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Production of algal biomass, chlorophyll, starch and lipids using aquaculture wastewater under axenic and non-axenic conditions

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ABSTRACT

Although co-cultivation of algae with aquaculture products has the potential to reduce water use and pollutant discharges while producing energy feedstocks and other end-products, little research has been carried out in this area. Maintaining axenic conditions (algal monocultures without other microorganisms) in algal culturing systems using aquaculture wastewater as a nutrient source would not be practical. This study examined the effects of the use of aquaculture wastewater as a nutrient source on biomass development of three algae cultures (indigenous mixed species consortium, *Chlorella* sp. and *Scenedesmus*) under axenic and non-axenic conditions. Biomass development was assessed by cell growth, chlorophyll, starch and lipid production. The presence of aquaculture microorganisms decreased biomass productivity in *Chlorella* but not in the other algae cultures. Non-axenic conditions had no effect on overall starch and chlorophyll production; however, significantly higher lipid contents were achieved under non-axenic conditions for *Chlorella* and the indigenous culture. The higher algal lipid content for these cultures under non-axenic conditions may have been due to competition with bacteria for nutrients. The presence of bacteria was required for effective removal of organics, while effective nitrogen removal was observed in all systems containing algae. Results from this study also show that algae harvesting should be timed to coincide with the peak production of the desired target end-product (biomass, chlorophyll, starch or lipid).

1. Introduction

The aquaculture industry has grown to meet increasing worldwide fish and protein demands [1]. As the scale and intensity of production increase, the volume and concentration of pollutants in the wastewater from aquaculture systems also increase. In addition, there is increasing emphasis on the need for aquaculture facilities to meet effluent standards for wastewater contaminants, such as solids organics, nitrogen (N) and phosphorus (P). However, conventional wastewater treatment processes have high capital, energy and chemicals costs and do not recover nutrients to produce useful or commercially viable end-products. Therefore using an integrated, biological approach that facilitates energy and cost savings and produces useful end-products, such as algal biomass, and intracellular products should be favored [2,3].

Aquaculture wastewater has been used previously to support symbiotic photoautotrophic growth using various co-cultivation approaches, such as aquaponics [3–6]. A potential alternative for integration of algae cultivation with aquaculture is shown in Fig. 1. Algal co-cultivation may be more advantageous than aquaponics because it has the potential to improve water quality and increase dissolved oxygen concentrations, which improves the target species' health, while producing a feedstock for onsite energy production and/or feed supplementation [3–5,7,8]. Drapcho and Brune [5] used algae in a partitioned aquaculture system to reduce ammonia concentrations and increase dissolved oxygen concentrations required for fish health. Haglund and Pedersén [7] used macrospecies algae, *Gracilaria tenuistipitata*, for wastewater treatment and epiphyte control in a rainbow trout system. Several prior studies produced algae for use as an onsite aquaculture feed supplement and found that algae grown on aquaculture wastewater had higher growth rates and protein contents and were more nutritious (containing a more complete amino acid profile) than non-leguminous plants such as oats, barley and rye [3,8–10]. Bioflocs technology (BFT) is an example of co-cultivation that takes advantage of the synergy between aquaculture, algae and microorganisms [6]. Bioflocs are an aggregate combination of heterotrophic bacteria, algae, colloidal particles and polymeric substances that can be used to supplement fish feed. The process also facilitates N immobilization and recovery [11].

The use of aquaculture wastewater as a nutrient feed for algae production increases the chances of contamination by microorganisms and non-target algal species. Many prior studies of algae photobioreactor systems have used axenic conditions (i.e. algal monocultures without other microorganisms) [12–15]. However, it would not be practical or economically

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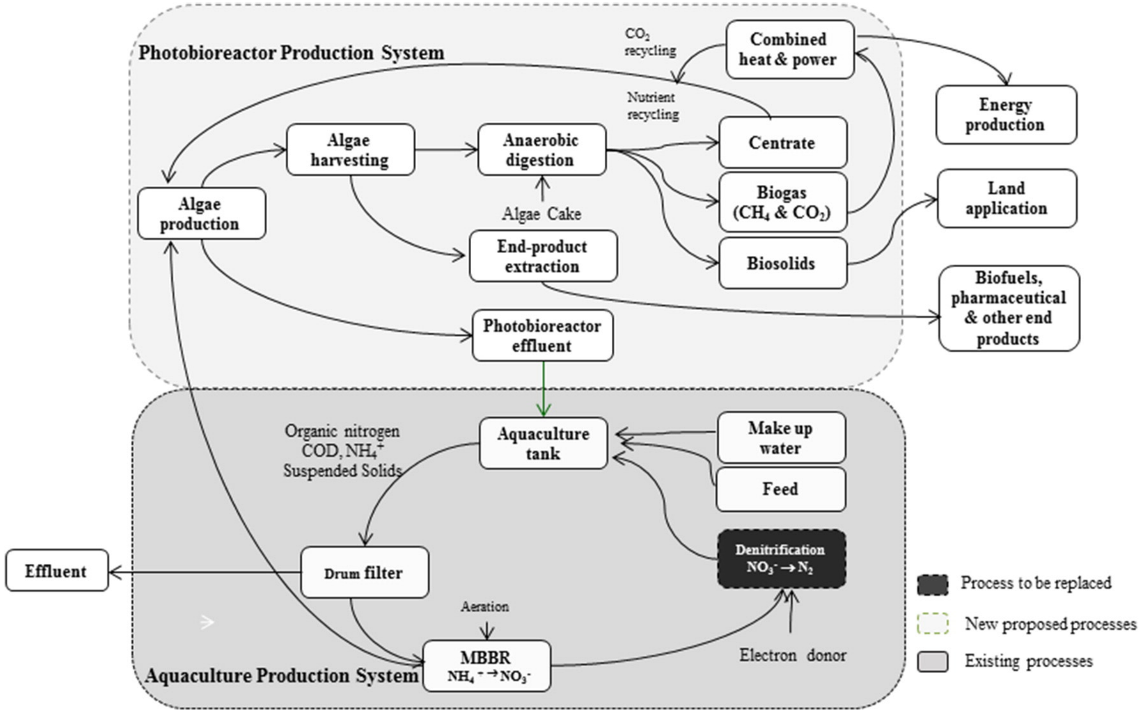

Fig. 1. Proposed integration of algae co-cultivation with aquaculture.

viable to maintain axenic conditions in large-scale open pond systems [13,14]. Non-target algae, bacteria or protozoans may compete with the target algal species for nutrients and light or may be toxic or predatory in nature [13,15–17]. However, some prior studies have shown that the presence of bacteria can improve algae production by making the system more resilient [7,17,18] (i.e., able to maintain its function although external stress and disturbances were present [18]). This increased resilience may be due to the ability of indigenous microorganisms to: 1) mineralize organic substrates to inorganic forms that are more bioavailable to algae [19,20]; 2) produce growth factors and micronutrients that support algal growth; and/or 3) convert toxic ammonia to nitrite and nitrate through nitrification [21–23]. In addition, the use of algae–bacteria consortia has the potential to reduce downstream processing costs. When cultures contain a mixture of algae and bacteria, algal cells have been shown to produce a matrix of carrageenan or alginate, which facilitates autoflocculation [24].

This is the first peer reviewed study to examine how indigenous microorganisms present in aquaculture wastewater affect algal biomass and end-product production. Three algal cultures were studied: a mixed indigenous consortium and pure cultures of *Chlorella* and *Scenedesmus*. The effects of axenic and non-axenic conditions on the ability of the system to maintain function and resilience were assessed. Two success criteria were used to examine system resilience: productivity of desirable end-products (biomass, chlorophyll, starch and lipids) and removal of nitrate and organic matter from the wastewater.

2. Materials and methods

Experiments were conducted at the Norwegian University of Life Sciences (UMB), Ås, Norway. Algae biomass, chlorophyll, starch and lipid production were investigated using wastewater from a recirculating aquaculture system (RAS). Algal system performance was compared under axenic and non-axenic conditions for an indigenous consortium and two pure algae cultures.

2.1. Aquaculture wastewater feed

Approximately 10 L of wastewater was collected from a UMB campus tilapia RAS, which has a total volume of 4200 L. The flow rate in the RAS was approximately 150 L/min, with 98–99% recirculation. The RAS included a drum filter with a 40 micron screen mesh size (Hydrotech HDF 501) and a moving bed bioreactor (MBBR) containing extruded plastic media for nitrification. The mean annual tilapia biomass produced was 300 kg/year. Tilapia are fed Aller 37/10 FLOAT daily, which has a protein content of 37%. For the axenic treatments, aquaculture wastewater was filter sterilized using a 0.2 μm glass fiber filter (AP 1504700). In order to

Table 1
Aquaculture wastewater feed characteristics.

Mean concentrations	Axenic	Non-axenic
TN (mg/L)	17.9	18.5
NO_3^- (mg/L as N)	17.6	18.1
COD (mg/L)	238	253
TP* (mg/L)	17.0	17.5
PO_4^{3-}-P* (mg/L)	16.9	17.1
pH	6.94	6.97
Transmissivity (%)	99.0	97.8
HPC (CFU/100 mL)	0	183
Potassium (K) (mg/L)	66	65
Calcium (Ca) (mg/L)	62	64
Sodium (Na) (mg/L)	21	21
Sulfur (S) (mg/L)	15	16
Magnesium (Mg) (mg/L)	10	11
Iron (Fe) (mg/L)	0.016	0.069
Zinc (Zn) (mg/L)	0.011	0.022
Copper (Cu) (mg/L)	0.006	0.007
Manganese (Mn) (mg/L)	0.002	0.003
Aluminum (Al) (mg/L)	<MDL	0.006

*TP and PO_4^{3-}-P concentrations given after supplementation with 15 mg/L of TP.
MDL = method detection limit.