Comparative analysis of two microalgae-bacterial symbiotic association growth in papermill effluent enriched through food waste digestate

TALAPATRA K N[1], GHOSH U K[2,*]

Department of Polymer and Process Engineering, IIT Roorkee Saharanpur Campus, Saharanpur – 247001, India

*corresponding author: U K Ghosh
e-mail: namitatalapatra@pp.iitr.ac.in

Abstract New treatment methods are needed to better recycle waste towards the circular economy. Microalgae can be used as an asset to treat effluent and simultaneously produce biofuel. This paper aims to evaluate the growth of microalgae-bacterial symbiotic association in papermill effluent enriched through food waste digestate. Besides that further removal capacity of nutrients from effluent were also evaluated. Two microalgae strains were observed: *Auxenochlorella protothecoide*, *Tetraselmis indica* in combination with mixed bacterial inoculation versus the monoculture of microalgae.. *Auxenochlorella protothecoide-bacterial symbiotic system* growth in the effluent was 1.6 times more than monoculture growth of *Auxenochlorella protothecoide*. On the 10th day, the symbiotic *Auxenochlorella protothecoide* system removed 86.95%, 85.67%, and 78.53% of chemical oxygen demand (COD), total dissolved nitrogen (TDN), and total dissolved phosphorus (TDP) respectively. The paper mill effluent after treatment can be recycled to use further for irrigation purposes.

Keywords: Algal biomass, Microalgae-Bacterial Consortium, Pulp and paper mill effluent

Abbreviations – PPE- Paper Mill Effluent, COD- Chemical oxygen demand, TDN- Total dissolved nitrogen, TDP- Total dissolved phosphorus

1. Introduction

Based on the COD of the water, the cultivation of microalgae is a water-demanding practice since 607 to 1944 L of water is necessary to yield 1 L of biodiesel of algae (Quinn & Davis, 2015). The pulp and paper industry, on the other hand, has significant environmental concerns. It consumes a significant volume of resources such as water, wood, and agricultural waste each year and emits extremely contaminated effluent such as extractive, lignin, and chlorinated compound degradation products that need to be properly treated (Generation et al., 2019).

A significant process needs to be set up which is significant for integrating microalgae-mixed bacteria culture and treatment of pulp and paper mill effluent (PPE) through a single stage. This will have several advantages, including increased recovery of water for reuse, algae dry weight, nutrient recycling, energy savings during treatment of effluent, and a lower CO_2 footprint.

According to various studies, PPE is low in nitrogen and phosphorus but high in carbon nutrients, making it unsuitable for microalgae cultivation (Gentili, 2014) (Bhatti et al., 2021). As an alternative, food waste digestate can be mixed with effluent as a source of Nitrogen and Phosphorus to support the growth of the microalgae consortium, resulting in significant cost savings and simultaneous treatment of effluent. FWD is the byproduct of the anaerobic digestion of various food wastes. Nitrogen and Phosphorus are two critical nutrients of digestate, which can be recycled to promote the growth of a symbiotic microalgae-bacterial system.

The microalgae and bacteria symbiotic system has shown greater benefits than monoculture microalgae (Talapatra et al., 2021). The presence of some bacteria will improve the process of effluent treatment while also lowering the high capital cost associated with maintaining the monoculture of microalgae (Talapatra et al., 2021). Untreated effluent has enormous potential for microalgae cultivation.

In this study,the percentage of nutrients removal capacity of the symbiotic association of microalgae were evaluated. Also, the growth rate of microalgae in four different batch culture in the presence of food waste digestate in effluent were evaluated.

2. Materials and Methods

2.1. Microalgae, bacteria culture, and the sample collection

Microalgae *Auxenochlorella protothecoide*, *Tetraselmis indica*, were obtained National Chemical Laboratory (NCL). The cultures were grown in light intensity of 180 $\mu mol\ m^{-2}\ s^{-1}$ for 10 days in a 1000 mL conical flask with a period of light/dark-16h/8h cycle and maintaining the temperature at 27 ± 0.5 °C.

The activated sludge and PPW (secondary) were collected from Khanna Paper Mill Ltd., India. The activated sludge was centrifuged at 3500 rpm for 10 minutes, and the settled particles were washed twice with 0.9% NaCl solution to be used as inoculum of mixed bacteria (Talapatra & Ghosh, 2022a). The inoculum of microalgae and activated sludge were added in a ratio of 3:1 to achieve symbiotic association in the culture medium (Nguyen et al., 2020)

2.2. Food waste digestate collection

The waste food was collected from the mess of IIT, Roorkee. The collected food waste digested anaerobically in the MEC reactor (Gautam et al., 2021). After anaerobic digestion, the collected solid digestate was dried in the sun, and powdered in a laboratory Wiley Mill (Miko India GOC35, India).

Table 1. PPE composition after the supplementation of food waste digestate dose

FWD Dose (g/l)	COD (mg/l) solution	Nitrogen (mg/l) solution	Phosphorus (mg/l) solution	Molar ratio N/P
0.836	485	39.7	6.7	13.45

2.3. Experimental setup

Fig.1. Experimental setup for culture were i)*Tetraselmis indica*, ii) *Auxenochlorella protothecoide*, iii) *Tetraselmis indica* consortium, and iv) *Auxenochlorella protothecoide* consortium.

2.3. Analytical methods

2.3.3. Dry weight

Before being weighed with an electronic balance, a filter paper with a pore size of 0.45 m was dried for 6 hours in a dryer at 105 ° C to determine dry cell weight (W1). After filtering the 10 mL (V) sample, the filter paper was dried to a constant weight (W2). The dry weight of the samples was calculated by subtracting the original weight from the final weight. Eqs. (1) and (2) were used to calculate the dry cell weight (mg L^{-1}) (Islam et al., 2013).

$$Dry\ cell\ weight\left(\frac{mg}{L}\right) = \frac{W2-W1}{V} \quad (1)$$

2.3.3. Measurement of effluent nutrients

0.45 m membrane filters were used to filter the STW. The filtrate was used to determine the parameters of water quality. COD, TDN, and TDP were calculated using the Hanna multiparameter photometer (HI839800).

3. RESULTS AND DISCUSSIONS

The initial growth rate of all batch cultures of microalgae appears to be lower during the initial growth period. These observations of biomass accumulation suggested that the microalgae were in an adaptation phase at the beginning of the culture due to high nutrient load (Fig. 2). The chemical constituents of PPE were given in Table 1. Fatty acids, adsorbable organic halides (AOX), lignin-degraded products, Carbohydrates, fatty acids, and other constituents contribute to the high COD (Nagarajan et al., 2020). As a result, Nitrogen and Phosphors were mixed with effluent as a source of nutrients in the form of food waste digestate to enhance the microalgae growth in PPE. A 100 g L^{-1} amount of digestate contains 3.59 g L^{-1} of nitrogen and 0.48 g L^{-1} of phosphorus (Talapatra & Ghosh, 2022b). The desired N/P molar ratio can be easily achieved through solid digestate. Furthermore, Solid digestate can be stored in powdered form and is more stable.

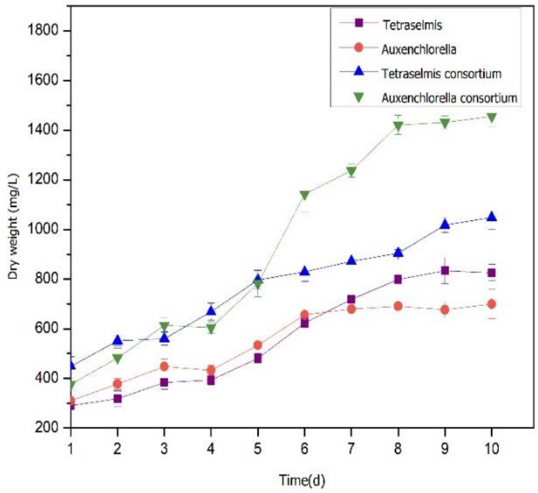

Fig.2. Changes in dry cell weight in pure and consortium of microalgae.

The *Auxenochlorella protothecoide-bacterial* system showed the highest biomass i.e. 1599.83 ± 33.06 mg L^{-1} growth as compared with other symbiotic systems. The maximum increase in biomass was 1.6 times more than the monoculture culture growth of the same microalgae. It is worth noting here that symbiotic system *Auxenochlorella protothecoide-bacterial* system play an crucial factor to increase growth rate of algae as compared to monoculture of same.

The removal efficiency of COD, TDN, and TDP were 86.95%, 85.67%, and 78.53% respectively for *Auxenochlorella protothecoide-bacterial* system in PPE is far more than monoculture of *Auxenochlorella protothecoide* (Fig. 3).

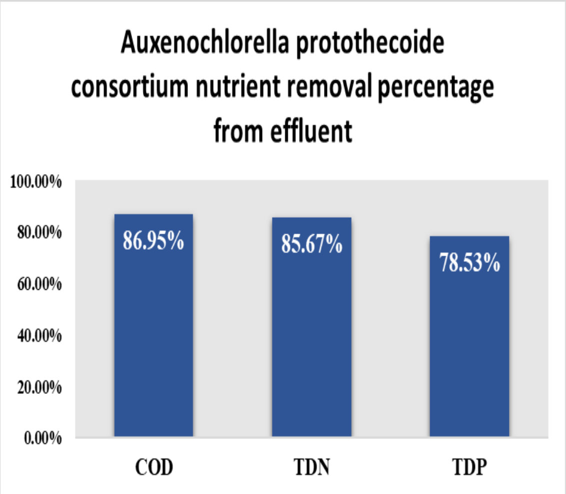

Fig. 3 Symbiotic *Auxenochlorella protothecoide* nutrient removal percentage

This is clear that the symbiotic relationship of microalgae-bacteria demonstrated superior COD, TDN, and TDP removal efficiency when compared to pure microalgae culture (Fig.2).

4. Conclusion

The entire process is integrated into an efficient means of circular economy to produce algae biomass utilizing negative value industrial effluent. For achieving a higher growth rate of microalgae, PPE was enriched through the secondary source of recycled nutrients i.e. food waste digestate. In this study, four systems of microalgae growth were investigated. When compared to the other symbiotic systems, the *Auxenochlorella protothecoide-bacterial* system in PPE achieved the highest removal efficiency of COD, TDN, and TDP were 86.95%, 85.67%, and 78.53% respectively. Also, the A. protothecoide-bacterial system showed the highest biomass i.e.1599.83 ± 33.06 mg L^{-1} growth as compared with the growth of other symbiotic systems or pure monoculture.A. protothecoide biomass can be used to produce biofuel in further studies. PPE contains harmful toxins like dioxin and metal contamination. Microalgae cultivation can reduce these toxins and metal contamination so that PPE can be used for irrigation purposes also.

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