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Bioremediation of synthetic intensive aquaculture wastewater by a novel feed-grade composite biofilm

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ABSTRACT

The current challenge in intensive aquaculture is to control the level of nutrient pollutants in the wastewater and provide sustainable sources of proteins for feed. A synthetic lichen type biofilm was developed to have the fungus *Mucor indicus* and the microalga *Chlorella vulgaris* grow together on a polymer matrix referred to as a “mycoalgae” biofilm. When the biofilm grows, it takes up phosphorus and nitrogen compounds and converts them to proteins and other cellular products. It cleans the water from nutrient pollutants as the algae are attached to the biofilm leaving purified water at the end of the process. Under 25 mg L^{-1} total ammonia-N (TAN) conditions, the biofilm reduced TAN to undetectable limits within 48 h with over 69% of the TAN reduction taking place by 24 h. The biofilm reduced levels of phosphate-P from 15 mg to undetectable limits within 24 h. Under the same conditions, 860 mg of dry mycoalgae biomass was generated at the end of the process on 16 cm^2 of mesh and 100 ml of culture media. This process allows for easy harvesting of the algae with no energy intensive process of separating the algae from the supernatant. The generated biofilm is composed of two organisms that have been shown to positively aid fish health when included as a feed supplement.

1. Introduction

Aquaculture has grown at an impressive 8% rate each year over the past three decades, which is critical as fish represent 16.7% of all animal protein consumed globally, meeting food security needs (Fisheries, 2016). The main drawback is that the formulated fish feeds in aquaculture rely heavily on wild caught forage fish to provide amino acids that are essential for proper fish nutrition. Wild capture fisheries output has plateaued over the past 20 years while aquaculture supplied fish has steadily increased (Fisheries, 2016). Additionally, significant nutrient pollution can take place when wastewater from aquaculture farms are discharged directly to receiving bodies of water in the forms of nitrogen and phosphorus (Hu et al., 2013). The challenge in intensive aquaculture is to produce fishes sustainably without nutrient pollution, and recycling the nutrients as microbial proteins in a way to close the nutrient cycle, which is addressed in this research.

Recirculating aquaculture systems (RAS) were widely researched as a possible solution in intensive aquafarming in order to recycle the water in a multi-step process (solids removal, biological TAN removal, CO_2 removal, and oxygenation), but nitrogen is “wasted” by nitrification/denitrification (Van Den Hende et al., 2011) as a large portion of the added feed goes uneaten. Water quality deterioration is caused by high concentrations of excreted metabolites and low feed utilization. Uneaten feed and waste can accumulate in the water supply when the water is not cleaned properly which will negatively impact fish and ecosystem health. Major concerns with aquaculture wastewater include removal of dissolved organic matter and nutrients, specifically: suspended solids, oxygen depletion, nitrogen (particularly un-ionized ammonia and nitrate), and phosphorus. Bio-filters, which remove nitrogen compounds from water, also produce N_2O, a powerful greenhouse gas with a global warming potential 310 times that of CO_2. Production of N_2O-N from aquaculture in 2003 accounted for 5.72% of all anthropogenic N_2O-N produced (Hu et al., 2012, 2013). If aquaculture as an industry continues to grow at a comparable rate, then understanding and limiting of N_2O must be performed. While nitrifying biofilters are effective at reducing the levels of total nitrogen in the water they are inefficient and do not allow for recovery of nitrogen compounds. Recent advancements in aquaculture include the development of Biofloc technology (Fareza Hanis Mohd Yunos et al., 2017) for nutrient recycling as animal feed. The system maintains a consortium of microscopic organisms that grow in the same culture tank as the aquaculture species. The microbes consume excreted organic waste and convert TAN and other organic waste products to microbial biomass (Rajendran and Hu, 2016; El-Sheekh et al., 2013; Perazzoli et al.,

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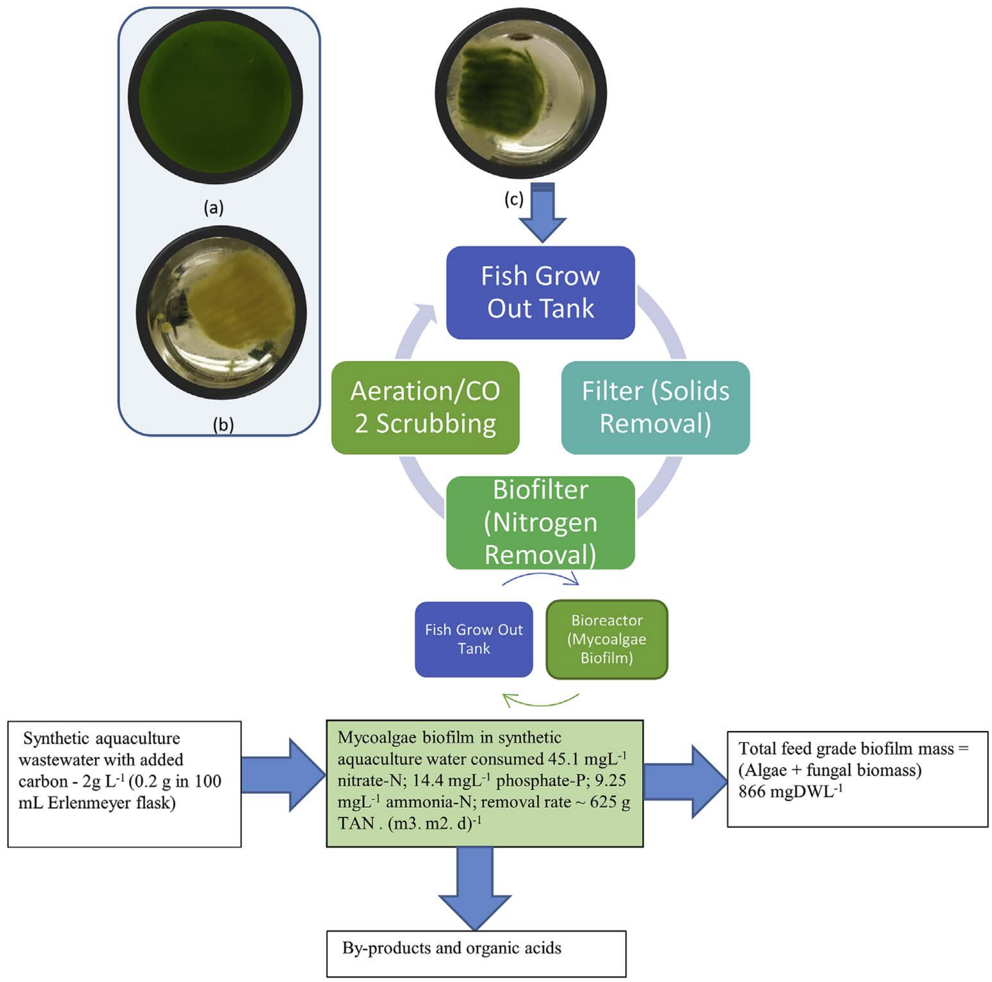

Fig. 1. A picture of the (a) suspended algae culture (b) fungal biofilm and (c) mycoalgae biofilm, in the presence of a matrix in medium (Pictures taken from the bottom of the flask).

2016). The microbial biomass is taken by the reared aquaculture species thus completing the nutrient cycle. This system needs energetically intensive aeration and mixing in order to keep particles in suspension as well as to ensure adequate levels of aeration for the reared aquatic species. Bioflocs also require intervention through either water exchange or drainage of sludge when suspended solids concentrations become too high (Crab et al., 2012).

Most commercial fish feed has a protein content of 28–50% depending on the growth stage of the reared fish. Fry and fingerling stages require a higher percentage of protein (35–50%) in their diet; while fish in the grow-out stage of production require lower amounts of protein (28–32%) (Ng, 2013). Historically fish meal and other fish derived products have made up the bulk of the protein content in fish feed as they possess a complete mixture of essential amino acids and other required nutrients. The main drawback of using fish as a major component of fish feed is that is a non-sustainable resource, if aquaculture is to grow as an industry, there is an urgent need to find other sustainable and scalable protein sources. Other protein sources have been utilized e.g. soybean meal, poultry byproducts, as well as many other vegetable and animal byproducts. These alternative feeds do have drawbacks, as they can contain anti-nutrients or they lack essential amino acids required for robust growth (Spinelli, 1980). One issue common to grains, legumes, and oil seed protein sources is the content of phytic acid. Phytic acid is a storage molecule for phosphorus; when it is not digested it passes through the gastrointestinal tract intact and leads to increased levels of phosphorus in wastewater. Phytic acid also binds calcium, iron, and zinc which negatively impact the growth rate of reared fish as they cannot metabolize these minerals readily without the enzyme phytase added to the feed, which serves to break down the phytic acid and makes the phosphorus bioavailable (Dendougui and Schwedt, 2004). Incomplete feeds are generally fortified with wild caught forage fish, which poses a problem as the rate of wild capture fish, has plateaued. If we want to see an increase in aquaculture reared fish, we therefore must find an alternative protein source that is sustainable, renewable, and nutritionally complete for reared fish.

A novel biofilm showing promising results in removing different forms of N, P, suspended solids and other organics from simulated aquaculture waste water in a single-step reactor with easy biomass recovery and high efficiency was developed recently at the University of Minnesota (Rajendran and Hu, 2016). The biofilm, formed by the fungus *M. indicus* along with the unicellular microalgae *C. vulgaris* on a polymer surface, exhibits complete attachment to the polymer surface, leaving behind clean water for recirculation and the composite biomass for nutrient recycling. The fungal and algal species are generally regarded as safe (GRAS) and have been shown to have positive effects on fish growth parameters and are readily eaten as part of a formulated diet (Cheunbarn and Cheunbarn, 2015; Karimi and Zamani, 2013). The successful demonstration in lab scale to remove the nutrients from synthetic aquaculture effluent using this composite biofilm will facilitate developing a consolidated single step bioreactor converting pollutants to proteins. Thus limiting nutrient pollution while sustainably providing healthy fish feed through recycling of nutrients. The literature on aquaculture wastewater was consulted and compiled to specify a range of working TAN concentrations that might be typical in various aquaculture environments. In this current work, the mycoalgae biofilm was developed in the synthetic aquaculture water, and different levels of TAN was tested to illustrate the nutrient removal efficiency in the typical intensive aquaculture and shrimp wastewater which contained