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Research paper

Reverse osmosis concentrate conditioning for microalgae cultivation and a generalized workflow

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ABSTRACT

Previous studies have demonstrated the feasibility of using wastewater as the nutrient source for microalgae cultivation. However, there is still a lack of guidance in wastewater conditioning to achieve optimum microalgae growth rate and complete N and P consumption in wastewater. In this study, a reverse osmosis concentrate (ROC) from the wastewater treatment unit of a dyeing company had been evaluated and conditioned for the phototrophic cultivation of *Chlorella* sp., with its toxicity, nutrient profile, salinity, pH and N/P ratio adjusted where necessary. After the wastewater was conditioned, *Chlorella* sp., which could not grow in the original ROC, achieved a growth rate comparable to that in an artificial medium. The percentages of N and P consumption were also balanced so that the nutrient utilization efficiency could be maximized. A generalized workflow and guidelines to develop wastewater conditioning plans for microalgae cultivation have also been proposed to guide the conditioning of wastewater streams for microalgae cultivation.

1. Introduction

Phototrophic microalgae cultivation has raised great interest as it allows CO_2, a greenhouse gas, to be converted into biomass which is an ideal feedstock for biofuels and nutritious food. Compared with the photosynthetic terrestrial plants, microalgae have much higher CO_2 conversion efficiency and do not require arable land [1–3]. However, the current cost of microalgae production is still high, partly due to the high nutrient cost in microalgae cultivation. Water and commercial fertilizer are estimated to account for 10–30% of the total cost in microalgae cultivation [4]. Therefore, to achieve affordable microalgae products, low-cost nutrient sources are indispensable.

Wastewater with nitrogen (N) and phosphorous (P) can be an ideal nutrient source for microalgae cultivation. In a study, *Chlorella* sp. was mixotrophically cultivated in wastewaters sampled from a municipal wastewater treatment plant [5]. The average specific growth rates of *Chlorella* sp. were 0.412, 0.429, 0.343 day^{-1} in the samples taken before primary settling, after primary settling, and after activated sludge tank, respectively. This value doubled to 0.948 day^{-1} when it came to the sample taken from the supernatant of the sludge centrifugation tank due to the higher nutrients concentration. The author also found that microalgae were more suitable to be used for N, P, and heavy metal removal from wastewaters than COD removal, which was probably because microalgae actually excrete small photosynthetic organic molecules during their growth. In another study, Lim et al. [6] suggested that using Bold's Basal Medium to enrich a treated textile wastewater could improve *Chlorella* sp. growth in it. The highest specific growth rate of 0.21 day^{-1} was achieved with 20% artificial medium supplemented. Apart from the wastewaters aforementioned, *Chlorella* sp. had also been successfully cultivated in domestic secondary effluents, diluted primary piggery wastewater, equalized soybean processing wastewaters, among others [7–9]. Similar studies have also been conducted to demonstrate the growth and nutrient removal performance of other axenic microalgae cultivation in wastewaters, such as *Nanochloropsis* sp. in untreated municipal wastewater, *Auxenochlorella* sp. in concentrated municipal wastewater, *Aphanothece* sp. in equalized fish processing wastewater, and *Scenedesmus* sp. in secondary effluent from a domestic wastewater treatment plant [10–13]. On top of axenic cultures, a consortium of three mixotrophic algal strains was cultivated in polybags using an untreated wastewater from carpet industry as the nutrient source [14]. An overall areal biomass productivity of 21.1 g m^{-2} d^{-1} was

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achieved, which could lead to a potential biomethane productivity of 12.13 $dam^3\ ha^{-1}\ y^{-1}$.

Despite the various types of wastewater studied, there have not been many studies using reverse osmosis concentrate (ROC) for microalgae cultivation [15]. Reverse osmosis is commonly used in wastewater treatment plants to remove ions from secondary treated wastewater to produce reuse water for the manufacturing process. The concentrate stream that does not pass through the membrane has a high concentration of ions. This stream is barely treated and often left to evaporate in the industry as the treatment cost would be high. However, similar to the aforementioned centrate of sludge centrifugation, ROC can be good nutrient sources for phototrophic microalgae growth, as it contains high concentrations of bioavailable N, P and metal ions [16,17]. Because carbon is often supplied by a polluted gas stream containing CO_2 in phototrophic microalgae cultivation, ROCs are ready to supply the majority of nutrients required by phototrophic microalgae if not all of them. Also, there is little suspended solids in ROCs so that there is no need to do filtration before use, and the chance of severe bacterial contamination during microalgae cultivation is rare [18,19].

The use of ROC for phototrophic microalgae cultivation was evaluated in this study. The purpose of this research is to answer how to condition a certain type of ROC for the best of microalgae growth as well as maximize the removal of nitrogen and phosphorous. *Chlorella* sp. was used as the model species in this study. Different from previous studies where the use of a wastewater for microalgae cultivation was completely based on trial and error, this work proposed to first acquire the key medium requirements of the microalgal species to be cultivated, namely, the toxicity limit, essential trace elements, required salinity, initial pH, and desired N/P ratio. The effect of these parameters on microalgae cultivation are summarized below.

In addition to carbon (C), N, P, and H_2O required by all microalgal species to form the basic building blocks of cells, trace elements such as metal ions are also essential for microalgae growth. A trace element that is vital to the microalgae or can significantly reduce the microalgae growth rate if absent is considered to be essential for microalgae cultivation [20]. The essential trace element profile is different among microalgal species, and an artificial medium often provides more elements than that are essential to a specific microalgal species, as it is intended to be applicable to as many microalgal species as possible. The essential trace elements required by *Chlorella* sp. have to be determined through experiments, as this is critical to determine if a wastewater contained enough nutrients for *Chlorella* sp. cultivation and the minimum number of trace elements to be replenished.

Salinity is a parameter defined based on the electrical conductivity of a solution. It also represents the osmotic pressure of the solution and affects the metabolic network of a microorganism. For microalgae, high medium salinity can be dangerous as exosmosis may occur and both cellular substrates and metabolic pathways could be damaged [21]. Although marine microalgae can usually live under a salinity range higher than the freshwater microalgae can do, there is still an upper limit [22,23]. On the other hand, low salinity may cause reduced growth rate in some microalgal species due to the disturbed ion flux at the cell boundaries [24].

The initial pH of the medium affects microalgae growth through influencing the enzymatic activities of microalgae. Some microalgae can only survive within a narrow range of pH, while others may have a greater tolerance and survive over a wider range of pH [25,26].

Nitrogen and phosphorous are the basic nutrients for microalgae growth. Although microalgae can usually grow over a wide range of N/P ratio, only if the N/P ratio in the wastewater matched the N/P uptake ratio for microalgae growth could both N and P be fully consumed. If the N/P ratio of a wastewater stream is much higher than the uptake ratio, N in the wastewater cannot be fully consumed as microalgae growth is limited by P. Similarly, if the N/P ratio of a wastewater stream is much lower than the uptake ratio, P will be left in the wastewater by the time N is completely used up. Therefore, an optimal N/P ratio is preferred so that N and P in a wastewater stream can be utilized to the largest extent by the microalgae. Note that the N/P uptake ratio of different microalgal species may vary due to their different cell compositions [27].

With the knowledge of the above nutrient requirement of a specific microalgal species, the potential outcome of using the characterized wastewater stream for cultivating the microalgal species could be estimated, and wastewater conditioning measures could be proposed to improve the specific growth rate where necessary.

In addition, a workflow for wastewater conditioning has been developed to facilitate future studies of microalgae cultivation using wastewater. Following this generalized workflow, many sets of medium requirements of different microalgal species could be collected to set up a database. The future queries of using wastewater for microalgae cultivation can simply compare the characterized wastewater features with the corresponding data in the database, and then, one can easily determine if a wastewater is suitable for cultivation of a specific microalgae, or which microalgae species can make the best use of the nutrients in a given wastewater steam. Surely, the required wastewater conditioning measures to improve the specific growth rate and nutrient removal efficiency are ready to be designed as well.

2. Materials and methods

2.1. Reverse osmosis concentrate

The ROC from a wastewater treatment unit in Guangdong, China, was considered for *Chlorella* sp. cultivation. The wastewater treatment unit received dyeing wastewater and treated it with advanced oxidation processes and reverse osmosis. The reverse osmosis unit was designed to reuse 60% of the influent. A sample of the ROC stream was characterized. COD and pH value of the ROC was 120 mg L^{-1} and 8.0, respectively, and the ROC appeared transparent. In addition, it contained sodium (Na), calcium (Ca), potassium (K), copper (Cu), chloride (Cl^-), sulfate (SO_4^{2-}), nitrate (NO_3^-), phosphate (PO_4^{3-}), silicate (SiO_3^{2-}), and fluoride (F^-) in the concentrations listed in Table 1.

2.2. *Chlorella sp.* and preparation of the inoculum

Chlorella sp. used in this study was obtained from Division of Life

Table 1
Characteristics of the ROC.

COD (mg L^{-1})	120
pH	8.0
Color	10
Salinity (g kg^{-1})	26.78
Ion concentration (mg kg^{-1})	
Sodium (Na^+)	2562.2
Calcium (Ca^{2+})	94
Potassium (K^+)	201.2
Copper (Cu^{2+})	12.8
Chloride (Cl^-)	3350
Sulfate (SO_4^{2-})	1171.2
Nitrate (NO_3^-)	310
Phosphate (PO_4^{3-})	47.5
Silicate (SiO_3^{2-})	76
Fluoride (F^-)	1.9