Influence of nitrogen on simultaneous treatment of fuel synthesis wastewater and PNSB biofilm formation for resource recovery.

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Introduction

Purple non-sulfur bacteria (PNSB) have been increasingly studied to produce various biomolecules, including polyhydroxyalkanoates, single cell protein, carotenoids, bacteriochlorophylls, and coenzyme Q10; however, the production cost of these various biomolecules is still high, in part due to the high costs of substrate and biomass separation. Biofilm provides a suitable means to concentrate biomass in-situ prior to harvesting, reducing separation costs. This study therefore aims to utilize fuel synthesis wastewater (FSW) as a low-cost carbon-rich substrate, while providing simultaneous wastewater treatment of this industrial stream. Moreover, the focus is towards PNSB biofilm formation triggers for economic biomass harvesting and resource recovery.

Materials and Methods

Two PBRs, one with nitrogen sufficient (N^+) and one with nitrogen-deficient (N^-) conditions was operated in a batch process with a piece of green shade (30.48 cm x 15.24) as biofilm support material. To ensure anaerobic conditions, both PBRs were flushed with nitrogen. Each PBR was operated at room temperature, agitated at 300 rpm, and with a constant average light incidence of 100 W/m^2. Illumination was provided with a 30 W LED white floodlight kept at 15 cm from the wall of the PBR. To check the performance of the PBR, different parameters, including COD, absorbance (PNSB growth), and pH were analyzed daily. COD was measured using Hach digestion kit tubes. The PNSB growth in suspended culture was measured by absorbance at 420 nm using UV-3600 plus spectrophotometer (Shimadzu). pH was measured by a pH meter. At the end of the experiment total and volatile suspended solids, extracellular polymeric substances (polysaccharides and proteins), carotenoids, bacteriochlorophyll, and hydrophobicity of the suspended and biofilm culture were determined. Total and volatile suspended solids were determined by standard methods (APHA, 2012). Extracellular polymeric substances (EPS) from suspended and biofilm culture was extracted by using the formaldehyde-heating method. EPS polysaccharides (PS) were determined by phenol sulfuric acid method using glucose as standard and EPS proteins (PN) using a Lowry protein assay with bovine serum albumin as standard. Hydrophobicity was measured by microbial adhesion to hydrocarbons (n-hexane). Suspended and biofilm biomass carotenoids (Crts) and bacteriochlorophyll (BChl) were extracted using acetone and acetone/methanol (7:2 v/v) solvent, respectively, and was quantified by measuring absorbance at 480 nm and 771 nm by UV-vis spectrophotometer. Single cell protein (SCP) was extracted using an alkaline extraction and quantified using the Lowry protein assay previously described. The tests in this study were carried out in analytical duplicates. The average results were shown as means ± standard deviation values. Independent t-test was used to analyze the data.

Results and Discussion

Higher PNSB biofilm is obtained in N^- condition as compared to the N^+ condition (Figure 1a-b). The COD removal in N^- and N^+ condition was 3530 ± 14 mg/L and 3225 ± 49 mg/L, respectively, indicating a significant difference ($p<0.05$) (Figure 1c). The biofilm in the N^- condition had higher VSS (831 ± 47 mg) as compared to N^+ condition VSS (518 ± 0 mg). However, the suspended biomass had a much higher VSS in the N^+ condition (1659 ± 234 mg) compared to the N^- condition (442 ± 156 mg) (Figure 1d). Hence, nitrogen-deficiency promotes biofilm formation, while nitrogen availability promotes suspended and overall growth. The hydrophobicity for both cultures and biomass forms ranged between 4.4 – 21.1%, with maximum and minimum hydrophobicity obtained in biofilm culture of N^+ and N^- condition, respectively (Figure 1e). N^- biofilm had higher EPS-PS (86.2 ± 4.8 mg/g) than N^+ biofilm (76 ± 0.1 mg/g). In the case of suspended culture, the observation was inversed with 139.2 ± 19 mg/g production of EPS-PS in N^+ and 114.9 ± 40 mg/g in N^- condition. The maximum production of EPS-PN was 278 ± 99 mg/g obtained in the N^- suspended culture and the minimum production of 109 ± 5 mg/g was obtained in N^- biofilm culture (Figure 1f). The maximum production of BChl was obtained from the suspended culture of N^+ (2.65 ± 0.3 µg/g) and minimum production from biofilm culture of N^- condition (0.37 ± 0 µg/g). The maximum and minimum Crts production was obtained from the suspended culture of N^- (3.75 ± 1 µg/g) and suspended culture of N^+ (1.72 ± 0.2 µg/g), respectively, while N^- biofilm Crts production was also larger than N^+ biofilm. Except the suspended culture of N^+ and N^- for Crts, all other conditions and cultures showed significant difference ($p<0.05$) for Crts and BChls production (see Figure 1g). The SCP content in both cultures and condition varies from 35-37 % with maximum and minimum in biofilm culture of N^+ and N^-, respectively (Figure 1h). An interesting point is the similarity in the protein content between the nitrogen-deficient and sufficient conditions since nitrogen is an essential component of amino acids that comprise proteins (Reihani and Khosravi-Darani, 2019). In this study, no aqueous nitrogen source was added under the nitrogen-deficient condition. This indicates the bacteria are highly

capable nitrogen fixers and that nitrogen limitation only slows the overall growth without significantly influencing cellular composition. No studies have yet systematically assessed nitrogen deficiency on SCP, so further investigation is warranted.

Conclusion

The PBR with nitrogen deficient condition had higher COD removal with higher PNSB biofilm formation, despite lower overall growth. Moreover, the results indicate that a nitrogen deficient biofilm based system has the potential for recovery of various high value-added resources including carotenoids and single cell protein.

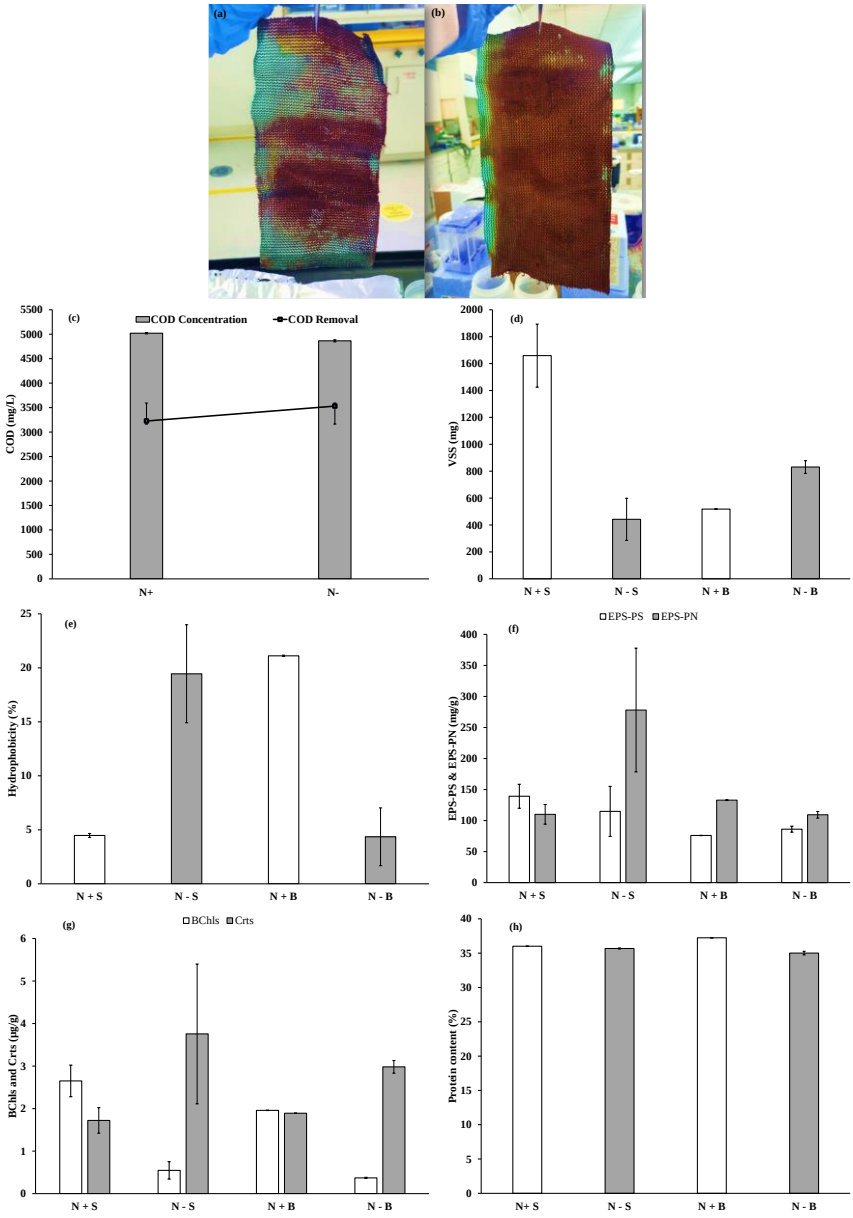

Figure 1: Biofilm formation in (a) N^+ and (b) N^- condition (c) COD concentration and removal (d) VSS (e) Hydrophobicity (f) EPS-PS and EPS-PN (g) BChls and Crts and (h) Protein content in N^+ and N^- condition.

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