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# Microalgal cultivation using aquaculture wastewater: Integrated biomass generation and nutrient remediation

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ABSTRACT

Microalgal cultivation using aquaculture wastewater is a promising biorefinary concept for integrated biomass generation and subsequent nutrient removal. In this study, potential of aquaculture wastewater (AWW) as a nutrient substrate for cultivation of *Scenedesmus obliquus*, *Chlorella sorokiniana* and *Ankistrodesmus falcatus* was investigated. Nutrient removal efficiencies were also investigated for selected microalgal strains. Sodium nitrate supplementation strategy is applied to enhance the productivities of biomass, lipid, carbohydrate and protein. Biomass productivities of *A. falcatus* (198.46 mg $L^{-1}$ $d^{-1}$) with 400 mg $L^{-1}$ sodium nitrate supplementation and *C. sorokiniana* (157.04 mg $L^{-1}$ $d^{-1}$) with 600 mg $L^{-1}$ sodium nitrate supplementation in AWW were comparable to the synthetic medium. Comparable lipid, carbohydrates and proteins productivities were observed in microalgal biomass cultivated using AWW to the productivities in the synthetic medium. Microalgal cultivation in AWW showed removal efficiencies in the range of 86.45–98.21% for ammonia, 75.76–80.85% for nitrate, 98.52–100% for phosphate and 42–69% for COD.



## 1. Introduction

Microalgae have attracted much attention from researchers, government bodies and industry due to their ability to remove nutrients from wastewater with the concomitant production of valuable biomass [1,2]. Microalgae are single cell photosynthetic organisms that utilize solar light energy, inorganic nutrients and environmental $CO_2$ (carbon dioxide) to generate biomass that can be used as a feedstock for biofuels, animal or aquaculture feed and other value added products [1]. Microalgae offers attractive advantages such as minimal requirement of arable land, fast growth rate than terrestrial plants, utilization of wastewater, $CO_2$ sequestration. Microalgae can be cultivated at large scale in closed systems (photobioreacors) or open systems (raceway ponds) to improve the productivity for desired products [3]. Microalgae have great potential for the production of high amount of lipids, proteins, carbohydrates, pigments etc. [4,5]. The yields of these products from microalgae are species dependent and could be improved by inducing stress and adapting certain cultivation strategies. Microalgal lipids can be converted to fatty acid alkyl esters (biodiesel). Whole algal biomass or lipid extracted algal biomass can be used as a feedstock for biomethane, biohydrogen, bioethanol, biobutanol and syngas production [6–9]. Whole and lipid extracted microalgal biomass are rich in proteins, reduced sugar, carbohydrates, pigments and thus can be used as a supplement or whole feed for human nutrition and in feed industry for animal and aquaculture [4].

Despite of the several benefits microalgae offer, its commercial scale production is still challenging due to the high production cost. Nutrients supplied in form of chemicals are major contributor towards the cost of production. Utilization of nutrients from the waste streams could improve the economics of microalgal biomass generation [10,11]. Microalgae are natural food of aquaculture ecosystem and widely used for larvae, crustacean and molluscs [12]. Microalgae based feed can provide proteins, essential amino acids, fatty acids, pigments etc. required for high quality aquaculture produce. Aquaculture and microalgae could form a promising biorefinary where the wastewater generated in aquaculture can be utilized for biomass generation and generated biomass can be directed towards the aquaculture feed production. Use of aquaculture wastewater minimizes the dependency of microalgae cultivation on fresh water and chemical nutrients. The generated biomass can also be used for various other purposes such as biodiesel, biomethane generation. Aquaculture wastewater has nutrients in the range of 100–150 mg $L^{-1}$ for COD, 3–7 mg $L^{-1}$ for ammonia, 2–110 mg $L^{-1}$ for nitrates and 2–50 mg $L^{-1}$ for phosphates [13–15]. Most of other agricultural and food industry wastewaters are comprised of high concentrations of these nutrients. Swine wastewater has COD of 500–60,000 mg $L^{-1}$, ammonia of 80–4800 mg $L^{-1}$ and phosphates 30–190 mg $L^{-1}$ [13]. Dairy wastewater has COD of 900–38,000 mg $L^{-1}$, ammonia of 130–2200 mg $L^{-1}$ and phosphates 30–300 mg $L^{-1}$ [13]. High nutrient concentrations in WW, limit light penetration due to turbidity

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and often result in toxicity, negatively impacting microalgal growth. Dilution prior to use as a microalgal growth medium is usually required in order to overcome these challenges. Conversely aquaculture wastewater contained an intermediate range of all the nutrients and can therefore be used directly for microalgal cultivation.

According to FAO (2014), the Aquaculture industry is one of the fastest growing food production industries globally, with 34% growth from 2006 to 2011. This fast growing industry also leads to production of huge amount of nutrient rich aquaculture wastewater and brought several negative impacts to the environment and economics. The aquaculture wastewater comprised of nitrogenous components like ammonia, nitrite, nitrate, phosphorus and organic carbon [14,16–18]. Discharge of this untreated aquaculture wastewater in clean water body could cause eutrophication which deteriorate the natural ecosystem [14]. To improve the economic prospects and sustainability of aquaculture industry it is important to focus on treatment of aquaculture wastewater and its reuse. Many methods have been developed for treatment of aquaculture wastewater such as denitrification process employed for release of nitrogenous compound in atmosphere [17], Chemical precipitation used to remove phosphorus by using ferrous chloride [19]. These methods are cost incurring and also produce toxic compounds as byproducts [14]. Bioremediation of aquaculture wastewater by microalgal cultivation is emerging technology. This technique could be cost effective, environmentally friendly and also produce valuable biomass. Microalgae utilize the nutrients present in aquaculture wastewater and convert them into biomass. The cultivation of algae in aquaculture wastewater provide dual advantages such as treating wastewater to reduce cost of wastewater treatment and produce cell biomass that are rich in lipids, proteins, carbohydrates and many other value added products [20].

Microalgal cultivation using domestic wastewater has been investigated extensively. However, very few studies explore the potential of using aquaculture wastewater for microalgal biomass propagation. Most of the previous studies focus either on nutrient removal or the biomass generation and do not elucidate the biochemical composition of the biomass. The present study provides a comprehensive account of this proposed biorefinary concept exploring the potential strains, biomass enhancement strategy, nutrient removal potential and biochemical composition of the microalgal biomass. In this study, *Scenedesmus obliquus*, *Chlorella sorokiniana* and *Ankistrodesmus falcatus* were grown using aquaculture wastewater (AWW). Nutrient removal potential is evaluated for selected microalgal strains. Nitrate supplementation strategy is also investigated for improvement of biomass, lipids, carbohydrates and protein yields of microalgae grown using aquaculture wastewater. The nitrate supplementation strategy developed in this study for effective use of aquaculture wastewater as a microalgal nutrient medium has not been reported in previous literature. This strategy can be easily applied at commercial scale for microalgal cultivation using aquaculture and other similar wastewater streams for efficient and economical microalgal biomass generation.

## 2. Materials and methods

### 2.1. Wastewater collection and characterization

The aquaculture wastewater was collected from aquaculture research facility, Durban, South Africa. In this aquaculture research facility Nile tilapia were reared in 5000 L black painted tanks in controlled temperature (27–32 °C) with continuous aeration. On regular interval water was recycled via biofiltration to remove nutrients and organic load. The wastewater used for current study was collected from the collection tank before the treatment. Aquaculture wastewater was brought to laboratory in 25 L containers and transported to laboratory within 30 min. Collected wastewater was stored in cold room at 4 °C for further utilization.

### 2.2. Wastewater analysis

The pH, electrical conductivity, temperature, salinity, dissolve oxygen (DO), dissolve oxygen percentage were measured at the time of sample collection by YSI MP - AES. The total solid and total dissolve solid were calculated according to APHA 2005 [21]. The chemical oxygen demand (COD) was analyzed by closed refluxed method. Centrifuged sample were used to determined ammonia ($NH_4^+$), nitrates ($NO_3^-$), nitrites ($NO_2^-$) and phosphates ($PO_4^{3-}$) by Gallery™ Automated Photometric analyzer (Thermo Scientific, USA). For the heavy metals analysis, sample were digested in microwave (Milestone S.R.L., Italy, output power 1200 W) at 180 °C for 20 min at 1000 W using acid mixture (15 mL $HNO_3$ and 4 mL $HCLO_4$). After cooling of digested sample the solution was allowed to evaporate until the volume reduced to 5 mL. The remaining portion of the sample was filtered and further diluted to 50 mL using deionized water for heavy metals analysis using microwave plasma atomic emission spectrometry (Agilent Technologies 4200 MP-AES).

### 2.3. Microalgal cultivation

*Chlorella sorokiniana*, *Scenedesmus obliquus* and *Ankistrodesmus falcatus* were selected for cultivation using aquaculture wastewater (AWW). The AWW was filtered using 0.45 μm filter papers followed by autoclaving prior to microalgal inoculation [14]. The initial concentration of inoculum for *C. sorokiniana* was 0.22 g $L^{-1}$; for *S. obliquus* was 0.19 g $L^{-1}$ and *A. falcatus* was 0.2 g $L^{-1}$ respectively. The microalgal cultures were maintain in 1 L conical flask with 500 mL working volume. Cultivation conditions were temperature 25 °C at 120 mol $m^{-2}$ $s^{-1}$ light intensity under a 16:8 light dark cycle [22]. These conditions were kept constant for all the experiments. Microalgae were also cultivated in BG11 nutrient medium for comparative analysis. Each set of experiment was done in duplicate for 14 days cultivation period. The flasks were placed on continuous orbital shaker (110 rpm) throughout the experimental period. Supplementation experiments were carried out with selected microalgal strains. For supplementation experiments 200, 400, 600 and 1500 mg $L^{-1}$ sodium nitrate was added in AWW.

### 2.4. Analytical methods

#### 2.4.1. Growth, photosynthetic performance and biomass analysis

Microalgal growth was monitored daily at 680 nm using spectrophotometric method. Biomass was estimated by gravimetric method at early, middle and late log phases of microalgal growth. Biomass productivity (mg $L^{-1}$ $d^{-1}$) was calculated at late log phase gravimetrically using Eq. (1) [23]. Physiology of photosynthesis was analyzed by pulse amplitude modulated (PAM) fluorometer. Quantum efficiency of photosystem II (Fv/Fm) and relative electron transport rate (rETR) were determined as describe by Ramanna et al. [24]. Biomass was harvested using centrifuge and freeze dried using lyophilizer (Mini lyotrap, LTE scientific Ltd., United Kingdom) for further analysis.

$$\text{Biomass productivity}\left(\text{mgL}^{-1}d^{-1}\right) = \frac{\text{Biomass yield}\left(mgL^{-1}\right)}{\text{Number of days}} \quad (1)$$

#### 2.4.2. Nutrient removal

The nutrients removal efficiency was monitored every 48 h. Briefly, 10 mL of sample were collected from culture flask and centrifuged followed by filtration using 0.45 μm syringes filter. These samples were used for analysis of nitrates ($NO_3^-$), nitrites ($NO_2^-$), total oxidizable nitrogen (TON), ammonia ($NH_4^+$) and phosphates ($PO_4^{3-}$) using Gallery ™ Automated Photometric analyzer (Thermo Scientific, USA) [25]. The chemical oxygen demand (COD) was analyzed by closed refluxed