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Nutrient Recovery of Aquaculture Sludge based on Phototrophic Bioconversion in Aquaponics：A Review

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Aquaponics is considered a new industrialized food production approach to meet the increasing needs of expanding urbanization. In this system, nutrient recovery from aquaculture sludge can achieve zero solid waste discharge and supplement nutrients to hydroponics system, which yield environmental and economic benefits. Anaerobic and aerobic mineralization are common approaches for the nutrient conversion of aquaculture sludge, but they are challenged by low-value products, nutrient loss, and large amounts of biomass by-products produced during the process. In this review paper, phototrophic bioconversion of aquaculture sludge by anoxygenic phototrophic bacteria (APB) is proposed to minimize carbon and nutrient dissipation, through which the resulting mineral nutrients can be taken up by plants and the assimilated protein-rich biomass can be utilized by aquaculture animals as food. Pre-treatment to solubilize aquaculture sludge is essential to obtain bioavailable substrates, because aquaculture sludge cannot be utilized by APB in the organic solid form. The characteristics of anaerobic and aerobic solubilization processes are discussed, and the performance of phototrophic bioconversion by APB under anaerobic and aerobic conditions is compared. A multi-loop aquaponic system with four ecological coupling models was developed for the recovery of APB-based nutrients. The symbiotic mechanism between the solubilization and phototrophic bioconversion process should be focused in the future.

1. Introduction

Aquaponics, a land-based system that combines recirculating aquaculture systems with hydroponics, is considered a new industrialized food production approach that can help to meet the increasing needs of expanded urbanization (Goddek et al., 2019). Within this system, the metabolized products obtained via microbial conversion and biodegradation from residual aquaculture effluent serve as nutrient sources to fruit or vegetable plants in the hydroponics unit. The effluent from the hydroponic unit is recycled as a clean water source for the aquaculture unit. In existing aquaponics, only liquid effluent is utilized, and solids (aquaculture sludge) from the system are removed (Yep and Zheng, 2019). Aquaculture sludge is mainly composed of animal excrement and residual feed. The recovery of nutrients from aquaculture sludge in aquaponics could provide environmental and economic benefits because of the zero solid waste discharge and supplementation of additional nutrients to hydroponics (Zhang et al., 2021). Anaerobic or aerobic mineralization is the main approach used to treat aquaculture sludge (Goddek et al., 2019).

Anaerobic mineralization (or anaerobic digestion) using anaerobic heterotrophic bacteria does not require aeration, consumes less energy, and produces biogas. Methane yield may be low due to the lower total solid (TS) content and C/N ratio. The organic acids produced can cause a reduction in pH, leading to an increase in the extraction and chelation of P, Fe, and other metals under acidic conditions (Jung and Lovitt, 2011). Goddek et al. (2018) found that after anaerobic mineralization and mobilization using a sequential upflow anaerobic sludge blanket (UASB) and expanded granular sludge bed (EGSB), the nutrients P, K, Ca, and Mg could be increased remarkably when the pH was below 6. Under acidic conditions, almost no nitrogen nutrients occur after anaerobic mineralization, and the concentration of total ammonium nitrogen (TAN) in effluents is high and nitrate is absent, which may be limiting for plant growth (Goddek et al., 2018). Higher nitrogen loss might occur during anaerobic processes through ammonia (NH_3) volatilization (Khiari et al., 2019) or through emission in the form of N_2 and N_2O by denitrification and anaerobic ammonium oxidation (Monsees et al., 2017). Jung and

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Lovitt (2011) observed a diminished performance in a low-pH reactor with respect to organic sludge reduction. The nutrients in anaerobic mineralization effluent require an optimized treatment process, such as reoxygenation of anaerobic water for subsequent hydroponic application (Monsees et al., 2017). Pang et al. (2007) reported that anaerobic secondary metabolites such as volatile fatty acids (VFAs) are phytotoxic. In general, anaerobic mineralization has several limitations such as a long reaction period, high process variability, and poor nutrient recovery rates (Mirzoyan et al., 2010).

Aerobic mineralization (or aerobic digestion) utilizes a diverse group of heterotrophic organisms, along with ammonia-oxidizing bacteria, nitrite-oxidizing bacteria, to breakdown organic matters in solid, and release minerals (Khiari et al., 2019). Heterotrophic organisms decompose organic matters in aquaculture sludge, producing ammonium (NH_4^+) (called ammonification), which is followed by nitrification to nitrate (NO_3^-) by autotrophic bacteria. Nitrate (NO_3^-) is the preferred source of nitrogen for a wide variety of plant species. Aerobic mineralization can achieve a high nitrogen nutrient recovery efficiency, in which nitrogen loss still occur because the accumulation of nitrate may promote denitrification or anaerobic ammonium oxidation in a local anaerobic environment (Monsees et al., 2017). Other plant macronutrients and micronutrients are also mobilized during aerobic bioprocesses, creating a well-balanced nutrient formulation. Monsees et al. (2017) found that the P concentration could be increased by 330 % and the K^+ concentration by 31 % during aeration treatment (AT) of sludge from RAS increased within 14 d of incubation. Aerobic mineralization consumes alkalinity owing to nitrification, resulting in a drop in pH. When the pH drops below 6, microbial activity will be significantly inhibited. To enhance the mineralization process and maximize the recovery of nutrients, aerobic mineralization under slightly acidic conditions is suggested to minimize nitrogen losses through ammonia volatilization and other losses through ion precipitation (Khiari et al., 2019). Monsees et al. (2017) compared aerobic and anaerobic mineralization of aquaculture sludge in aquaponic systems, and concluded that the aerobic mineralization showed better recovery of P, Ca, Mg, B, Cu, Zn, and Mn than anaerobic conditions, and nutrients (water-sludge mixture) obtained in aerobic treatment can be easily integrated in aquaponic systems. The main disadvantages of aerobic mineralization are the high energy consumption due to aeration, and large amount of biomass produced.

In summary, anaerobic and aerobic mineralization only focus on mineral nutrient recovery available for plants in aquaponics. The loss of nutrient, such as CO_2, CH_4, N_2, and N_2O via emission, and P or metal elements (macronutrients/micronutrients) through precipitation, is inevitable. Furthermore, biomass by-products bring undesired risks, which cannot be further utilized by animals or plants and can have negative effects on the aquaponic system.

The application of anoxygenic phototrophic bacteria (APB) would be a promising approach to recover nutrients from aquaculture sludge in aquaponics. APB are a diverse collection of eco-friendly organisms that grow using energy from light without releasing oxygen, using a variety of organic/inorganic electron donors (George et al., 2020). They can be simply categorized as purple phototrophic bacteria (PPB) and green phototrophic bacteria (GPB), which include purple sulfur bacteria (PSB) and purple non-sulfur bacteria (PNSB), whereas the latter includes green sulfur bacteria (GSB) and green non-sulfur bacteria (GNSB) (Frigaard, 2016). PPB, especially PNSB (such as *Rhodospirillum*, *Rhodopseudomonas*, *Rhodomicrobium*) are the most commonly used in wastewater treatment and resource recovery (Capson-Tojo et al., 2020). Phototrophic bioconversion by APB can produce a wide range of metabolic and cellular products of value, including bioavailable mineral nutrients for plants (Sakarika et al., 2020), and the biomass nutrients that can be used as animal feed (Delamare-Deboutteville et al., 2019). APB is used as a probiotic because it produces multiple value-added substances, such as proteins, coenzyme Q10, indole-3-acetic acid, 5-aminolevulinic acid, bacteriochlorin, carotenoids, and polyhydroxyalkanoates (Cao et al., 2020), which are beneficial to the growth of animals and plants (Sakarika et al., 2020). From an economic perspective, the recovery of biomass nutrients in aquaponics does not require separation via a membrane, which is indispensable in the effluent of photobioreactors (PBRs) used for resource recovery from waste streams. However, APB cannot directly utilize complex organic carbon sources (Chen et al., 2020). The nutrients contained in the sludge should first be mobilized through a solubilization process to provide the substrates available for APB which comprises two steps: liquefaction and conversion of macromolecules to smaller molecules. An anaerobic or aerobic approach can be selected to complete the solubilization process.

In this study, nutrient recovery based on the phototrophic bioconversion of aquaculture sludge is proposed to improve the nutrient recovery efficiencies (NREs) of aquaponics. Three aspects are discussed to fill the research gap: the solubilization process under anaerobic or aerobic conditions for the pre-treatment of aquaculture sludge, phototrophic bioconversion by APB under different light-oxygen conditions (light-anaerobic or aerobic, dark-aerobic), and a multi-loop aquaponic system with four ecological coupling models for the recovery of APB-based nutrients.

2. Pre-treatment to solubilize aquaculture sludge

The characteristics of aquaculture sludge may vary widely, depending on the species. Typically, fish sludge is characterized by a low total solid (TS) content (1.5–3 %) and a lower C/N ratio (Jung and Lovitt, 2011). The volatile (organic) fraction ranged from 50 to 92 % (Mirzoyan et al., 2010). Fish sludge contains large amounts of available N and P elements, which are essential for plant growth (Monsees et al., 2017).

The nutrients contained in aquaculture sludge are difficult to utilize directly by anoxygenic phototrophic bacteria (APB). The common carbon sources for APB are simple organic compounds, including low-molecular weight organic acids (acetate, propionate, butyrate, malate, succinate, and lactate), sugars (glucose and fructose), and alcohols (primary and secondary alcohols). Therefore, pre-treatment (defined as the solubilization process) is necessary to provide the substrates available for APB. The solubilization process includes two steps: the conversion of solids to soluble organic matter (SOM) and further conversion to low-molecular substrates. Both anaerobic and aerobic approaches can be considered to fulfil the solubilization process, which are characterized as incomplete anaerobic or aerobic mineralization with a short conversion time and have their own characteristics of products and environmental factors.

2.1 Anaerobic solubilization process

The anaerobic solubilization process converts insoluble organic matter into low-molecular-weight fatty acids and alcohols using anaerobic heterotrophic bacteria without aeration. It can be considered as incomplete anaerobic digestion process. Compared with the anaerobic digestion process with four main phases, only the hydrolysis phase and the fermentation phase are suitable for anaerobic solubilization process, while the acetogenesis phase should be minimized and the methanogenesis phase should be avoided. (Mirzoyan et al., 2010). The microorganisms responsible for hydrolysis break down organic solids (containing lipids, polysaccharides, proteins, and nucleic acids) into soluble matter (sugars, amino acids, and fatty acids) under the action of extracellular enzymes. In the fermentation (acidogenesis) phase, the solubilized matter is further degraded into low molecular weight matter, such as VFAs, alcohols, ammonia, CO_2, H_2S, and other by-products by acidogenic (fermentative) bacteria. The anaerobic solubilization process does not require completion of the fermentation (acidogenesis) phase, as APB can directly utilize low-molecular-weight fatty acids (short-chain fatty acids). In the acetogenesis phase, VFAs are further digested into acetic acid, H_2, and CO_2 which leads to unnecessary loss of the carbon source though acetic acid can be easily utilized by APB. Therefore, acetogenesis phase should be minimized during the anaerobic solubilization process. In the methanogenesis phase, the production of methane also caused the loss of carbon source which should be avoided during solubilization. The anaerobic solubilization process requires a shorter conversion period and produces fewer biomass by-products compared with the anaerobic digestion process. The main challenge for nutrient recovery of anaerobic solubilization process is the nitrogen loss caused by denitrification or anaerobic ammonium oxidation.

The anaerobic solubilization process does not require the oxidation reduction potential (ORP) to be too low, thus avoiding the promotion of methane production. VFA, TAN, and soluble chemical oxygen demand (SCOD) can be used to characterize the degree of anaerobic solubilization. According to the research by Conroy and Couturier (2010), during hydrolysis of fish waste over 60 % of the biodegradable solids could be converted to soluble organic matter (SOM) in terms of the maximum SCOD concentration in less than 2 d. The generation of VFA leads to a drop in pH, resulting in an increase in the concentration of dissolved P and Ca (especially below 6.5). Meanwhile, the measured TAN increased linearly with the proportional production rate of VFA, and slightly acidic conditions could be formed under the co-action of ammonium accumulation and VFA generation. The results are similar to those of Monsees et al. (2017), in which pH slightly increased from 6.2 to 7.0 in an anaerobic (unaerated, DO<0.1 mg/L) treatment of aquaculture sludge for nutrient mobilization. This finding indicates that an appropriate pH could be maintained in the anaerobic solubilization process for subsequent APB growth, which can be used as a parameter to reflect the extent of anaerobic solubilization.

2.2 Aerobic solubilization process

The aerobic solubilization process converts organic solids into simple substrates available for APB using a diverse group of heterotrophic organisms, along with ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB) under aeration. The aerobic solubilization process is similar to the aerobic hydrolysis (AH) stage, in which aerobic microorganisms convert insoluble macromolecular organic matter into small molecular organic matter under the action of extracellular enzymes to provide substrates for subsequent bacterial fermentation (Qu et al., 2021). The aerobic solubilization process is superior to the anaerobic one, as it has a high conversion rate within a short period owing to the metabolic characteristics of aerobic heterotrophic bacteria. The suitable oxygen supply rate is the key factor to ensure the good effect of aerobic solubilization process. The soluble organic matter obtained in the aerobic solubilization process was quite different from that in the anaerobic solubilization process. As for N conversion, the aerobic solubilization process does not require the conversion of ammonia to nitrate, as APB can assimilate ammonia efficiently. This is benefit for the reduction of nitrogen loss, and saving the supplementation of alkalinity due to nitrification. The pH in the aerobic solubilization process can be maintained at approximately 7 under the compromise of ammonification (ammonium production) and the respiration of heterotrophic microorganisms (CO_2 release) (Delaide et al., 2019), which is suitable for the subsequent phototrophic bioconversion by APB. For the aerobic solubilization process, the media used in the reactor can enhance the conversion efficiency due to the formation of biofilms, which has been verified in a previous study by Gao et al. (2021), a modified biological aerated filter (MBAF) filled with sponge media for nutrient conversion from fish sludge.

The extent of the aerobic solubilization process can also be monitored by tracking the concentrations of SCOD and TAN. Besides, biological oxygen demand (BOD) based on the PNSB-inoculated method might be good way to evaluate the quantity of substrate available for APB. The PNSB that grow under aerobic conditions can be used as inoculated bacteria to replace aerobic heterotrophic bacteria to monitor BOD.

3. Phototrophic bioconversion process

APBs contain bacteriochlorophylls (Bchls) and carotenoids (CDs), which use either type I (iron-sulfur type) or II (quinone type) photochemical reaction centers for photosynthesis, generating ATP and cellular reductants used for CO_2 fixation (Frigaard, 2016). The APB has three catabolic pathways: fermentation, photosynthesis, and aerobic respiration. The predominant metabolic growth mode depends on environmental conditions such as light-oxygen conditions, electron acceptors and donors, leading to diverse metabolic products (Sakarika et al., 2020).

3.1 Phototrophic bioconversion under anaerobic conditions

Under anaerobic conditions, purple phototrophic bacteria (PPB) have three catabolic pathways for phototrophic bioconversion for resource recovery: photoautotrophy, photoheterotrophy and chemotrophy (fermentation without light). The photoautotrophy (e.g., purple sulfur bacteria) uses light as an energy source, CO_2 as a carbon source, and various inorganic electron donors (such as H_2, H_2S, and S). The photoheterotrophy uses light as an energy source and short-chain organic carbon (such as acetate, propionate, butyrate, malate, succinate, and lactate) as the carbon source. The chemotrophy (fermentation without light) uses organic moieties (such as glucose, sucrose, lactose, and ethanol) as energy and carbon sources.

Under light-anaerobic conditions, an oxidation reduction potential (ORP) below -200 mV is beneficial for the efficient photoheterotrophic growth of PPB (Capson-Tojo et al., 2021). Phototrophic bioconversion under anaerobic conditions using PPB has the advantages of high biomass yields and value-added products (Sakarika et al., 2020). For example, the biomass yield of PNSB can reach 0.8 –1.2 g $COD_{biomass}$/g$COD_{removed}$ using simple substrates (Capson-Tojo et al., 2021). Among PPB, purple non-sulfur bacteria (PNSB) are commonly used for resource recovery from waste streams (Capson-Tojo et al., 2020). PNSB mainly utilizes light in the infrared region to grow photoheterotrophically, and the main absorbance waveband of bacteriochlorophylls lies between 805 and 1035 nm.

Both anaerobic and aerobic solubilization processes can be combined with anaerobic phototrophic bioconversion. In terms of the ORP, the anaerobic solubilization process and anaerobic phototrophic bioconversion seem to optimize the symbiotic relationship. The symbiotic mechanism of the bacteria should be investigated in the future.

3.2 Phototrophic bioconversion under aerobic conditions

For most groups of APBs, the presence of oxygen hinders the formation and function of photosynthetic machinery and pigments. Under aerobic conditions, phototrophic bioconversion by PPB has the metabolic characteristics of chemoheterotrophy (respiration) or chemoautotrophy (nitrification), with oxygen as an electron acceptor. Aerobic metabolism is dominant owing to the suppression of the synthesis of Bchls and carotenoids, regardless of the availability of light. Some PPB perform nitrification and denitrification (Capson-Tojo et al., 2020). According to the research by Capson-Tojo et al. (2021), PPB cannot thrive under aerobic or micro-aerobic conditions and yields lower amounts of biomass (0.15 –0.31 g $COD_{biomass}$ /g $COD_{removal}$) when growing chemoheterotrophically. In this case, phototrophic bioconversion under aerobic conditions is similar to the performance achieved in common aerobic reactors for wastewater treatment, for example, considerable COD decrease, fast consumption of COD and nutrients, and lower nutrient removal efficiencies is achieved. Even under illumination, aerobic chemoheterotrophy dominates, rather than photoheterotrophy, although it was reported that photoheterotrophic growth occurred under microaerobic (DO 0.5-1.0 mg/L) or aerobic conditions (DO 2.0-8.0 mg/L). This indicates that phototrophic bioconversion under aerobic conditions can yield more mineral nutrients than biomass nutrients.

Integration of the aerobic solubilization process and aerobic phototrophic bioconversion would be significant for improving the nutrient conversion efficiency. If a local anaerobic environment can be formed under aerobic conditions (with illumination), a mixed microbial community with photoautotrophy, photoheterotrophy, chemoheterotrophy, and chemoautotrophy might coexist to improve the efficiency of phototrophic bioconversion. Related research should be conducted in the future.

3.3 APB-based nutrient recovery in aquaponics

APB-based nutrient biomass has a high resource utilization potential in aquaponics. First, APB (such as PPB) can be used as an aquaculture feed supplement with high single-cell protein content (Delamare-Deboutteville et al., 2019). In addition, carotenoids and bacteriochlorin present in APB have antioxidant and disease-preventing properties (Capson-Tojo et al., 2020). They have been used in aquaculture production as dietary ingredients, disease-preventive agents, and bioremediation agents (Caigoy et al., 2016). Second, PPB can produce high-value compounds that are beneficial to plant growth (Sakarika et al., 2020). Third, APB (such as PNSB) can enhance plant growth performance and increase the yield of edible plant biomass owing to the accumulation of polyphosphate and the production of plant growth-promoting substances, including pigments and vitamins (Sakarika et al., 2020). Finally, APB (such as PPB) can act as a rhizosphere microbiome to enhance the resistance of plants to environmental stresses (Sakarika et al., 2020). This finding indicates that APB-based nutrients contain minerals available for plants and biomass nutrients of APB for animals, which is beneficial for the improvement of aquaponics products (Sakarika et al., 2020).

A question that remains is how to utilize APB-based nutrients in aquaponics. As shown in Figure 1, a multi-loop aquaponic system with four ecological coupling models was developed for the recovery of APB-based nutrients. The APB-based nutrients produced in coupling-2 were reused through two routes. The coupling-1 plus coupling-2 mainly focuses on the reuse of biomass by fish. The APB-based nutrient was purified through biofilter-1 (ammonium to nitrate) and “Low nutrient hydroponics” unit, and then recycled to the fish tank. The coupling-3 plus coupling-4 mainly focuses on the reuse of mineral nutrients. The APB-based nutrient after biofilter-2 (ammonium to nitrate) enters the unit of “high nutrient hydroponics” to provide mineral nutrient for the plants with high nutritional requirements, and then enters into unit of “Mesotrophic hydroponics,” to reduce the residual nutrient by the herbaceous plants. The purified water with a low concentration of nutrient can be recycled to the fish tank. Through multi-loop aquaponic system, the yields of animals and plants is expected to be improved. Future research should be focused on the evaluation of the whole system quantitatively.

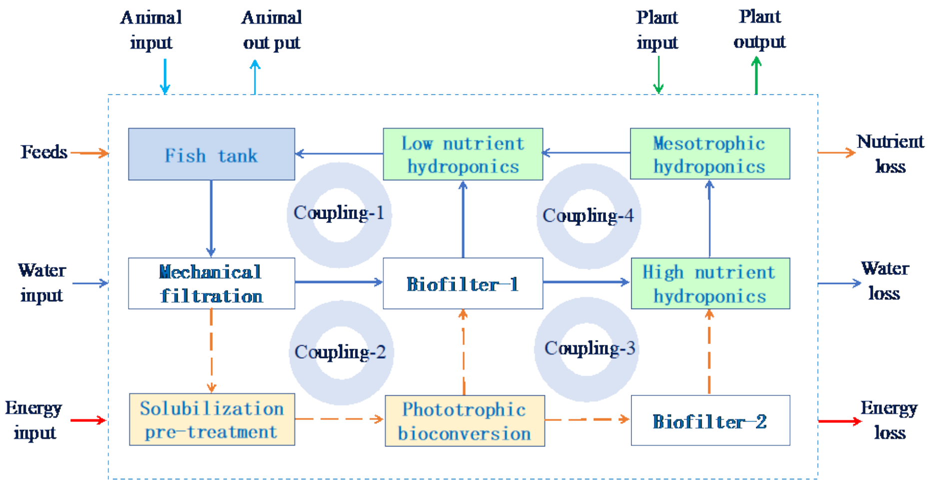

Figure 1: Ecological coupling model of multi-loop aquaponic system for APB-based nutrient recovery

4. Conclusions

In this study, the phototrophic bioconversion of aquaculture sludge by anoxygenic phototrophic bacteria (APB) is proposed to improve nutrient recovery in aquaponics. The APB-based nutrient includes mineral nutrients that can be taken up by plants, as well as the biomass nutrients that can be supplied to aquaculture animals as feed. A pretreatment to solubilize aquaculture sludge under aerobic or anaerobic conditions is essential to obtain the substrates available for APB. Phototrophic bioconversion processes under different light-oxygen conditions (anaerobic and aerobic) can yield different value-added products. A multi-loop aquaponic system with four coupling models for the recovery of APB-based nutrients can be developed for the recovery of APB-based nutrients. Future research should focus on the symbiotic mechanism between the solubilization process and phototrophic bioconversion process.

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