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INFLUENCE OF CARBON SOURCE COMPLEXITY ON PROKARYOTIC COMMUNITY COMPOSITION IN BIOFLOC SYSTEMS: A REVIEW

Pengaruh Kompleksitas Sumber Karbon pada Komposisi Komunitas Prokariotik Sistem Bioflok: Sebuah Review

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ABSTRACT

Biofloc technology (BFT) relies on the management of microbial communities to improve water quality and recycle nutrients in aquaculture systems. This review summarizes findings from 16 studies that analyzed prokaryotic (bacterial) communities in biofloc systems using different carbon sources, categorized by structural complexity: (i) simple (glucose, molasses), (ii) mid-complex (starch), and (iii) complex (digested plant cellulose, wheat bran). Dominant phyla commonly found across all carbon categories include *Proteobacteria*, *Bacteroidota/Bacteroidetes*, *Actinobacteriota*, *Cyanobacteria*, *Firmicutes*, and *Planctomycota/Planctomycetes*. Unique phyla associated with simple carbon were *Chlamydiae*, *Acidobacteriota*, *Dadabacteria*, *Patescibacteria*, and *Xanthomonadaceae*, while *Fusobacteria* dan *Gemmatimonadetes* appeared only in systems using mid-complex and complex carbon sources. *Chloroflexi* appeared in all carbon categories but was more frequently mentioned in studies when systems using mid-complex and complex carbon sources. These results suggest differences in substrate utilization, respiratory pathways, and bacterial groups involved in degrading carbon of varying complexity. However, quantitative comparisons of bacterial communities could not be made due to variations in C:N ratios, carbon dosages, and cultured species among studies. A more standardized approach is needed to better understand how carbon complexity shapes the structure and function of microbial communities in biofloc systems.

Keywords: Bacteria, Glucose, Molasses, Starch, Aquaculture

ABSTRAK

Teknologi bioflok (BFT) bergantung pada pengelolaan komunitas mikroba untuk meningkatkan kualitas air dan daur ulang nutrien dalam sistem akuakultur. Artikel review ini merangkum hasil dari 16 penelitian yang menganalisis komunitas prokariotik (bakteri) pada sistem bioflok dengan berbagai jenis sumber karbon, dikategorikan berdasarkan kompleksitas strukturnya: (i) sederhana (glukosa dan molase), (ii) menengah (pati), dan (iii) kompleks/tinggi (selulosa tanaman tercerna dan dedak gandum). Filum dominan yang umum ditemukan di semua kategori karbon meliputi *Proteobacteria*, *Bacteroidota/Bacteroidetes*, *Actinobacteriota*, *Cyanobacteria*, *Firmicutes*, dan *Planctomycota/Planctomycetes*. Filum unik penyusun komposisi bakteri pada gula sederhana yaitu *Chlamydiae*, *Acidobacteriota*, *Dadabacteria*, *Patescibacteria*, dan *Xanthomonadaceae*. Sementara itu, filum *Fusobacteria* dan *Gemmatimonadetes* ditemukan hanya pada sistem dengan karbon kompleksitas menengah dan tinggi (kompleks). Chloroflexi ditemukan pada semua kategori karbon namun lebih sering pada karbon kompleksitas menengah dan tinggi. Hasil ini mengimplikasikan adanya dugaan perbedaan substrat, jalur respirasi, dan jenis bakteri dalam mengurai karbon dengan kompleksitas struktur berbeda. Studi ini tidak bisa menyimpulkan perbandingan kuantitatif komunitas bakteri akibat adanya perbedaan rasio C:N, dosis karbon, serta spesies budidaya antarpenelitian. Diperlukan pendekatan yang lebih terstandar untuk memahami secara mendalam bagaimana kompleksitas karbon memengaruhi struktur dan fungsi komunitas mikroba dalam sistem bioflok.

Kata kunci: Bakteri, Glukosa, Molase, Pati, Aquaculture

INTRODUCTION

Biofloc technology (BFT) has been well-known as an ecological intensification-friendly for fish culture (Avnimelech, 2015; Zaki *et al.*, 2020). Biofloc technology enables the maintenance of water quality, enhances animal growth and production, and improves nutrient utilization efficiency (Crab *et al.*, 2007; Hargreaves, 2013; Kumar *et al.*, 2018; Panigrahi *et al.*, 2019). BFT systems exhibit a higher carrying capacity than conventional aquaculture systems, allowing producers to increase stocking density without compromising water quality, system stability, or animal welfare (Widanarni *et al.*, 2012; Zaki *et al.*, 2020; Oliveira *et al.*, 2022; Tinh *et al.*, 2023).

Biofloc helps the systems maintaining water quality such as by degrading both organic and inorganic N-species that excreted in form of faeces and urine, respectively. Beyond regulating total ammonia nitrogen (TAN) and nitrite (NO_2–N) concentrations (Crab *et al.*, 2007; Ray *et al.*, 2011; Avnimelech, 2015), biofloc also serves as an in situ natural feed source produced from recycled waste nutrients (Cardona *et al.*, 2015). This process can reduce the feed conversion ratio (FCR) by up to 30% (Hargreaves, 2006; Wasielesky *et al.*, 2006). The protein-rich biofloc biomass contributes substantially to the nutritional requirements of cultured species, allowing for reduced protein levels in formulated feeds (Xu and Pan, 2013b; Jatobá *et al.*, 2014). In shrimp BFT systems, biofloc can supply up to 37% of nitrogen and approximately 60% of carbon retained in shrimp biomass (Avnimelech and Kochba, 2009; Cardona *et al.*, 2015; Emerenciano *et al.*, 2017; Ray *et al.*, 2017). Additional advantages of biofloc include the presence of beneficial bacteria that suppress pathogens through competitive exclusion (Emerenciano *et al.*, 2013; de Souza Valente *et al.*, 2020), antioxidant compounds that may enhance immune response (Liu and Fang, 2003; De Schryver and Verstraete, 2009), and extracellular digestive enzymes that assist nutrient assimilation in the host gut (Xu and Pan, 2013; Wang *et al.*, 2016; Chakrapani *et al.*, 2021).

Biofloc systems can be operated in freshwater, brackish, or marine environments, supporting a wide range of species such as the herbivorous piracanjuba (*Brycon orbignyanus*) (Sgnaulin *et al.*, 2018), omnivorous species like crucian carp (*Carassius auratus*) and common carp (*Cyprinus carpio*) (Wang *et al.*, 2015; Ebrahimi *et al.*, 2020), Nile tilapia (*Oreochromis niloticus*) (Khanjani, 2021), and carnivorous crustaceans such as black tiger shrimp (*Penaeus monodon*) (Kumar *et al.*, 2017) and Pacific white shrimp (*Litopenaeus vannamei*) (Tinh *et al.*, 2023).

Biofloc itself is a heterogeneous aggregation of microorganisms—including bacteria, archaea, algae, protozoa, zooplankton, and fungi—embedded within a matrix of extracellular polymeric substances (EPS) composed primarily of polysaccharides (Hargreaves, 2006; De Schryver *et al.*, 2008; Wilen *et al.*, 2008). Among these, heterotrophic bacteria play a pivotal role in nitrogen assimilation and water quality control by converting dissolved organic and inorganic nitrogen compounds into microbial biomass, using organic carbon as an energy source (Hargreaves, 2013; Martins *et al.*, 2020).

To support bacterial heterotrophic assimilation, a C:N ratio of 15–20 in the total nutrient input (feed plus carbohydrate) is generally recommended (Avnimelech, 1999; De Schryver *et al.*, 2008; Xu and Pan, 2012). Various carbon sources—including molasses, tapioca starch, and corn starch—are commonly used to maintain the desired C:N ratio and promote heterotrophic bacterial activity (Ekasari *et al.*, 2014b; Panigrahi *et al.*, 2019; Khanjani *et al.*, 2021).

Over the past decade, carbon supplementation strategies in biofloc systems have shifted from the use of easily degradable carbon sources toward more slowly degradable ones. This transition aims to minimize sudden fluctuations in water quality and to promote more stable and diverse microbial activity. Slowly degradable carbon sources are hypothesized to support a richer and more complex microbial community within the biofloc matrix; however, this premise has not yet been conclusively proven. Several studies have explored how different carbon sources influence the microbial composition of biofloc systems. Building upon these findings, this review aims to summarize and compare the effects of various carbon types—differing in structural complexity—on the composition and diversity of key microbial groups, including phytoplankton, zooplankton, bacteria (primarily), and fungi.

METHODS

This review adopts a structured and systematic approach to synthesize current knowledge on the microbial composition of biofloc systems in aquaculture. In this context, *microbial communities* refer to, but are not limited to, phytoplankton, zooplankton, bacteria (primarily), and fungi. The reviewed studies encompass biofloc environments associated with various cultured species, including *Litopenaeus vannamei*, *Oreochromis niloticus*, and *Clarias gariepinus*, covering both laboratory-scale and commercial-scale systems.

The review process was designed to ensure comprehensive coverage, transparency, and reproducibility of literature selection and analysis. Relevant publications were retrieved from Science Direct (Scopus). The search covered articles published between 2015 and 2025, corresponding to the period of rapid development and application of biofloc technology (BFT) in aquaculture. The following combinations of keywords were used:

- “Biofloc” OR “BFT”, and
- “Carbon” or “C:N ratio”
- “Prokaryotic community” OR “microbial community” OR “microbiome” OR “bacterial composition” OR “metagenomics”.

In addition to peer-reviewed articles, grey literature—such as conference proceedings, theses, and institutional reports—was also screened when relevant to the microbial composition of biofloc systems. To ensure quality and relevance, only peer-reviewed studies written in English or Bahasa Indonesia were included.

Studies were considered eligible if they met the following criteria:

1. Reported microbial composition or community diversity in biofloc systems
2. Employed external carbon-dependent system management
3. Utilized 16S rRNA sequencing or metagenomic approaches and presented phylum-level compositions
4. Provided clear descriptions of experimental conditions or system configurations

5. After data collection, studies using carbon sources uncommon in field-scale biofloc management (e.g., glycerol and PHBV) were excluded. Consequently, we classified the remaining carbon sources into four categories based on structural complexity: (i) simple sugars (glucose and sucrose), (ii) molasses, (iii) starch, and (iv) digested plant cellulose and wheat bran
6. The top five phyla reported in each study were listed and counted for comparison across studies

A qualitative synthesis approach was applied to integrate findings across studies. Where available, quantitative data, such as the relative abundance of dominant phyla, were summarized descriptively. Differences in microbial community structures across systems with varying carbon source complexities were analyzed to identify key determinants of microbial assembly in biofloc environments. Finally, the review highlights research gaps, methodological limitations, and emerging directions for future studies, particularly regarding functionality of the prokaryotic community in biofloc systems.

RESULTS

Table 1 summarizes the prokaryotic community compositions in biofloc water samples from 16 studies, classified according to the structural complexity of the carbon sources, arranged from the simplest to the most complex:

a) Glucose – simple monosaccharide – (5 studies)
b) Molasses – mixture of simple sugars, slightly more complex – (6 studies)
c) Starch – polysaccharide composed of glucose chains – (3 studies)
d) Digested plant cellulose – partially broken-down cellulose; complex polysaccharide fragments – (1 study)
e) Wheat bran – highly complex matrix of cellulose, hemicellulose, lignin, proteins, and other compounds – (1 study)

Across all carbon types, several bacterial phyla were commonly observed (in no particular order): Proteobacteria, *Bacteroidota/Bacteroidetes*, *Actinobacteriota*, *Cyanobacteria*, *Firmicutes*, *Planctomycota/Planctomycetes*, *Verrucomicrobia*, *Chlamydiae*, *Acidobacteriota*, *Dadabacteria*, *Patescibacteria*, *Chloroflexi*, *Fusobacteria*, *Gemmatimonadetes*, *Peridiniaceae*, and *Xanthomonadaceae*.

For glucose, the dominant prokaryotic phyla reported across the selected studies, ranked from highest to lowest number mentioned in observed studies, were: *Proteobacteria*, *Bacteroidota/Bacteroidetes*, *Actinobacteriota*, *Verrucomicrobia*, *Cyanobacteria*, *Firmicutes*, *Planctomycota/Planctomycetes*, *Chlamydiae*, *Bacteroiota*, *Acidobacteriota*, *Dadabacteria*, and *Patescibacteria*. For molasses, the most represented phyla were: *Proteobacteria*, *Bacteroidota/Bacteroidetes*, *Actinobacteriota*, *Planctomycota/Planctomycetes*, *Verrucomicrobia*, *Chloroflexi*, *Cyanobacteria*, *Firmicutes*, *Peridiniaceae*, and *Xanthomonadaceae*. In systems using starch as the carbon source, the dominant phyla were: *Proteobacteria*, *Bacteroidota/Bacteroidetes*, *Cyanobacteria*, *Planctomycota/Planctomycetes*, *Actinobacteriota*, *Chloroflexi*, *Fusobacteria*, and *Gemmatimonadetes*. Studies employing more complex carbon sources were limited; only one study each was found for digested plant cellulose and wheat bran. In the digested plant cellulose-based system, the major phyla were *Proteobacteria*, *Bacteroidota/Bacteroidetes*, *Actinobacteriota*, *Fusobacteria*, and *Gemmatimonadetes*. Meanwhile, in the wheat bran-based system, the predominant phyla were *Proteobacteria*, *Bacteroidota/Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, *Planctomycota/Planctomycetes*, and *Chloroflexi*.

Figure 2a shows the prokaryotic phyla observed across five different carbon sources. Most of the detected phyla were shared among multiple carbon types, indicating that similar microbial groups can occur in systems supplied with different carbon sources. Some unique phyla observed were *Chlamydiae*, *Acidobacteriota*, *Dadabacteria*, *Patescibacteria*, *Peridiniaceae*, and *Xanthomonadaceae*. Figure 2b presents the distribution of phyla across carbon sources classified by structural complexity (without quantitative values). The carbon sources were grouped into three categories based on their compositional nature and bond complexity: simple (glucose and molasses), mid-complex (starch), and complex (digested plant cellulose and wheat bran). Figure 2b also illustrates the phyla shared among the three carbon categories. *Chlamydiae*, *Acidobacteriota*, *Dadabacteria*, *Patescibacteria*, *Peridiniaceae*, and *Xanthomonadaceae* were observed only in systems with simple sugars, whereas *Fusobacteria* and *Gemmatimonadetes* were unique to mid-complex and complex carbon sources. *Chloroflexi* appeared in all carbon categories but was more frequently mentioned in studies when systems using mid-complex and complex carbon sources. Overall, Figure 2b indicates that mid-complex and complex carbon sources support more similar microbial community patterns compared to simple sugars.

Table 1. Biofloc microbial compositions in various aquaculture species different carbon types

Carbon type	**Observed microbial phyla****	**Source**
Glucose	*Proteobacteria (45.8 %), Bacteroidetes (21.1 %), Planctomycetes (13.5 %), Chlamydiae (10.3 %) and Firmicutes (6.8 %)*	Huang *et al* 2022a
Glucose	*Bacteroidetes, Proteobacteria, Planctomycetes, Verrucomicrobia, and Cyanobacteria*	Wei *et al.*, 2016
Glucose	*Proteobacteria (44%), Actinobacteriota (22%), Bacteroiota (8%), Acidobacteriota, and Dadabacteria*	Chen *et al* 2025
Glucose	*Proteobacteria, Bacteroidota, Verrucomicrobia*	Chen *et al.*, 2023
Glucose	*Proteobacteria, Bacteroidota, Actinobacteriota, Patescibacteria and Verrucomicrobiota*	Ma *et al.*, 2025
Molasses	*Bacteroidetes, Proteobacteria, Actinobacteria, Verrucomicrobia and Planctomycetes,*	Rajeev *et al.*, 2023
Molasses	*Proteobacteria, Bacteroidetes, Planctomycetes, Chloroflexi and Actinobacteria*	Xu *et al* 2021
Molasses	*Bacteroidetes and Proteobacteria (74%), Cyanobacteria, Actinobacteria and Verrucomicrobia*	Jiang *et al.*, 2020
Molasses	*Proteobactera, Peridiniaceae and Xanthomonadaceae*	Panigrahi *et al.*, 2018
Molasses	*Proteobacteria, Bacteroidota, Actinobacteriota, Firmicutes and Plactomycota*	Yang *et al.*, 2025
Molasses	*Proteobacteria, Bacteroidetes, Actinobacteria, Chloroflexi and Planctomycetes*	Xu *et al.*, 2024
Starch	*Proteobacteria (35%), Planctomycetote (15%), Bacteroidota (15%), Cyanobacteria (12%) and Chloroflexi (10%)*	Tinh *et al* 2021
Starch	*Proteobacteria, Bacteroides, Fusobacteria, Gemmatimonadetes and Actinobacteria*	Deng *et al.*, 2018
Starch	*Proteobacteria, Cyanobacteria, Bacteroidetes and Planctomycetes*	Wei *et al.*, 2016

Wheat bran	*Proteobacteria, Chloroflexi, Planctomycetota, Bacteriodota and Cyanobacteria*	Vinasyiam *et al* 2025
Digested plant cellulose	*Proteobacteria, Bacteroides, Actinobacteria, Fusobacteria and Gemmatimonadetes*	Deng *et al.*, 2018

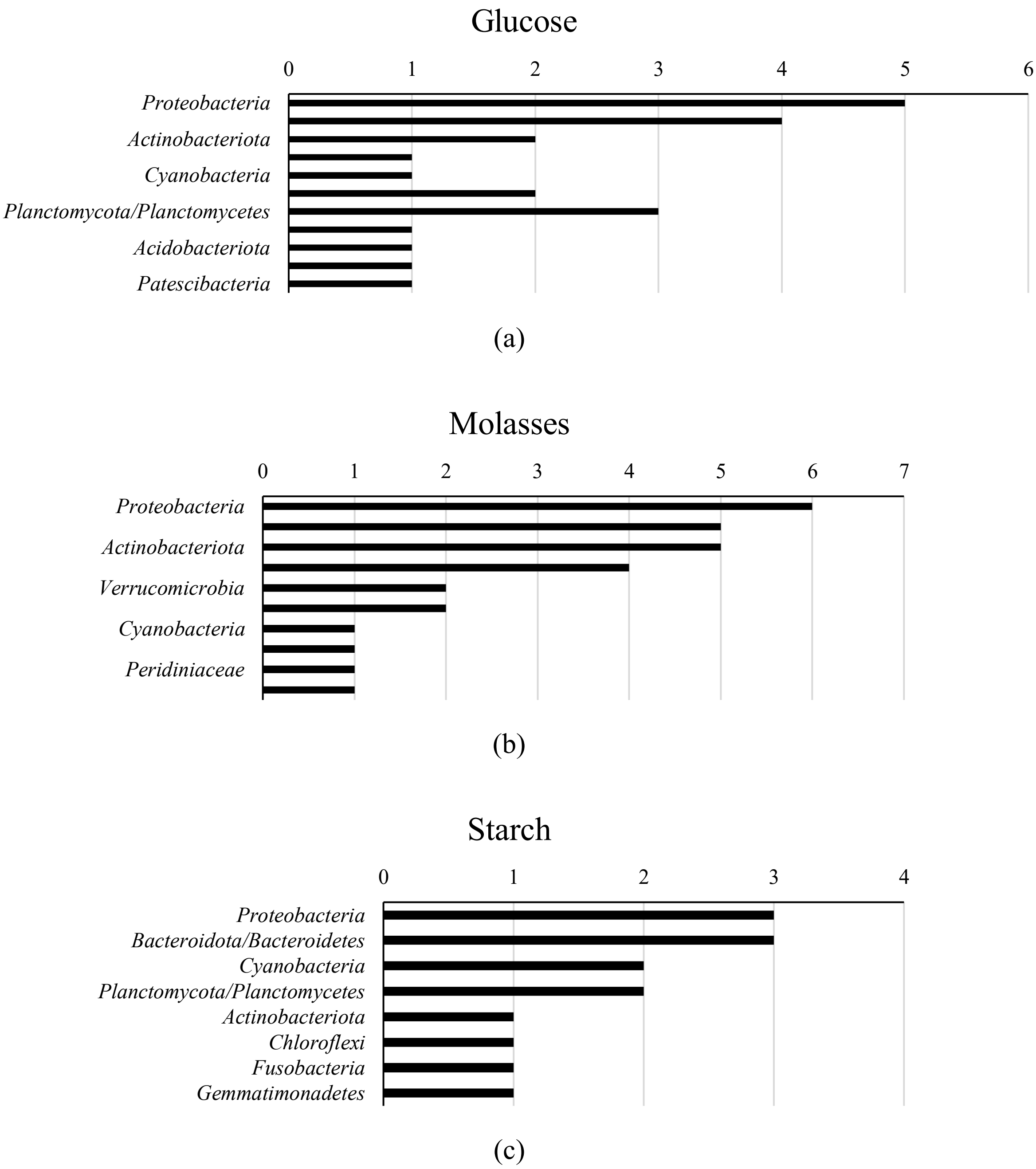
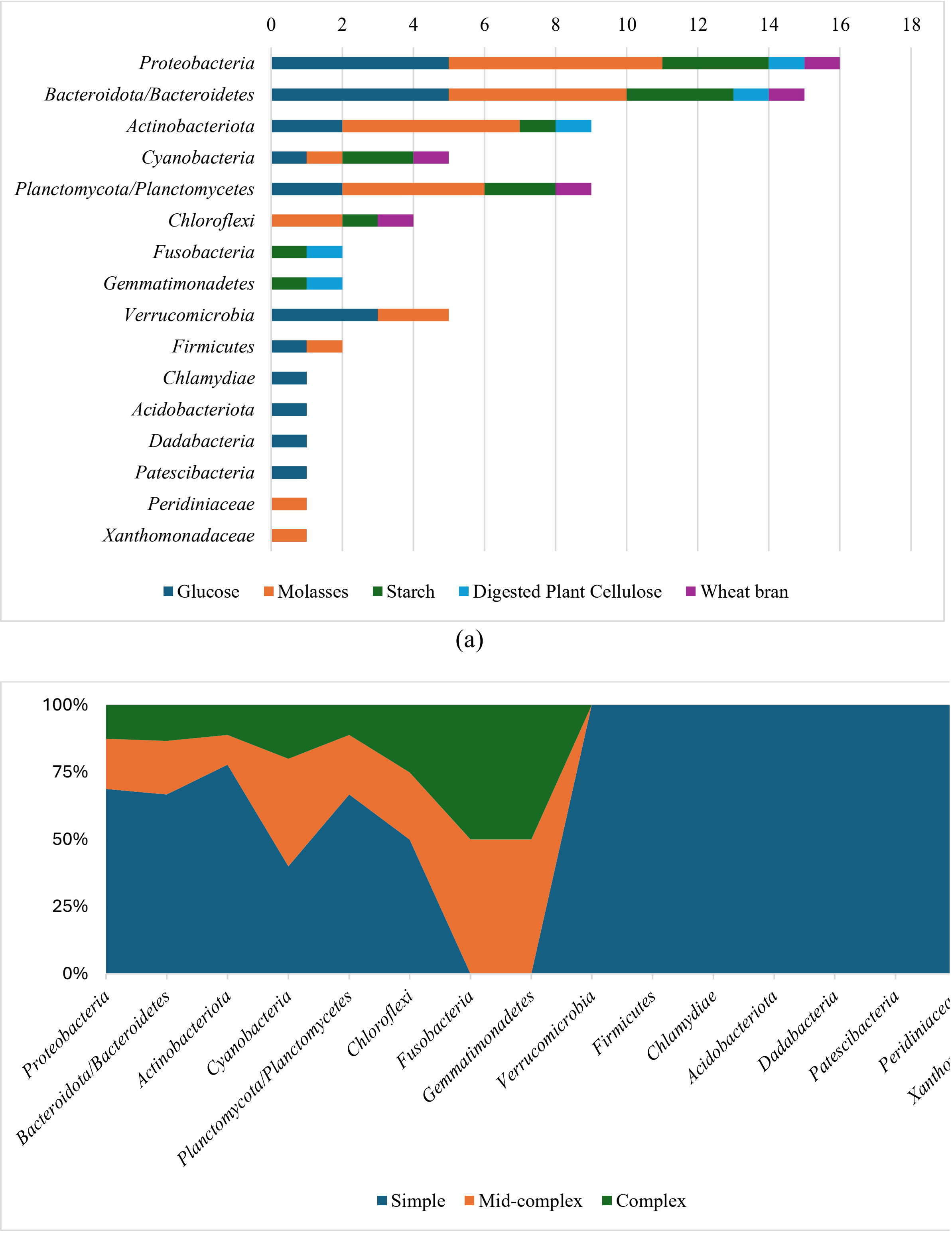


Figure 1. Prokaryotic community compositions in biofloc water fed with different carbon types (a) sucrose, (b) molasses, and (c) starch.



(a)

(b)

Figure 2. Prokaryotic phyla distribution in different (a) carbon sources, and (b) carbon classification simple, mid-complex and complex).

DISCUSSION

Previous studies have investigated the microbial composition of biofloc systems to address the key challenge of effectively managing and directing microbial community dynamics within these environments. Under conventional practice, biofloc formation is managed by adding simple sugars (e.g., molasses) or starch-rich carbon sources (e.g., corn starch, wheat flour) to maintain a high C:N ratio during culture. Continuous supplementation with carbohydrates promotes ongoing biofloc production, primarily through heterotrophic bacterial growth. Heterotrophic bacteria are widely recognized as the main drivers of floc formation, as they secrete extracellular polymeric substances (EPS) that act as binding agents, enhancing aggregate stability and contributing to biomass accumulation within the floc. Consequently, this bacterial group has become the central focus of most microbiological investigations in biofloc systems. Among microbial communities, prokaryotic assemblages are the most frequently studied due to their central role in nutrient cycling and floc structure formation (Cardona *et al.*, 2016; Chakrapani *et al.*, 2021); Tinh *et al.*, 2021; Xu *et al.*, 2022; Zhu *et al.*, 2022)

The present review found that the dominant prokaryotic phyla varied across carbon sources, and that these carbon sources differed in structural complexity. Mid-complex and complex carbon sources tend to share more-similar phyla compared to those found in systems supplemented with simple sugars (Figure 2b). While most observed phyla were generalists, some appeared unique to systems using simple sugars such as glucose and molasses—namely *Chlamydiae*, *Acidobacteriota*, *Dadabacteria*, *Patescibacteria*, *Peridiniaceae*, and *Xanthomonadaceae* (Figure 2a). In contrast, *Fusobacteria* and *Gemmatimonadetes* were found exclusively in mid-complex and complex carbon systems. Notably, *Chloroflexi* species are known to contribute to floc structure by producing extracellular polysaccharides (EPS) (Yu *et al.*, 2018; Zhu *et al.*, 2022).

It is hypothesized that more complex carbon sources require bacterial consortia capable of multi-step enzymatic degradation processes to break down complex molecular bonds, whereas simple sugars can be directly utilized by most heterotrophs. Therefore, both the relative abundance and the types of prokaryotic phyla are expected to differ depending on the carbon complexity, linear as reported in previous studie (Chakrapani *et al.*, 2021; El-Husseiny, 2018; Kabir *et al.*, 2020; Serra *et al.*, 2015; Vinasyiam *et al.*, 2025). The results in this study suggest differences in substrate utilization, respiratory pathways, and bacterial groups involved in degrading carbon of varying complexity. The findings of this review support this hypothesis; however, several knowledge gaps remain. For instance, this study did not consider quantitative analyses of carbon input, and variations in the C:N ratio, due to the limited number of literatures. These factors are known to strongly influence microbial community composition (Addo *et al.*, 2021; Deng *et al.*, 2018; Panigrahi *et al.*, 2018). Moreover, differences in fish species, feeding regimes, and nutrient excretion among the reviewed studies likely contributed to further variation in microbial assemblages. For example, in a freshwater African catfish (*C. gariepinus*) biofloc system, Yun *et al.* (2017) reported that the microbiota consisted of 35% bacteria, 29% microalgae, 24% fungi, and 12% zooplankton. However, the relative proportions of these microbial groups are highly variable and context-dependent (Ray *et al.*, 2010). Ju *et al.*, 2008 reported only 3% bacterial biomass in the biofloc collected from Pacific white shrimp (*Litopenaeus vannamei*) culture. Furthermore, not only the accumulation proportion compared to other microbiota, but the difference may also include the bacterial composition. The limited number of studies on biofloc microbial communities also constrained the extent to which factors other than carbon sources could be examined in this review.

CONCLUSION

The reviewed evidence demonstrates that the biochemical complexity of carbon sources plays a decisive role in structuring microbial communities within biofloc systems. A clearer understanding of these relationships will be essential for developing targeted carbon management strategies that enhance biofloc functionality and efficiency in sustainable aquaculture.

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