**บทความวิชาการ**

# การประยุกต์ใช้พอลิไฮดรอกซีอัลคาโนเอตเป็นแหล่งคาร์บอนเพื่อบำบัดสารประกอบไนโตรเจนในระบบเพาะเลี้ยงสัตว์น้ำแบบหมุนเวียน

# Application of Polyhydroxyalkanoates as Carbon Source for Nitrogen Compound Treatment in Recirculating Aquaculture System (RAS)


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## บทคัดย่อ

สารประกอบไนโตรเจนในระบบเพาะเลี้ยงสัตว์น้ำเกิดจากของเสียจากการขับถ่าย อาหาร และซากของสิ่งมีชีวิต การบำบัดสารประกอบไนโตรเจนนิยมใช้กระบวนการทางชีวภาพโดยตรึงแบคทีเรียบนวัสดุ แบคทีเรียจะนำสารประกอบไนโตรเจนเข้าไปในเซลล์และเปลี่ยนเป็นสารอื่นเพื่อสร้างพลังงานไว้ใช้ในการเจริญเติบโต กระบวนการดีไนตริฟิเคชันเป็นหนึ่งในกระบวนการทางชีวภาพในการบำบัดสารประกอบไนโตรเจน จุลินทรีย์จะใช้ไนเทรตเป็นตัวรับอิเล็กตรอนและใช้สารอินทรีย์ (แหล่งคาร์บอน) เป็นตัวให้อิเล็กตรอนในกระบวนการหายใจ ซึ่งมีข้อเสีย คือ การเติมสารอินทรีย์ในปริมาณน้อยส่งผลให้เกิดการสะสมของไนเทรต แต่หากเติมในปริมาณมากส่งผลให้สารอินทรีย์ในน้ำมีปริมาณเพิ่มขึ้น ดังนั้นจึงต้องมีการควบคุมโดยผู้เชี่ยวชาญพอลิเมอร์ย่อยสลายได้ทางชีวภาพ เช่น พอลิไฮดรอกซีอัลคาโนเอต จึงเป็นทางเลือกหนึ่งในการหลีกเลี่ยงปัญหาดังกล่าว เนื่องจากมีข้อดี คือ ย่อยสลายได้ทางชีวภาพ สามารถใช้เป็นตัวกรองชีวภาพและเป็นแหล่งคาร์บอนได้โดยไม่มีผลกระทบต่อปริมาณไนโตรเจนในน้ำ บทความวิจัยนี้จึงกล่าวถึงการประยุกต์ใช้พอลิไฮดรอกซีอัลคาโนเอตเป็นแหล่งคาร์บอนเพื่อบำบัดสารประกอบไนโตรเจนในระบบเพาะเลี้ยงสัตว์น้ำแบบหมุนเวียน



**คำสำคัญ:** ระบบเพาะเลี้ยงสัตว์น้ำ สารประกอบไนโตรเจน พอลิไฮดรอกซีอัลคาโนเอต ดีไนตริฟิเคชัน พอลิเมอร์ชีวภาพ



## Abstract

Nitrogen compounds in aquaculture systems resulted from excretion waste, feed and aquatic organism remains. The treatment of nitrogen compounds is commonly used biological process by fixing the microorganism on the surface of the material. Afterwards, the bacterial cell uptakes the nitrogen compound, which is changed to other substances to create energy for bacteria growth. Denitrification is



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one of the biological processes in nitrogen compound treatment. In this process, microorganisms use nitrate and organic carbon (carbon source) as electron acceptors and electron donors, respectively, in the respiration process. However, the disadvantage of low carbon addition may lead to nitrate accumulation. On the other hand, the excess carbon addition increased the organic content in the system. Therefore, the addition of carbon should be controlled by the specialist. Biodegradable polymers such as polyhydroxyalkanoate (PHA), are considered as an alternative to avoid the problem since there were advantages, which are, for example, biodegradation, using biofilter and being carbon sources, without any effects on quantity of nitrogen in water. In this research, we discussed the application of PHA as a carbon source to eliminate nitrogen compounds in recirculating aquaculture systems.

**Keywords:** Aquaculture Systems, Nitrogen Compound, Polyhydroxyalkanoates**,** Denitrification, Biopolymer

## Introduction

As an increasing of aquaculture industry also increase the environmental problem. Huge amount of wastewater containing high nitrogen was discharged to environment. The removal of nitrogen and phosphorus from wastewater has become an emerging worldwide concern because these compounds cause eutrophication in natural water. Moreover, nitrate is a risk to human health, especially as a possible cause of infant methaemoglobinaemia. Nitrogen removal technology, a cost effective and advanced wastewater treatment technology, is growing in importance in Thailand. There are many techniques for nitrogen treatment such as rotating biological contactors, trickling filters, bead filters and fluidized sand filters. However, the applicable to extensive system and environmentally friendly process are still required. The nitrogen removal using biopolymer is a novel technology. It has the advantage over the other techniques that it is eco-friendly and relatively inexpensive. This make it an economically viable approach for sustainable aquaculture.

## Nitrogen Compound Treatment in Recirculating Aquaculture System

Fisheries are important to the economics of Thailand. In 2017, The Department of Fisheries reported gross domestic product (GDP) of fisheries sector was 81 billion baht, increased to 9.32 % in the same time on 2016 [1]. At present, aquatic animals in nature have decreased. The Department of Fisheries has promoted aquaculture to solve such problems. Aquaculture is the process of raising aquatic organisms, include plants (both small phytoplankton and large macrophytes such as seaweed), molluscs, crustaceans, amphibians, and reptiles, in an enclosed area (e.g., earthen ponds, raceway, and net-pen) for use in the fishing industry [2, 3]. Traditional aquaculture methods are low labour, easily managed systems, but they can sustain fairly low stocking densities [4]. In order to improve the fish yield and profit, traditional aquaculture has been transformed to intensive aquaculture system. However, intensification demands increased inputs, such as fish and feed per unit culture area and, therefore, increased waste generation from the aquaculture production systems. The impact of waste products from aquaculture has increased public concern and threatens the sustainability of aquaculture practices [5].

Among the many existing aquaculture systems, the recirculating aquaculture system (RAS) seems to overcome these limitations and can provide a form of sustainable farming for both marine and freshwater species [6]. Nevertheless, recirculating water results in decreased water quality, primarily through the accumulation of fish waste and uneaten food. The nitrogen budget of fish culture ponds showed that fish only absorbed 11.6-46.5% of the nitrogen input in the feed [7]. Ammonia can be generated from protein deamination in residual feeds or via metabolism by fish (e.g., gill and renal excretion). Total ammonia nitrogen is of importance in aquaculture, because ammonia is toxic. To effectively control ammonia concentration in RAS, three processes such as physical, chemical, and biological processes are used. However, biological nitrogen removal is a major process for ammonium removal in the wastewater treatment. The advantages and disadvantages of biological process for the treatment of nitrogen compounds in RAS is shown in Table 1.

**Table 1** Advantages and limitations of conventional biological nitrogen removal processes [8].

| Process | Advantages | Limitations |
|---|---|---|
| Bardenpho (4-stage) | Total N concentration less than 3 mg/L possible | - Large reactor volume is required<br>- Second anoxic tank has low efficiency |
| Predenitrification | - Very adaptable to existing activated sludge processes 5-8 mg/L total N is achievable<br>- Reparation of alkalinity because of denitrification | - N-removal capability is a function of internal cycles<br>- DO control is required before recycle |
| Postdenitrification | Capable of achieving total N levels less than 3 mg/L | - Higher operating cost due to additional carbon dosage<br>- Carbon dosage control is requireds |
| Bio-denitro | Large reactor volume is resistant to shock load 5-8 mg/L total N is achievable | - More complex to operate<br>- Two reactors are required so as to increase construction cost |
| Sequencing batch reactor | - Process is flexible and easy to design<br>- Quiescent settling provides low effluent TSS concentration<br>- Mixed liquid/solids cannot be washed out by hydraulic surges<br>- Secondary settling tank is not required | - More complex to operate<br>- Effluent quality depends on reliable decanting facility<br>- Be not suitable for large plants |
| Oxidation ditch | - Highly reliable process<br>- Simple operation<br>- Capable of treating shock/toxic loads without affecting effluent quality<br>- Economical process for small plants<br>- Well-stabilized sludge: low biosolids production | - Large structure, greater space requirement<br>- Low F/M bulking is possible<br>- Some modifications are proprietary and license fees may be required<br>- Plant capacity expansion is more difficult<br>- Nitrogen removal capability is related to skills of operating staff and control methods |

**Table 1** Advantages and limitations of conventional biological nitrogen removal processes [8]. (continue)

| Process | Advantages | Limitations |
|---|---|---|
| Step feeding | - Distributes load to provide more uniform oxygen demand<br>- To minimize high clarifier solids loading in peak wet weather flows<br>- Adaptable to existing activated sludge process<br>- With internal recycle in last stage, total N less than 5 mg/L possible | - N-removal capability is a function of flow distributions<br>- Flow split is not measured or known accurately<br>- DO control is required before recycle<br>- Flow split control is required to optimize operation |

TSS, total suspended solid; DO, dissolved oxygen

Based on the microbial nitrogen cycle and the metabolism of inorganic nitrogen compounds (Fig. 1), many biological technologies and processes have been developed and implemented for nitrogen removal from wastewater [8].

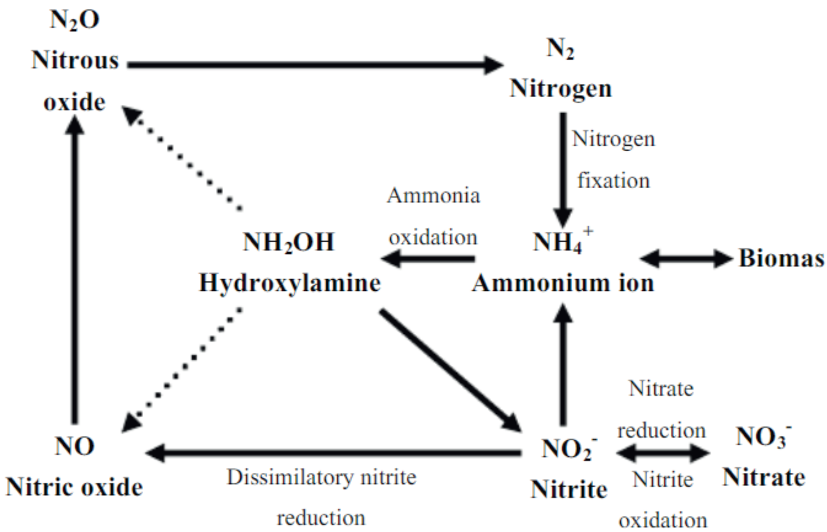


**Figure 1** Microbial nitrogen cycle [8].

As a side effect, nitrate accumulation has been observed in RAS. Though significantly less toxic than ammonia, nitrate is also toxic to aquatic organisms and, specifically, it is a threat to aquaculture fish and shrimp at elevated levels. Therefore, a biofilter is usually installed as an integral part of a RAS to mitigate ammonia accumulation via biological nitrification (nitrifying bacteria oxidizing ammonia to nitrite then nitrate) [9]. The biological denitrification processes can be considered two steps processes, consisting of the reduction of nitrate to nitrite followed by the reduction of nitrite to nitrogen gas. In the absence of oxygen, nitrate becomes the terminal electron acceptor of choice, with a single oxygen atom removed from each nitrate ion, releasing one nitrite ion. This results in an undesirable release of nitrite, but in the presence of excess organic electron donors (carbon), nitrite is converted first to nitrous oxide, immediately to nitric oxide, and then just as quickly to nitrogen gas [10]. The carbon source used as an electron donor for the denitrification process impacts the conversion rates of nitrate to nitrogen. The most commonly used carbon sources for denitrification culture are methanol, acetate, glucose, and methanol. These sources are water soluble and are readily available to bacteria. The addition of an insufficient carbon source may lead to nitrate accumulation for incomplete denitrification, whereas the addition of an excess carbon source may lead to an increase in the effluent organic matter. The management of liquid or water soluble carbon sources for denitrification increases the cost of the process, and it will sometimes be overlooked in busy aquaculture operations. A passive control system, requiring less supervision, can reduce the management needed for its successful operation. Biodegradable non-water soluble polymers such as polyhydroxyalkanoates (PHA), can be suitable carbon sources for denitrification [11]. For denitrification with PHAs as solid carbon sources, microorganisms use the biopolymer in the form of pellets simultaneously as a biofilm carrier and as a water insoluble carbon source for denitrification, which is accessible only by enzymatic attack [12]. The application of PHA as carbon source to eliminate nitrogen compounds in aquaculture systems has been reviewed. The utilization of PHA for nitrogen treatment has never been reviewed before.

## Application of Polyhydroxyalkanoate (PHA) for Nitrogen Compound Treatment in Recirculating Aquaculture System (RAS)

PHA is biopolymers synthesized by microorganisms as lipid inclusions for energy storage in granular forms within the cellular structure. PHAs are natural polyesters of 3-, 4-, 5-, and 6-hydroxyalkanoic acid, which are thermoplastics [13]. PHA has properties similar to petrochemical polymers, such as polypropylene or polystyrene [14], and excellent biodegradability through enzyme activity by microorganisms. Therefore, PHA is used in industry to replace petrochemical plastics [15]. PHA is solid, non-water-soluble biopolymers that can be used as alternate self-regulating carbon sources [11]. In nitrogen compound treatment processes, PHA is often used as solid substrates in denitrification. There are various types, but the most popular PHAs are poly-β-hydroxybutyrate (PHB) and poly (3- hydroxybutyrate-*co*-3-hydroxyvalerate) (PHBV), and the monomers are poly-3-hydroxybutyrate and poly (3- hydroxybutyrate-*co*-3-hydroxyvalerate), respectively.  Advantages and disadvantages of using PHA in aquaculture systems are shown in Table 2.

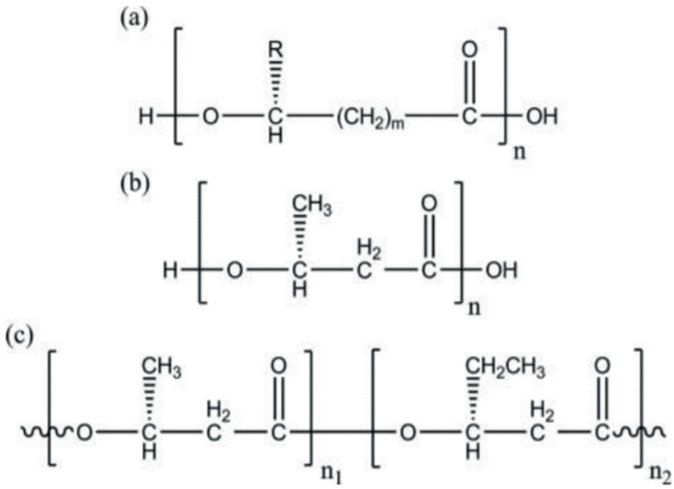


**Figure 2** Chemical structure of polyhydroxyalkanoates (a), poly-β-hydroxybutyrate (b) and poly (3- hydroxybutyrate-*co*-3-hydroxyvalerate) (c) [16].

**Table 2** Advantages and disadvantages of using polyhydroxyalkaniate (PHA) in aquaculture systems.

| Advantages | Disadvantages |
|---|---|
| • Non-toxic<br>• Biodegradable by naturally occurring bacteria<br>• Safe and easy process<br>• The use of PHA has no potential risk of release of dissolved organic carbon with the resultant deterioration of effluent water quality | • The polymers are highly expensive<br>• Degradation of PHA is incomplete, methane can result as an end product<br>• Gradually degraded by biological process which may lead to carbon source lacking for denitrification |

Boley *et al*. (2000) [12] was the first to report the application of biodegradation polymers as solid substrate and biofilm carriers for denitrification. The denitrification process with biodegradable polymers presented here is a simple process. Microorganisms simultaneously use the biopolymer in the form of pellets as a biofilm carrier and as water insoluble carbon source for denitrification, which is accessible only by enzymatic attack (Fig. 3). There are two key steps involved mechanism of biofilm bacteria degrade bioplastic: (i) the polymer cleavage backbone, named depolymerization, where hydrolysis and oxidation are the main responsible, together with the extracellular enzyme, responsible for the cleavage on internal linkage (endo-action) and on the terminal polymer molecules (exo-action); (ii) the mineralization, which occurs inside the cell responsible for microbial degradation. Bioplastic could degrade aerobically in wild nature, anaerobically in sediments and landfills and partially aerobically/anaerobically in compost and soil. During the aerobic biodegradation, carbon dioxide and water are produced, while during anaerobic biodegradation, water and methane is produced [8].

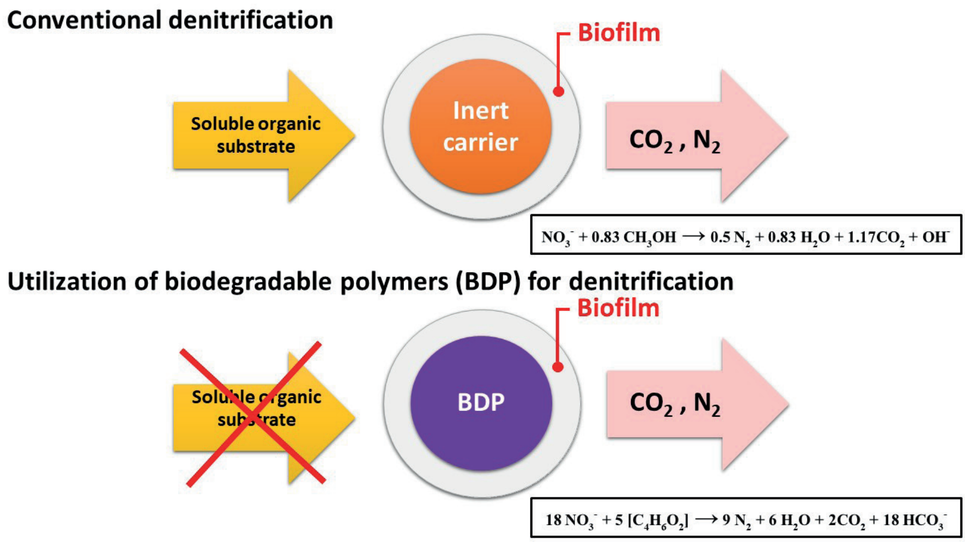


**Figure 3** The comparison of different carbon source for nitrogen compound treatment [12].

The study indicated that denitrification using biodegradable biopolymers is a safe and easy process whereas the polymers are highly expensive. In addition, Qiu *et al*. (2016) [17] detected the effects of solid-phase denitrification on nitrate removal. The data showed that nitrate levels decreased and were maintained below 10 mg/L after 100 days of running in a RAS. In contrast, the nitrate concentration of the control increased, reaching 100 mg/L. Thus, PHA is considered a good choice as an organic carbon source for denitrification. The treatment of nitrogen compounds using biodegradable polymers as carbon sources is better than soluble organic carbons because the addition of soluble organic carbon would result in organic carbon loss and water dissolved oxygen (DO) drop, which causes stress for the aquatic organism and it might cause increase in mortality. Meanwhile, less amount of soluble organic cannot achieve acceptable denitrification performance [18]. In RAS, soluble carbon, such as glucose or molasses, was added when ammonium increased. The external carbon sources are added in order to improve the ratio of chemical oxygen demand (COD) to nitrogen and consequently to increase the efficiency of denitrification processes. Pérez-Fuentes et al. (2016) [19] found that adding molasses at concentrations of > 0.12 g/L caused a dramatic decrease in oxygen (from 3.2 to 1-1.5 mg/L), but this was recovered after 1 hour. The suddenly and temporarily lowered the DO concentration, leading to stress, and increased oxygen demand to the aquatic organism. On the other hand, denitrification with biodegradable polymers have been used as organic carbon sources and biofilm carriers. The release of organic carbon from biodegradable polymers is the result of enzymatic attack, and the released amount was determined by bacteria demand. In the presence of microorganisms, such as bacterial and fungi, the macromolecular chains are broken, starting the biodegradation process. It is likely that the use of biodegradable polymers carries no potential risk in causing deterioration of the effluent quality by releasing dissolved organic carbon (DOC) [20].

## Future Prospects for the Utilization of PHA for Nitrogen Removal

PHA is a kind of polymer that is receiving attention because it is a material that can be degraded by the action of microorganisms in the environment. However, the application of PHA as carbon sources in the treatment of nitrogen compounds in aquaculture systems has obstacles because PHA is more expensive than other biofilters. At this time, genetic improvement of microorganisms has been made in order to increase the production of PHAs. Bacteria used for PHA production include *Cupriavidus necator*, *Pseudomonas* sp., and *Escherichia coli* [20-21]. The use of PHAs as the solid substrate for denitrification has several advantages over the conventional system supplemented with liquid organic substrate. PHAs serve not only as constant source of reducing power for denitrification but also as solid matrices favorable for development of microbial films. Moreover, the use of PHAs has no potential risk of release of dissolved organic carbon with the resultant deterioration of effluent water quality. If the production cost of PHAs can be brought down, its application to the denitrification process will become economically more promising.

## Conclusion

Biological processes for nitrogen compound treatment include nitrification and denitrification. PHAs can be applied in biological processes as carbon sources and biofilm carriers because they have the advantage that can being biodegradable. However, the high price of PHA is still a major barrier. The reduction of PHA cost by the genetic improvement of microorganisms has been made in order to increase the production of PHAs. In the cooperation with utilize of PHAs combined with other biofilters, such as sponges, to increase the efficiency of the bioreactor and to reduce the cost of building the nitrification reactor. However, research on PHA for nitrogen compound treatment should be studied in greater detail.

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