Aquaculture Waste: Potential Synthesis of Polyhydroxyalkanoates

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ABSTRACT: Petroleum-based plastics commonly and widely used on a daily basis are a threat to ecological health as they do not degrade in an ecologically feasible time frame. A class of natural polymers known as polyhydroxyalkanoates (PHAs) represents an up-and-coming alternative to petroleum-based materials, as they share properties similar to those of commodity plastics, such as polyethylene, polystyrene, among others, with the advantage of being biodegradable. PHAs are naturally produced by microorganisms under stress, and various farming practices have been proposed to be used for the synergistic and sustainable production of PHA for commercial purposes. Aquaculture has demonstrated particular potential for the production of PHA; however, a large struggle in commercializing these polymers is in procuring necessary feedstocks for manufacture outside of the laboratory environment. Through the coupling of PHA production and biofloc technology in aquaculture, the impediments to commercial exploitation can be potentially surmounted, while also providing for higher production efficiency in aquafarms. This mini-review covers the basic aspects of biofloc technology applied to aquaculture for the commercial production of PHA in large scale and offers a brief perspective on the next steps associated with the research and implementation of PHA production with biofloc technology.

1. INTRODUCTION

Increased carbon footprints of countries going through intense development in recent history has shined a light on the detriment of petroleum-based polymeric materials on ecological health and the imperative nature of finding an alternative option. In investigating substitutes, one should explore the promise of bio-based polymers and composites with increased potential for biodegradability and recyclability.1,2 Within this context, polyhydroxyalkanoates (PHAs) are versatile biopolymers suitable for various applications and are readily biodegradable, among other advantages.3 Recent advancements in the production and synthesis of PHAs call for attention for further investigation. While large-scale production has been one of the largest holdbacks for many biopolymers,4 production pathways in aquaculture using biofloc technology and aquaculture waste can be potentially paired with PHA production, offering a sustainable, efficient, and effective alternative. These combined methods can lead to a natural and sustainable pathway for the production of versatile and biodegradable plastic alternatives, as illustrated in Figure 1. The discussion covered in this mini-review addresses aquaculture, biodegradable polymeric materials, and PHA. Herein, a sustainable, complementary alternative for the production of PHA is presented. It is possible that aquaculture waste alone may not be sufficient to meet the global demand on PHA, but its use has great potential for complementing PHA production, offering a symbiotic system that benefits both aquaculture and PHA production. The methods described in this mini-review may also be interesting to aggregate value to farming activity, offering an additional stream of revenue for local rural communities.

Biofloc technology is an increasingly common method for the management of aquaculture facilities.5 At its core, the biofloc method of aquaculture has water quality control as its central focus. The importance of water management is apparent in areas where land is expensive or water is scarce, and high intensity aquaculture must be practiced ensuring cost-effective production. To maximize profits, production inputs, such as feed, water, and land, must be used as efficient as possible. It is therefore common to resort to rearing fish in higher densities, requiring waste treatment infrastructure. As a result, many producers make use of costly and space-consuming mechanical methods for treatment. In addition, a common issue related to aquaculture systems is the management of disease in the colony, which often originates from the use of water exchange as a water quality control method. In areas where farms are in close proximity to one another, such as estuarine areas for shrimp farming, disease can quickly spread between neighboring facilities, causing mass infection.

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Biosecurity in production can be improved by reducing water exchange. Biofloc systems circumvent these quandaries by handling waste treatment and water quality control in a closed system. In not preforming water exchange, solids and microbes are allowed, or even encouraged, to promote the growth of bacterial biomass. This can be properly managed and controlled in part by a sufficient amount of mixing and aeration, along with other factors that will be further discussed in section 2. In biofloc aquaculture, bacteria proliferate, being sustained by detritus and excess feed, which form microaggregates, sometimes even large enough to be spotted by the naked eye. These “particulates” are also called biofloc. An example is shown in Figure 2. Biofloc can be used in a grand assortment of ways. While most use biofloc to provide nutrients back to the incumbent fish in their environment, the interest of this review is to highlight usage relating to biodegradable polymeric materials (BPMs).

BPMs encompass a large array of bioplastics. Due to the proliferation of polymeric materials in everyday life, BPMs have been proposed as a way for society to transition into more sustainable practices. While petroleum-based polymers can last seemingly in nitely longer than the period for which they were initially used, BPMs are fashioned to last a comparatively short life, often only lasting as long as they are in use. Notably, there are two main types of BPMs, native or synthetic, depending on their source. While some common BPMs, such as poly(lactic acid) (PLA) and poly(glycolic acid) (PGA), are considered to be synthetic, polyhydroxyalkanoates are natural. PHAs are natural polyesters that originate from organisms that were first investigated by Maurice Lemoigne, who isolated poly(3-hydroxybutyrate), or poly(3HB), from Bacillus megaterium in 1925. This was the first bioplastic discovered to be derived from bacteria and, at the time, was thought to be the only type of polyester formed in such a manner. In 1974, polymers with longer 3-hydroxyalkanoate units were isolated from microorganisms in sewage. Those polymers contained 3-hydroxyvalerate (3HV) as the major repeating unit, with 3-hydroxyhexanoate (3HHx) and possibly 3-hydroxyheptanoate (3HHp) present in small amounts, as well. Nowadays, there exists a profusion of different types of PHAs, as illustrated in Figure 3.

As mentioned hitherto, biofloc technology can be used to make BPMs, more speciﬁcally, PHA. Bacteria strains that thrive in the aquaculture medium can consume carbon and produce various types of PHA, following a well-known biosynthesis pathway. The speciﬁc chemical structure of the PHA produced can be controlled by selecting the carbon sources introduced in the system. Indeed, the structures of carbon substrates consumed by the bacteria are tightly related to the structure of the PHA generated. One of the advantages of utilizing biofloc for the production of PHA is that the
polymer produced by the bacteria has an antibacterial effect in the guts of specimens being farmed in the aquaculture system (fish and shrimp), which can be used to enhance fish production in tandem to generating harvestable BPMs.4 One limitation of PHA production in this environment is that bacteria will only produce PHA under stress caused by deficiency in nitrogen, which can be attained by closely controlling C/N ratios in the medium.4 The bacterial competition for ammonium in soil has been studied in a system that evaluated the growth of two heterotrophic species and Nitrobacter.8 Alcaligenes eutrophus, Azotobacter vinelandii, and Pseudomonas oleovorans are among a few varieties of bacteria that synthesize PHA through the process of bioflocs9 and accumulate it intracellularly in the form of granules, as shown in Figure 4.

Table 1 lists a variety of select plant and bacterium species, along with the corresponding feedstock that has been used to produce PHA. Various other organisms are reported in the literature for the production of PHA from carbon-rich waste material, such as activated sludge, hydrocarbons, and water treatment waste.11 A series of potential waste raw materials for the microbial production of PHA has also been reported.12 The bacteria Alcaligenes eutrophus, Azotobacter vinelandii, and Pseudomonas oleovorans have been used for the synthesis of PHA with bioflocs, whereas sugar cane molasses and agricultural wastes served as the carbon sources for Bacillus subtilis and Escherichia coli.15−18 The results obtained with the use of petrochemical wastewater as a feedstock for Bacillus axaraqunsis demonstrate the feasibility of using this bacteria in the industrial processing of petrochemicals, helping to reduce their environmental impact while also improving the productivity of the process, and rendering it more sustainable.16 Another interesting source of PHA is through nonmicrobial means, such as the plants Arabidopsis thaliana, Camelina sativa, Nicotiana tabacum, and Saccharum officinarum. In these instances, plants cultivated in large scale, such as the tobacco plant, can enable the mass production of PHA in large quantities.17

Merging the generation of PHA and the aquaculture industry has promise in overcoming the largest economical obstacles faced by many bioplastics.1,18 By combining these two industries, benefits arise in both improving possible efficiency in aquafarms while minimizing inputs and cost and creating a synthesis pathway for a promising strand of biodegradable polymer alternatives, which can be extracted and processed for large-scale commercial use. This mini-review offers a brief and updated overview of biofloc technology systems and the generation and extraction of polyhydroxyalkanoates, as well as its properties and possible future applications.

2. SYNTHESIS METHODS FOR THE PREPARATION OF BIOFLOC AND PHA

2.1. Natural Production of Biofloc. Biofloc aquaculture, or biofloc technology (BFT), is, as previously mentioned, primarily focused on how waste is dealt with within the farming system. It involves the usage of natural processes and bacteria to sustain the required upkeep for high-density fish to
be successfully maintained. While this is the basic description, several intricacies within the BFT setup are implemented to ensure its successful operation.

2.1.1. General Biofloc Method. BFT setups are, in essence, held up by the microorganisms that they cultivate. As mentioned previously, the limited water exchange with the outside environments provides biosecurity while also providing a stable host ecosystem for assorted microorganisms to thrive. Additionally, BFT helps limit monetary and environmental costs associated with constant water intake and expulsion. The specific bacteria strain used in a given biofloc system depends on the BFT setup employed. There exists two main types of BFT setups. Those exposed to natural light and those which are not. Outdoor systems are commonly installed in coated pools or tanks or sometimes even in circular tanks in greenhouse structures, like the BRASYS structure depicted in Figure 5. The presence of sunlight allows for the cultivation of algae, causing the water to be green. Such systems are often referred to as green water systems or recirculating aquaculture system (RAS). In contrast, in indoor systems without the presence of natural light and addition of probiotics, the controlling factor for water quality is that of purely bacterial origin, leading to brown water biofloc systems.

The biological activity of the cultivar serves as an internal waste treatment, while also providing the basis for the formation of “bioflocs”, discussed in more detail later in the text. Another key aspect of BFT setups is aeration, which is achieved through water exchange and mechanical mixing, also having an impact on solid buildup management. In order to guarantee a sustainable BFT setup, all solids must be in a state of suspension to maintain adequate oxygen levels, as deposits of bioflocs can absorb dissolved oxygen, leading to zones in which anaerobic organisms can form and produce toxic compounds to the fish within the system. Even under ideal mixing conditions, buildup of solids can eventually become too great to remain in suspension, therefore, excess solids need to be removed regularly to maintain healthy living conditions (Figure 6). With oxygen demand being higher for suspended solids, large tanks require an entire array of paddlewheels to ensure aeration and proper mixing, while small tanks and shrimp raceways are usually aerated by other methods, such as diffused aeration and airlift pumps. The need of aeration represents an inconvenient large draw on power demand, which is not ideal for situations in which electricity cannot be obtained in reasonable supply.

In this context, in Central Brazil, a superintensive production system for fish and shrimp has been validated. The BRASYS (biofloc, RAS, and aquaponic system) allows the biofloc technology to be practiced in the culture water and the excess biomass to be filtered for waste treatment. Therefore, it is possible to increase the population density (fish or shrimp), increase aeration energy efficiency, and decrease cultivation time, among other gains.

It should be noted that BFT systems are, in a sense, a balancing act of many different factors, such as stocking densities and feeding rates, that directly affect the output. For example, on the one hand, it has been observed that as stocking density increases, survival rate decreases. On the other hand, a higher stocking density translates into higher spatial efficiency, illustrating the need to manage all factors of a BFT system in order to achieve peak productivity.

Another factor of high importance in BFT systems is ammonia buildup, as elevated levels of ammonia can be fatal to fish. While ammonia builds up from many natural processes in any setup, water exchange is normally the method by which this is solved in aquaculture farms. Alternatively, microorganisms (Nitrobacter) can be utilized to help adjust ammonia levels, conserving water usage and improving the overall sustainability of the process.

If feeding levels are generally low, the top contributor to ammonia removal becomes algae uptake. In the case of BFT systems.
systems, the source of nutrients captured by algae consists of uneaten feed, animal excrements, and dead algae. In times of intense shade, where algae growth is hindered, ammonia buildup can become problematic. If feeding levels are high, or if nitrogen remains in the system for a long time, nitrification can eventually take over. During nitrification, ammonia is oxidized into less toxic nitrate by \textit{Nitrobacter}.\textsuperscript{5}

Bacterial assimilation is one of the most relevant processes in the production of PHA in BFT systems. In this process, heterotrophic bacteria capture nitrogen from the medium and convert it into proteins, immobilizing it in the form of excess solids. The growth of these heterotrophic bacteria is entirely reliant on outside sources; therefore, organic carbon must be added to stimulate their growth and to keep appropriate C/N ratios.\textsuperscript{5} Control of C/N ratios can also be used to specifically facilitate the growth of PHA-producing bacteria, which allows the use of BFT systems for the sustainable production of PHAs.\textsuperscript{5}

2.1.2. \textit{Microbiological Synthesis of Biofloc}. Bioflocs consist of a suspension of organisms, such as algae, bacteria, protozoans, as well as their secreted proteins, and other solids, such as animal excrements and uneaten feed. The specific composition of biofloc can be varied based on the desired effect. While most bioflocs are microscopic, some can actually be observed by the naked eye. Besides having notable enhancing effects inside their natural environment, these particles can also be used in the production of desired products, such as PHAs. The production of PHAs from biofloc is the focus of the second half of this mini-review.

Most of the \textit{in situ} advantages related to the activity of bioflocs stem from the reuptake of nutrients from feed inputted into the system. In fact, only 20–30\% of the nitrogen in feed is typically taken up by fish or shrimp directly, while the

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Figure 7. Synthetic pathway for the \textit{in situ} formation of P3HB.

Figure 8. Possible methods used for the extraction of PHA from bacteria.\textsuperscript{18} Reprinted with permission from ref \textsuperscript{18}. Copyright 2018 C. L. Nielsen.
remaining 70−80% is released as waste. The waste can then be converted into proteins and other nutrients by cultured microorganisms to be finally reabsorbed by fish, completing the reuptake of microbial proteins. This has been shown to enhance the growth of simultaneously cultured fish and shrimp species by providing an almost entirely additional food source that can be consumed between normal feeding schedules. It has been shown that, in a BFT setup, for every “unit of growth” derived from feed alone, an additional 0.25−0.50 “units of growth” can be attributed to microbial protein intake.5

It has been proposed that biofloc can be used to produce the biodegradable polymeric material known as PHA outside of its natural environment. One of the most common biosynthetic pathways for PHB formation in situ is shown in Figure 7, where acetate is catalyzed by acetyl-CoA synthase to form acetyl-CoA. Two acetyl-CoAs are then catalyzed by β-ketothiolase, forming acetoacetyl-CoA, which is then reduced into (R)-3-hydroxybutyryl-CoA, and finally added onto an existing chain of P3HB by P(3HB) synthase.23 It should be noted that other pathways, not discussed in this text, also exist for the production of other types of PHAs. Despite its antibacterial properties in the realm of aquaculture applications, PHA has traits that make its recovery from the system desirable, as will be discussed later in the text.

2.2. PHA from Bioflocs. 2.2.1. Methods for Recovering PHA from Bioflocs. While PHAs could be incorporated solely inside BFT systems as a supplementary food for fish and shrimp, it is also possible to recover it from bioflocs for use as a material in commercial applications. Because PHA is produced and stored intracellularly, external use involves appropriate separation steps in order to recover it for general usage. Various methods are currently in use for the extraction of PHA from bacteria, as shown in Figure 8 and described in further detail in the text.

It is important to note that PHA recovery occurs in three stages, namely, pretreatment, extraction, and purification. While pretreatment and purification are not necessarily required, their incorporation in the process is desirable to ensure efficiency in recovery, leading to a product with high purity at a low cost.18 Centrifugation must be initially performed, prior to any other pretreatment, to separate out the desired PHA-containing bacterium from undesirable solids, other bacterium, and algae. Subsequently, other treatments, such as heating, freezing, and osmotic shock, can be applied to make the disruption of host PHA cells easier in later steps. During extraction, the actual lysing takes place through the use of solvents, such as methylene chloride or other chlorinated hydrocarbons, as well as through chemical digestion, mechanical disruption, biological extraction using other bacteria, or spontaneous liberation using supercritical CO2.18 Finally, purification can be used to increase the final purity of processed PHA. Purification methods rely on techniques, such as the usage of enzymes with hydrogen peroxide or ozone to oxidize contaminants.18 The method of choice for acquiring PHA from bioflocs depends greatly on the desired properties of the PHA obtained, with no single best protocol. Many combinations have been employed in an attempt to balance costs and the desired degree of purity.

The technology associated with PHA production from aquaculture waste can be sized according to the amount of waste generated. For example, the waste generated in highly intensive systems with an animal density of 75 kg/m3, such as BRASYS (described in this mini-review), can vary from 75 to 150 g of dry mass for each liter of water extracted from the cultivation tank. The concentration of PHA in the dry biomass can vary between 2.3 and 4.5 wt %, depending on the microorganism used. Thus, in a production model comprising a 150 m3 cultivation tank, in which 126,000 L of water is processed at each production cycle of 210 days, 9450−18,900 kg of organic matter can be generated, leading to a total amount of PHA recovered ranging from 2835 to 17,010 kg. The same principle would apply to the combined production of 10 smaller farms of 15 m3. In terms of PHA extraction, the methodology can be dimensioned to fit the demand. There are biopolymer extraction equipment in the industry that suit the volume of organic matter produced. The stream of organic matter for a given PHA may either come from a large producer, or the stream from 10 smaller production farms can be gathered and combined to be refined in a single large extractor, with greater extraction capacity, fixed, and operational costs.

With respect to the PHA purification process in intensive production systems, such as BRASYS, a biofilter retains the raw material from the production tank waste. The organic matter from the biofilter enters the PHA extractor with a high degree of purity, eliminating contaminants and increasing extraction efficiency and PHA purity. Simple, low-cost bioreactors, usually present in production farms, are used to enrich the microbiology of the crop and increase the purity of the organic matter. Finally, the manure resulting from the extraction of PHA from crude organic matter can be used as fertilizer or for fertirrigation of pastures.

2.2.2. Physicochemical Characterization of PHA. Physical and chemical properties of PHAs allow one to properly determine their adequate use. Because PHAs are a category of polymers with variable chemical structures and characteristics, the discussion presented in this text will be associated with specific types of PHA, such as, for example, polyhydroxybutyrate (PHB), one of the most prevalent forms of PHA reported. Table 2 summarizes previously published properties of PHAs, in general.24 It is noteworthy that certain types of PHA exhibit material properties comparable to those of polypropylene (PP), such as odor and water resistance. Pure PHB is relatively brittle, having an elongation at break of 15% and stiffness of 1.0 GPa. These properties can change over time due to recrystallization and aging at room temperature. For example, elongation at break has been reported to decrease...
over the course of 30 days. Blending of PHB and other PHAs with other materials has been done in order to complement the positive traits of PHAs, such as biocompatibility and UV resistance, with an improvement of characteristics, such as crystallization, flexibility, and elongation. It is interesting to note that PHA copolymers, such as PHB-co-PHHex (the chemical structures of PHB and PHHhex can be found in Figure 2), also exhibit lower stiffness (∼0.1−0.5 GPa) and elongations varying between 5 and 850%. Additionally, PHB-co-PHHex has a degree of crystallinity (35%) approximately 50% lower than that of typical PHB (60–80%).

3. POTENTIAL USES FOR PHA

Due to their similar properties, PHB could be employed, after proper extraction, to service industry spaces where PP is currently used, such as many single-use plastics, as shown in Figure 9. Currently, the largest use of bioplastics globally (approximately 53% of the total annual production) lies in packaging, which matches the most common current application of PP. Due to its biodegradability, sustainable production, and renewable nature, PHAs can be considered ideal candidates for use as a temporary food packaging material. Other specific potential applications include polymer blends, such as the combination of PHA and polyactic acid (PLA), which results in an overall improvement of heat tolerance and mechanical strength, overcoming some of the weaknesses of PHAs, such as brittleness, and therefore rendering it suitable for microwave-safe applications or containers for hot liquids, like coffee cups and soup bowls.

Another proposed use of PHA is as a coating for calcium phosphates to improve compressive strength, bioactivity, and cell proliferation rate when used in the realm of bone tissue engineering. Due to its biodegradability, a PHA coating is highly effective for temporary scaffolding, gradually degrading over time. While structural usage has been already examined, PHA also has promise in the fields of drug delivery and textiles. Indeed, a silk fabric has been prepared from a PHA/PLA fiber blend. The advantages of these fibers are that they possess a level of wrinkle resistance and “breathability”, while still exhibiting the appearance of pure silk.

4. CONCLUSIONS AND FUTURE PROSPECTS

This mini-review summarizes the basic aspects of biofloc technology, as well as PHA production from bioflocs. As the concept of biofloc technology and the use of bio-based plastics become popular, improved methodology is expected to emerge. For example, one could envision the use of genetic engineering in order to develop novel bacteria capable of improving overall efficiency by optimizing nitrogen removal, bacterial assimilation, and/or PHA production. Along these lines, some progress has been reported allowing the PHA production by eukaryotes. While biofloc aquaculture has been used both to output fish and PHA in relatively small settings, future focuses should also lie in the development of an economically feasible model for large-scale commercialization of this method. The synergistic combination of PHA synthesis with biofloc aquaculture still depends on business incentives, such as carbon credits, in order to overcome the competition of traditional petrochemical plastics and offer a sustainable, cleaner alternative production method. The environmental benefits of PHA production are still hindered by straining conditions associated with nitrogen deficiency requirements, therefore requiring improvements on cultivation methods. Nevertheless, a promising approach has been proposed for the commercially competitive production of PHB from sugar cane waste.

The choice of the renewable resource to be used for the production of PHA relies heavily on local biomass availability. Diversification of feedstock is beneficial because it allows multiple farming activities (including aquaculture) to contribute to PHA production, making the process more versatile, scalable, and sustainable. In that context, biofloc technology complements a portfolio of possible feedstocks. It is worth noting that PHA production scale may be adjusted according to the volume of production of the systems implanted on the farm. Low, medium, or large volume farms producing fish in intensive systems can be associated with low, medium, and large extraction industries. Additionally, the organization of producers in the form of associations or cooperatives to gather waste represents a lower cost option for the extraction of PHA on a large scale.

Further investigation is also necessary to promote commercialization of PHAs, as well as to examine how they could be best implemented into our current societal needs, while ensuring minimal processing adjustments. Ultimately, PHAs will complement the portfolio of bio-based polymer alternatives to offer possible ways to diminish society’s dependence on petroleum-based plastics and materials, and to improve sustainable growth and development.

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