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Supplemental carbon sources applied in biofloc technology aquaculture systems: types, effects and future research

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Abstract

Biofloc technology (BFT) systems have been driven towards increased sustainability in the last decade. BFT depends on maintenance of the optimal carbon-to-nitrogen (C/N) ratio through supplementation with organic carbon sources. The types of carbon sources and addition strategies are critical considerations in BFT systems. Thus for the purpose of this review, a thorough search of the literature was conducted to gather relevant information from reliable sources, ranging from reputable journals to books and useful reports in the field of BFT. Keywords used for the literature search include: 'biofloc technology systems', 'carbon sources', 'solid carbon sources', 'effects of carbons sources', 'carbon source addition strategies', 'nutritional quality of bioflocs', 'carbon sources and water quality', 'C/N ratio in BFT', and 'carbon sources and bacterial community'. Among the several peer-reviewed articles, books and technical reports consulted, 147 (dated from 1987 to 2020) were relevant for the preparation of this review. The current review thus examines the subject of supplemental carbon sources in BFT systems and discusses the various effects of their application with respect to the culture organism, microorganisms, water quality and the nutritional quality of flocs. The benefits and challenges associated with the types of carbon sources used in BFTs are also presented. Suggested organic carbon sources and their addition strategies are provided, and further research directions are proposed.

Key words: addition strategy, biodegradable polymers, biofloc technology, carbon sources, heterotrophic bacteria.

Introduction

The protein content of most fish biomass has been reported to be greater than 65% (Hertrampf & Piedad-Pascual 2000). The protein consumed by fish is mainly used for energy production, while terrestrial animals mostly use carbohydrates and lipids for energy production (Hepher 1988; National Research Council 2011). Therefore, fish in general have greater dietary requirements for protein than do cattle or sheep (Crab *et al.* 2007). Additionally, the length ratio of fish intestines to body is commonly <3, which results in a short storage time for chyme in the intestines and discharge of large amounts of undigested feed (Amirkolaie 2005). In feed-driven aquaculture systems, approximately 70% of the dietary dry matter will be unharvested and discharged as waste (Isam 2005; Emerenciano *et al.* 2017).

The fate of waste in the effluent water from the various types of aquaculture systems is different. For open systems, such as cage aquaculture and flow-through systems, both the dissolved waste and solid waste are discharged to the receiving water bodies directly (Isam 2005; Sharifinia *et al.* 2018, 2019). In aquaculture ponds, most waste is retained in the culture water or the bottom, and little is discharged (Boyd *et al.* 2002; Hargreaves 2013). In recirculating systems without a denitrification unit, most waste substances are collected and discharged in the backwash effluent of the mechanical filter or the biofilter (van Rijn 2013; Ahmad *et al.* 2017). For aquaculture systems based on biofloc technology (BFT), almost all waste kept in the fish tank is converted into micro-biomass through manipulating the ratio of carbon to nitrogen (C/N; Ebeling *et al.* 2006; Khanjani & Sharifinia 2020a; Khanjani *et al.* 2020c). This microbial biomass becomes available to the aquaculture organism (e.g. shrimp) as a secondary protein source and therefore influences their growth performance significantly (Khanjani *et al.* 2020b).

The underlying mechanism of BFT involves the use of heterotrophic bacteria to assimilate ammonium nitrogen (NH_4^+–N) into the microbial biomass, which can be accomplished within five hours with an appropriate C/N ratio (Avnimelech 1999; Ebeling *et al.* 2006). By relying on the activities of microorganisms (phytoplankton, bacteria, fungi and zooplankton), BFT aquaculture systems do not require sophisticated equipment or instruments, such as external biofilters and mechanical filters, to run in zero-exchange water mode (Crab *et al.* 2009, 2012; Hargreaves 2013; Wei *et al.* 2016; Emerenciano *et al.* 2017). In addition, BFT has been described to have the potential of solving most of the problems associated with the use of conventional aquaculture technologies (Khanjani & Sharifinia 2020a). Therefore, the BFT aquaculture systems are considered a type of sustainable technology and are particularly notable for their environmentally friendly approach and cost-effectiveness (Azim & Little 2008; Crab *et al.* 2012; Emerenciano *et al.* 2017; Khanjani & Sharifinia 2020a).

Organic carbon supplies the energy to heterotrophic bacteria to grow and multiply (Avnimelech 1999). A C/N ratio in the substrate ranging from 10 to 20 is considered optimal for heterotrophic bacteria to assimilate NH_4^+–N (Goldman *et al.* 1987; Avnimelech 1999; Avnimelech 2006; Avnimelech 2007; Crab *et al.* 2012). Recently, supplying molasses (C/N of 15) to a shrimp based biofloc system resulted in an increase in the populations of heterotrophic bacteria, which caused a corresponding decline in the concentrations of ammonia and nitrite, respectively (Khanjani *et al.* 2020c). Theoretically, 6.07 parts of organic carbon are required for heterotrophic bacteria to convert each part of NH_4^+–N to biomass ($NH_4^+ + 1.18\ C_6H_{12}O_6 + HCO_3^-$- $+ 2.06\ O_2 \rightarrow C_5H_7O_2N + 6.06\ H_2O + 3.07\ CO_2$; Rittmann & McCarty 2001; Ebeling *et al.* 2006). Considering that organic carbon in the substrate is consumed during the respiration and metabolism processes of heterotrophic bacteria, their C/N ratio should be greater than that of the cell composition of the bacteria (~5; Rittmann & McCarty 2001). However, the C/N ratio in an aquaculture system is typically less than 6, and the C/N ratio of most artificial feeds is <10 (Avnimelech 1999; Cao *et al.* 2020). Consequently, it is imperative to increase the C/N ratio of the aquaculture system to meet the respiratory and metabolic needs of heterotrophic bacteria. Hence, applying supplemental carbon sources or elevating the carbon content of the input feed should be carried out to increase the C/N ratio in BFT aquaculture systems (Avnimelech 1999; Crab *et al.* 2012; Li *et al.* 2018; Liu *et al.* 2018a).

The carbonaceous substrates added to a given BFT aquaculture system are mainly related to the types of the organic sources and the addition strategies; both are known to influence the performance of BFT aquaculture systems, including biofloc characteristics, water quality parameters, and growth performance or welfare of the cultured species (Table 1). Extensive studies have been conducted to characterize the mechanisms of the BFT aquaculture system by using different supplemental organic carbon sources (Azim & Little 2008, Nootong *et al.* 2011; Hu *et al.* 2014; Ekasari *et al.* 2010; Luo *et al.* 2017; Luo *et al.* 2019a,b; Ferreira *et al.* 2020; Ebrahimi *et al.* 2020). Therefore, the current review presents information about the various organic carbon sources used in BFT aquaculture systems and analyses the associated benefits and challenges. Suggested organic carbon sources and their addition strategies are provided, and some potential areas for future research are highlighted. These suggestions will help improve the practice of BFT aquaculture systems.

Types of carbon sources used in BFT aquaculture systems

In a zero-exchange BFT aquaculture system, all faeces and unused feed remain in the fish or culture tank. Consequently, the nutrients unconsumed by the animals are potentially available for heterotrophic bacteria and other microbial organisms. Owing to the wide variations in feed composition, the assimilation rates of the fish or shrimp, the amount of the organic carbon leaching from the solids, etc., it is often difficult to estimate the real-time available organic carbon retained in the tank. Therefore, the inert organic carbon source is always neglected, and only the external organic carbon source is considered when determining the optimal amount of organic carbon required for the heterotrophic bacteria to assimilate NH_+^-–N.

To date, numerous carbonaceous substrates with rich organic carbon have been applied to increase the C/N ratio in BFT aquaculture systems. These substrates can be categorized on the basis of the chemical compositions or the speeds with which they release DOC into the water.

Categorization based on chemical compositions

Carbohydrates

Carbohydrates are perhaps the most abundant organic carbon source for most heterotrophic bacteria. Glucose, sucrose, starches, molasses and cellulose are typical carbohydrates and are composed of carbon, hydrogen and oxygen atoms (Merriam-webster 2020). Compared with other

Table 1 Organic carbon sources, addition strategies, effects on culture organism, water quality and biofloc characteristics

Organic carbon sources types (C/N ratio)	Additional strategies	Cultured species	Effect on the growth performance and/or welfare of the fish or shrimp	Effects on the water qualities	Effects on the characteristics of bioflocs	Reference
Total of 4.33 kg of feed was added to each tank to start-up; no other form of fertilizer was used during the stable period	Relied on the input carbon content of feed. Therefore, no extra carbon source was added	*Litopenaeus vannamei*	The survival rate was similar for all the treatments with the mean value being 71 ± 8%. FCR showed no significant difference between the different feeds employed. However, FCR was significantly lower in groups with solid removal	Water parameters did not accumulate to detrimental levels for shrimp although no extra carbon was added. pH slightly declined but this was corrected by with $NaHCO_3$	In this study, proximate composition of floc was not determined. However, controlling floc levels (TSS and VSS) resulted in better growth performance of shrimp	Ray *et al.* (2010)
Maida, wheat, gram, millet, rice, corn, molasses and multigrain flour, respectively C/N ratio >15	Carbon sources were added based on the derivation of Avnimelech (1999), that is 0.465 × feed	*Litopenaeus. vannamei*	Growth indices such as; ADG, FCR, Survival, SGR and ADW were similar for millet, molasses and multigrain treatments Generally, mortality was low in millet and multigrain treatments. Up-regulation in immune parameters such as; SOD, MnSOD and BGBP were also observed in these treatments groups	TAN and nitrites were mainly oxidized to nitrates and subsequent reduction in the levels of nitrates was observed in the carbon supplemented groups. This maintained the water quality parameters in the optimal range for shrimp growth	Flocs in millet, molasses and multigrain treatments were of high protein quality since limiting amino acids (arginine, methionine and lysine) were detected	Panigrahi *et al.* (2019)
Corn flour, wheat flour, sugar, 12:1	About 1 g per 100 L of carbon source was administered to set the appropriate C/N ratio for heterotrophic bacteria growth. Specifically, 3.7 g of wheat flour, 2.7 g of sugar and 3.5 g of corn flour was added	*Oreochromis niloticus*	Among the carbon treatments, the highest fish weight was recorded in the sugar group (0.44 g ind^{-1}). The final FCR was within the range 1.28–1.51 with significant differences detected among the different carbon source treatments. Overall, similar zootechnical characteristics were observed in the sugar and corn flour groups, respectively	No significant difference was found for TAN (0.33, 0.30, 0.15 mg L^{-1}, respectively) and nitrite levels among the corn flour, wheat flour and sugar treatments, respectively	Carbon addition resulted in higher accumulation of TSS, with wheat flour group recording the highest TSS levels (226.6 mg L^{-1}). However, proximate composition or other floc characteristics were not reported	García-Ríos *et al.* (2019)

Table 1 (continued)

Organic carbon sources types (C/N ratio)	Additional strategies	Cultured species	Effect on the growth performance and/or welfare of the fish or shrimp	Effects on the water qualities	Effects on the characteristics of bioflocs	Reference
Molasses, corn flour, wheat bran C/N >16	Various combinations of the carbon sources were assessed but the best addition for shrimp growth was; 60% of molasses plus 20% of corn flour plus 20% of wheat bran	*L. vannamei*	FCR and survival rate was similar among the biofloc treatments. However, in terms of the different carbon source combinations, the best growth performance was observed in the 60% (molasses), 20% (corn flour) and 20% (wheat bran) group	All carbon groups maintained the water quality within acceptable range for shrimp growth with the exception of pH which decreased occasionally to non-optimal levels due to carbon source addition	Carbon source influenced the protein content of bioflocs (23.95–32.32%). Lipid content of flocs was also influenced by the carbon source (2.92–5.33%). Activities of amylase, protease, lipase and cellulase were recorded in flocs from all carbon treatments	Wang *et al*. (2016)
Molasses, added every fourth day, 10:1–20:1	The C/N ratios were determined based on the protein content of the feed and carbon was added in a four-day interval till the end of the experiment	Juveniles of *O. niloticus*	Although C/N ratios were different among the bioflocs treatments, the survival of tilapia juveniles were similar but higher, since molasses was added as extra carbon source. Growth response parameters such as weight gain, final biomass, weight gain per day and final weight was highest in some of the biofloc treatments using molasses as carbon source	An initial trial with pulse (>0.12 g L^{-1}) addition of molasses resulted in a decline in O_2 levels (3.2-1-1.5 mg L^{-1}). Water parameters were subsequently maintained (TAN, nitrites, nitrates) at tolerable levels for tilapia. However, a gradual decline in pH was observed due to molasses addition	Floc concentration was highest in the treatment with C/N ratio 20:1 employing which determined by molasses addition. This was in a magnitude of 200% higher than the control without molasses addition	Pérez-Fuentes *et al*. (2016)
Sucrose used only for start-up period or floc establishment period	During start-up period, about 15 g of sucrose was added anytime ammonia reached 1.5 mg L^{-1} or higher	*L. vannamei*	The survival rate of shrimp was highest (86.2 ± 1.7%) in the BFT treatment compared to other groups. Additionally, FCR (1.1 ± 0.1) and SGR (1.4 ± 0.1%) were better in BFT group compared non-BFT treatment	Turbidity was higher in biofloc treatment (15.1 ± 5.7 NTU). TAN, NO_2^- and NO_3^- concentrations were higher (1.5 ± 0.8, 9.2 ± 4.5 and 21.4 ± 8.8 mg L^{-1}, respectively) in the BFT group compared to the non-BFT group. This might have been because sucrose was only applied during start-up	Higher N isotope levels in BFT treatment reveal that the flocs served as a supplemental protein source for the shrimp. Therefore, bioflocs were of high nutritional value	Tierney and Ray (2018)

Table 1 (continued)

Organic carbon sources types (C/N ratio)	Additional strategies	Cultured species	Effect on the growth performance and/or welfare of the fish or shrimp	Effects on the water qualities	Effects on the characteristics of bioflocs	Reference
No supplemental carbon	No extra carbon source was added to the biofloc group, but in situ carbon flocs were relied upon as the carbon source	*L. vannamei*	Growth parameters such as total biomass, harvest weight and FCR were better in the RAS group (1.8 ± 0.1) compared to the BFT group (1.5 ± 0.1) with no supplemental carbon source. The final average weight of shrimp in RAS treatment was higher than BFT treatment	Lower level of ammonia was recorded in BFT treatment compared to RAS treatment. However, pH declined in the BFT treatment compared to the RAS. Turbidity, nitrite and nitrate were higher in BFT group than RAS	Flocs served as carbon source in BFT group since C^{13} was higher in BFT group than in RAS group. Isotope analysis reveals bioflocs provided about 18% and 60% of C and 1–16% of N to the shrimp	Ray *et al.* (2017)
Molasses was used in the first 4 weeks Sugar cane molasses used anytime TAN was above 0.6 mg L^{-1}, 15:1	Molasses was added based on Avnimelech (1999) according to input N. that is anytime TAN exceeded 0.6 mg L^{-1}, molasses was added to control nitrogen	Juveniles of *L. vannamei*	The shrimp attained an SGR of 6.9% per day and final biomass was 2.17 ± 0.05 g with FCR of 0.87 ± 0.08	Organic nitrogen, phosphorus and phosphate consistently accumulated in the system. NO_3^- reached up to 80% of the total inorganic nitrogen level in treatments	Low FCR implies the flocs were of high nutritional value for shrimp	da Silva *et al.* (2013)
Sugarcane molasses, 15:1	Carbon source was added anytime TAN exceeded 1 mg L^{-1} to maintain C/N ratio	*L. vannamei*	Shrimp survival was >95% in all treatments. However, final biomass, final weight and weight gain values differed significantly among treatments with different floc sizes with the exception of 150 and 300 μm floc size group	Ammonia fluctuated between 5 and 0 mg L^{-1} throughout the study. Nitrite oscillated after day 13 until the end of the study. Nitrate accumulated up to 50 mg L^{-1} at the end of the study	Floc sizes (e.g. 50, 150 and 300 μm) did not affect the nitrification process	Souza *et al.* (2019)
Molasses 6 g C VS 1 g TAN, C/N 15:1	Carbon source was added anytime the TAN concentration was 0.8 mg L^{-1} or higher. The derivation was based on Avnimelech (1999)	*L. vannamei*	The highest final biomass and productivity estimation was recorded in BFT groups employing Na_2CO_3 and Ca $(OH)_2$ as agents for pH correction. That is the final biomass amounted to 630.0 ± 61.1 and 615.7 ± 9.3 g, respectively. Also, the productivity values for two groups were 2.3 ± 0.1 and 2.2 ± 0.1 kg m^{-3}, respectively	TAN levels fluctuated widely in the group with no carbonate manipulation. NO_2^- concentration was maintained below 25.7 mg L^{-1}, which was still within tolerable levels for shrimp irrespective of alkalinity manipulation	The microbial flocs concentration exceeded the levels suitable for shrimp growth among all treatments, which were later controlled by removing some floc and TSS	Furtado *et al.* (2011)

Table 1 (continued)

Organic carbon sources types (C/N ratio)	Additional strategies	Cultured species	Effect on the growth performance and/or welfare of the fish or shrimp	Effects on the water qualities	Effects on the characteristics of bioflocs	Reference
Molasses 20 g C VS 1 g TAN	Molasses was added anytime TAN exceeded 1 mg L^{-1} and calculated based on Avnimelech (1999)	Juveniles of *L. vannamei*	Shrimp survival rate was better in lower floc levels than higher floc level. However, average weight gain and final weight was similar among all floc treatments	pH was higher (7.8) in lower floc level (200 mg L^{-1}) group than higher floc level groups (400–600 and 800–1000 mg L^{-1}). PO_4^{3-} was higher in higher floc level groups. Alkalinity (218.4 mg L^{-1}) was higher in low floc level (200 mg L^{-1}) group	Biofloc concentration influenced the protein quality of flocs. Higher CP (28%) was recorded in lower floc concentration group than higher floc level groups (20.2% and 18.6%, respectively)	Schveitzer *et al.* (2013)
Sucrose CN 15:1 6 g C VS 1 g TAN	Daily addition of sucrose was based on Avnimelech (1999) and Ebelimg *et al.* (2006) in which 6 g of carbon was needed to convert 1 g of TAN	Juveniles of *L. vannamei*	Final ind. weight was higher in pH 8.1 (14.51 g) group than pH 7.6 (13.76 g) group. Survival rate was higher in pH 8.1 (87.5%) than pH 7.6 (80.8%) and Control (71.7%). FCR was lower and did not differ in groups with pH adjustment (7.6 and 8.1) but differed in the control BFT group with no pH adjustment. THC and phagocytic activity in shrimp was higher in pH 7.6 and 8.1 groups than the control which was significantly lower	Alkalinity was higher in high pH (8.1) group. Lower TAN concentration (0.25 mg L^{-1}) was recorded in pH (8.1) group. NO_2^- was significantly lower in pH (8.1) group than pH 7.6 group (3.77 mg L^{-1}) and control group (9.08 mg L^{-1})	PHB content in the flocs was higher in pH 8.1 and pH 7.6 groups compared to the control. However, contents of polysaccharides and carotenoids in flocs were similar in all groups	Zhang *et al.* (2017)

Table 1 (continued)

Organic carbon sources types (C/N ratio)	Additional strategies	Cultured species	Effect on the growth performance and/or welfare of the fish or shrimp	Effects on the water qualities	Effects on the characteristics of bioflocs	Reference
Wheat milling by-product and rice bran CN 15:1	Daily addition of carbon sources 2 h after feeding	*O. niloticus*	Fish growth performance was better in biofloc treatment than clear water group. Specifically, weight gain, FCR etc were higher in the group in which wheat milling by-product was used as the carbon source. Non-specific immune parameter such as; albumin, globulin, total protein and humoral innate immunity parameters (e.g. lysozyme) were significantly higher in biofloc groups	Carbon source did not affect DO, temperature and salinity. However, different carbon source affected TAN, NO_2^-, and NO_3^- significantly. Nitrogen level was higher in biofloc treatment than clear water group	Floc volume was high in the group employing wheat milling by-product as carbon source	Mansour and Esteban (2017)
Glucose C/N 10, 15 and 20	Glucose (40% C) was added every 3rd day based on the protein content of feed. 15.6 g of glucose to 10 g of feed was required	*O. niloticus*	Growth performance of tilapia was better in C/N 10 and 15 than C/N 20 group. Enzyme (trypsin and lipase) and immune parameters (lysozyme, alkaline phosphatase etc.) were also better in C/N 10 and 15 than C/N 20	NO_2^- levels were lower in CN 20 than the CN 10 and CN 15 groups. TAN fluctuated widely in all groups	CN ratio impacted the colour of flocs. Intensity of browner flocs corresponded with increasing CN ratio	Liu *et al.* (2018c)
Tapioca starch, 16:1	Daily addition of tapioca starch according to the input N from feed	*O. niloticus*	Daily average growth rate was higher (1.4 day^{-1}) in the tapioca treatment than the control (1.0 day^{-1}). Also, survival rate was higher in tapioca treatment (96%) than the control	TAN and NO_2^- accumulated at slower rates in carbon group than the control. This implied that carbon addition delayed the establishment of nitrification	Addition of tapioca increased floc concentration and also increased nitrogen and carbon content in the flocs than the control treatment	Nootong *et al.* (2011)

Table 1 (continued)

Organic carbon sources types (C/N ratio)	Additional strategies	Cultured species	Effect on the growth performance and/or welfare of the fish or shrimp	Effects on the water qualities	Effects on the characteristics of bioflocs	Reference
Sodium acetate	Sodium acetate was added at the rate of 75% of the feed based on the methodology of Gao *et al*. (2012) in which carbon source was calculated as; 0.465*feed*protein content of feed	*O. niloticus*	Fish weight/individual was 22% higher in BFT treatment than RAS treatment SGR and total weight gain in fish from BFT treatment were, respectively, 128% and 112% more than RAS treatment. FCR was 18% lower in BFT than RAS Total superoxide dismutase activity was high in fish from BFT system than RAS system	Compared to the RAS treatment, BFT recorded higher mean levels of TAN (60 ± 0.45 mg L^{-1}) and NO_2^- (119 ± 2.01 mg L^{-1}). NO_3^- build up was not observed in BFT treatment	The crude protein content of flocs in BFT (30.90 ± 9.04%), which was suitable for tilapia nutrition	Luo *et al*. (2014)
Glucose added after each feeding, PHB and PCL CN of 16.84	Glucose addition was done after each feeding at a rate of feed × 46.5% PHB and PCL was added by filling 8 nylon bags with 20 g of the granules and hanged in the tanks for carbon supplementation	*O. niloticus*	Final biomass was similar between the PHB group (37.93 kg m^{-3}) and PCL treatment and similar between PHB treatment and glucose treatment (44.1 kg m^{-3})	TAN and NO_3^- concentrations were below 3.0 mg L^{-1} and 0.8 mg L^{-1}, respectively, during steady state. Ongoing nitrification, TAN and NO_3^- assimilation and nitrification was observed in all groups	Crude protein (CP) content of flocs was similar and ranged from 31% to 39% foe PHB and PCL treatment. Additionally, CP did not differ between PHB group and glucose treatment	Luo *et al*. (2017)
Carbon source additions were of 2 categories; that is molasses was used only during the initial weeks (INI) Daily sugarcane molasses additions (CONT), 12:1	In INI group, molasses was only added during the initial weeks of the biofloc development In the CONT treatment, molasses was added every day to the system	Juveniles of *L. vannamei*	The survival rate of shrimp in INI was higher (76.9 ± 6.7%) than in the CONT group (57.0 ± 8.6%). Also FCR was lower in the INI group (1.5 ± 0.1) than CONT group (2.4 ± 0.0). Final biomass in INI (1.8 ± 0.3 kg m^{-3}) was significantly higher than in the CONT group (0.8 ± 0.0 kg m^{-3})	CN ratio during steady state was lower in both treatments (i.e. INI and CONT), which, respectively, were 6.41 ± 1.41 and 7.5 ± 1.6. Also, the ratio of BOD:TSS was low in both groups. Sludge accumulation in INI was more stable than CONT group. Values of TAN, NO_2^-, NO_3^-, temperature and oxygen were similar in both groups	TSS accumulated higher in group CONT (i.e. 0.25 kg kg^{-1} of feed supplied) than group INI The accumulation TSS in group INI was 0.16 kg kg^{-1} of feed	Arantes *et al*. (2017)

Table 1 (continued)

Organic carbon sources types (C/N ratio)	Additional strategies	Cultured species	Effect on the growth performance and/or welfare of the fish or shrimp	Effects on the water qualities	Effects on the characteristics of bioflocs	Reference
Mature bioflocs used No supplemental carbon was used.	Mature biofloc with already established nitrification condition and which was also in the stage of chemotrophy or mixotrophy was used. Thus, no additional carbon was added	*Litopenaeus. vannamei*	Biofloc treatment fed with lower protein content feed (24.3%) resulted in lower weight gain, SGR and final weight values than biofloc groups fed with higher protein content feed (i.e. 30.3%, 32.9% and 36.7%)	In all biofloc treatments, levels of pH (7.48–8.56), DO (4.7–6.3 mg L^{-1}), temperature (27.9–30.2°C) and salinity (20–21‰) were all suitable for shrimp culture. Similarly, TAN, orthophosphate (3.36–4.91 mg L^{-1}), NO_2^- (0.05–0.31 mg L^{-1}) and NO_3^- (7.4–79.9 mg L^{-1}) were within the tolerable limits for shrimp	Biofloc volume and TSS was high and showed significant difference on day 14 of the experiment resulting in initiation the removal of some flocs	Jatobá *et al.* (2014)
Sucrose, molasses and glycerol CN 22:1	Each type of carbon source was added twice daily between the feeding times to maintain CN ratio of 22:1 in heterotrophic based tanks	*L. vannamei*	Survival rate for shrimp similar in all groups with carbon addition (sucrose, molasses and glycerol) with values of 53.2%, 21.6% and 49.2%, respectively, except that the values for sucrose and molasses groups differed significantly. Growth rate by week was 0.7 ± 0.1 g for CA, 0.3 ± 0.2 for molasses treatment, 0.7 ± 0.0 g for sucrose treatment and 0.6 g for glycerol treatment	TAN was significantly higher (0.3–1.1 mg L^{-1}) in the heterotrophic groups (H) compared to the chemo-autotrophic (CA) based group (0.2 mg L^{-1}) group. NO_2^- levels did not differ in all the treatments (0.2–1.5 mg L^{-1}). NO_3^- levels was significantly higher (9.08 mg L^{-1}) in CA group compared to H group (0–1.4 mg L^{-1}). Alkalinity was higher in H group (276–352 mg L^{-1}) than the CA group (169 mg L^{-1})	Specific characteristics of flocs were not reported. However, a pronounced rise in solids was observed in the sucrose and glycerol treatment during the latter stages of the experiment. Variability in settleable solids was relatively high; however, there was no significant differences detected among groups	Ray and Lotz (2014)
Wheat flour, input 20:1 (CHO/TAN)	Wheat flour was added at a rate of 60% of the added feed based on Avnimelech (1999)	Mixed sex *O. niloticus*	Indicators of welfare of fish including: gill histology, plasma cortisol, blood haematocrit, fin condition and proximate composition did not differ among all treatments (35% feed, 24% feed and control groups). In BFT tanks, net fish yield/production amounted to 45% more than in the control group	Wide variations in alkalinity were observed in the biofloc groups (8–250 mg L^{-1}). However, stability in the levels of total alkalinity (18–27 mg L^{-1}) in the control with no carbon addition was observed. TAN, NO_2^- and NO_3^- fluctuated widely in BFT groups than the control	Nutritional parameters of flocs were similar in all BFT groups. Flocs constituted 27–28% polyunsaturated fatty acids and 28–29% monounsaturated fatty acids in BFT groups	Azim and Little (2008)

Table 1 (continued)

Organic carbon sources types (C/N ratio)	Additional strategies	Cultured species	Effect on the growth performance and/or welfare of the fish or shrimp	Effects on the water qualities	Effects on the characteristics of bioflocs	Reference
Tapioca starch (TS), Plant cellulose (PC) and TS + PC CN range. 13.0–14.7	Daily addition of carbon source 0.6 g per gram of feed added 2 times a day	*Pelteabaggrus vachelli*	Weight gain ratio showed significantly higher value in TS (94.47%) than the other treatments. SGR was significantly higher in TS (1.58 ± 0.20 %) and lowest in the control group (1.19 ± 0.20%). On the other hand FCR showed similar values among all treatments (2.02–3.11) including the control group	TAN was generally lower in carbon supplemented groups (i.e. 2.4, 1.8 and 2.2 mg L^{-1}, respectively, for TS, PC and TS + PC) than that of control (3.6 mg L^{-1}). NO_2^- remained below 1.5 mg L^{-1} till the end. DOC was significantly higher in TS (16 mg L^{-1}) than the other groups in order of TS > TS + PC > PC > Control	Biofloc volume (BFV) did not differ among carbon supplemented groups and the control. TSS was higher in BFT groups (90.1 ± 30.3 mg L^{-1}) than the control (51.9 ± 22.6 mg L^{-1}). sludge volume index (SVI) was higher in PC treatments (PC was 51.7 and TS + PC was 53.2 mg L^{-1}) than the control group (34.6 mg)	Deng *et al*. (2018)
Molasses	Molasses was added to the BFT tanks anytime TAN exceeded 0.5 mg L^{-1}. Specifically, molasses were added based on the addition of 6 g of carbon for each 1 g TAN according to Ebeling *et al*. (2006) and Avnimelech (2009)	*L. vannamei*	Final mean weight of shrimp was higher in the biofloc-enriched (25%, 50%, 75% and 100%; i.e. 8.01–8.42 g) compared to that of the control group with 0% enrichment (7.37g) Survival rate was not significantly different among all treatments and ranged from 90.93–99.06% FCR was significantly lower in the biofloc-enriched groups (0.84–1.23) compared to the control group (1.52)	TAN concentration was significantly lower in the biofloc-enriched groups (ranged from 0.0001–1.5 mg L^{-1}) compared to that recorded in control group. NO_2^- concentration was significantly higher in the control group (10.11 mg L^{-1}) compared to the biofloc-enriched groups (0.54–1.85 mg L^{-1})	TSS concentrations were similar among all treatments (383–636 mg L^{-1}) except the 100% biofloc-enriched group which was significantly higher in TSS (714 mg L^{-1}). Levels of settleable solids were significantly higher in the biofloc-enriched groups (12.51–16.20 mg L^{-1}) than the control (8.48 mg L^{-1})	Krummenauer *et al*. (2014)

Table 1 (continued)

Organic carbon sources types (C/N ratio)	Additional strategies	Cultured species	Effect on the growth performance and/or welfare of the fish or shrimp	Effects on the water qualities	Effects on the characteristics of bioflocs	Reference
Carbon source was based the method of Ebeling *et al.* (2006) and Avnimelech (2009)	Carbon supplementation was carried out according to the procedures of Ebeling *et al.* (2006) and Avnimelech (2009)	*L. vannamei*	Final weights of shrimp were similar between BL (12.96 g) and PR (12.81 g) groups, respectively, but both were significantly higher than that in the VP (10.93) group. Survival rate and final biomass were statistically lower in the PR group compared to the others. FCR was similar between group BL (1.71) and PR (1.99) and between BL and VP (1.56) but not between VP and PR	Levels of TAN were lower in the Blower, BL group (1 mg L^{-1}) and Vertical Pump group (2.13 mg L^{-1}), which together differed significantly differed from that of the Propeller (PR) group (5.91 mg L^{-1}). NO_2^- concentration was significantly higher in BL group (8.97 mg L^{-1}) and VP group (4.54 mg L^{-1}) than the PR group (1.97 mg L^{-1}). Also, NO_3^- was higher in BL group (14.88) mg L^{-1} which differed from that of VP (4.53 mg L^{-1}) and PR (3.97 mg L^{-1}) groups, respectively	Dry matter content of flocs in BL group was the lowest (16.19%) but was equal for group VP (19.18%) and PR (19.18%), respectively. The crude protein content of flocs was higher in BL treatment compared to VP (27.88%) and PR (26.59%). Crude lipid content in the flocs was lower in BL (0.79%) compared to that VP (1.08%)	Lara *et al.* (2017)

BGBP, β-1,3-glucan binding protein; CL, crude lipid; CN, carbon: nitrogen ratio; CP, crude protein; FCR, feed conversion ratio; PCL, polycaprolactone; PHB, poly-beta hydroxybutyrate; SGR, specific growth rate; SOD, superoxide dismutase; TAN, total ammonia nitrogen; TSS, total suspended solids.

organic carbon sources, glucose ($C_6H_{12}O_6$) and dextrose may be economically prohibitive at a commercial scale because they are relatively expensive (Zhang *et al.* 2016b). Sucrose ($C_{12}H_{22}O_{11}$) occurs naturally in most plants, is available in many markets and has demonstrated success at facilitating the bacterial assimilation of NH_4^+–N (Kuhn *et al.* 2009; Ray *et al.* 2011; Merriam-webster 2020).

There are other types of complex carbohydrates in BFT aquaculture systems including starch, molasses and cellulose. Starch $(C_6H_{10}O_5)_n$ is a naturally abundant nutrient carbohydrate (Merriam-Webste 2020). The starch used in BFT aquaculture systems commonly comes from corn, wheat, sweet potato or cassava starch (Ekasari *et al.* 2010; Fugimura *et al.* 2015). With sucrose as the main ingredient, molasses is commonly made from processed cane or beet sugar (Merriam-webster 2020). Molasses is a less expensive organic carbon that has also been demonstrated to be effective at stimulating heterotrophic assimilation of NH_4^+–N (Burford *et al.* 2004; Emerenciano *et al.* 2012). For example, using soya bean and sugarcane molasses as external organic carbon sources maintains water quality in the super-intensive culture of *L. vannamei* in BFT systems (Fugimura *et al.* 2015; do Espírito Santo *et al.* 2017). Cellulose $(C_6H_{10}O_5)_x$ is a polysaccharide of glucose units occurring naturally in fibrous products (Merriam-webster 2020). Plant-based cellulose is considered one of the most important carbon sources for bioprocesses (Nowak *et al.* 2005; Ge *et al.* 2012). Natural cellulose-rich materials, for example chopped straw, have been demonstrated to be able to control NH_4^+–N in BFT aquaculture systems effectively (Serfling 2006). Additionally, these cellulose-rich materials do not require frequent additions and are less expensive than other soluble substances (Serfling 2006).

Organic acids and alcohols

Glycerol ($C_3H_8O_3$) can be obtained from the biodiesel manufacturing process (Merriam-webster 2020). Owing to its greater degree of reduction potential, glycerol is considered an alternative carbon source in industrial bioprocesses and has been proven to facilitate production of nutritious bioflocs (Crab 2010b; Clomburg & Gonzalez 2013; Xiberras *et al.* 2019). Acetic acid follows a simple bio-degradation pathway and can be directly used by the β-oxidation process to form acetyl-CoA in denitrifying bacterial systems (Elefsiniotis & Li 2006). As an easily biodegradable carbon source, sodium acetate (CH_3COONa) has been used for BFT aquaculture systems in some studies (Luo *et al.* 2013). Sodium acetate is always used for growing or culturing bacteria in lab-scale studies or processes (Schneider *et al.* 2006). However, on a commercial scale, the use of sodium acetate may be limited due to the associated high cost of production (Schneider *et al.* 2006).

Biodegradable polymers

Biological degradable polymers (BDPs) are polymeric materials that can release DOC through the action of microbial enzymes (Song *et al.* 2009). Polyhydroxyalkanoate (PHA), poly(3-hydroxybutyrate) (PHB), polycaprolactone (PCL) and polybutylene succinate (PBS; Fig. 1) have been used to influence denitrifying conditions or processes as external organic carbon sources (Müller *et al.* 1992; Boley *et al.* 2000; Chu & Wang 2011). The main advantage of using BDPs as organic carbon sources is reducing the risk of overdosing or shortage with little management (Boley *et al.* 2000; Li *et al.* 2016). PHAs are polyesters of several kinds of hydroxyalkanoates synthesized by various bacteria (Reddy *et al.* 2003). PHB is composed of small ($C_4H_6O_2$) monomer units produced by bacteria as an energy storage compound, especially under limited nutritional conditions (Reddy *et al.* 2003; Bhuwal *et al.* 2013; Wang *et al.* 2013). It is known that PHB positively affects aquatic organisms by improving growth and anti-infective ability (Defoirdt *et al.* 2004; Schryver & Verstraete 2009). PCL is a thermoplastic synthetic polymer composed of caprolactone ($C_6H_{10}O_2$) monomers (Hosni *et al.* 2019). PCL is available for most microorganism and is less expensive than PHB (Chu & Wang 2011). PBS is a novel biodegradable aliphatic polyester composed of butylene succinate ($C_8H_{12}O_4$) monomers (Hosni *et al.* 2019). Compared with PCL and PHB, PBS is a relatively inexpensive BDP carbon source for heterotrophic bacteria compared with PCL and PHB (Luo *et al.* 2014). Recently, PHB and PCL have been demonstrated to be effective organic carbon sources for BFT aquaculture systems (Luo *et al.* 2017, 2019a; Li *et al.* 2018). Additionally, biodegradable plastics blended with starch or any other degradable material were recently used to improve the DOC release rate and lower the cost of BFT design and management (Zhang *et al.* 2016b; Luo *et al.* 2017). Li *et al.* (2018) found that blending biodegradable polymers with longan powder significantly improved the removal efficiency of inorganic nitrogen compared with that of longan powder alone in a Nile tilapia (*Oreochromis niloticus*) BFT aquaculture system.

Categorization based on the speed of releasing DOC

Carbonaceous substrates releasing DOC instantly

Glucose, glycerol and acetate are three simple and directly soluble carbon sources that have been extensively used in BFT aquaculture systems (Avnimelech 1999; Burford *et al.* 2004; Hari *et al.* 2004; Luo *et al.* 2019b). Simple water-soluble carbonates, such as glucose and sucrose, dissolve and are decomposed quickly, providing greater levels of DOC for heterotrophic bacteria to assimilate NH_4^+–N in a short time and resulting in rapid NH_4^+–N removal. More complex carbohydrates, such as starch and molasses, require more time to be degraded into simple sugars, resulting in a slower removal of NH_4^+–N (Serra *et al.* 2015; Wei *et al.* 2016).

Supplying water-soluble substrates as organic carbon has been criticized for its complexity and cost, as additions should occur a few times per day or per several days with careful calculation and constant supervision to avoid overdosing or starvation (Serfling 2006; Emerenciano *et al.* 2012; Luo *et al.* 2017; Luo *et al.* 2019a). Additionally, dissolved oxygen is required for bacteria to decompose organic matter substrates (Azim *et al.* 2007). The addition of water-soluble carbohydrates to the fish tank may induce a sudden reduction in dissolved oxygen (Schryver & Verstraete 2009; Pérez-Fuentes *et al.* 2016).

Carbonaceous substrates that release DOC relatively slowly

Unlike water-soluble substrates, solid external carbonaceous substrates must be degraded to DOC first. Therefore, compared with soluble substrates, water-insoluble carbonaceous substrates release DOC more slowly, and subsequently, the removal of ammonia is relatively slower.

The usually water-insoluble solid-phase organic carbon sources include BDP_S and plant-based materials (Lee & Wang 2006). The mechanism governing microbial degradation of BDPs is understood to a certain extent (Hosni *et al.* 2019). However, few studies have focused on the bio-degradation of solid-phase carbonaceous substrates in BFT aquaculture systems. Zhang *et al.* (2016b) proposed the following hypothesis: a typical BFT aquaculture system exhibits aerobic and suspension conditions; the free bacteria attach to the surface of the solid substrates and form colonies; enzymes are secreted to decompose the BDPs into small, water-soluble monomers; and heterotrophic bacteria then consume the available DOC to assimilate NH_4^+–N (Table 2).

Effects of different carbon sources on BFT system performance

Carbonaceous substrates in BFT systems have been found to produce a wide range of effects on BFT systems and influence features such as bacterial communities, water qualities, culture organisms and characteristics of bioflocs. These influences may be due to the efficiency of maintaining the C/N ratio, degraded products or other unknown factors. To date, no studies have focused on the mechanism of these effects. The following subsections delve into some of the known effects of carbon sources on the components mentioned above.

Bacterial community

Generally, BFT aquaculture systems attempt to control water quality through the manipulation of the C/N ratio to encourage the activities and growth of heterotrophic bacteria that assimilate inorganic nitrogen into their biomass (Avnimelech 1999; Ebeling *et al.* 2006; Crab *et al.* 2012). In particular, this action results in the development of conglomerates of microorganisms including bacteria, algae and protozoa which form a community with other organic components, such as detritus and particulate matter (Wei *et al.* 2020). The microbial community constitutes a vital aspect for both shrimp and finfish development in aquaculture, as they influence the physiological performance of the fish, and preference may be given to some specific microbial groups due to their functional roles (Zhang *et al.* 2016a; Garibay-Valdez *et al.* 2020a, 2020b). Although carbon sources stimulate the growth of heterotrophic bacteria, they influence bacterial communities in different ways (Avnimelech 2012; Panigrahi *et al.* 2019). For example, they can influence bacterial numbers, relative abundance and diversity.

Bacterial numbers

Since carbonaceous substrates affect the metabolism of bacteria in BFT systems (Wei *et al.* 2020), they can also influence their cell counts or numbers (Panigrahi *et al.* 2019). However, studies describing the relationship between carbon source type and bacterial numbers in biofloc aquaculture systems are currently inadequate. Earlier reports indicated that carbon supplementation can maintain heterotrophic bacterial abundance in the range of 10^7–10^8 cells mL^{-1} (Avnimelech 2009). The effects of carbon source on bacterial populations may be due to certain bacterial groups, including heterotrophic bacteria and certain autotrophic bacteria, obtaining their energy from organic carbon compounds (Ebeling *et al.* 2006; Hargreaves 2006). This process contrasts with the effects of organic carbon compounds on chemosynthetic nitrification bacteria that obtain their energy from inorganic compounds (Ebeling *et al.* 2006). Along these lines, carbon-supplemented systems exhibit greater bacterial biomass than do systems without carbon supplementation. Studies are needed to characterize the specific bacterial communities dominating BFT systems (Luo *et al.* 2020). This line of research should also include the specific effects on their numbers or populations with respect to the types of carbonaceous substrates used. Clarity on these aspects of bacteria communities in BFT and the associated carbon sources will advance manipulative strategies to favour the most relevant bacterial species.

Relative abundance

Although biofloc systems are expected to be purely heterotrophic, activities of chemo-autotrophic bacterial population have been observed (Nootong *et al.* 2011; Luo *et al.* 2019a, 2020). Therefore, populations of these two groups of bacteria may both find ideal growing conditions in BFT systems. This phenomenon could result from the type of carbon source used, the C/N ratio of the culture water or levels of suspended particles (Liu *et al.* 2018c; Luo *et al.* 2020). However, the use of glucose and glycerol has been found to promote the population of heterotrophic bacteria in BFT systems (Wei *et al.* 2020). Additionally, molasses and dextrose, which are highly soluble carbonaceous substrates, are reported to heavily favour the populations of heterotrophic bacteria in BFT aquaculture (Wasielesky Jr *et al.* 2006).

The development of molecular and high-throughput sequencing techniques has enabled an advanced understanding of the relationship between carbon sources and microbial composition in biofloc systems (Lv *et al.* 2014; Li *et al.* 2018).Wei *et al.* (2020) recently found that glucose, glycerol and starch promote the dominance of bacteria groups, such as *Proteobacteria* and *Bacteroidetes*, which together compose more than 70% of the overall bacterial community, while other less represented groups include *Planctomycetes*, *Actinobacteria* and *Verrucomicrobia* (Table 3). Using brown sugar as the sole carbon source, Deng *et al.* (2019) also reported *Proteobacteria as* being the dominant bacteria phylum (>67%). Prior to these findings, other reports also indicated the dominance of these bacterial groups in biofloc systems with respect to soluble carbon sources (Cardona *et al.* 2016; Luo *et al.* 2017). On the other hand, Li *et al.* (2018) reported *Firmicutes* as the dominant bacteria in all groups; these bacteria accounted for 96.69%, 96.51% and 97.13% of all bacteria when Longan powder, PHB and PBS, respectively, were used as the carbonaceous substrates (Table 3). In a recent study using amaranth and wheat as carbon sorces, a total of 22 phyla were identified with *Planctomycetes*, *Proteobacteria*, *Firmicutes* and *Bacteroidetes* representing the dominant bacterial groups in the two treatments (Vargas-Albores *et al.* 2019).

Interestingly, the effects of carbon source on bacterial communities in flocs may also produce corresponding effects on fish/shrimp. For example, the bacteria communities present in bioflocs are reported to have the potential to significantly influence the abundance and activities of the gut microbiota of the aquaculture animal, which can subsequently affect their physiological processes, welfare and growth (Chaiyapechara *et al.* 2012; Cardona *et al.* 2016; Li *et al.* 2018).

Aside from the bacteria communities, carbon source may also influence the phytoplankton communities in BFT systems. For example, compared with the control group (with no glucose addition), a combination of glucose and *Bacillus* spp. In a BFT system resulted in greater abundance of *Chlorophyceae* and *Cryptophyceae* (Du *et al.* 2018). *Chlorophyll a* was also found to accumulate more in BFT treatments utilizing molasses as an organic soluble carbon source, signifying an abundance of microalgae communities in the system (Ju *et al.* 2008; Fugimura *et al.* 2015). The abundance of these phytoplankton communities may result in greater levels of crude lipids and fatty acids in the resulting bioflocs and thus increase the nutritional value of bioflocs (Ju *et al.* 2008; Ballester *et al.* 2010; Emerenciano *et al.* 2012; Godoy *et al.* 2012). Table 3 lists the common bacteria groups and their dynamics reported by recent studies using different carbonaceous substrates.

The aforementioned groups of bacteria are known to be involved in organic matter or substrate degradation and nitrogen transformation processes (Tu *et al.* 2014; Liao *et al.* 2015; Cardona *et al.* 2016; Vargas-Albores *et al.* 2019), functions that are vital for the success of the BFT process. These reports imply that microbial density, structure and diversity can be significantly influenced by the choice of carbon source applied to the system (Najafpour *et al.* 2006; Li *et al.* 2018; Deng *et al.* 2018; Panigrahi *et al.* 2019; Wei *et al.* 2020). These findings could also be influenced by different conditions and abiotic factors including variations in C/N ratio (Panigrahi *et al.* 2018), temperature, levels of total suspended solids (TSS), oxygen, feeding regimes and the culture species in question. Specifically, the management of suspended solid levels in minimal exchange systems, such as BFT, can also significantly influence bacterial communities (Ray *et al.* 2010a,2010b).

In terms of the influence of the C/N ratio, Panigrahi *et al.* (2018) found that microbial diversity was greater in systems with greater C/N ratios (20) than in systems with lower C/N ratios (<10) with *Psychrobacter* (26%), *Proteobacteria* (25%) and *Peridineaceae* (20%) as the dominant groups. Additionally, glucose is said to eliminate the virulent mechanisms of pathogens, such as *Vibrio harveyi*, and protect *Artemia* from vibriosis (Crab 2010b). However, these conclusions require in-depth investigations by future studies to better understand the mechanisms involved. Additionally, in current BFT practices, a major caveat is the inability to effectively manipulate bacterial communities while providing optimal water quality and health of the culture species (Cardona *et al.* 2016). Therefore, understanding the relationship between carbon sources and microbial community structure may advance manipulative strategies and selection of carbon for biofloc systems. Additionally, the choice of carbonaceous substrate should be based on not only its effects on the bacteria but also its ability to enhance the nutritional value and quality of the floc and growth of the aquaculture animal (Kuhn *et al.* 2009; Sakkaravar & Sanker 2015).

Bacterial diversity

The indices for describing bacteria diversity dynamics in most environments including BFT systems include the Chao1 index and the Simpson and Shannon estimators.

Generally, owing to the zero-exchange mode of operation of BFT systems, their bacterial groups are more diverse than those of other conventional aquaculture systems (Martínez-Córdova *et al.* 2015). This phenomenon could be due to the direct influence of carbon additions in BFT systems. It was reported that plant cellulose and plant cellulose plus tapioca starch resulted in a more diverse community of ammonia-oxidizing bacteria (AOB) compared with treatment without carbon addition, as detected by the Simpson and Shannon diversity estimators (Deng *et al.* 2018).

Recently, Wei *et al.* (2020) reported that use of glucose, glycerol and starch as carbon sources in BFT resulted in Shannon diversity values of 5.64 ± 0.07, 5.83 ± 0.35 and 4.66 ± 0.15, respectively, implying greater but similar bacterial diversity in the glucose and glycerol treatments but significantly lower diversity for starch treatment. However, prior to this report, it was found that, compared with treatments lacking sugarcane molasses, treatments involving molasses did not significantly influence the Shannon indices, the values of which were 2.86 ± 0.32 and 2.66 ± 0.39, respectively (Cardona *et al.* 2016). Additionally, with PHB, PCL and glucose as extra carbon sources, the Chao1 and Shannon indices were greater under the PHB treatment than under the glucose and PCL treatments (Luo *et al.* 2017). This finding may imply that, compared with other carbon materials, PHB is more preferable to most bacteria. These findings of bacterial diversity in relation to the carbon source type may lead to the conclusion that different carbon sources have distinct effects on the bacterial diversity in biofloc systems. In other words, some carbon sources may be preferred by bacteria communities. Hence, this finding could influence selection of a carbon source type for use in BFT in the field of aquaculture.

Water quality

One major goal of utilizing carbonaceous substrates in BFT systems is the maintenance of optimal water quality for the target culture species through C/N manipulation. However, maintaining the appropriate C/N ratio is known to play varied roles in processes, including development of nutritious bioflocs, reducing the concentration of total ammoniacal nitrogen (TAN) and eventually improving water quality (Pérez-Fuentes *et al.* 2016; Dauda *et al.* 2017; Panigrahi *et al.* 2019; Liu *et al.* 2019; Hoang *et al.* 2020). Variations in the C/N ratio due to supplied carbon substrates affect the competition between autotrophic and heterotrophic bacterial communities and thereby affect water quality (Nootong *et al.* 2011; Wei *et al.* 2016; Luo *et al.* 2020). Carbon sources, such as glucose, glycerol, acetate, molasses and starch, are commonly used as carbonaceous substrates in BFT systems for controlling the C/N ratio (Schneider *et al.* 2006; Emerenciano *et al.* 2012; Deb *et al.* 2017). Notably, to maintain appropriate water quality, these carbon materials are often required to be added several times daily or over a couple of days (Khatoon *et al.* 2016). Owing to their high solubility in aquatic systems, controlling water quality by using soluble carbon sources produces quick results compared with those from the use of insoluble or complex carbon materials (plant cellulose and biopolymers), which often require microbial degradation prior to carbon release. Soluble carbon sources provide greater levels of dissolved organic carbon (DOC) for the activities of heterotrophic bacteria (Serra *et al.* 2015) and thereby improve water quality. Carbon source types influence the water quality of biofloc systems in different ways which may depend on conditions of the system in terms of specific treatments and system variations (Table 1). The critical water quality parameters influenced by carbon source additions or C/N ratio manipulations (10, 15 and 20) include (TAN), NO_2^-, NO_3^- and orthophosphate (PO_4^{3-}; Avnimelech 1999; Azim & Little 2008; Zhang *et al.* 2016; Luo *et al.* 2017).

Dissolved inorganic nitrogen

In aquaculture practice, controlling TAN at low levels or at concentrations less detrimental to the culture organism is a major concern (Boyd & Tucker 2014). In light of this, biofloc aquaculture systems utilize carbonaceous substrates to maintain TAN at safe levels. The effects of carbon sources and the additions strategies influence TAN levels in BFT systems. For example, by using molasses, Panigrahi *et al.* (2019) maintained TAN at safe levels (0.096 ± 0.02) for shrimp growth. Similarly, Liu *et al.* (2018b) observed good results when sugarcane molasses was used to maintain the appropriate level of TAN for shrimp development. In another study, similarities in the levels of TAN were observed when sugarcane and soya bean molasses were compared for their effectiveness as carbon sources in BFT (do Espírito Santo *et al.* 2017). Notably, the similar effects produced by some carbon sources may imply that they are replaceable or interchangeable in terms of maintaining water quality. However, it should be clarified that other carbon sources produce distinct effects on TAN concentrations in BFT environments. For example, according to Caipang *et al.* (2015), sweet potato flour produces flocs with the ability to lower TAN levels in the culture environment more than those treated with wheat flour. Additionally, Deng *et al.* (2018) demonstrated the influence of carbon additions (tapioca starch, plant cellulose and their combination) on reducing TAN concentrations in BFT systems, as all carbon supplementation treatments showed lower levels of TAN compared with those of the control treatment without carbon addition. These effects of different carbon sources in controlling TAN in BFT systems still require in-

Figure 1 Examples of biodegradable polymers (BDPs) used as carbon sources in biofloc technology aquaculture systems: (a) Granules of polycaprolactone (PCL); (b) polybutylene succinate (PBS); (c) poly-β-hydroxybutyrate (PHB).

depth investigations to provide reliable data to influence the decision-making of practitioners on the best choice of carbonaceous material.

Nitrite (NO_2^-) is formed as an intermediate product of the nitrification process in most environments. NO_2^- accumulation is toxic to aquaculture organisms and can compromise their growth and welfare (Bussel *et al.* 2012; Furtado *et al.* 2015). The nitrification process occurs in most BFT systems (Nootong *et al.* 2011; Luo *et al.* 2020; Robles-Porchas *et al.* 2020). Therefore, monitoring and controlling NO_2^- levels have become important considerations in BFT practice. Carbon source additions in BFT systems seem to greatly affect the concentration of this compound. For example, by increasing the C/N ratio in a BFT system to 20 using glucose as the extra carbon material, Liu *et al.* (2018a) found that NO_2^- was almost always at very low concentrations (<0.2 mg L^{-1}) and sometimes not even detected (0 mg L^{-1}) in an *O. niloticus*-based culture. Additionally, using rice bran, glycerol and sucrose as extra carbon sources, Dauda *et al.* (2017) observed that nitrite-N was significantly lower in all carbon-supplemented groups compared with that in the control group in which there was no carbon addition. The authors also found that, compared with rice bran and sucrose treatments, glycerol treatment resulted in a consistently lower level of nitrite, which all resulted in greater concentrations at certain points. By comparing glucose and PHB as extra carbon sources for BFT systems, Luo *et al.* (2019a) observed that PHB was better at maintaining NO_2^- at a relatively low level (0.16 ± 0.24 mg L^{-1}) compared with the levels resulting from glucose (0.20 ± 0.22 mg L^{-1}) and a combination of glucose and PHB (0.19 mg L^{-1}). However, in the study by Deng *et al.* (2018), unlike the noticeably lower levels of TAN observed in carbon-supplemented treatments described earlier, levels of NO_2^- were not significantly different in the carbon-supplemented groups compared with the control group with no carbon treatment. This outcome may have been influenced by factors other than the carbon source, or it could imply that carbon supplementation and its corresponding effects on nitrite levels may depend on the carbon type used. Owing to these concerns, further investigations are required to better understand the dynamics of nitrite with respect to carbon additions in BFT systems.

As previously mentioned, biofloc systems are theoretically expected to be completely based on heterotrophic bacterial or predominantly based on ammonia assimilation rather than being based on autotrophic bacterial systems. However, several studies have uncovered the phenomenon of nitrate (NO_3^-) accumulation in biofloc aquaculture systems (Shang *et al.* 2018; Huerta-Rábago *et al.* 2019; Vargas-Albores *et al.* 2019). Robles-Porchas *et al.* (2020) have

Table 2 Summary of some of the beneficial effects of soluble and insoluble carbon sources in BFT systems

Benefit	Reference
Soluble carbon sources	
Improves the nutritional quality of bioflocs	Azim and Little (2008), Ekasari *et al*. (2010) and Emerenciano *et al*. (2012)
Stimulates the production of the bacterial storage compounds	De schryver *et al*. (2012)
Elicits quick response to spikes in ammonia and improving water quality	Zhang *et al*. (2016) and Luo *et al*. (2019a)
Defend against pathogens by disruption of pathogen quorum sensing	Crab (2010b)
Insoluble carbon sources	
Lower feed conversion ratio	Luo *et al*. (2019a)
A more stable ability to release carbon for the maintenance of water quality	Zhang *et al*. (2016) and Luo *et al*. (2017)
Controlling overdosing of carbon into the culture water	Luo *et al*. (2017) and Li *et al*. (2018)
Ensures reduced supervision and management, thereby reducing labour requirements	Luo *et al*. (2017) and Luo *et al*. (2019a)

extensively reviewed the important role of nitrification in BFT systems for the removal of nitrogen compounds from the culture water. The end product of the nitrification process in any given system is nitrate nitrogen (Ward *et al.* 2011; Robles-Porchas *et al.* 2020). Nitrate accumulation in BFT systems is an indication of ongoing nitrification and signifies the completion of this process. Irrespective of this evidence, the carbon source addition strategy tends to influence the levels of NO_3^- in BFT systems (Luo *et al.* 2017; Li *et al.* 2018; Luo *et al.* 2019b). However, it was recently reported that the carbon source type used for supplementation does not significantly affect the levels of NO_3^- in BFT systems (Arantes *et al.* 2017; García-Ríos *et al.* 2019). Although nitrate is generally not toxic to the culture organisms at certain concentrations, it is an issue when it accumulates to extremely high levels (>75 mg L^{-1}; Furtado *et al.* 2015). Therefore, inventing strategies targeted at the removal or reduction in this compound in BFT systems through carbon source addition is necessary. Such attempts have been made by Li *et al.* (2018), who described the possibility of controlling nitrate levels (0.14–0.37 mg L^{-1}) by employing an ex situ carbon source (PHBVL and PBSL) supplied with a peristaltic pump. However, more practical strategies for nitrate removal and further understanding are required regarding the relationship between carbon addition and nitrate accumulation in BFT systems.

Total nitrogen (TN) includes the nitrogen present in both the dissolved form and that attached to solids in biofloc systems. TN has been found to accumulate in BFT systems, although little is known about its toxicity or effects on aquaculture organisms (Luo *et al.* 2017). However, mechanisms that control TAN, nitrite and nitrate in BFT systems may eventually lead to reduction in TN. The relationship between carbon source addition and TN has yet to be adequately described in BFT systems.

Effects of carbon source on TSS or floc volume

Owing to the influence of carbon addition on the flocculation process in BFT aquaculture systems, greater floc levels or TSS have been observed in biofloc systems (Pérez-Fuentes *et al.* 2016; Zhang *et al.* 2017). Floc volume (FV) or TSS are found to influence the dynamics, processes and nutrient pathways in BFT systems (Luo *et al.* 2019b). In terms of floc volume, Deng *et al.* (2018) demonstrated that carbon source did not significantly affect this parameter; however, a significant effect was observed for the concentration of TSS (Table 1). Some carbon sources may release carbon more slowly (BDPs) compared with the release rates of other types (e.g. glucose, sucrose, glycerol). This phenomenon may therefore have an influence on the concentration of TSS or floc volume depending on which carbon type is chosen. There have been varying reports on this aspect of BFT aquaculture systems (Table 1).

Specific details on the nexus of different carbon sources and the corresponding effects on overall water quality in BFT systems reported by some studies are presented in Table 1.

Cultured organisms

Studies in BFT demonstrated the essential role of different carbon sources on the performance of the culture organism, including the growth (see Table 1), welfare, immune status and health of the aquaculture species (Dauda *et al.* 2017; Ahmad *et al.* 2019; Panigrahi *et al.* 2019).

Growth performance

The choice of the carbonaceous material in BFT systems affects the zootechnical performance of the culture animal in specific ways. In a related study, corn flour produced the greatest weight gain results in a *Labeo rohita*-based biofloc system when different carbon sources were tested (Ahmad *et al.* 2016). Similarly, Deng *et al.* (2018) described the effectiveness of tapioca starch in improving the growth of *Pelteobagrus vachelli* in terms of the weight gain ratio compared with the effectiveness from plant cellulose and their combinations (plant cellulose + tapioca starch). Additionally, the effectiveness and preference of tapioca starch in improving growth indices of fish compared with those of sugarcane bagasse have been demonstrated (Irshad *et al.* 2016). On the other hand, compared with the control treatment with no carbon addition, treatment involving sucrose application resulted in increased growth (SGR, survival rates and lower FCR) of *Penaeus monodon* (Huang *et al.* 2017). This finding adds to the evidence that different carbon sources differentially influence the growth of fish or shrimp. Therefore, the choice of carbonaceous material in BFT is a critical decision in the practice of aquaculture. Essentially, Dauda *et al.* (2017) evaluated the effects of sucrose, rice bran and glycerol on the growth of *Clarias gariepinus;* the best survival rate (90.6%) was found in the glycerol treatment compared with the others (76.3% for sucrose and 22.6% for rice bran), although overall growth was unaffected by the carbon source type. Additionally, Luo *et al.* (2019a) showed that PHB (an insoluble carbon source) is a more convenient carbon source for *Litopenaeus vannamei* BFT culture since it resulted in a greater survival rate (62%), final weight (0.87 g) and lower FCR (1.52) compared with those resulting from the glucose treatment. For tilapia culture, Luo *et al.* (2017) reported no differences in terms of fish growth (final biomass) between PHB (37.93 kg m^{-3}) and PCL (34.29 kg m^{-3}) treatments or PHB and glucose (44.14 kg m^{-3}) treatments, which demonstrates the effectiveness of insoluble carbon sources in BFT performance.

Growth may be linked to the quality of bioflocs produced, as they are a supplementary feed source for fish or shrimp species. Additionally, compared with simple and soluble carbon sources, complex carbon sources such as starch may result in higher crude lipid content (Wei *et al.* 2016; Rajkumar *et al.* 2016). However, these results are not exhaustive; Dauda *et al.* (2017) asserted that other relevant studies have reported contrasting results.

Welfare of culture organisms

These results suggest that different carbon sources may have different effects on the levels of immunostimulants, nutritional quality and bioactive compounds in flocs generated, which eventually influence the growth and physiological integrity of culture species (Crab *et al.* 2012; Ahmad *et al.* 2016; Ahmad *et al.* 2019). For example, Ahmad *et al.* (2019) tested the effectiveness of tapioca, corn, sugarcane bagasse and wheat as external carbon sources on the performance of *Labeo rohita* in a BFT system. The authors found that tapioca produced the best results in terms of haemoglobin content (6.61 ± 0.03 g dL^{-1}), total leucocyte count (109.66 ± 0.06 thousand cells mm^{-1}), antioxidant status, and lactate and malate dehydrogenase enzymes. Table 1 provides a summary of carbon source type and the corresponding effects on fish or shrimp growth and welfare as reported by studies in BFT.

Effects of carbon source on nutritional quality of bioflocs

Importantly, the influence of the carbon source on the microbial community may also influence the nutritional quality of bioflocs. It has generally been demonstrated that bioflocs from a well-designed BFT facility have acceptable proportions of ash, carbohydrate, lipid and protein content for use as fish/shrimp feed (Crab *et al.* 2010a). In essence, the nutritional composition of bioflocs can be affected differently by different carbon sources (glucose, acetate and glycerol; Crab *et al.* 2010a). This finding implies that the carbonaceous material chosen to develop or produce flocs in BFT systems could directly or indirectly influence the nutritional quality of bioflocs. Therefore, with an understanding of the influence of different carbon sources on the nutritional characteristics of flocs, the choice of carbon material with respect to the nutritional requirements of the culture species in question may be standardized and not based on discretion. This decision may be based on both the level of carbon in the carbonaceous material and the effects on the nutritional quality of flocs.

The protein content of bioflocs is of prime importance because protein is a major nutritional requirement for energy and development of most aquatic animals, and it should be in the range of 20–50% (Tacon 1987). A recent report found that heterotrophically produced bioflocs recorded a protein content of 46.7% compared with autotrophically produced flocs which can meet the protein requirement of most aquaculture organisms (Martinez-Porchas *et al.* 2020). Thus, bioflocs developed using various carbon sources should meet the protein needs of the culture organism. For example, molasses application produces flocs of relatively high protein quality: 30.4% (Emerenciano *et al.* 2012) and 31–31.2% (Tacon *et al.* 2002; Wasielesky *et al.* 2006). Additionally, Luo *et al.* (2017) reported the effectiveness of PHB, PCL and glucose in producing flocs with high crude protein (34 ± 1, 31 ± 4 and 39 ± 1%, respectively) which meet the protein requirement of tilapia. In this study, glucose treatment produced flocs that had significantly greater protein contents compared with those under PCL treatment, but the contents were similar to the content under the PHB treatment.

In terms of the lipid content, Wasielesky *et al.* (2006) reported 0.47% when molasses was used as the carbon source, which is similar to the level when molasses, sugar or jaggery (0.5%) were used (Sakkaravar & Sankar 2015). However, Fugimura *et al.* (2015) reported even greater levels of crude lipid (2.39%) for molasses treatment. Additionally, utilizing PCL and PHB as carbon sources produced flocs with high crude lipid levels (>5%; Luo *et al.* 2017). Specifically, jaggery is reported to favour the activities of fungi, yeast and heterotrophic bacterial growth

Table 3 Organic carbon sources and the associated dominant bacteria community in BFT aquaculture systems described by some studies

Carbon source and C/N	Species (salinity)	Abundant bacterial groups at the phylum or/and genus level	Notes	Reference
Amaranth and wheat grains 12:1 (input)	*Litopenaeus vannamei* (36)	*Proteobacteria*, *Planctomycetes*, *Bacteroidetes* *Marinobacter*, *Myroides*, *Cellulomonas*, *Clostridium*, *Pelagibaca*, *Planctomycetes*, *Arcobacter*, *Flavobacterium*, *Candidatus*, *Protochlamydia*, *Opitutus*, *Hyphomonas*, *Vibrio*, *Ketogulonicigenium*, *Tenacibaculum Cyclobacterium*, *Isospharea* and *Microbacterium Peptostreptococcus*	Type of substrate affected the initial biofouling process, but favoured the same heterotrophic communities	Martínez-Córdova *et al*. (2018)
Molasses 1 kg of molasses m^{-3}	*L. vannamei* (35.3–36.8)	*Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Actinobacteria*, *Firmicutes*, *Chlamydiae*, *Cyanobacteria*, *Candidatus Sacc*, *Rhodobacter*, *Ketogulonicigenium Ruegeria*, *Sulfurimonas*, *Croceibacter*	Autochthonous bacteria had the greatest influence on the diversity	Huerta-Rábago *et al*. (2019)
Sucrose (S), Cassava dregs (CD) and enzyme-hydrolysed cassava dregs (EH) 20:1 (input)	*L. vannamei*	*Proteobacteria*, *Fusobacteria*, *Bacteroidetes*, *Firmicutes*, *Saccharibacteria*, *Chlorobi*, *Gemmatimonadetes*, *Planctomycetes*, *Actinobacteria*, *Verrucomicrobia* *Piscirickettsiaceae*, *GR-WP33-58*, *Halomonadaceae*, *Flavobacteriaceae*, *Rhodobacteraceae*, *Vibrionaceae*, *Shewanellaceae*, *Nannocystaceae*, *Saprospiraceae*, *1G93*	No significant difference was found between S and CD. EH differed S and CD significantly	Shang *et al*. (2018)
Wheat and amaranth 12:1 (input)	*L. vannamei* (35)	*Planctomycetes*, *Proteobacteria*, *Bacteroidetes*, *Chlamydiae*, *Verrucomicrobia*, *Fusobacteria* *Bacillariophyta*, *Fibrobacteres*, *Tenericutes*, *Streptophyta*, *Euglenida*, *Aquifiace*, *Phaeophyceae*, *Chloroflexi*, *Synergistetes*, *Spirochaetes*, *Chlorophyta*, *Chlorobi*, *Cyanobacteria*, *Thermotogae*, *Actinobacteria*, *Firmicutes*	Micro-environmental conditions of the culture units shaped the microbiota of biofloc regardless the type of seed used	Vargas-Albores *et al*. (2019)
Longan powder (LP); polyhydroxybutyrate-hydroxyvalerate and poly (butylene succinate)/C/N >15 (in water)	*Oreochromis niloticus* (freshwater)	*Firmicutes*, *Proteobacteria*, *Bacteroidetes*, *Actinobacteria*, *Verrucomicrobia*, *Chlorobi*, *Cyanobacteria*, *Deferribacteres*, *Euryarchaeota*, *Chloroflexi*, *Fusobacteria*, *Planctomycetes Bacillus*	Solid carbon source had a significant effect on the microbial community	Li *et al*. (2018)
Glucose and polycaprolactone (PCL) C/N of 20:1 (in water)	*Clarias gariepinus* (Freshwater)	*Proteobacteria*, *Bacteriodetes*, *Firmicutes*, *Cyanobacteria*, *Nitrospirae*, *Parcubacteria*, *Chloroflexi*, *Armatinonadetes*, *Planctomycetes*	The diversity of bacteria were similar among groups, however, the relative abundance of some bacterial phyla showed significant differences among the treatments considered	Luo *et al*. (2020)
Addition of Molasses and its combination with algicidal bacteria (CZBCI)	*L. vannamei* (post larvae, PL12) (20)	*Cyanobacteria*, *Proteobacteria*, *Bacteroidetes*, *Planctomycetes*, *Verrucomicrobia* and *Actinobacteria*	The addition of molasses had some effect on the bacterial richness and abundance. Combination of molasses and algicidal bacteria (CZBCI) controlled the abundance of cyanobacteria in the culture system. The abundance of total bacteria and culturable heterotrophic bacteria increased gradually in all groups	Xu *et al*. (2019)

Table 3 (continued)

Carbon source and C/N	Species (salinity)	Abundant bacterial groups at the phylum or/and genus level	Notes	Reference
Molasses C/N:5/1.10/1.15/1. 20/1 (input)	*L. vannamei* (32)	*Vibrio*, *Halomonas*, *Pseudoalteromonas*, *Alphaproteobacteria*, *Rahnella*, *Gammaproteobacteria*, *Syntrophus*, *Xanthomonadaceae*, *Thauera*, *Peridiniaceae*, *Achromobacter*, *Alcaligenes*, *Microbacterium*, *Attheyaceae*, *Desulfomicrobium*, *Caldilinea*, *Psychrobacter*, *Proteobacteria*	C/N ratio manipulation significantly influenced the bacteria groups and abundance in the BFT system. The bacterial diversity was more spread in C/N = 20 group, with the major bacteria communities being representing 26%, 25% and 20% for *Psychrobacter*, *Proteobacteria* and *Peridiniaceae*, respectively	Panigrahi *et al*. (2018)
Glucose, starch and glycerol C/N ratio of 15 for culture water	*L. vannamei* (5)	Most of the shared OTUs representing 86.1% were composed of *Proteobacteria*, *Bacteroidetes* and *Planctomycetes*, in a proportion of 54.3%、18.4% and 13.4%, respectively	The bacterial community was significantly influenced and shaped by the carbon source used	Wei *et al*. (2020)
Glucose and no glucose addition (NCA) CN >15 for glucose treatment (water)	*O. niloticus* (fresh water)	*Fusobacteria*, *Proteobacteria*, *Bacteroidetes* and *Planctomycetes*	Bacteria communities were similar in both glucose treatment and NCA treatment	Liu *et al*. (2018d)
Glucose, poly-β-hydroxybutyric, polycaprolactone >15 (in water)	*O. niloticus* (freshwater)	*Proteobacteria*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, *Planctomycete*, *Firmicutes*, *Fusobacteria*, *Tenericutes*, *Chlamydia*e	Bacterial community was similar between the three groups	Luo *et al*. (2017)

The analytical method for the bacteria community of all reports presented here was based on high-throughput 16S-rRNA gene sequencing. Bacteria group in italics mean abundance at Genus level.

(Sakkaravar & Sankar 2015). Therefore, the nutritional composition of bioflocs is linked to the composition of the microbiota present in the flocs.

Recently, it was reported that longan powder, polyhydroxybutyrate-hydroxyvalerate (PHBVL) and polybutylene succinate (PBS) produced high-quality bioflocs with an essential amino acid index (EAAI) of 0.969 ± 0.011, 1.007 ± 0.014 and 0.995 ± 0.012, respectively, which meet the nutritional requirements of tilapia (Li *et al*. 2018). These findings, along with others (Table 1), indicate that some carbon sources may be preferred under certain BFT systems or conditions with respect to the culture species. This is because different aquaculture organisms have different nutritional requirements (National Research Council 2011). Therefore, more investigations regarding the influence of different carbon sources on the nutritional quality of bioflocs are needed to standardize the use and choice of carbon material in BFT systems. Additionally, information on the influence of carbon source type on the nutritional quality and biofloc characteristics is presented in Table 1.

Addition strategies for carbonaceous substrates

For a given BFT aquaculture system, the addition strategy of the carbonaceous substrate influences the C/N ratio, which allows heterotrophic bacteria to assimilate NH_4^+–N.

The strategies to administer extra carbon material to BFT systems are a critical aspect that should be considered during the start-up and the steady-state phases of BFT systems. However, there is little understanding on the best strategies for carbon addition, as few studies have explored this aspect of BFTs. In biofloc systems, carbon materials or substrates for controlling C/N ratios are either added as external substances or included in the feed through adjustment of the C/N ratio of the feed (Avnimelech 1999, 2009; Crab *et al*. 2012; Hargreaves 2013; Bakar *et al*. 2015). However, of these two methods of carbon supplementation, external addition is the most widely applied (Azim & Little 2008; De Schryver *et al*. 2008; Ekasari *et al*. 2010; Kundu *et al*. 2013; Li *et al*. 2018). Several addition strategies have been reported (Table 1).

The most common organic carbon addition strategy is based on calculating the amount of the organic carbon to supplement according to the nitrogen content in the feed. Ebeling *et al*. (2006) proposed that the external organic carbon is required only when TAN concentrations exceed a threshold concentration (e.g. 2 mg L^{-1}). The efficiency of BDPs as the sole carbon source and their ability to slowly release DOC characterize the third carbon addition strategy for BFT aquaculture systems.

Addition amount of organic carbon according to the feed N content

Determining the amount of external organic carbon source to add on the basis of the feed N is the most common strategy. This strategy was proposed by Avnimelech (1999). The strategy is described as follows: The theoretical C/N ratio in the bacteria biomass is 4 ($C/N_{bacteria} = 4$), the assimilation rate of C for bacteria is 40% ($ARC_{bacteria} = 40\%$), the C content of the added carbohydrate is 50% (% $C_{CH} = 50\%$), and this implies that 20 g carbohydrate containing 10 g C is required to reduce 1 g N. In a zero-exchange aquaculture system, 50% of the feed N consumed is retained in the water in the form of NH_4 ($\%N_{waste} = 50\%$). Therefore, for a feed containing 30% crude protein ($\%N_{feed} = 4.65\%$ N), the amount of the external carbonaceous substrate (A_{CH}) containing 50% C is 46.5% of the fish feed (A_{feed}; Eqn 1). In this instance, the final input C/N (C in feed and carbohydrate, N in feed) is given as 15.75.

$$\frac{A_{CH}}{A_{feed}} = \frac{\%N_{feed} \times \%N_{waste} \times (C/N)_{bacteria}}{\%C_{CH} \times ACR_{bacteria}} = \frac{4.65\% \times 50\% \times 4}{50\% \times 40\%} = 46.5\% \quad (1)$$

The amount of the external carbohydrate depends on the N content of the feed, C content of the carbohydrate, and the waste rate or discharge rate of the feed N.

The premise of this strategy is that C and N should be presented in the form available for the bacteria to assimilate TAN. However, in practice, both C and N exist in many forms. Initially, most waste N is trapped in faeces, and unused feed takes some time to be converted to TAN. When water-soluble carbon sources are used, C will be quickly decomposed to the form available for bacteria to utilize. Therefore, even if the initial ratio of total C to total N may be sufficient for bacteria to assimilate TAN, C will be present in excess due to the lag time of the mineralization of organic solid N to dissolved inorganic N. When the waste N is decomposed to TAN, all C may be decomposed to CO_2. Therefore, it is nearly impossible to have the proper C/N ratio for heterotrophic bacteria even when the calculation is done correctly. Additionally, this strategy focuses on the C/N ratio of the input material, not the C/N ratio of the aquatic systems in which the bacteria live. As previously discussed, almost all faeces and unused feed are retained in the fish tank. Even if all TAN is assimilated into bacteria biomass, the bacteria will senesce and decay to form TAN again. Therefore, along with additional N from the feed at each feeding time, TAN is replenished by dead bacteria or other microbes. It is therefore difficult to ensure that the C/N ratio in water is appropriate for heterotrophic bacteria to assimilate TAN. This challenge may be one of the reasons that nitrification occurs in BFT aquaculture systems (Luo *et al.* 2020; Robles-Porchas *et al.* 2020).

If the carbohydrate is added after each feeding or most feeding times, there will be a great amount of carbon required. This raises the cost of production not only because of the carbon source but also because of the cost of the addition activity. Therefore, this strategy is commonly used to produce bioflocs only in the initiation of BFT aquaculture systems.

Addition amount of the organic carbon according to TAN level in the water

To avoid wasting organic carbon, some studies supplied the organic carbon according to the TAN level over time in the water. On the basis of the stoichiometry of heterotrophic removal of TAN in aquaculture systems, Ebeling *et al.* (2006) reported that 6.07 g of organic carbon would be required to heterotrophically convert 1 g of NH_4^+–N. Organic carbon in the studies of Furtado *et al.* (2011), Zhang *et al.* (2017) and Liu *et al.* (2019) was added based on 6 g C for 1 g TAN when the TAN level was above the threshold level (1 or 2 mg L^{-1}). The method described by Schveitzer *et al.* (2013) states that when TAN is above 1 mg L^{-1}, molasses should be added according to the procedure of Avnimelech (1999), in which 20 g carbon source is required to convert 1 g TAN.

The premise of this strategy is the continuous determination of TAN levels. If determination of TAN is conducted intermittently, there is risk that TAN may accumulate to levels detrimental to fish and may occur in the interval between two test periods of TAN. Therefore, organic carbon will be in short supply for the heterotrophic bacteria because no organic carbon was added during the interval.

BDPs as a slow-release carbon source

The real-time monitoring of the C/N ratio in BFT aquaculture systems has not yet been described. The amount of soluble carbohydrate is calculated based on the feed N or TAN level in the water, both of which vary widely for a commercial-scale BFT aquaculture system. These considerations complicate control of C/N ratio for a BFT aquaculture system. The objective of using BDPs to maintain the C/N ratio in BFT aquaculture systems centres on omitting frequent calculations of the amount of soluble organic carbon and continuous determination of TAN levels. When BDPs are used, they can be put into nylon bags and hung inside fish tanks at the beginning of production; no other additions are required during the entire production period (Luo *et al.* 2017). Related studies demonstrated the potential of PHB or PCL as a sole external carbon source to maintain water quality of BFT aquaculture systems stocking white shrimp (*L. vannamei*), tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*; Zhang *et al.* 2016b; Luo *et al.* 2019a; Chen *et al.* 2020).

Some combined strategies of supplemental carbon sources

Owing to the associated advantages and disadvantages of the major classification of carbon sources, some recent studies have described the best carbon addition strategies for BFT to achieve more desired results (Deng *et al.* 2018; Li *et al.* 2018; Luo *et al.* 2019a, 2020). Until recently, external carbon materials in BFT including soluble (e.g. molasses, glycerol, glucose) and insoluble (e.g. biodegradable polymers and plant cellulose) carbon sources had mostly been applied individually to BFT systems for C/N ratio manipulation. However, some researchers have begun to explore other means of administering different categories of carbon materials (soluble and insoluble) in an effort to offset the challenges associated with their sole application (Li *et al.* 2018; Luo *et al.* 2019a).

As described in the previous sections, for a commercial-scale BFT aquaculture system, it is difficult to maintain the appropriate C/N ratio for heterotrophic bacteria to assimilate TAN at all times. An increasing number of studies have adopted different external carbonate addition strategies for different phases of a BFT aquaculture system (Ferreira *et al.* 2020). For example, to initiate a BFT system, sufficient organic carbon is supplied to stimulate heterotrophic bacteria to grow dominantly and form bioflocs (Luo *et al.* 2019a). The addition of organic carbon source is then reduced or even paused to establish the nitrification process for the remainder of the time.

Luo *et al.* (2019a) investigated the performance of three carbohydrate addition strategies to establish the nitrification process for new BFT aquaculture systems. One-time glucose addition (C/N = 20) established the best nitrification process. Supplying glucose when TAN levels exceed 2 mg L^{-1} extended the initiation of the nitrification process. PHB as the sole organic carbon source resulted in good establishment of nitrification plus NH_4^+–N assimilation. Ferreira *et al.* (2020) proposed that for a mature BFT aquaculture system, an organic carbon source is required only when TAN levels exceed the tolerable concentration of the culture species.

For *L. vannamei* biofloc nursery BFT systems, the three strategies of supplemental organic carbon include daily addition of glucose at a rate of 75% of the feed, hanging PHB in the fish tank directly plus adding glucose at a proportion of 6 g C to 1 g NH_4^+–N when NH_4^+–N exceeds 2 mg L^{-1}, and adding PHB as the sole organic carbon source. The second strategy (PHB plus glucose) yielded the best results (Luo *et al.* 2019a).

Additionally, a combination of tapioca starch and plant cellulose as the carbon source resulted in the development of bioflocs with more diverse microbial communities and richness (Deng *et al.* 2018). Li *et al.* (2018) attempted to add carbon with an ex situ peristaltic pump instead of the conventional in situ BFT systems. This strategy maintained the C/N ratio slightly above 20 for the proper system functioning and resulted in maintaining water quality (TAN, nitrite and nitrates) at safe levels for tilapia culture. It is important to note that the carbon addition strategy employing ex situ BFT systems aided by the peristaltic pump resulted in some removal of NO_3^- which is known to accumulate in BFT systems (Li *et al.* 2018). However, Luo *et al.* (2020) found that these strategies of carbon addition could not maintain the appropriate C/N ratio required by the heterotrophic bacterial groups. This finding implies that in-depth investigations are still required to determine the best carbon addition strategy for BFT systems. Thus, the carbon addition strategy as well as the choice of carbon may influence different features of the biofloc system, such as bacterial communities, water quality and species growth and performance (Liu *et al.* 2018d; Panigrahi *et al.* 2019).

Overall, carbon addition strategies in BFT are not well described, and little information is available. Therefore, future investigations should focus on illuminating this subject to determine the best carbon addition strategy for BFT systems.

Primary cost analysis of the different carbon sources

Many factors may influence the selection of carbonaceous substrates for BFT systems including accessibility, cost, biodegradability and bacterial utilization of the material (Fugimura *et al.* 2015; Sakkaravar & Sanker 2015). Among these factors, the cost of the carbon source can significantly affect the decision of the farmer about which carbon addition strategy to adopt. Relatively expensive carbon materials will eventually affect the overall cost of production of the aquaculture products. On the other hand, affordable carbon substrates may also influence the carbon addition strategies of the farmer among other related issues. The cost factor may also affect carbon consumption aspects of any BFT operation (see Table 4). Therefore, the cost-effectiveness of these materials must be evaluated for each category of carbon sources currently applied in BFT systems. However, studies describing the cost of organic carbon sources are scarce in the current stock of literature (Fugimura *et al.* 2015; Zhang *et al.* 2016). Both the carbon source and the addition strategies determine the total cost of organic carbon sources used for a given BFT aquaculture system.

Usually, simple water-soluble carbohydrates (e.g. glucose and sodium acetate) are more expensive than complex water-insoluble materials (e.g. plant-based materials). For commercial-scale BFT aquaculture systems, using a carbon source with low economic value, such as industrial by-products (e.g. brewery residues) and plant-based material, is preferred because these sources may significantly reduce the operational costs (De Schryver *et al.* 2008; Fugimura *et al.* 2015). Luo *et al.* (2014) determined that 0.9 kg sodium acetate was consumed per kg of fish harvested for a lab-scale BFT tilapia in which sodium acetate was supplied at a rate of 75% of the feed. Zhang *et al.* (2016b) found that the average cost of PHB-based BFT tilapia systems was only one-tenth that of glucose-based systems. The operating cost is also related to the types of carbon sources. As described above, using water-soluble carbon requires constant calculations based on the feed N and monitoring the TAN level in the aquaculture system. This approach could thus result in increased labour costs. On the other hand, applying slow-release carbon sources such as BDPs may offset the operational costs associated with frequent carbon addition or supply strategies (Zhang *et al.* 2016b).

In addition to the carbon source, the addition strategies influence the cost of carbon sources to a considerable extent because different addition strategies results in different amounts of organic carbon source being consumed. Additionally, excess carbon source not only results in wastage but also increases dissolved oxygen consumption. For example, compared with one-time additions and combinations of various sources, multiple daily additions of external carbon materials means that more carbon will be consumed, and therefore, more cost will be incurred. Specifically, Fugimura *et al.* (2015) estimated the cost of consumption of three carbon sources including brewery residuals, cassava flour and sugar cane molasses, to be 0.18, 1.76 and 2.81 USD kg^{-1}, respectively. This finding suggests implies that molasses may be more expensive than the other two substrates in BFT application in the long run because of its greater solubility and faster consumption (Fugimura *et al.* 2015).

In light of these concerns, a number of carbon materials, especially those obtained from food waste, agriculture waste and by-products, are inexpensive to acquire and may be easily prepared by the farmer without incurring further cost. Notable examples are maize flour, rice flour, molasses, wheat flour, millet flour, maida flour, gram flour (Pangrahi *et al.* 2019), tapioca starch, plant cellulose (Deng *et al.* 2018) and other agriculture-derived materials. Compared with molasses and sugar, jaggery is known to produce flocs of greater protein content and is more inexpensive (Sakkaravar & Sanker 2015). Additionally, residues and cassava flour from brewery industries are another relatively inexpensive alternative source of carbonaceous substrates for application in BFT systems (Fugimura *et al.* 2015). Other natural materials, such as chopped straw and other cellulose-based carbonaceous substrates (Boley *et al.* 2000; Serfling 2006), can be acquired relatively inexpensively or without incurring any cost. However, soluble carbonaceous substrates, such as glucose, sucrose, sugar, acetate, glycerol, molasses and longan powder, and insoluble substrates, such as polybetahydroxybutyrate (PHB), polycaprolactone (PCL) and polybutylene succinate (PBS), are often made commercially and are available for purchase, thus introducing an element of cost when these sources are employed in any BFT system. Comparatively, Chu and Wang (2011) observed that PCL was much more inexpensive than PHB. Additionally, in terms of cost, PHB and PBS cost approximately 3.23 and 2.69 USD kg^{-1}, respectively, compared with that of longan powder (a soluble carbon source), which costs 0.46 USD kg^{-1} (Li *et al.* 2018).

Although insoluble carbon sources are expensive on a per-kilogram basis, on a long-term basis, they may be more affordable since they may be used multiple times because of their slow degradation compared with that of soluble carbon sources which are used up immediately when applied to the system. Additionally, on an experimental scale and in terms of a cost evaluation of PHB and glucose consumption, Zhang *et al.* (2016b) reported that the cost of application and consumption of PHB (0.21 ± 0.02 USD kg^{-1}) are significantly lower than those of glucose (1.69 ± 0.06 USD kg^{-1}) during the experimental period in the BFT system. The cost of consumption of PHB in this study was found to be 1/10 that of glucose, thus making PHB a relatively inexpensive alternative in the long run. Importantly, the cost implications of carbon substrates for BFT still require intensive evaluation in future studies.

The use of carbon materials of low economic value, such as by-products of food industries, which can still produce good-quality bioflocs, may be preferable due the potential reductions in production cost (De Schryver *et al.* 2008). Therefore, determining the appropriate strategies for organic carbon source supplementation is important for a successful BFT system. Cost analysis of five carbon sources and their corresponding cost of consumption are presented in Table 4.

Future research dimensions

To date, most BFT studies related to organic carbon sources focused more on the potential of controlling TAN or on the positive influence on the cultured fish. To fully use the advantages of BFT aquaculture systems, the following potential research should be carried out in the future (see the subsequent sections).

Table 4 Carbon source consumption and cost of addition described for 1 kg feed containing 30% protein (4.65% N)

Carbon source type	Theoretical carbon content	1 kg of feed required to add carbon source	Price of carbon source (USD)	Price of adding carbon source for 1 kg feed/ (USD kg^{-1})
Glycerine	39.1	0.45 L	2.049/500 mL	1.844
Glucose	40.0	0.44 kg	2.191/500 g	1.927
Sucrose	42.1	0.41 kg	3.181/500 g	2.607
Wheat/ corn starch	44.4	0.39 kg	324.95–536.87/ tonne	0.127–0.209

USD, United States Dollars.
Adapted from data from a previous study and supported by data from Food Business Network and China National Test Agent.

The mechanism of carbon source type on the bacteria community

The objective of adding organic carbon is to increase the C/N ratio to stimulate dominant growth of heterotrophic bacteria (Avnimelech 1999). Most studies have demonstrated that the type of organic carbon source influences the microbial community (see section 3.1 and Table 3). However, it is not understood whether this mechanism occurs through maintenance of the C/N ratio or intermediate products from organic substrate decomposition. Is there some relationship between the specific beneficial bacteria and types of organic carbon source used?

Improving carbon addition strategies for specific BFT aquaculture systems

Given the abundant research on carbon sources, there have been relatively few studies focusing on addition strategies for a specific BFT aquaculture system. The addition strategy may determine the performance organic carbon in BFT systems. For example, continuous feeding induces an increase in biofloc level (Van Wyk 2006). Biofloc levels are mainly related to the C/N ratio in the system and the number of attached bacterial cells. If nitrification needs to be established, the C/N and biofloc levels should be balanced. If the biofloc level is low and C/N in the water is high, nitrification will be inhibited to some extent (Zhu & Chen 2011). The addition strategy for this situation focuses on both the input C/N and the C/N in the water.

Prospects of synthetic carbon sources

As described in the analysis in section 4, each type of organic source has pros and cons. Wang *et al.* (2016) found that 60% molasses, 20% corn flour and 20% wheat bran are a better carbon source addition for *L. vannamei* than using molasses, corn flour or wheat bran individually. The results of Li *et al.* (2018) showed that blending longan powder with PHBV or PCL results in better performance of BFT systems than when longan powder is the only carbon source. A synthetic carbon source that combines the advantages of its components is predicted to perform well.

Conclusion

It is now evident that the success of BFT aquaculture systems depends on an adequate supply of supplemental carbonaceous substrates to stimulate the activity of the microbial communities that convert toxic nitrogenous compounds into microbial biomass. These carbon sources may be categorized into two broad categories – soluble carbon sources and insoluble carbon sources – with further specified subcategories. Different carbon sources affect BFT systems differently, and this review discusses the effects on microbial communities, culture organism, water quality and nutritional quality of bioflocs. There are more intrinsic benefits associated with carbon sources currently employed in BFT systems that are yet to be explored, which presents an avenue for potential research in the near future. The review also discussed some current carbon addition strategies employed in BFT systems, and related future research areas are suggested.

A cursory look at the literature reveals that research on insoluble carbon sources in BFT systems has recently emerged, but there remains little information on this form of carbon sources. Although some notable studies have been carried out, the results presented so far are interesting, and further studies are thereby warranted. Future research should also focus on exploring other carbon sources that may yield better results than the traditional ones currently in use. These studies will be helpful to standardizing the application of carbon sources in BFT systems to achieve the desired results and generate a more pragmatic aquaculture technology.

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Conflict of interest

The authors declare that they have no conflict of interest.

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