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Fuel-synthesis wastewater treatment and purple non-sulfur bacteria biomass and pigments production: Effect of vitamin concentration

SULTAN Shaikh[1,a*], NAIM Rashid[1,b], GORDON McKay[1,c] and HAMISH R. Mackey[1,2,d*]

[1] Division of Sustainable Development, College of Science and Engineering, Hamad bin Khalifa University, Qatar Foundation, Doha, Qatar

[2] Department of Civil and Natural Resources Engineering, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

[a]sushaikh@hbku.edu.qa, [b]narashid@hbku.edu.qa, [c]gmckay@hbku.edu.qa, [d]hamish.mackey@canterbury.ac.nz*

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Abstract. The effect of different concentrations of ATCC vitamin supplement on the production of purple non-sulfur bacteria biomass (suspended and biofilm), carotenoids (Crts) and bacteriochlorophylls (BChls) from fuel-synthesis wastewater (FSW) was investigated in this study. The results suggest that maximum COD removal (3465 ± 125 mg/L) from FSW was obtained at a vitamin media concentration of 10 mL/L. The best optimal condition for increased biomass production from suspended and biofilm culture is the addition of vitamin at concentrations of 20 mL/L and 10 mL/L, respectively. The optimum condition for maximum Crts and BChls production from suspended and biofilm culture was found as 5 mL/L and 0 mL/L, respectively. Hence, the biomass response to vitamins is complex and vitamin addition can be used to prioritize different end goals with purple-non sulfur bacteria treatment systems and biomass utilization.

Introduction

Photosynthetic bacteria (PSB) have been the focus of research since the 1960s for their potential use in treating different wastewaters including olive mill wastewater, poultry slaughterhouse wastewater, palm oil mill effluent wastewater, and dairy wastewater [1]. PSB have been found extremely effective at removing COD, phosphate, nitrate, and hydrogen sulfide from different wastewaters and have a high conversion ratio of organic carbon present in wastewater into valuable biomass [2]. PSB can attain high cellular concentrations of proteins, carotenoids (Crts), bacteriochlorophylls (BChls), biopolymers, antibacterial compounds, and pantothenic acid compared with many types of bacteria [3].

Among these products, Crts and BChls are of particular interest since they are useful pigments for numerous industries. They are applicable to food, drug, and cosmetic products. Crts, for instance, have been utilized as food coloring agents and cosmetic additives, while BChl is a potential chemical for photodynamic therapy [4–6]. These natural biochemicals are in growing demand in the modern industrial sector. To address this requirement, researchers are focusing on increasing the Crts and BChls content of PSB.

PSB are usually anoxygenic and have four different functional groups: purple non-sulfur bacteria (PNSB), purple sulfur bacteria, green non-sulfur bacteria, and green sulfur bacteria [8]. The most commonly used PSB are PNSB bacteria since they are heterotrophs allowing conversion of organic substrates in wastewater. The synthesis of pigments by bacterial culture in culture medium has already been reported [9]. However, the cost of culture medium contributes significantly to the cost of PNSB biomass and pigments production. One way to cut costs is to use

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non-hazardous wastewater instead of the culture medium as the substrate. Therefore, integrated application of PSB to treat relatively benign wastewaters with Crts and BChls production from the biomass is gaining attention [7]. However, few studies have examined the biological synthesis of PNSB cells and pigments from wastewater [2,7].

In this study fuel-synthesis wastewater (FSW) is selected as culture medium. FSW is the byproduct of the Fischer-Tropsch process where hydrogen and carbon monoxide are converted over a catalyst at high temperature and pressure to synthetic alkanes. It is a major industry in Qatar, the study location. FSW is transparent, having low levels of colloidal or settleable particles, and is deficient in nutrients and has a very high chemical oxygen demand (COD). Due to the synthetic conversion process and pure inputs, FSW contains very few chemicals apart from organics consisting of C, H, and O; mainly volatile fatty acids, alcohols and alkanes [10].

As PNSB also requires other nutrients like nitrogen, phosphate, trace elements, and vitamins for growth [11], these components were added to the FSW. Since no study has been conducted to optimize the vitamin composition for mixed culture PNSB biomass and pigments production, this study investigated the impact when the vitamin concentration was varied. The goal of this research is to treat FSW while producing maximum biomass and pigments. Key questions are a) What will be the effect of different vitamin concentrations on fuel synthesis wastewater treatment? b) What will be the effect of different vitamin concentrations on PNSB biofilm formation? c) What will be the effect of different vitamin concentrations on Crts and BChls production?

Materials and Methods

Microorganisms and Growth Media. This study used a mixed culture of bacteria dominant with PNSB. The substrate used in this experiment was fuel synthesis wastewater (FSW) with the addition of KH_2PO_4 (3.03 g/L), NH_4Cl (3.03 g/L), $NaHCO_3$ (4.29 g/L), ATCC trace minerals supplement (MD-TMS) (10 mL/L), and different concentrations of ATCC vitamin supplement (MD-VS) (0, 5,10, and 20 mL/L).

Experimental Setup and Culture Condition. In this study eight 250 mL DURAN bottles with a working volume of 235 mL were used as photobioreactors (PBRs) with four different ATCC vitamin (MD-VS) concentrations. To provide mixing and light all PBRs were placed in an incubating shaker (New Brunswick Innova 44, Canada) at 150 rpm, 35 °C temperature, and continuous light supply of 0.7 W/m^2. The initial pH of all conditions was 7.10 ± 0 with no control of pH during the study duration. A piece of green shade (25 cm^2) was suspended in each bottle as a supporting material for biofilm development. Green garden shade was used as it is porous, economical, thin, and readily available in the market and has previously been demonstrated in our group as an effective PNSB support stratum. The experiment was conducted for 30 days, and each condition was run in duplicate.

Analytical Methods. An UV-3600 plus spectrophotometer (Shimadzu, Japan) was used to assess the growth of PNSB in a suspended culture and at the end of the experiment volatile suspended solids (VSS) and total suspended solids (TSS) were determined using standard procedures [12]. PNSB biofilm biomass from the green shade at the end of experiment was released with distilled water and measured by TSS and VSS.

To extract the supernatant for COD, samples of effluent wastewater were centrifuged at 23,366 g for 10 minutes in a centrifuge (Sorvall LYNX 6000, Thermo Scientific, USA) and filtered using 0.45 µm polyethersulfone syringe filters (Nalgene). The COD was determined using Hach high range Test'N'tube kits and the USEPA Reactor Digestion Method 8000 [13].

Suspended and biofilm biomass Crts and BChls were extracted using acetone and acetone/methanol (7:2 v/v) solvent, respectively, and were quantified by measuring absorbance at 480 nm and 771 nm by UV-vis spectrophotometer as previously described [14].

Statistical Analysis. Given the uncertainties in equality of variance associated with small sample sizes, Welch's analysis of variance (ANOVA) was used to analyze statistical differences between samples. For similar reasons, the conservative Bonferroni test was used for post-hoc testing of significance. JASP software was used to conduct the analysis and α = 5% was used as the level of significance threshold.

Results and Discussion

Fuel Synthesis Wastewater Treatment. The COD removal was maximum (3465 ± 125 mg/L) at 10 mL/L vitamin concentration followed by 0, 20 and 5 mL/L. It shows that PNSB have the ability to remove enough COD (2355 ± 95 mL/L) even without the addition of vitamin (0 mL/L). A strange drop in COD removal was observed at 5 mg/L of vitamin solution, to less than half that of either the 0 or 10 mg/L conditions (Table 1). One possible explanation for the observed behavior is the selection pressure for different organisms at different vitamin concentrations and the resulting microbial community that is established.

Table 1. COD removal and biomass production at different vitamin concentrations. Alphabetical letters indicate significant differences from a Bonferroni post-hoc test conducted following a significant Welch's ANOVA test.

Vitamin concentration [mL/L]	COD Removal [mg/L]	Biomass [mg]	
		Suspended	Biofilm
0	2355 ± 95^{a}	200 ± 0.0^{a}	15 ± 5^{bcehi}
5	1065 ± 235^{bc}	75 ± 25^{ac}	12.5 ± 2.5^{bcehi}
10	3465 ± 125^{ade}	112.5 ± 62.5^{ace}	45 ± 20^{acehi}
20	2205 ± 235^{ace}	300 ± 25^{adfg}	10 ± 0.0^{bcehi}

PNSB Growth at Different Vitamin Concentration. At 20 mL/L vitamin concentration the biomass production from suspended culture was found to be higher (300 mg) than at any other concentration. However, when comparing the vitamin concentrations examined in biofilm cultures, the 10 mL/L concentration yields the highest biomass output of 45 mg. Except for the vitamin concentration of 0 mL/L in suspended culture, all other concentrations demonstrated an increase in biomass production with increasing vitamin concentration. However, in biofilm culture, biomass increases and decreases as the vitamin concentration increases (Table 1). The significant difference between all conditions is shown in table 1 which suggests that suspended biomass production from 20 mL/L vitamin concentration is different ($p<0.05$) from 5 mL/L and 10 mL/L but is similar to ($p>0.05$) 0 mL/L vitamin concentration. However, there is no significant difference between biomass of the biofilm cultures under the different vitamin concentrations.

Suspended culture biomass production can be confirmed from the absorbance value of the suspended culture. However, absorbance is not the accurate measurement for the biomass present in the suspended culture as PNSB cells get attached and detached from the green shade and can change the absorbance value. PNSB growth in the suspended culture was nearly the same (0.8 ± 0.0) across all vitamin concentrations on day 4. On day 8, PNSB growth was somewhat greater at a concentration of 0 and 20 mL/L compared to 5 and 10 mL/L of vitamin concentration. On day 12 and 30, the PNSB growth was found to be a maximum when the vitamin concentration was 20 mL/L, followed by 0 mL/L (Fig. 1a). Likewise, biofilm VSS can be confirmed from the visual observation of biofilm formation on green shade. Higher PNSB biofilm formation was observed at 10 mL/L vitamin concentration followed by 0, 5 and 20 mL/L vitamin concentration (Fig. 1b-d). Therefore, it is suggested that the best optimal condition for increased biomass production for suspended and biofilm growth is the addition of vitamin at concentrations of 20 mL/L and 10 mL/L, respectively.

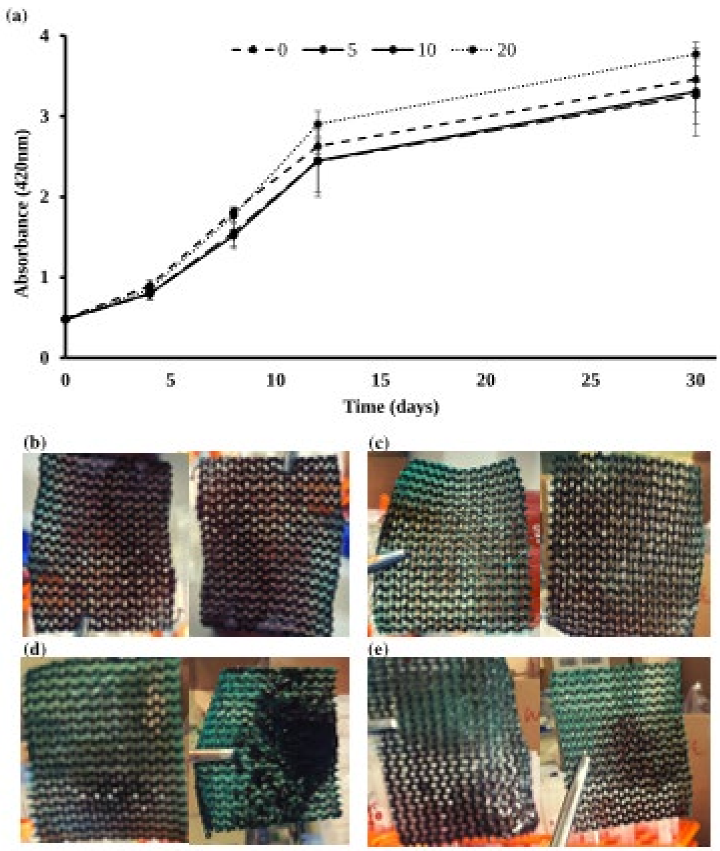

Fig. 1 (a) PNSB suspended growth at different vitamin concentrations. Biofilm formation on green shade at vitamin concentration of (b) 0 mL/L (c) 5 mL/L (d) 10 mL/L, and (e) 20 mL/L

Content of Carotenoids and Bacteriochlorophylls. The content of Crts and BChls in the suspended culture was found higher at a vitamin concentration of 5 mL/L and it was greater than in all other concentrations. However, from the biofilm culture, the maximum Crts content was observed from the condition where no vitamin was added. Except 0 mL/L vitamin concentration, the Crts concentration in the suspended culture decreased with an increase in vitamin concentration. Whereas, in the biofilm culture, the Crts concentration increases with an increase in vitamin concentration. However, BChls were always higher in suspended culture as compared to the biofilm culture. This was most noticeable with the 5 mg/L vitamin condition. Hence the optimum condition for maximum Crts and BChls production from suspended and biofilm culture was 5 mL/L and 0 mL/L. However, it should be noted that there was no significant difference between the biofilm and suspended culture values or between vitamin concentrations for both pigments (Table 2).

Table 2. PNSB Crts and BChls contents at different vitamin concentrations. Alphabetical letters indicate significant differences from a Bonferroni post-hoc test conducted following a Welch's ANOVA test.

Vitamin concentration [mL/L]	Crts content [µg/g]		BChls content [µg/g]	
	Suspended	Biofilm	Suspended	Biofilm
0	7.4 ± 1.4[a]	15.4 ± 3.7[a]	2.90 ± 0.2[a]	2.60 ± 0.4[a]
5	12.5 ± 6.9[a]	5.1 ± 1.4[a]	4.40 ± 2.3[a]	1.24 ± 0.0[a]
10	7.5 ± 5.5[a]	6.8 ± 0.2[a]	1.34 ± 0.5[a]	1.05 ± 0.2[a]
20	5.0 ± 0.9[a]	10.5 ± 0.7[a]	1.73 ± 0.3[a]	1.68 ± 0.0[a]

The Crts/BChls ratios are shown in Table 3. The Crts/BChls ratio of suspended culture for all conditions is in the range of 2.5 – 4.6. Biofilm cultures showed a slightly higher range of 4.1 to 6.5. However, there is no significant difference between all the vitamin concentration conditions and suspended and biofilm cultures. Wang et al. [2], while studying the influence of various concentrations of NaCl on the production of Crts and BChls by photosynthetic bacteria (PSB), discovered a ratio of 2.1 – 2.8. The ratio of PSB Crts/BChls was reported by Zhou et al. [15] to be between 1.15 and 1.42 when the authors treated wastewater using a variety of light sources. However, there is a lack of in-depth studies in the literature on PNSB Crts and BChls production from various substrates/wastewaters with different vitamin concentrations.

Table 3. Crts/BChls ratio of PNSB suspended and biofilm culture at different vitamin concentrations. Alphabetical letters indicate significant differences from a Bonferroni post-hoc test conducted following a Welch's ANOVA test.

Vitamin concentration [mL/L]	Crts/BChls	
	Suspended	Biofilm
0	2.5 ± 0.3^{a}	5.8 ± 0.4^{a}
5	2.8 ± 0.0^{a}	4.1 ± 1.2^{a}
10	4.7 ± 2.1^{a}	6.6 ± 0.8^{a}
20	2.9 ± 0.0^{a}	6.3 ± 0.7^{a}

Summary

It was demonstrated that culture media without addition of ATCC vitamin can be used to grow PNSB for COD removal as well as pigments production. The culture, without the addition of vitamin, removed around 55% of COD and produced Crts in the range of 7.4–15.4 µg/g and BChls in the range of 2.6–2.9 µg/g. Furthermore, it has also the potential to induce PNSB biofilm growth on green shade.

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