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Application of enzyme-hydrolyzed cassava dregs as a carbon source in aquaculture

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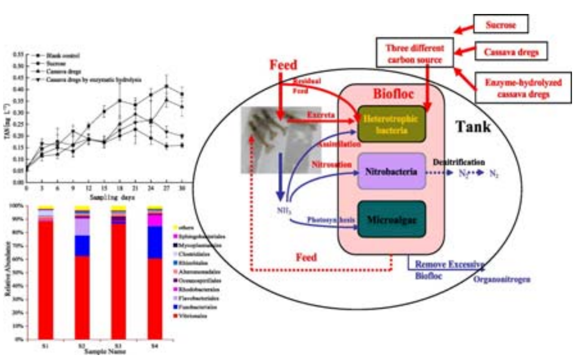
HIGHLIGHTS

- High yield of reducing sugar was produced by enzyme hydrolysis of cassava dregs.
- Enzyme-hydrolyzed cassava dregs as a substitute of sucrose applied in biofloc aquaculture system.
- Enzyme-hydrolyzed cassava dregs was more easily assimilation by heterotrophic bacteria.
- Microorganism in biofloc will affect the indigenous microflora in shrimp intestines.

GRAPHICAL ABSTRACT



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ABSTRACT

As a kind of tropical agricultural solid waste, cassava dregs had become a thorny nonpoint source pollution problem. This study investigated the feasibility of applying cassava dregs as a substitute for sucrose in biofloc technology (BFT) systems. Three types of biofloc systems (using three different carbon sources sucrose (BFT1), cassava dregs (BFT2) and enzyme-hydrolyzed cassava dregs (BFT3) respectively), and the control were constructed in this experiment in 200 L tanks with a C/N ratio of 20/1. The comparison of the water quality indicators (The total ammonia nitrogen (TAN), nitrite (NO_2^--N), nitrate (NO_3^--N), chemical oxygen demand (COD)), biofloc for the above four groups was performed, and the results indicated that BFT3 showed greater potential to the formation of biofloc, which was beneficial for the water quality control. So the shrimp survival rate was the highest and the feed conversion rate was the lowest in BFT3. Besides, the high-throughput sequencing results showed that the relative abundance of heterotrophic bacteria in the top 30 dominant microbial communities in BFT3 was higher than those in BFT1 and BFT2 by 20.70% and 1.19%, respectively, which could decrease TAN to improve the water quality. Overall, the results had proved that the cassava dregs of enzymes hydrolysis could be used as an ideal and cheap carbon source in BFT.

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1. Introduction

Litopenaeus vannamei (*L. vannamei*), commonly known as *Pacific white shrimp*, is widely cultured in Asia, South America and North America. The production of *L. vannamei* is responsible for approximately 76% of the total global shrimp yield in 2011 (Yu et al., 2014). Presently, traditional *L. vannamei* aquaculture faces serious problems. The scarcity and expense of water have limited aquaculture development. With the expansion of the shrimp industry, the discharge of nutrient-rich effluents from culture ponds to coastal waters is becoming a major environmental concern (Naylor et al., 1998; Shang et al., 1998). Moreover, severe outbreaks of infectious diseases require more stringent biosecurity measures, such as the reduction of water exchange rates (Avnimelech and Kochba, 2009).

Currently, biofloc technology (BFT) is considered as an environmentally friendly alternative for the development of intensive aquaculture because it offers several advantages over conventional systems, such as lower water renewal rates and a lower level of maintenance (Haslun et al., 2012). Bioflocs are composed of algae, fungi, bacteria, flagellates, ciliates and other microorganisms that adhere to an organic matrix and present as floating flocs in the water column (Schryver et al., 2008). In BFT technology, microorganisms maintain water quality and serve as food for aquatic products. A BFT culture system contributes to water quality by removing nitrogen compounds, supplementing the diet of the aquatic animals and allowing for high stocking densities (Krummenauer et al., 2016).

During the aquaculture period, shrimp excrete ammonia nitrogen, which accumulates in ponds. A major source of ammonia nitrogen is the typically protein-rich feed (Avnimelech, 1999). Mineralization of accumulated organic matter under anaerobic conditions leads to the formation of toxic metabolites, such as NH_3 and NO_2^--N, and exposure to toxic materials endangers the well-being of the cultured shrimp (Avnimelech and Ritvo, 2003). However, nitrogen is used to produce microcells that are rich in protein, and inorganic nitrogen is immobilized into bacterial cells at a high C/N level. Controlling C/N levels is a major focus of BFT research. Maintaining the C/N ratio within a certain range is a prerequisite for floc formation (Avnimelech, 2006). The C/N level of bacterial cells is 5:1 (Mooyoung et al., 2001), and the carbon is used for respiration; thus, the optimal C/N level of the organic substrates may be higher than that in the body composition under aerobic conditions. The C/N level in an aquaculture system could be increased by adding different locally available inexpensive carbon sources or by reducing the protein content in feed (Avnimelech, 1999; Hargreaves, 2006).

At present, the mature carbon sources of BFT are sucrose, starch and corn. However, the high cost of these carbon sources limits their use in practice. Cassava dregs, a by-product of cassava starch (*Manihot esculenta* Crantz) production, are thrown into water courses or left in ditches, which overflow and carry much of the organic load towards bodies of water. Their high water content and rich nutrients make cassava dregs highly likely to cause microbial contamination of the surrounding environment, leading to undesirable fermentation. Rainfall create a serious non-point source pollution problem, which can pose a threat to surrounding soil, rivers and human. (Fiorda et al., 2015) Surprisingly, cassava dregs are an inexpensive carbohydrate a potential carbon source for BFT systems. Cassava dregs can be biologically converted to various high-value products to maximize the effective utilization of this important bioresource (Zhang et al., 2016b). >6 million tons of cassava have been harvested for starch extraction since 2009 in Guangxi, China (Sun et al., 2012), and approximately 700 kg of cassava dregs with 70% water content are produced per 1000 kg cassava. These dregs are difficult to degrade using natural environmental methods alone; thus, it is extremely important to find more efficient and more environmental friendly ways to use cassava dregs. The composition of cassava dregs is complex, and starch, cellulose and lignin account for 61.3%, 10.5% and 8.6% respectively (Table A.1); however, utilizing all of these contents is difficult. Studies has reported that cassava dregs can be decomposed into small-molecule reducing sugars via enzymolysis, the effects of which were distinct (Zhou et al., 2014). In this study, we hydrolyzed cassava dregs using enzymolysis and then added the innocuous and nonpoisonous products of this reaction to a biofloc aquaculture system to replace the more expensive carbon source, sucrose. The aim of this work was to explore the feasibility of using cassava dregs as an alternative carbon source in biofloc aquaculture.

An important step towards understanding and utilizing BFT was to characterize and control the microbial community. In this study, we explore the changes in microbial aquaculture populations, investigate the structure and diversity of the microbial communities in BFT and shrimp intestine samples from *L. vannamei* culture ponds using high-throughput sequencing technology. Our results clarified the relationship among environmental factors and the microbial communities in a biofloc aquaculture system as well as the microbial communities in the intestine of *L. vannamei*. Our results provide novel information that can be used to improve the microflora in aquaculture environments and inhibit pathogens to reduce *L. vannamei* disease by adding new carbon sources to control the C/N ratio. These findings provide novel insights into the feasibility of using cassava dregs as a carbon source in biofloc aquaculture and reference the theory of BFT using the latest microbial identification technology and analysis methods.

2. Materials and methods

2.1. Materials

L. vannamei were purchased from the breeding base of the Beihai Fishery Seed Co. Ltd., Guangxi, China. Healthy shrimp of similar size were chosen. The initial body weight and body length of the shrimp were 8.01 ± 0.14 g and 9.03 ± 0.20 cm, respectively. Before the formal experiment, the shrimp were fed basic feed for 14 days to adapt to the environment and feed. The diet consisted of commercial shrimp feed with a crude protein level of 42%. The feeding levels accounted for 3% of the shrimp body weight per day. To balance the C/N ratio and encourage the development of heterotrophic bacteria in the BFT, three carbon sources were added to obtain a C:N of 20:1 (Avnimelech, 1999). In addition, the experimental groups were fed basic feed supplemented with sucrose (BFT1), cassava dregs (BFT2) and cassava dregs treated with enzymes (BFT3), moreover, the control was fed without an additional carbon source. The calculation of the C:N ratio considered the nitrogen content in the feed, the quantity of food distributed in the tanks and the rate of nitrogen excretion by the shrimp, as mentioned in the description the biofloc culture method (Hari et al., 2006). The amounts of sucrose, cassava dregs and enzyme-hydrolyzed cassava dregs were 190%, 206% and 206% of the shrimp feed, respectively (Wang et al., 2015).

Cassava dregs were provided by the Nanning Fushu Starch Factory, Nanning, China. The water content of the fresh cassava dregs was 70%. We sterilized the cassava dregs for 30 min at 121 °C in high-temperature and high-pressure sterilization pots and dried the cassava dregs for 2 h at 120 °C in a thermostatic drum wind drying oven. A 20–40 mesh screen was used to filter the cassava dregs after processing in a crusher. After physical processing, the cassava dregs were hydrolyzed for 24 h at 60 °C by cellulase, which had an enzyme activity of 400 U/mg; for 12 h at 90 °C by α-amylase, which had an enzyme activity of 4000 U/g; and for 6 h at 60 °C by a saccharifying enzyme with an enzyme activity of 100,000 U/mg (Table A.2). The reducing sugar contents of the sucrose, cassava dregs, and enzyme-hydrolyzed cassava dregs were 100%, 14.7% and 83.8%, respectively.

2.2. Experimental setup

The aquaculture experiments were performed at the aquaculture center of the School of Marine Sciences, Guangxi University. Twelve