of microbial community that co-exist in a complex multi-functional interaction to create a culture system that promotes optimum growth supported by auto-synthesized food sources and enhanced health conditions. BFT system is a zero-water exchange system that relies on the microbial community not only in detoxifying the ammonia generated from fecal deposits and unconsumed feed. Sustainable aquaculture is deterred by an upsurge in the formation of lethal nitrogenous waste caused by organic residue under intensive culture [1–4]. BFT is a sustainable aquaculture technique that relies on the *in-situ* production of

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https://doi.org/10.1016/j.heliyon.2024.e25228
Received 9 June 2023; Received in revised form 28 December 2023; Accepted 23 January 2024
Available online 1 February 2024

Table 1
Water quality conditions in the biofloc and clearwater systems.

Culture system		Species	Culture period	References
BFT	CLW			
Temperature (°C)				
29.10	29.10	*L. vannamei*	55 days	[30]
27.20	29.00	*L. vannamei*	80 days	[31]
27.90	*28.00*	*L. vannamei*	48 days	[32]
27.00	*27.80*	*P. monodon*	127 days	[33]
26.20	*26.50*	*L. vannamei*	30 days	[34]
26.20	*26.40*	*M. japonicus*	106 days	[4]
26.50	*26.60*	*P. satiferus*	45 days	[35]
23.12	*23.39*	*L. vannamei*	60 days	[36]
32.30	*32.00*	*F. indicus*	120 days	[37]
Summary:				
Biofloc (BFT): 27.28 °C;				
Clearwater (CLW): 27.64 °C				
Dissolved Oxygen (mg.L^{-1})				
6.40	6.30	*L. vannamei*	83 days	[13]
5.04	5.37	*L. vannamei*	80 days	[31]
5.70	*6.00*	*L. vannamei*	48 days	[38]
5.02	6.12	*P. monodon*	127 days	[33]
7.60	8.10	*L. vannamei*	30 days	[34]
5.60	7.80	*M. japonicus*	106 days	[4]
7.10	7.20	*P. satiferus*	45 days	[35]
5.99	6.57	*L. vannamei*	60 days	[36]
4.13	4.20	*F. indicus*	120 days	[37]
Summary:				
*Biofloc: 5.*84 mg L^{-1};				
*Clearwater: 6.*40 mg L−1				
pH				
7.70	7.90	*L. vannamei*	83 days	[13]
6.61	6.77	*L. vannamei*	80 days	[31]
7.59	*8.02*	*P. monodon*	127 days	[33]
7.87	*8.03*	*L. vannamei*	30 days	[34]
7.80	*8.30*	*M. japonicus*	106 days	[4]
7.40	*8.20*	*P. satiferus*	45 days	[35]
7.80	*7.80*	*L. vannamei*	60 days	[36]
8.22	*8.23*	*F. indicus*	120 days	[37]
Summary:				
Biofloc: 7.62;				
Clearwater: 7.90				
Salinity (g.L^{-1})				
28.40	28.80	*L. vannamei*	83 days	[13]
25.00	*25.00*	*L. vannamei*	48 days	[38]
31.60	31.90	*L. vannamei*	80 days	[31]
16.85	*16.94*	*P. monodon*	127 days	[33]
31.80	*31.50*	*L. vannamei*	30 days	[34]
22.70	*22.90*	*M. japonicus*	106 days	[4]
34.70	*35.00*	*P. satiferus*	45 days	[35]
41.20	*39.90*	*F. indicus*	120 days	[37]
Summary:				
*Biofloc: 29.*03 g L^{-1};				
Clearwater: 28.99 g L−1				
Total Ammonia- Nitrogen, TAN (mg.L^{-1})				
0.10	0.30	*L. vannamei*	83 days	[13]
1.50	*0.30*	*L. vannamei*	48 days	[38]
0.43	1.15	*P. monodon*	127 days	[33]
0.13	0.09	*L. vannamei*	30 days	[34]
0.26	0.42	*L. vannamei*	21 days	[39]
0.57	0.78	*L. vannamei*	60 days	[36]
Summary:				
*Biofloc: 0.*49 mg L^{-1};				
*Clearwater: 0.*50 mg L^{-1}				
Nitrite (mg.L^{-1})				
2.20	0.90	*L. vannamei*	83 days	[13]
9.20	*2.50*	*L. vannamei*	48 days	[38]
0.58	*1.15*	*P. monodon*	127 days	[33]
0.43	*0.13*	*L. vannamei*	30 days	[34]
0.15	*0.45*	*L. vannamei*	21 days	[39]
0.93	*1.89*	*L. vannamei*	60 days	[36]

(*continued on next page*)

Table 1 (*continued*)

Culture system		Species	Culture period	References
BFT	CLW			
0.52	*0.03*	*F. indicus*	120 days	[37]
Summary:				
*Biofloc: 2.*00 mg L^{-1}*;*				
*Clearwater:1.*00 mg L^{-1}				
Nitrate (mg.L^{-1})				
39.30	20.50	*L. vannamei*	83 days	[13]
21.40	*11.30*	*L. vannamei*	48 days	[38]
1.89	*3.02*	*P. monodon*	127 days	[33]
0.949	*1.617*	*L. vannamei*	21 days	[39]
2.42	*3.21*	*L. vannamei*	60 days	[36]
0.08	*0.03*	*F. indicus*	120 days	[37]
Summary:				
*Biofloc: 11.*00 mg L^{-1}*;*				
*Clearwater: 6.*61 mg L^{-1}				
Turbidity (NTU)				
90.10	6.10	*L. vannamei*	83 days	[13]
15.10	*3.80*	*L. vannamei*	48 days	[38]
18.20	58.20	*L. vannamei*	21 days	[39]
Summary:				
Biofloc: 41.13 NTU;				
Clearwater: 22.70 NTU				

microorganisms, including bacteria, algae, protozoa, and nematodes, majority being heterotrophic bacteria. The function of the biofloc is to reduce the nitrogenous metabolic waste (ammonia, nitrite) produced by shrimp feeding and production. Through their metabolism, these bacteria liberate many inorganic compounds to the environment that can be used by other living organisms, also they produce exoenzymes that decompose diverse compounds such as cellulose, lignin, keratin and other molecules that are hard to transform [5]. In a biofloc closed system, certain species of microorganisms play a crucial role in maintaining water quality and promoting the growth and health of the culture animals. Innovative aquaculture systems using BFT have been applied to many fish farms due to increasing concern about environmental pollution [6]. Heterotrophic bacteria in BFT assimilate inorganic nitrogen and synthesis them into useful energy-rich bio-materials for the trophic utilization of the cultured animals [7–10], consequently detoxifying the culture system of the deleterious waste generated from the fecal pellets and unconsumed feeds left in the water medium. In this way, BFT act as a self-sustaining aquaculture system enhancing the re-use of waste as well as the creation of resources from harmful waste [11].

Compared to normal aquaculture, biofloc based systems promote higher shrimp growth rates and better water quality due to the presence of biofloc, which is essentially a microbial community [12]. Unlike many recirculating systems, BFT does not rely on external biological filtration, but rather on a dense microbial community that develops in the water column [13]. The operating cost of growing animals in BFT is also drastically cut down as feeding is partially supplemented and the cost of water supply is completely eliminated. In addition, biofloc systems have advantages in terms of ventilation through consistent oxygen supply to aid the metabolic activities of the microbial community as well as the direct utilization by the culture animals. Compared to clean water, biofloc systems require higher aeration because the microbial communities in biofloc systems require oxygen supply for metabolism [14]. The growth and overall health of shrimp reared in a biofloc system is enhanced by the gut microbiota of different scales which is usually different from that in clear water systems [15]. The nitrogenous waste generated through the metabolic activities of shrimp stimulate develops and sustains the microorganisms in a biofloc community which is dominated by bacteria species. It has earlier been established by researchers that only 20–30 % of nitrogen administered in the diet of aquatic organisms is obtained at harvest [16–18]. Every aquaculture system is confronted with the nitrogen balance whose residual by-products constitute great harm to the grown animals. The build-up of $NH4^{+}$-N and NO2- is typical of closed system [19,20]. Considered as blue revolution, BFT makes intensive production of shrimps possible under limited growing space which gives rise to a corresponding discharge of waste. However, the heterotrophic bacteria in BFT take up these wastes thereby promoting nutrient, water reuse and enhancing the conducive conditions of the system [21,22]. Nutrient supply in this system is obtained from activities of organic decomposers that operate under optimum CN ratio where carbon is often augmented with the application of additional carbohydrate sources [23]. Microbial protein is synthesized from the trophic activities of the microorganisms on the nitrate which is obtained from the oxidation of ammonia arising from organic waste in the system. The introduction of carbon into the system aids use up of the nitrate and carbon to produce biomass comprising proteins, carbohydrate, lipids and nucleic acids [24] which is made available as food source for the grown aquatic animals [25–28].

The main purpose of farmers using BFT system is to utilize the microbial community that provides immunity in the culture system and synthesizes food to meet the nutritional needs of the farmed animal. Biofloc systems are designed to foster the growth and wellbeing of aquatic creatures, including fish and shrimp. Through photosynthesis and the organic growth of macro-aggregates, BFT harmonizes carbon and nitrogen levels, promoting self-nitrification in culture water [29]. Determining the microbial makeup of a successful aquaculture production in BFT can be quite challenging. The functionality of the entire BFT system hinges on understanding the roles played by each microbial species. Inadequate knowledge of this information poses an even greater challenge to farmers who may incur significant losses due to pathogenetic bacteria in the biofloc microbial community, leading to disease outbreaks, shrimp

Table 2
Zootechnical variables in the biofloc and clearwater systems.

Culture system		Species	Culture period	References
BFT	**CLW**			
Final weight (g)				
11.10	11.60	*L. vannamei*	83 days	[13]
9.55	7.06	*L. vannamei*	80 days	[31]
6.70	5.90	*L. vannamei*	48 days	[38]
17.97	12.95	*P. monodon*	127 days	[33]
0.62	1.26	*L. vannamei*	42 days	[40]
0.73	0.70	*L. vannamei*	110 days	[41]
10.78	9.52	*L. vannamei*	30 days	[34]
4.00	3.60	*L. vannamei*	21 days	[39]
11.33	9.98	*M. japonicus*	106 days	[4]
9.28	8.08	*P. satiferus*	45 days	[35]
6.64	5.97	*L. vannamei*	60 days	[36]
20.50	18.00	*F. indicus*	120 days	[37]
Summary:				
Biofloc: 9.10 g;				
Clearwater:7.88 g				
Net yield (kg.m^{-3})				
1.60	1.90	*L. vannamei*	83 days	[13]
3.66[a]	2.36[a]	*L. vannamei*	80 days	[31]
1.96[a]	1.13[a]	*P. monodon*	127 days	[33]
1.30	0.92	*M. japonicus*	106 days	[4]
0.683[b]	0.613[b]	*F. indicus*	120 days	[37]
Summary:				
Biofloc1.84				
Clearwater: 1.38				
SGR (%.day^{-1})				
2.85[c]	3.05[c]	*L. vannamei*	83 days	[13]
0.12[c]	0.08[c]	*L. vannamei*	80 days	[31]
2.08[c]	2.06[c]	*L. vannamei*	48 days	[38]
0.14	0.10	*P. monodon*	127 days	[33]
1.06	0.46	*M. japonicus*	106 days	[4]
0.09	0.07	*L. vannamei*	110 days	[41]
1.48	1.08	*L. vannamei*	30 days	[34]
1.40	0.92	*L. vannamei*	21 days	[39]
0.07	0.06	*L. vannamei*	60 days	[36]
1.09	1.03	*F. indicus*	120 days	[37]
Summary:				
Biofloc: 1.03				
Clearwater:0.89				
FCR				
1.80	1.50	*L. vannamei*	83 days	[13]
1.00	1.50	*L. vannamei*	80 days	[31]
1.10	*1.40*	*L. vannamei*	48 days	[38]
1.42	*2.30*	*P. monodon*	127 days	[33]
1.60	*1.10*	*L. vannamei*	42 days	[40]
1.67	*1.80*	*M. japonicus*	106 days	[4]
1.41	*1.86*	*L. vannamei*	21 days	[39]
2.60	*2.90*	*F. indicus*	120 days	[37]
Summary:				
Biofloc: 1.51				
Clearwater: 1.70				
Survival (%)				
69.00	78.00	*L. vannamei*	83 days	[13]
46.07	40.22	*L. vannamei*	80 days	[31]
86.20	*80.20*	*L. vannamei*	48 days	[38]
81.87	*65.73*	*P. monodon*	127 days	[33]
98.40	*85.00*	*L. vannamei*	42 days	[40]
65.70	*52.30*	*M. japonicus*	106 days	[4]
56.67	*60.00*	*P. satiferus*	45 days	[35]
82.20	*86.90*	*L. vannamei*	60 days	[36]
81.00	*83.00*	*F. indicus*	120 days	[37]
Summary:				
Biofloc: 78.31 %				
Clearwater: 74.74 %				

units converted from: a = kg.3 m^{-3} to kg.m^{-3}; b = kg.ha^{-1} to kg.m^{-3}; c = g.wk^{-1}to %.day^{-1}.

mortality, and economic losses. To facilitate efficient BFT, it is necessary to understand the fundamental water quality conditions, zootechnical variables, and microbial communities involved in the floc and amphibolic synthesis of nutrients from organic deposits. This review paper examines the crucial genera responsible for supporting a productive BFT system, and provides a functional characterization of the most commonly reported microorganisms in BFT.

2. Materials and methods

Information presented in the review was obtained from relevant scholarly publications on BFT. The summary values for biofloc and clearwater in Table 1 are the means of the findings of various researchers. Values marked "a-c" presented in Table 2 are converted figures reported by the respective researchers in various units as indicated in the footnote. Figs. 1 and 4 were created by modifying templates obtained from MindPro drawing tool version 9.0.10 for windows (www.edrawmind.com) while Figs. 2 and 3 were plotted on MS Excel from information presented in Table 3.

3. BFT vs clearwater shrimp culture

Reports from research repositories have tested various aspects of comparison between BFT and clearwater in shrimp [111]. compared sugar beet molasses, refined sugar and corn starch in the grow-out culture of *Cyprinus carpio* and reported corn starch having the least total ammonia nitrogen concentration with corresponding higher fish yield. In a study by Ref. [112] *Litopenaeus vannamei* growth performance and water quality were observed after administering glucose, molasses, and starch [113]. The findings revealed that glucose and molasses were the most effective in both growth performance and water quality. Additional studies that investigated carbon sources are [114–116]. Similarly, more researchers studied the impact of certain bacteria species to show their efficacies in BFT [117]. inoculated *Bacillus infantis* in the culture of *L. vannamei* and recorded a significantly better water quality with corresponding lower population of *Vibrio* sp. than in the clearwater (control) unit [118]. inoculated the biofloc culture of *L. vannamei* with microalgae and probiotic bacteria and confirmed that better growth and lower infestation of *Vibrio* sp. was obtained from the treatment units than in the control that had no microalgae and probiotic bacteria [119]. isolated 125 bacteria in BFT and emphasized that *Halomonas* sp. and *Bacillus* sp. were crucial in biofloc formation. Other researches that have carried out extensive work on microbial composition in BFT are [34,120–124].

Another aspect of BFT that has received extensive research is stocking density [125]. tested water quality, growth performance and

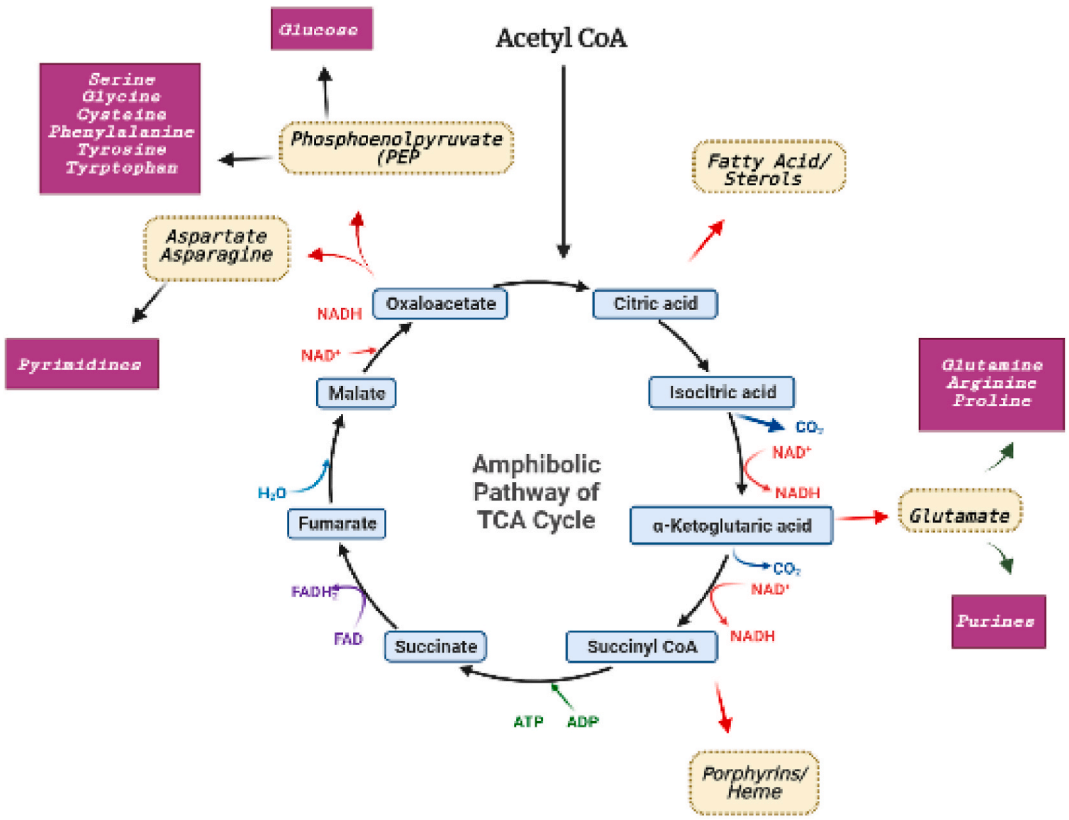

Fig. 1. Amphibolic pathway of TCA cycle by heterotrophic bacteria in biofloc system.

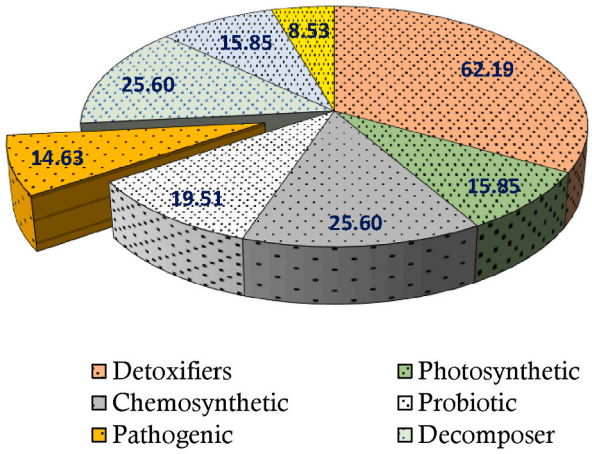

Fig. 2. Percentage roles of microorganisms in BFT.

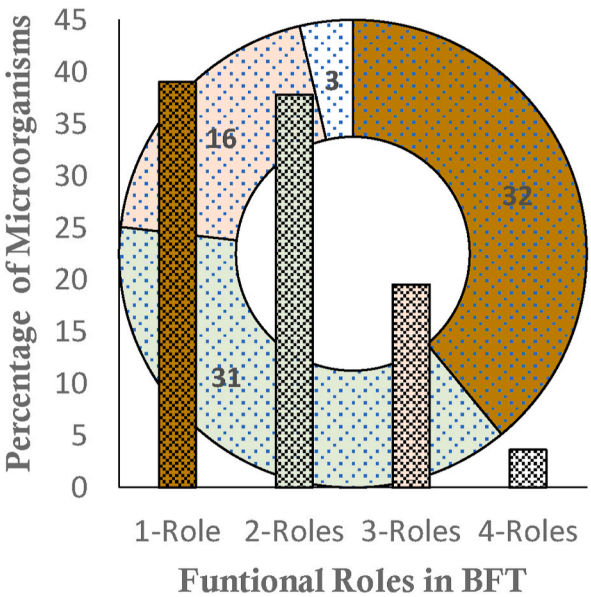

Fig. 3. Percentage roles of microorganisms in BFT.

microbial community of *L. vannamei* at stocking densities of 2000, 4000 and 6000 m^3. Findings from the research showed that the highest yield (0.133 kg m^{-3}) in the highest stocking density with corresponding least survival (71.66 %). Other reports on stocking density in BFT are those of [126–129]. Over 80 % of these reports have however dwelt on the water quality and growth performances of various species of cichlids and shrimps. Tables 1 and 2 are compilations of some research reports on the water quality and zootechnical parameters respectively of shrimps which is in focus in this review. Although not all the reports have clear-cut comparison between biofloc and clearwater in their designs, some values presented are extracted from biofloc units having conditions such as stocking density, C:N ratio, carbon source (molasses) and other experimental/culture conditions such as salinity level, type of rearing facilities, grow-out period etc. same as in the clearwater (control) units.

Information from the water quality and zootechnical parameters summarily considers BFT having more acceptable figures as supported by the presence of microorganisms. Ammonia, nitrite and nitrate are expectedly higher in BFT as documented by researchers and keeping them as low as possible is one of the most important concerns for the practice of aquaculture [130]. Detoxification of ammonia and its intermediate products remains pivotal in BFT and indicative of the productive microbial composition in floc [77, 131–133]. The application of probiotic has been confirmed to speed up the immobilisation of ammonia much faster than the traditional nitrifying bacteria. High turbidity in BFT system is usually due to the floc accumulation. The impact of turbidity in BFT is usually of less effect as biosynthesis does not largely depend on photosynthesis that would require the penetration of sunlight in the water columns.

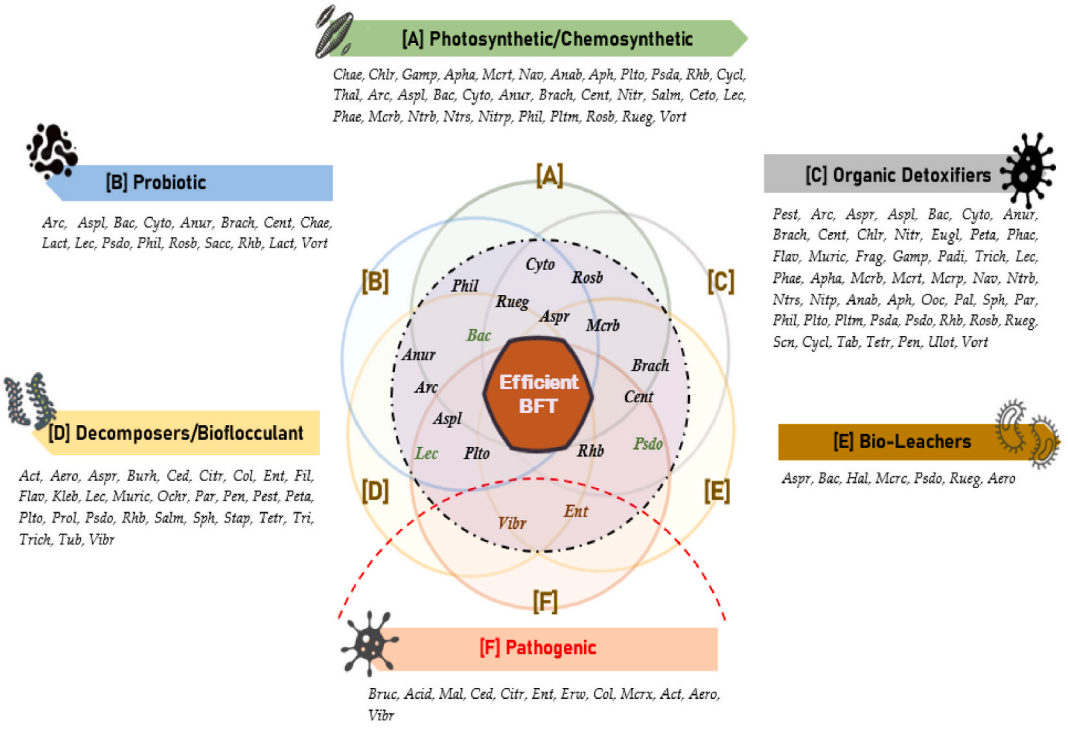

Fig. 4. Functional Characterisation of microbial community in BFT

*Note that *Acidovorax (Acid), Actinobacteria (Act), Aeromonas (Aero), Anabaena (Anab), Anureopsis (Anur), Aphanizomenon (Aph), Aphanocapsa (Apha), Arcella (Arc), Asplanchna (Aspl), Aspergillus (Aspr), Bacillus (Bac), Brachionus (Brach), Brucella (Bruc), Burholderia (Burh), Cedecea (Ced), Centropyixus (Cent), Cetobacterium (Ceto), Chaetoceros (Chae), Chlorella (Chlr), Citrobacter (Citr), Colurella (Col), Cyclotella (Cycl), Cytophaga (Cyto), Enterobacter (Ent), Erwinia (Erw), Euglena (Eugl), Filinia (Fil), Flavobacterium (Flav), Fragilaria (Frag), Gamphosphaeria (Gamp), Halomonas (Hal), Klebsiella (Kleb), Lactobacillus (Lact), Lecane (Lec), Microbacterium (Mcrb), Micrococcus (Mcrc), Microspora (Mcrp), Microcystis (Mcrt), Moraxella (Mcrx), Muricauda (Muric), Navicula (Nav), Nitrospira (Nitp), Nitrococcus (Nitr) Nitrobacter (Ntrb), Nitrosomonas (Ntrs), Ochrobactrum (Ochr), Oocystis (Ooc), Padiastrum (Padi), Palmella (Pal), Paramecium (Par), Pencillium (Pen), Pestalotiopsis (Pest), Petalomonas (Peta), Phacus (Phil), Plantomyces (Plto), Plantomicrobium (Pltm), Prolinoborus (Prol), Pseudoalteromonas (Psda), Pseudomonas (Psdo), Rhabsitis (Rhb), Rhodotorula (Rhd), Roseobacter (Rosb), Ruegeria (Rueg), Sacchromyces (Sacc), Salmonella (Salm), Scanedesmus (Scn), Sphaerocystes (Sph), Staphyloccocus (Stap), Tabellaria (Tab), Tetrahymena (Tetr), Thalassiosira (Thal), Trichocerca (Tri), Trichoderma (Trich), Tubifex (Tub), Ulothrix (Ulot), Vibrio (Vibr), Vorticella (Vort).*

4. Biochemical processes by microorganisms in BFT

Biofloc is a composition of organic residue and a microbial community deliberately introduced into the culture system in order to attract benefits arising from their metabolic activities. The trophic activities of the heterotrophic bacterial in the cellular breakdown of carbohydrate yields energy in BFT with appreciable amount of protein in the glycolytic pathway that is commenced with the oxidation of the deposit of fecal materials and unconsumed feed. High-energy compounds namely ADP and ATP as well as compounds with thioester bonds (acetyl-CoA or succinyl-SCoA) are synthesized from the catabolic reaction.

Amphibolic degradation by heterotrophic (or chemoorganotrophic) bacteria simultaneously produce energy and generates precursor molecules for the biosynthesis of new cellular constituents [134]. The amphibolic chain of reactions (Fig. 1) synthesizes fatty acids, glucose derivatives as well as proteins from the organic wastes in BFT to support the nutritional need of the culture animals and also rids the system of the accumulation of these residues. The carbon and nitrogen sources in the organic residue in the aquaculture system are biosynthesised into nicotinamide adenine dinucleotide (NADH + H^+) which provides the chemical energy for further catabolic breakdown of organic sources. Pyruvate is produced from glucose in the glycolytic pathway through a two-stage phosphorylation to form the pivotal intermediary product in the biosynthetic process in biofloc system. Pyruvate oxidation occur in prokaryotic heterotrophic bacteria is enhanced by pyruvate dehydrogenase complex to yield acetyl-CoEnzyme A (Acetyl-CoA) [135]. reported that Pyruvate formate-lyase (PFL) specifically plays the role in the breakdown of pyruvate to acetyl-CoA in prokaryotic bacteria. The formation of acetyl-CoEnzyme A is from the decarboxylation of pyruvate and covalent connection to Co-enzyme A by a thioester linkage to form a molecule referred to as acetyl-CoA.

Acetyl-CoA forms the base structure of entry into the Tricarboxylic Acid (TCA) cycle. As an electron acceptor, it reacts with oxaloacetate to form citrate. For prokaryotic heterotrophic bacteria in BFT, NADP-dependent enzyme, Isocitrate dehydrogenase ($NADP^+$) catalyses the dehydrogenation of D-*threo*-isocitrate to oxoglutarate converse to other eukaryotes that have NAD^+-dependent enzymes, Isocitrate dehydrogenase (NAD^+) catalysing reaction [136]. Fatty acid biosynthesis which is catalysed by acetyl-CoA carboxylase commences with the carboxylation of acetyl-CoA to malonyl-CoA. During this reaction, protein-bound acyl carrier

Table 3
Microorganisms composition and functions in BFT.

Family	Genera	Function in BFT	Selected References
Comamonadaceae	*Acidovorax*	v	[37,42,43]
Moraxellaceae.	*Actinobacteria*	v, vi, vii	[4,44,45]
Vibrionaceae	*Aeromonas*	v, vi, viii	[17,46,47,48]
Nostocaceae	*Anabaena*	i, ii	[48–50]
Brachionidae	*Anuraeopsis*	i,iii,iv	[13]
Nostocaceae	*Aphanizomenon*	i, ii	[51,52,50]
Merismopediaceae	*Aphanocapsa*	i, ii	[53,52,49]
Arcellidae	*Arcella*	i, iii, iv	[50]
Aspergillaceae	*Aspergillus*	i, vii, viii	[54,55,56]
Asplanchnidae	*Asplanchna*	i,iii,iv	[47]
Bacillaceae	*Bacillus*	i, iii. iv, viii	[57,42,58,59]
Brachionidae	*Brachionus*	i,iii,iv	[12,47]
Brucellaceae	*Brucella*	v	[57]
Burkholderiaceae	*Burholderia*	vi	[60]
Enterobacteriaceae	*Cedecea*	v, vi	[61,62,63]
Centropyxidae	*Centropyxis*	i, iii, iv	[50]
Fusobacteriaceae	*Cetobacterium*	iii	[64,65]
Chaetoceraceae	*Chaetoceros*	ii, iv	[66,67,68]
Chlorellaceae	*Chlorella*	i; ii	[55,69,69]
Enterobacteriaceae	*Citrobacter*	v, vi	[64,70]
Lepadellidae	*Colurella*	v, vi	[12,64]
Stephanodiscaceae	*Cyclotella*	i; ii	[46,47]
Bacteroidaceae	*Cytophaga*	i, iii, iv	[4]
Enterobacteriaceae	*Enterobacter*	v, vi, viii	[4,58,71]
Erwiniaceae	*Erwinia*	v	[64]
Euglenaceae	*Euglena*	i	[4], [64], [1124]
Trochosphaeridae	*Filinia*	vi	[57,47,64],
Flavobacteriaceae	*Flavobacterium*	i, vi	[43,49]
Fragilariaceae	*Fragilaria*	i	[57,42]
Gomphosphaeriaceae.	*Gamphosphaeria*	i, ii	[42,47–49,52]
Halomonadaceae	*Halomonas*	viii	[72]
Enterobacteriaceae	*Klebsiella*	vii	[58,59]
Lactobacillaceae	*Lactobacillus*	iv	[4,73]
Streptococcaceae	*Lactococcus*	iv	[74]
Lecanidae	*Lecane*	i, iii, iv, vii	[12,54,56,71]
Comamonadaceae	*Malikia*	v	[43]
Microbacteriaceae	*Microbacterium*	i, iii	[75]
Micrococcaceae	*Micrococcus*	viii	[5,42,58]
Microcystaceae	*Microcystis*	i, ii	[42,47–49,52]
Microsporaceae	*Microspora*	i	[47]
Moraxellaceae	*Moraxella*	v	[59]
Flavobacteriaceae	*Muricauda*		[23,76]
Naviculaceae	*Navicula*	i, ii	[47,67]
Nitrobacteraceae	*Nitrobacter*	i, iii	[77,75,63]
Ectothiorhodospiraceae	*Nitrococcus*	i, iii	[75]
Nitrosomonadaceae	*Nitrosomonas*	i, iii	[72,75,63,78]
Nitrospinaceae	*Nitrospira*	i, iii	[71,79,75,63]
Brucellaceae	*Ochrobactrum*	vi	[80]
Oocystaceae	*Oocystis*	i	[47,81,82]
Hydrodictyaceae	*Padiastrum*	i	[5,47,83,84]
Palmellaceae	*Palmella*	i	[[47,85]
Parameciidae	*Paramecium*	i, vii	[86,87,88]
Trichocomaceae,	*Penicillium*	i, vii	[54,55,56]
Amphisphaeriaceae	*Pestalotiopsis*	i, vii	[86,62,87]
Euglenaceae	*Petalomonas*	i, vii	[54,56]
Euglenaceae	*Phacus*	i	[47,89,88]
Lewinellaceae	*Phaeodactylibacter*	i, iii	[75,90,91]
Philodinidae	*Philodina*	i, iii, iv	[50]
Planctomycetaceae	*Plantomicrobium*	i, ii, vi	[57,92,91]
Plantomycetaceae	*Plantomyces*	i, iii	[70,75]
Neisseriaceae	*Prolinoborus*	vi	[93]
Pseudoalteromonadaceae	*Pseudoalteromonas*	i, ii,	[52,92,91]
Pseudomonadaceae	*Pseudomonas*	i, vi, vii; viii	[5,42]
Rhabditidae	*Rhabsitis*	i, ii, vi	[12,94,95]
Sporidiobolaceae	*Rhodotorula*	iv	[94]
Rhodobacteraceae	*Roseobacter*	i, iii, iv	[4,96,91]
Rhodobacteraceae	*Ruegeria*	i, iii,viii	[97,98,99]
Saccharomycetaceae	*Saccharomyces*	iv	[64,92]

(*continued on next page*)

Table 3 (*continued*)

Family	Genera	Function in BFT	Selected References
Enterobacteriaceae	*Salmonella*	iii, vi	[58,100]
Scenedesmaceae	*Scanedesmus*	i	[48,101,102]
Palmellaceae	*Sphaerocystes*	i	[57,66,47]
Sphingomonadaceae	*Sphingomonas*	vi	[103]
Staphylococcaceae	*Staphylococcus*	vi	[4,58,98]
Tabellariaceae	*Tabellaria*	i	[47,64,92,99]
Tetrahymenidae	*Tetrahymena*	i, vii	[54]
Thalassiosiraceae	*Thalassiosira*	ii	[94]
Trichocercidae	*Trichocerca*	vi, vii	[88,100,101]
Hypocreaceae	*Trichoderma*	i, vii	[54,55,56]
Tubificidae	*Tubifex*	vi,	[57,47,64]
Ulotrichaceae	*Ulothrix*	i	[57,47,64]
Vibrionaceae	*Vibrio*	v, vi	[96,102,104–109]
Vorticellidae	*Vorticella*	i, iii, iv	[110]

Roles in BFT.
(i) maintenance of water quality by utilizing nitrogenous compounds (**Organic detoxifiers**).
(ii) provision of natural food through photosynthesis (**Photosynthetic**).
(iii) protein-synthesis from the breakdown of nitrogenous waste (**Chemosynthetic**).
(iv) pathogen competition and inhibitory effect (**Probiotic**).
(v) Carriers of Disease pathogens (**Pathogenic**).
(vi) Saprophytic breakdown of organic residue (**Organic decomposers**).
(vii) Sedimentation of floc residue (**Bioflocculant**).
(viii) Solubilisation of nutrient to make bioavailable (**Bio-leachers**).

protein (ACP) is formed by acetyl-CoA transferring its acetyl group to the thiol group. A few series of carboxylation, hydrogenation, condensation and dehydrogenation produce series of intermediate products and eventually, b-hydroxydecanoyl-ACP dehydratase which produces the *cis*-b, y (or Δ^3)-decanoyl ACP in anaerobic heterotrophic bacteria [137].

Amino acids are produced from alpha-ketoglutarate (AKG). The precursor product, glutamate is further converted to glutamine, proline, arginine and purines. Also called 2-oxoglutarate, AKG are considered essential metabolites necessary in regulating all metabolic reactions that condition the physiological and genetic modifications in animals [138]. The biosynthesis of AKG during amphibolic reaction plays a vital role in antioxidative defence and other critical functions that bother on the proper cellular performance [139,140] and this serves a crucial role in the immunological functions of heterotrophic bacteria in BFT. Furthermore, succinyl-CoA syntheses porphyrin and hemes. Oxaloacetate synthesizes phosphoenolpyruvate (PEP) which is the intermediate product for glucose, serine, glycine, cysteine, phenylalanine, tyrosine and tryptophan as well as aspartate, asparagine which forms the pyrimidines.

5. Microbial community and their roles in BFT

BFT culture systems are driven by biofloc, which are communities of microbes namely bacteria, algae, fungi, protozoa, rotifers, grazing macroinvertebrates, and detritus [141,142] performing the overlying function of saprophytes, algae grazers and pathogenic bacteria, nitrifying bacteria and floc-farming organisms [54,57,61,143]. In an optimally functioning BFT system, all constituent species must be suspended in the water column, carrying out their roles and in a sustainably high connections with other microorganisms [57]. The breakdown and reuse of chemical waste is boosted by the existence of chemo-phototrophic and autotrophic microbes in biofloc. *Bacillus* sp., *Acinetobacter* sp., *Sphingomonas* sp., *Pseudomonas* sp., *Rhodopseudomonas* sp., *Micrococcus* sp., *Nitrosomonas* sp., *Nitrospira* sp., *Nitrobacter* sp., *Cellulomonas* sp., and yeast constitutes a large population of heterotrophic beneficial microbial in biofloc system. An improved growth and complete well-being of the grown animals is resident on the microbial aggregate in floc that plays the role of providing nutrients in the system [57].

Bacillus sp often produces enzymes and proteins, that provide nutritional benefits in the breakdown of organic matter in BFT, hence contributing to the overall dynamics in the microbial community in BFT [144]. *Saccharomyces cerevisiae*, a species of yeast has a well-developed secretory pathway that makes it suitable for production of proteins needed to be synthesized by chemo-autotrophs in BFT [145,146]. *Acinetobacter* sp are known for their metabolic diversity that serves in the degradation of organic compounds, thereby detoxifying the biofloc system of the build-up of harmful ions and compounds generated from accumulated waste [147]. *Sphingomonas* sp plays a crucial role of degrading a wide spectrum of organic pollutants, thus assisting in the removal of harmful substances from systems generating organic waste [148]. *Rhodopseudomonas* sp are photosynthetic bacteria and may contribute to oxygen production in the biofloc, promoting aerobic conditions that support detoxification processes [149]. The activities of *Nitrospira* sp, *Nitrobacter* sp and *Nitrosomonas* sp during nitrification aids the conversion of ammonia to nitrite and nitrate which detoxifies the biofloc system of ammonia toxicity [150]. The metabolic diversity of *Pseudomonas* sp aids the decomposition of this organic material in biofloc to release nutrients back the system [151,152].

The roles of biofloc are closely linked to the interactions of the community of microorganisms in their trophic co-existence in terms of acquisition of nutrients and metabolic processes [42]. This is because these various species of microorganisms exploit various organic substrates in floc and perform varying metabolic actions yielding different amount and nature of protein product as well as

their capacities to detoxify in the biofloc system. Essentially, the activities of this large spectrum bacteria are known to exhibit antioxidant activity along with health benefits [86] and also role in creating a competition in the pathogenic bacteria [42].

Bacteria in closed biofloc systems offer many benefits, including improved water quality, enhanced growth performance, and better disease resistance for aquaculture animals. In the biofloc system, flocs formed by aggregation of microorganisms serve as natural bioremediation [57,153]. Beneficial bacteria in biofloc systems plays the role by extenuating the activities of pathogenic bacteria and improving the immunity of aquatic animals [42]. The enrichment of the diet with beneficial bacteria in biofloc systems can further enhance water quality, growth performance and disease resistance [42,46]. The use of biofloc technology can also reduce input costs, improve biosecurity and control the concentration of ammonia in aquaculture ponds [57,51].

Heterotrophic bacteria thrive on carbon for the metabolic breakdown of ammonia and its eventual uptake. Optimal C:N ratios encourages the spread of useful bacteria and suppress the growth of dangerous bacteria, leading to enhance water quality and disease control [22,154,155]. Furthermore, nitrogen uptake and breakdown of biotoxins are boosted under suitable C:N ratios. Biofloc production, waste decomposition, and nitrogen uptake can be optimized by ensuring suitable C:N ratios, as highlighted in Ref. [57]. Maintaining these ratios is crucial in achieving success with biofloc technology in shrimp production. The trophic role of microbes in BFT is to aid the conversion of organic material into food sources for cultured animals during which time, lethal compounds and toxins are equally removed from the system [42]. Growth responses, digestibility of food, and enhancement of immunity against bacterial contaminations in cultured animals is reported to be improved by bacterial compound called poly-β-hydroxybutyrate (PHB) which is accumulated in biofloc [57]. Playing the role of nutrition and bio-accessible compounds, biofloc enhance aquatic growth and health [14]. However, if organisms begin to display signs of stress or disease, it may be an indication of harmful bacteria. As such, it is vital to regularly test the water quality and mount surveillance on the microbial community within the system to ensure optimal conditions for aquatic life [156]. By maintaining a robust microbial community and first-rate water quality, biofloc systems can facilitate effective and sustainable aquaculture.

Aquatic animals grown under BFT are known to exhibit a more robust immune capacity. Aquatic organisms perform better when there is food abundance leading to lesser competition and self-enabling immunity to environmental perturbation [157]. These improved immune responses have been attributed to the presence of microorganisms that induce the immune system [53,74,157,158] and an enhancement of the enzyme activities in floc [153,74,158]. Several findings suggest that *Bacillus* sp have the potential to be a valuable bioagent for improving the health and productivity of aquatic cultured organisms [159–161]. This function distinguishes the BFT from other RAS as its performance is promoted by the probiotic bacterial community. An aggregation of these heterotrophic bacteria plays a role in the bioremediation of the harmful waste generated in the system. Considered as a complex community of bacteria and other microbes, BFT contains abundant bioactive compounds that boosts shrimp tolerance to stress and stimulate their antioxidant activity [162,163]. [164] emphasized the cohabitation of several classes of microorganisms in biofloc systems, which create a symbiotic relationship with cultured aquatic animals and other microbial species. The composition of bacterial populations in biofloc systems is influenced by several factors, including the type of aquatic animal being cultured, the quality of feed used, and environmental conditions such as water quality.

Some microorganisms are however detrimental to biofloc technology, most commonly is *Vibrio* sp. which has been identified as the commonest bacteria that has caused serious economic loss in shrimp culture and particularly biofloc system of its culture [66,165]. Management of CN ratio has been confirmed to reduce acute hepatopancreatic necrosis disease (AHPND), a disease triggered by the presence of *Vibrio parahaemolyticus* in biofloc system [166]. A number of these vibrio diseases shown identifiable symptoms that are distinguishable from others. Generally, shrimps suffer from bacterial septicemia caused by *Vibrio alginolyticus, V. anguillarium,* and *V. parahaemolyticus*; necrosis with *Vibrio* sp as the pathogen, *Pseudomonas* sp, *Aeromonas* sp. and *Flavobacterium* sp; brown spot disease caused by *Aeromonas* sp. and *Flavobacterium* sp. filamentous bacterial disease caused by *Leucothrix mucor.* A range of challenges may arise in aquaculture when utilizing biofloc system. These include a rise in ammonia level, high turbidity, retarded growth, and the poor health of the grown aquatic animals. The aim of the BFT system is to convert ammonia into nitrate, with probiotic bacteria playing a key role in this process [167,168]. However, if the system experiences a sudden increase in ammonia or nitrite levels, it may indicate bacterial malfunction. Controlling ammonium levels is a significant concern in aquaculture, and chemoautotrophic bacterial nitrification (CBN) is a crucial process in achieving this [169]. The biofloc system itself creates the ideal environment for nitrifying bacteria to grow by accumulating flocculated matter, as well as ammonia and nitrite [170].

Fig. 2 show the percentage role of the various microorganisms in BFT system. Researchers have reported that the entire spectrum of microorganisms in BFT system are either organic detoxifiers, photosynthetic, chemosynthetic, pathogenic, saprophytic, bioflocculants or bio-leachers (Table 3). The activities of some of the microorganisms in BFT detoxify the ammonia in the system and other toxins while others play the trophic roles the synthesizing food photosynthetically or chemosynthetically. Some microorganisms in BFT system are probiotic in their functions which are inhibitory or competitive to the pathogens in the system. The pathogenic microorganisms in BFT are active when conditions supporting their actions are made possible in the system. During these activities, secondary processes such as saprophytic breakdown of organic residue and their sedimentation as well as the solubilisation of nutrients in the decomposed floc residue are triggered by other categories of microorganisms known as bio-leachers. The seamless operation of these microorganisms yields a balanced BFT where detoxification, food synthesis, bio-flocculation of floc residue and immunity are spontaneously provided by the BFT system.

The distribution and functional roles played by microorganisms in BFT is presented in Fig. 3. Out of the 82 genera reported in this review, 39.02 % are known to carry out only one role in BFT while 60.98 % played multiple roles in the system. Three genera: *Bacillus* sp., *Lecane* sp., and *Pseudomonas* sp., carried out four out of eight (50 %) in the multiplicity of these roles. This category of microorganisms occupies the most critical niche in the microbial composition in BFT. *Bacillus* sp, *Lecane* sp and *Pseudomonas* sp have been reported to be crucial microorganism in BFT [57,156,64].

Amphibolic degradation often results in the synthesis of certain intermediates that are useful both in anabolic and catabolic pathways. Heterotrophic bacteria in a biofloc system usually influence these specific metabolic pathways and conditions that produce varying end products during amphibolic degradation. During catabolic processes, heterotrophic bacteria break down organic compounds to produce ATP through oxidative phosphorylation (a source of cell energy and precursor) for the biosynthesis of synthesize amino acids, nucleotides, and other cellular building blocks [171,172]. The organic acids produced by *Bacillus* sp during the breakdown of organic matter in BFT generally serves as an energy source for other microorganisms in the system and biosynthesis of the various products in the pathway [14,173]. *Lecane* sp filter feeders whose activities contribute to the reduction of organic particles. This gives rise to the synthesis of organic acids such as acetic acid, lactic acid, or citric acid which are key intermediaries in the amphibolic pathway [83]. Some bacteria in biofloc systems produce complex extracellular polymers composed of proteins, polysaccharides, and nucleic acids that act in the formation and stability of floc. In addition, some heterotrophic bacteria in the biofloc system facilitate the nitrogen cycle in the system. This denitrification process facilitated by *Pseudomonas* sp supports the maintain proper nitrogen balance and prevents the accumulation of excess nitrate in the BFT system, which is harmful to shrimp and other aquatic organisms [82,84].

6. Detecting the borderline between the beneficial and harmful microbial community in BFT

The diversity of microorganisms, their relative abundance, and the changes in their numbers over time in biofloc technology aquaculture systems are impacted by several factors. Understanding these factors is crucial for farmers as they can use this knowledge to manage their systems better and promote healthier animals [95]. This is a critical aspect that has proved elusive to most farmers and researchers, thus posing significant management challenges in BFT. It is therefore essential to maintain an equilibrium within the biofloc system to ensure proper functioning of probiotic bacteria and to keep ammonia and other noxious intermediate products within acceptable limits [174]. The success of biofloc technology is dependent on the maintenance of proper water quality and the acknowledgment of the importance of microorganisms [175,176]. Water quality conditions serve as useful indicators of the productivity of the microbial community in a BFT system. Accordingly, a beneficial microbial community in biofloc can be inferred when optimum water quality conditions are maintained. Conversely, poor and unstable water quality conditions are triggered by the failure of the microbial community to maintain the concentration and ionic balancing of all the constituent nutrients in BFT.

Considering the roles played by microorganisms as reported by several researches, certain genera of microorganisms could be considered fundamental due to their ability to undertake multiple roles in effective functioning of BFT. Fig. 4 depicts such genera of microorganisms to include *Anureopsis* sp., *Arcella* sp., *Asplanchna* sp., *Aspergillus* sp., *Bacillus* sp., *Brachionus* sp., *Centropyixus Cytophag* sp., *Enterobacter* sp., *Lecane* sp., *Microbacterium* sp., *Plantomyces* sp., *Phacus* sp., *Pseudomonas* sp., *Rhabsitis* sp., *Ruegeria* sp., *Roseobacter* sp. and *Vibro* sp. Although other microorganisms may not be considered less important, the over-lapping roles of these so-called fundamental microorganisms may significantly cover for other microorganisms absent in BFT. In addition, their absence in floc might indicate a dysfunctional BFT, which might impair nutrient synthesis, system health, detoxification, organic breakdown and sedimentation, as well as nutrient extraction from organic deposits to release nutrients for additional biosynthesis in the BFT system. Due to their reported roles in nearly all of the metabolic activities that take occur in the BFT system, *Bacillus* sp., *Lecane* sp., and *Pseudomonas* sp. could be regarded as the three primary genera in BFT [177]. With a sufficient population of *Bacillus* sp., *Lecane* sp., and Pseudomonas sp. in the microbial community, a highly efficient BFT may be considered. Efficient BFTs can be considered as BFTs with sufficient numbers of Bacillus sp., *Lecane* sp. and *Pseudomonas* spp. in microbial communities. *Vibrio* spp. and *Enterobacter* spp. Under unsuitable water conditions, they can transform into harmful pathogens that thrive in nutrient-rich BFT systems. Under certain conditions, such as low oxygen and high temperatures, bacteria can multiply rapidly and cause fish diseases [178]. To avoid such consequences, it is important to monitor key water quality parameters such as dissolved oxygen, pH, and ammonia, nitrite, and nitrate levels. Closely related is the genus *Pseudomonas*, which poses a threat to biofloc under unfavourable water conditions, leading to reduced growth rates and disease outbreak [179].

7. Conclusion

BFT has proven to be a sustainable aquaculture system with enormous advantages. By promoting the growth of helpful bacteria, biofloc systems keep harmful bacteria at bay, resulting in improved growth and health of shrimp and other aquatic animals. As a veritable measure towards maintaining a healthy BFT system, optimum water quality condition is imperative in order to impede the thrive of pathogenic microorganisms. Furthermore, probiotic bacteria are reported to inhibit the activities of pathogenic bacteria and so, deliberate augmentation in the population of probiotic such as *Bacillus* sp. through inoculation could enhance a healthy BFT system.

A swing from the beneficial to harmful roles of microorganisms is inimical to an efficient BFT. Close attention on the water quality provides quite useful signal indicating when the microbial community no longer supports the effective operation of the system. Sharp alteration from acceptable ranges should prompt quick action by immediately incorporating probiotics if not already in use. Regular evaluation of the microbial community in BFT is equally valuable with a deliberate effort to keep the population of *Vibrio* and *Enterobacter* to the minimum (<5 %) in the microbial community of BFT system.

CRediT authorship contribution statement

Edward Terhemen Akange: Conceptualization, Software, Writing – original draft, Writing – review & editing. **Athanasius Aondohemen Aende:** Visualization, Writing – review & editing. **Hajar Rastegari:** Conceptualization, Writing – review & editing. **Olumide A. Odeyemi:** Conceptualization, Writing – review & editing. **Nor Azman Kasan:** Funding acquisition, Supervision, Writing –

review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

This work was funded by the Ministry of Higher Education (MOHE), Malaysia under Higher Institution Centre of Excellence (HICoE), Institute of Tropical Aquaculture and Fisheries (AKUATROP) program [Vot. No. 63933, JPT.S(BPKI) 2000/016/018/015 Jld.3 (23) and Vot. No. 56050, UMT/PPPI/2- 2/5 Jld.2 (24)]. All authors would like to dedicate special gratitude to whoever was involved and participated in this research project.

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