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Resource recovery through bioremediation of fuel-synthesis wastewater in a biofilm photobioreactor using purple non-sulfur bacteria: A circular bioeconomy approach

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ABSTRACT

In the current era of wastewater treatment, integrating reusable water production with resource recovery is a key goal. This study aims to treat fuel-synthesis wastewater (FSW), intending to recover various resources, including polyhydroxybutyrate (PHBs), single cell protein, bacteriochlorophylls, carotenoids, and coenzyme Q10 from suspended and biofilm growth to decrease the harvesting costs. The study considered the treatment process, biofilm growth, and resource recovery potential in a mixed-culture system enriched with purple non-sulfur bacteria for treating FSW. Specifically, the effects of four different FSW strengths (25–100 %) and nitrogen sufficiency (N^+) or deficiency (N^-) were evaluated in eight biofilm photobioreactors. This study observed a direct correlation between the concentration of FSW and PHB content; specifically, as the FSW content decreased from 100 % (undiluted) to 25 % the PHB content decreased. The undiluted condition achieved 17 % dry cell weight as PHB in the suspended growth and 22.6 % in the biofilm growth under N^- condition. The protein content ranged between 33 and 44 %, and the presence of nitrogen had a slight positive effect on higher protein content. No trend was observed for carotenoids or bacteriochlorophylls in the N^- condition. In contrast, for the N^+ condition, the concentration of bacteriochlorophylls increased with decreasing wastewater concentration under suspended growth, while it decreased with decreasing wastewater concentration under biofilm growth. Coenzyme Q10 concentration was enhanced under the most growth-limited condition (25 %, N^-). PHB and protein content of these resources seem most promising when using N^- and N^+ conditions, respectively.

1. Introduction

The circular economy model, responding to the demand for sustainable waste management, promotes the transformation of waste into valuable resources, a concept now integral to wastewater treatment [[1],[2]]. Various industries produce vast amounts of wastewater. One example is the production of synthetic fuels using the Fischer–Tropsch process, which results in large quantities of fuel-synthesis wastewater (FSW) at a rate similar to that of the produced fuel. This wastewater is an organic-rich, strongly acidic wastewater with high chemical oxygen demand (COD) up to 32 g/L [[3],[4]]. Given its high organic content, FSW presents an ideal candidate for resource recovery through biological treatment methods, recognized for their simplicity, cost-effectiveness, and environmental benefits [[5],[6]].

Among the microbes suited for this, purple non-sulfur bacteria (PNSB) stand out due to their versatile metabolism and ability to produce a range of valuable bioproducts from wastewater, including carotenoids (Crts), bacteriochlorophylls (BChls) [7], coenzyme Q10 (CoQ10) [8], single cell protein (SCP) [9], and polyhydroxybutyrates (PHB) [10]. Additionally, PNSB-based wastewater recovery processes are anaerobic without odour or direct greenhouse gas emissions [11], making it a sustainable treatment approach if natural lighting is sufficient.

BChls and Crts are pivotal for converting light to chemical energy in PNSB, finding extensive use across the food, cosmetics, and pharmaceutical industries due to their vibrant colors and health benefits, serving as natural alternatives to synthetic compounds [12–14]. Successfully produced from various industrial wastewaters, BChls, and Crts

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illustrate the potential of wastewater as a resource for valuable pigment production [15–20].

CoQ10 is a naturally occurring antioxidant found in the biological cell membranes of plants, animals, and microorganisms. It plays a critical role in cellular energy production and helps to prevent cellular damage. This makes it a highly desirable ingredient in a variety of fields, including health care, food additives [21], medicine [22], and cosmetics [23].

SCP represents a sustainable high-protein biomass, substituting traditional protein sources. With its comprehensive nutrient profile and lower environmental footprint, SCP from PNSB highlights an innovative approach to enhancing global food security and provides a viable alternative for aquaculture feed [24–26].

PHBs are biodegradable polymers with properties similar to petrochemical plastics. They offer an eco-friendly alternative for a wide range of industrial applications. Their biodegradability and commercial value make PHBs a promising target for resource recovery from wastewater, supporting the transition towards more sustainable materials [27–30].

To date, PNSB has demonstrated the ability to recover these resources from various wastewaters, and recently, FSW has been demonstrated as a potentially suitable wastewater source to recover PHBs, SCP, Crts, and BChls [31–33]. The strength of the substrate can significantly affect bioresource production, but its effects have not been well studied with PNSB for the production of various bioproducts from the same wastewater source. In a study conducted by Özgür et al. [34], the utilization of *Rhodobacter capsulatus* was explored for hydrogen production. Their study found that employing 100 % dark fermentor effluent of glucose as the substrate led to a decrease in hydrogen production compared to concentrations of 75 % and 25 %. Correspondingly, Lee et al. [35] researched the impact of various butyrate concentrations (10, 17, 25, 50, and 100 mM) on hydrogen production utilizing *Rhodobacter sphaeroides*. Their findings indicated that a butyrate concentration of 25 mM resulted in the highest total amount of hydrogen produced and the highest specific production rate. Interestingly, when the butyrate concentration reached 100 mM, hydrogen was not detected despite the occurrence of growth. These studies highlight the importance of considering the substrate strength when optimizing bioresource production.

The economic feasibility of resource recovery systems is a critical factor, with the costs associated with various bio-products demonstrating a wide range. For instance, synthetic Crts are priced between USD 250/kg to USD 2000/kg, while natural Crts fetch a higher price range of USD 350/kg to USD 7500/kg [36]. The price of BChls varies from USD 224 million/kg to USD 583 million/kg [37]. CoQ10 production via PNSB fermentation is significant, ranging from USD 36.9/kg to USD 154.9/kg of CoQ10, which is a substantial part of the total production cost [8]. On the other hand, the cost estimation for SCP production using wastewater in a closed photobioreactor is around USD 24/kg of protein [38]. In terms of PHBs, their cost is slightly higher compared to petroleum-based polymers, ranging from USD 26/kg to USD 33/kg, based on data from Goodfellow, a UK-based firm [39].

One significant expense associated with resource recovery systems, especially when using phototrophic cultures, is the separation of biomass [40]. Settling, flocculation, dissolved air flotation, filtration, or centrifugation [41] are different techniques used to separate biomass, with centrifugation generally required to dewater the biomass to a level suitable for further processing. The electrical costs of centrifugation could account for as much as 30 % of the overall cost of producing photosynthetic biomass [42]. Utilization of biofilms provides a means to deploy alternative harvesting techniques, such as simple scraping from the support strata due to the high concentration of biomass [41]. While microalgal attached cultivation has been well studied for biofuel applications [[43],[44]], biofilm systems for photoheterotrophic PNSB remain relatively unexplored and are a target for this study.

In the author's previous work, nitrogen limitation was found to promote biofilm growth and is considered in this study for its interaction with wastewater strength, which can also influence biofilm formation [32]. Moreover, it has also been reported that nitrogen deficiency promotes PHB synthesis in PNSB, while nitrogen availability may influence protein production within the cell [45]. Additionally, PNSB possesses the nitrogenase gene, which gives them an ecological advantage in reactor conditions under nitrogen limitation. This is particularly helpful for nutrient-limited wastewater like FSW. PNSB is, therefore a promising candidate for industrial effluent treatment, and a biofilm culturing approach may improve the cost-effectiveness of PNSB resource recovery routes. Therefore, this study explores the effect of different FSW concentrations with nitrogen deficient (N^-) and nitrogen sufficient (N^+) media on PNSB biofilm formation and bio-products production. The research questions in this study are: a) What will be the influence of different FSW concentrations and nitrogen conditions (N^- and N^+) on PNSB biomass production (suspended vs biofilm) b) What will be the effect of different FSW concentrations and nitrogen conditions (N^- and N^+) on value-added bio-products production c) What will be the best optimal condition for PHBs, SCP, Crts, BChls, and CoQ10 production.

2. Materials and methods

2.1. Microorganisms and growth media

This study used a mixed culture of bacteria grown previously on FSW for over 18 months as a seed source. The culture was grown under illuminated anaerobic conditions and was enriched with PNSB, predominantly *Rhodopseudomonas*, and anaerobic fermenting bacteria. The FSW was used as a carbon substrate for the seed source cultivation and the experiment. The FSW was collected from a large GTL plant in Qatar and was characterized before use in the experiment, as reported in Table A1. For the initial seed culture development, the following nutrients were added to the FSW: NH_4Cl at 3 g/L, KH_2PO_4 at 3 g/L, $NaHCO_3$ at 4.3 g/L, ATCC vitamin supplement (MD-VS) at 10 mL/L, and ATCC trace minerals supplement (MD-TMS) at 10 mL/L. The same additions were made for the experimental study, with the exception that NH_4Cl was excluded from the medium of the N^- condition.

2.2. Biofilm photobioreactor and culture condition

A 2 L wide-mouth glass bottle with a working volume of 1.7 L was used as the biofilm photobioreactor (BPBR). Agricultural shade cloth (mesh opening size: 600–800 μm) with dimensions of 30 cm x 15 cm was used to support biofilm formation in each BPBR and was formed into a cylinder that could rotate inside the BPBR. Agricultural shade cloth was selected as the biofilm support material as it is thin, porous, economical, and has been previously demonstrated as an effective support stratum [31]. In this study, eight different conditions with different fuel synthesis wastewater concentrations (A: 100 %, B: 75 %, C: 50 %, and D: 25 %) and nitrogen conditions (N^- and N^+) were tested in eight separate BPBRs (Table A2).

For dilution, a corresponding volume of freshwater was added to FSW, and then nutrients were added to all BPBRs. The BPBRs were run in a batch configuration. The BPBRs were inoculated with the mixed microbial culture enriched with PNSB; then, the BPBRs were flushed with nitrogen to remove dissolved oxygen. The BPBRs were tightly sealed and placed on magnetic stirrers operating at room temperature and at a speed of 300 rpm. The BPBRs were consistently lit with an average light intensity of 100 W/m^2, utilizing 30 W white LED floodlights positioned 15 cm from the wall of the BPBR (as shown in Fig. 1). The BPBRs functioned in a singular batch configuration. Owing to varying lag durations, the N^- tests lasted for 19 days and the N^+ tests for 8 days, each concluding when they reached the stationary phase, as identified by optical density evaluations. The same experiment with a different batch of FSW was repeated with BPBR A (100 % wastewater) and BPBR D (25 % wastewater) in N^- and N^+ conditions to confirm repeatability. These two conditions were selected for repetition as they showed

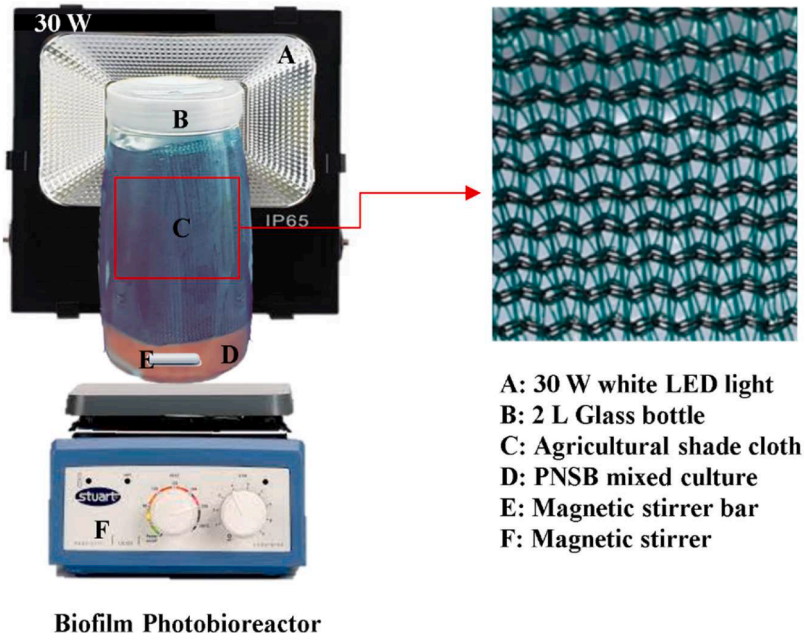

Fig. 1. Schematic diagram of biofilm photobioreactor (BPBR) used in the study.

maximum and minimum PNSB growth in the initial experiment. The seed was taken from the original seed source maintained in the laboratory.

2.3. Analytical methods

The biomass growth in suspended growth was measured daily by absorbance at 420 nm using a UV-3600 plus spectrophotometer (Shimadzu, Japan). Upon completion of the experiment, total suspended solids (TSS) and volatile suspended solids (VSS) were measured by using standard methods [46]. The amount of biofilm attached to the shade cloth was dislodged using a specified volume of distilled water. Subsequently, the total biofilm solids (TBS) and volatile biofilm solids (VBS) were quantified. All VSS and VBS values (collectively total and volatile solids, or TS and VS) are presented in terms of mass instead of concentration to facilitate easy comparison between suspended and biofilm growth. A pH multimeter was used to measure the pH (Orion, Thermo Scientific, USA). Samples of the effluent wastewater underwent centrifugation at 23,400 g for 10 min using a Sorvall LYNX 6000 centrifuge from Thermo Scientific, USA. Following centrifugation, filtration was performed through 0.2 μm polyethersulfone syringe filters supplied by Nalgene, Fisher Scientific, USA. The filtrate was analysed for COD and total nitrogen (TN). The COD of the supernatant was determined on the same day of sample collection, following the USEPA Reactor Digestion Method 8000 using high range COD vials (Hach, USA) [47]. Other parameters were determined within 48 h, and samples were stored at 4 °C until analysis. The TN was measured by a TNM-L unit fitted to a TOC-L Analyzer (TOC-L, Shimadzu, Japan). At the end of the experiment, PHB, SCP, BChls, Crts, and CoQ10 of the suspended and biofilm growth were extracted and analyzed.

PHB was extracted and quantified through the sodium hypochlorite dispersion method and UV spectrophotometry. Cellular protein was extracted via an alkaline extraction technique and quantified using the Lowry protein assay method with bovine serum albumin as the standard [48]. BChls and Crts were extracted using acetone/methanol (7:2 v/v) and acetone as the respective solvents, followed by quantification using equations detailed in our prior study [31]. Likewise, the comprehensive procedures for extracting and analyzing these components have been extensively documented in a previous publication [31]. CoQ10 levels were assessed using the Human Coenzyme ELISA Kit (MyBioSource, USA), with absorbance measured at 450 nm utilizing a microplate reader (Spark, Tecan, Austria). CoQ10 analysis was undertaken on the same alkaline extract used for protein analysis [8].

2.4. Statistical analysis

Analytical duplicates were performed for all the tests in this study, with a subsequent repeat (biological duplicates) of Condition A and D to assess repeatability. The average results were shown as means ± standard deviation values. One way analysis of variance (ANOVA) was used to analyze the variance of the results, with 5 % as the significance level.

3. Results and discussion

The aim of this research was to find the substrate and nitrogen conditions for the maximum production of value-added products such as PHB, SCP, BChls, Crts, and Q10 from the suspended and biofilm growth of PNSB while treating FSW. To determine the best optimal condition, eight different combinations of FSW concentration (100 %, 75 %, 50 %, and 25 %) denoted as A, B, C, and D, respectively, with nitrogen conditions (N^- and N^+) were investigated in BPBRs.

3.1. Effect of wastewater concentration and nitrogen on FSW treatment

The COD profile of all BPBRs with and without nitrogen (N^- and N^+) decreased over time, indicating that the bacteria in the BPBRs effectively break down the organic matter (Fig. 2a and b). The COD removal in all BPBRs of the N^- condition showed rapid COD removal after the lag phase (day 11) till the end of the experiment, where it became constant. The higher the COD removal result, the higher the suspended growth and the increase in pH. The pH fell in the range of 7.8–8.3 for all BPBRs. The complete pH profile of all BPBRs in both nitrogen conditions can be found in the supplementary file of this article (Fig. A1). At the start of the experiment, the COD removal was lower in all BPBRs in the N^- condition, likely due to the absence of nitrogen in the growth media. This slowed down the overall growth of the bacteria, as indicated by the absorbance values in Fig. 4c.

In contrast, the COD removal in all BPBRs of the N^+ condition began to decrease at the start of the experiment, and the COD removal increased after adding NH_4Cl to BPBR A and B. In contrast, the COD

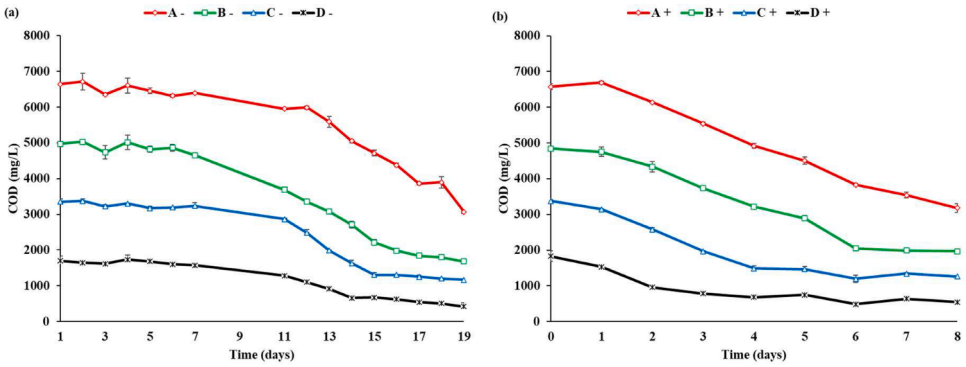

Fig. 2. COD profile in all BPBRs of (a) N^- and (b) N^+ conditions throughout the experiment. (A: 100 %, B: 75 %, C:50 %, D:25 % wastewater concentration, − indicates N^-, and + indicates N^+ condition).

removals were lower in BPBR C and D, most likely because they consumed their readily biodegradable COD (rbCOD). The higher COD removals in BPBR A and B were associated with higher absorbance values (Fig. 4d) and a pH level greater than 8.

The COD removal in BPBRs A and B under the N^- condition was higher than in the N^+ condition, but the difference was not statistically significant ($p > 0.13$). Meanwhile, the COD removal in BPBRs C and D was almost the same for both nitrogen conditions, with no significant difference ($p = 1.0$). The highest COD removal in both nitrogen conditions was observed in BPBR A, followed by B, C, and D (Table A3). This finding is consistent with other studies, such as the study by Myung et al. [49], which reported higher COD removal from undiluted swine

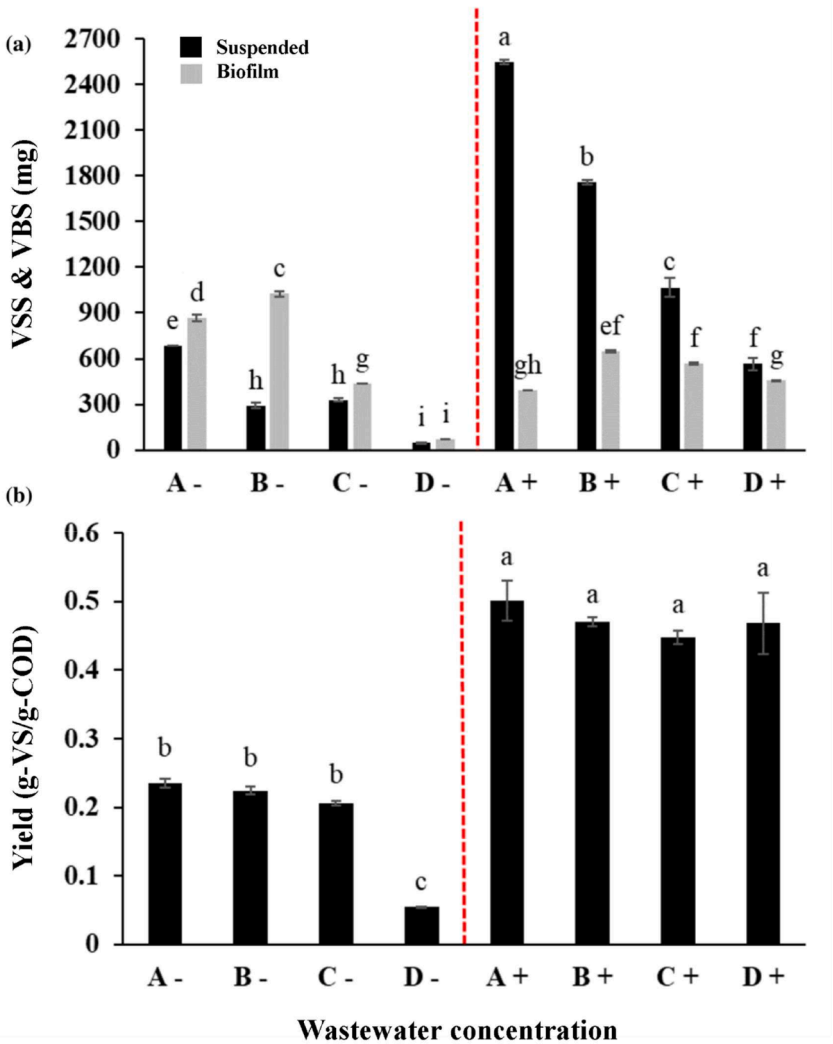

Fig. 3. (a) VS from suspended and biofilm growth of all BPBRs (b) Biomass yield in all BPBRs of N^- and N^+ condition (A: 100 %, B: 75 %, C:50 %, D:25 % wastewater concentration, − indicates N^-, and + indicates N^+ condition). Alphabetical letters highlight significant differences ($p < 0.05$) as determined by a Bonferroni post-hoc test conducted after a significant Welch's ANOVA test.

wastewater than from diluted wastewater. As expected, the maximum COD removal rate (>70 %) was observed in BPBR D for both nitrogen conditions and appeared to plateau, possibly due to the non-biodegradable components in the FSW or soluble microbial products. The repeat experiment validated the COD removal observations, with no significant differences ($p > 0.05$) to the corresponding values from the first test.

Fig. A2 shows the TN in all BPBRs of the N^+ condition. TN reduction was closely linked to organic removal, with A^+ being the first to consume available TN by day 3. On day 4, the TN reduced to less than 1 mg/L in all BPBRs except BPBR D (13.5 ± 0.06 mg/L), which had also plateaued regarding COD removal. By day 4, the residual TN concentration of all BPBRs was less than 1 mg/L except BPBR D, whose concentration was 13.4 ± 0.3 mg/L. Therefore, on day 5, 45 mL of 3.0 g/L NH_4Cl solution was added again in all N^+ BPBRs to differentiate between N^- and N^+ conditions. On day 8, the TN observed was 1.2 ± 0.1, 3.2 ± 0.1, 58.1 ± 0.6, and 89.7 ± 0.7 mg/L in BPBR A, B, C, and D. BPBRs C and D showed minimal COD or TN removal from day 5 onwards. The residual COD is in line with the findings of [50], where they found up to 60 % of COD removal from FSW by *Alcaligenes faecalis, Stenotrophomonas* sp., and *Ochrobactrum* sp. They reported that the remaining organic compounds in FSW were challenging to degrade or required different bacterial strains or treatment methods for removal.

3.2. Effect of nitrogen and wastewater concentration on PNSB growth

The biofilm VBS was highest in BPBR B^- (1025.8 ± 19.5 mg), followed by A^-, C^-, and D^-. Likewise, the maximum VBS in the culture of N^+ was highest in BPBR B^+ (649 ± 6 mg), followed by C^+, D^+, and A^+ (Fig. 3: a). Hence, BPBR B had the maximum biofilm formation under both nitrogen conditions. The comparison of VBS between the two nitrogen conditions indicates that higher VBS was obtained from N^- conditions under higher COD concentrations (A and B), while at lower COD concentrations (C and D), N^+ conditions lead to more significant biofilm formation between the nitrogen condition pairs. For the chemoheterotroph *Xanthomonas*, Ham and Kim [51] discovered that nitrogen source presence reduced biofilm formation, while its limitation promoted it. Yet, the literature has a gap regarding the combined effects of N availability and wastewater concentration.

For suspended biomass, the highest VSS concentrations were observed in reactor A and the lowest in reactor D for both N conditions. For N^+ conditions, the VSS and initial COD concentration were correlated. In the N^- condition, the pattern was a little different since the majority of growth was in the biofilm, with C having a higher VSS (329 ± 13.2 mg) than B (291 ± 19.6 mg). The presence of N strongly affected the quantity of VSS produced, with the N^+ condition having between 3.2 and 12 times the VSS levels of the corresponding N^- condition. In the A dilution, the A^- suspended growth reached 685 ± 0 mg, while in the A^+ condition, the growth reached 2548 ± 13 mg. Therefore, N availability had a much more apparent and consistent impact on suspended growth. The VSS of all BPBRs were statistically different from their respective VBS ($p < 0.013$). The statistical difference in VSS and VBS of all different BPBRs is shown in Fig. 3a.

The VBS measurements correlated with visual observations of the biofilm formation on the shade cloth (Fig. 4: a and b). Similarly, absorbance can be a good indicator of suspended biomass growth, particularly for PNSB, although it is not directly proportional as cells can alter their cell pigment levels. The PNSB growth was negligible at the start of the experiment in the N^- condition, with growth becoming obvious after day 8. However, in the N^+ condition, there was gradual growth from the outset of the experiment, and it reached a maximum value in 8 days (Fig. 4: c and d). Nitrogen led to higher suspended growth concentrations and more rapid growth curves. Comparing all conditions, it has been concluded that A^+ and B^- are the optimum conditions for maximum biomass production from suspended and biofilm growth, respectively. The repeated experiment with BPBR A and D showed consistent results to the initial experiment ($p > 0.05$) with the exception of BPBR A^+. Nevertheless, the trends were similar (Figs. A3, A4, and A5).

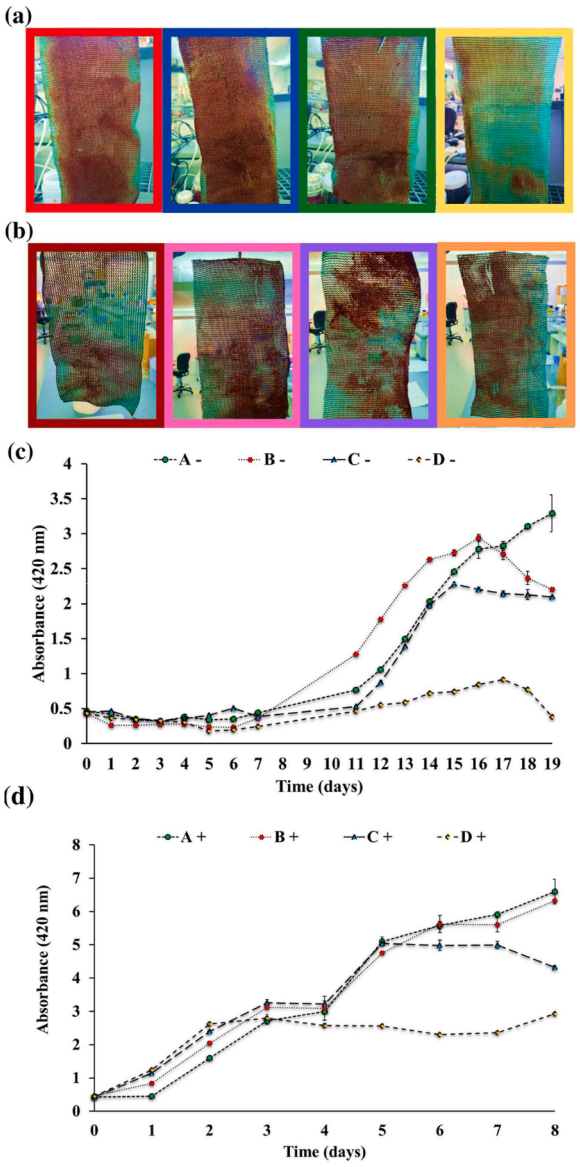

Fig. 4. (a) Biofilm formation in N^- condition BPBR A (red border), B (blue border), C (green border), and D (yellow border); (b) Biofilm formation in N^+ condition BPBR A (brown border), B (pink border), C (purple border), and D (orange border); (c) PNSB suspended growth in N^- condition of all BPBRs (d) PNSB suspended growth in N^+ condition of all BPBRs. (A: 100 %, B: 75 %, C:50 %, D:25 % wastewater concentration, + and − indicate nitrogen availability).

In the N^- condition, BPBR A produced the highest yield, having a value of 0.23 ± 0.0 g-VS/g-COD. Likewise, for the N^+ condition, BPBR A again showed the best result, measuring 0.50 ± 0.7 g-VS/g-COD, roughly double the yield of A^-. Using 1.42 as a typical biomass COD/VSS ratio, the yield for N^+ BPBRs falls between 0.64 and 0.71 g-COD/g-COD. These findings are consistent with other PNSB related studies but are relatively lower than the reported optimum yields of 1 g-COD/g-COD [51]. However, they significantly surpass the anticipated yields for anaerobic heterotrophs, underscoring the PNSB role in the organic conversion mechanism.

In contrast, the values for N^- conditions range between 0.08 and 0.33 g-COD/g-COD. These lower values are attributed to the higher energy obligations of nitrogen fixation and longer test duration for N^-

BPBRs. The statistical difference in yield of all BPBRs is shown in Fig. 3b, which indicates that BPBR D under N^- condition is significantly different ($p < 0.001$) from all the BPBRs of N^-. One possible explanation for the lower biomass yield in BPBR D is the exhausted readily biodegradable COD, resulting in a period of endogenous decay.

3.3. Effect of nitrogen and wastewater concentration on PHB

Polyhydroxybutyrate (PHB) is the most common PHA created from acetyl-CoA through a series of three enzymatic processes that are catalyzed by β-ketothiolase, acetoacetyl-CoA reductase, and PHB synthase [52]. The PHB cell concentration varied from 4.8 to 17.0 % and 2.4 to 11.6 % in suspended biomass of N^- and N^+ conditions, respectively. In the biofilm, the N^- biomass ranged between 9.7 and 22.6 % PHB, and N^+ between 4.5 and 9.5 % PHB. The PHB cell concentration was higher in the biofilm growth than in the suspended growth in both nitrogen conditions except A^+ and B^+ where a higher PHB content was observed in the suspended growth system (Fig. 5a). In the repeated experiment, the PHB cell concentrations, in almost all conditions, varied from those of the first test ($p < 0.05$). Nitrogen deficiency was still important in promoting PHB synthesis in the suspended biomass, while biofilm values were relatively similar irrespective of nitrogen. However, lower strength wastewater consistently led to higher PHB in the repeat test for both biomass types and nitrogen conditions (Fig. A6). Reasons for the differences between the two tests may include a different pH profile of test 1 and test 2 for BPBR D (Fig. A1), which can affect the way cells direct excess reducing equivalents between hydrogen and PHB [53]. Another possible reasons is a change in the microbial community, either in composition or metabolism, as COD removal rates ranged from 51 to 75 % in test 1 and 62–94 % in test 2 (Figs. A7 and A8).

Padovani et al. [54] reported that an N^- condition may contribute to creating a stressful growth environment and result in an increased polymer accumulation. When *Rhodopseudomonas* sp. S16-VOGS3 was cultured under different carbon sources (acetate, butyrate, and lactate) and nitrogen source (NH_4Cl); the content of PHB varied between 0.4 and 11.6 %. When the bacterium was grown with the same carbon sources and under N^- condition, the content of PHB ranged between 7.5 and 23.5 % [55]. While the values from this study are similar to Padovani et al. [54], some research indicates that PHA values can peak at 60 % within PNSB cultures [56]. One significant difference is the variation in specific light intensity. Fradinho et al. [66] initially used two specific light intensities of culture, including 5.6 W/g X and 3.4 W/g X, which resulted in a maximum PHB content of 60 % after 3 days. In contrast, the specific light intensity used in all conditions of this study was less than 1 W/g X, leading to a lower PHB content.

Under N^- condition, the biofilm produced a larger quantity of PHB compared to the suspended biomass in all BPBRs due to a combination of similar biomass quantity and higher cell concentration. However, under N^+ condition, except for D^+, all BPBRs had a higher concentration of PHB in suspended growth compared to biofilm growth. This was because in the N^+ condition, both suspended biomass growth and PHB cellular concentration were higher than for the biofilm. The PHB quantity obtained from the suspended growth of A^-, B^-, A^+, and B^+ differed significantly ($p < 0.017$) from their respective PHB quantities in biofilm growth. On the other hand, for C and D, no significant difference ($p = 1.0$) was observed between suspended and biofilm growth within the same reactor under both nitrogen conditions (Fig. 5b).

Total PHB production was highest in the 100 % wastewater concentration under N^- and N^+ conditions (312.6 ± 10.8 mg and 331.9 ± 62.5 mg, respectively), followed by 75 %, 50 %, and 25 % wastewater concentrations. These results align with expectations, as the higher carbon content in 100 % wastewater concentration leads to higher PHB production. Overall, total PHB production increased with increasing wastewater concentration in N^- and N^+ conditions (Fig. 5c). Fig. 5d shows the PHB yield, which again follows the same trend as total PHB and PHB content in both conditions. Overall, the PHB yield was higher under N^+ conditions than under N^- conditions. This may be due to greater enzymatic efficiency at higher substrate concentration gradients. Among all the reactors, BPBR A consistently had the highest PHB yield, and BPBR D had the lowest yield. The most likely cause for the decrease in PHB yields with decreasing wastewater concentration (especially BPBR B, C, and D) is the COD plateau phase caused by the more limited substrate in these reactors, during which the stored PHB is consumed.

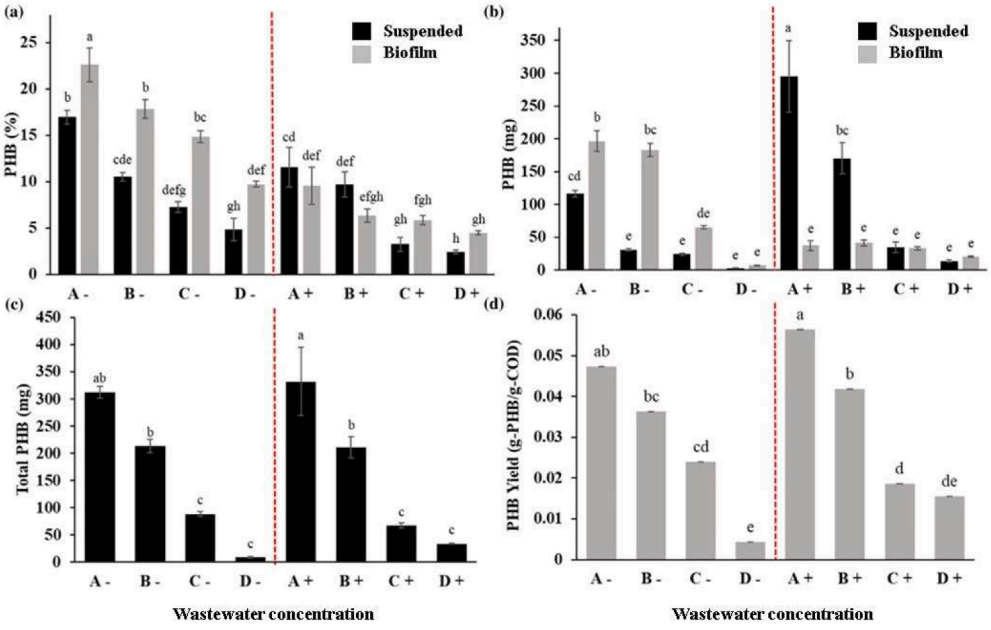

Fig. 5. (a) PHB cell concentration, (b) PHB mass quantity, (c) Total PHB mass quantity (suspended + biofilm growth), and (d) PHB yield of all BPBRs in N^- and N^+ conditions (A: 100 %, B: 75 %, C:50 %, D:25 % wastewater concentration, − indicates N^-, and + indicates N^+ condition). Alphabetical letters highlight significant differences ($p < 0.05$) as determined by a Bonferroni post-hoc test conducted after a significant Welch's ANOVA test.

3.4. Effect of nitrogen and wastewater concentration on single cell protein (SCP)

The protein content in the N^- conditions ranged between 35 and 41 %. In N^+ conditions, they ranged between 33 and 44 %. Nitrogen presence had a small but precise promotion of protein content. Conditions C had the highest protein among the wastewater dilutions, while A had the lowest overall. No clear trends were observed between biofilm and suspended growth (Fig. 6a). The protein content values align with expectations, as higher protein content corresponds to lower PHB content. BPBR C and D have higher protein contents in both conditions, which in the case of PHB is low in both BPBRs. This relationship was confirmed by calculating the Pearson correlation coefficient between PHB and protein content, resulting in an observed value of −0.55, indicating a moderate negative correlation between the two parameters. In the repeated experiment, Test 2 showed slightly higher protein values overall (by 3–20 %), except for D-, which showed lower protein content in test 2 than 1 (by 8–13 %) (Fig. A9). Test 2 also showed higher protein content in A^- compared to A^+, which is attributed to the negative correlation between PHB and protein content.

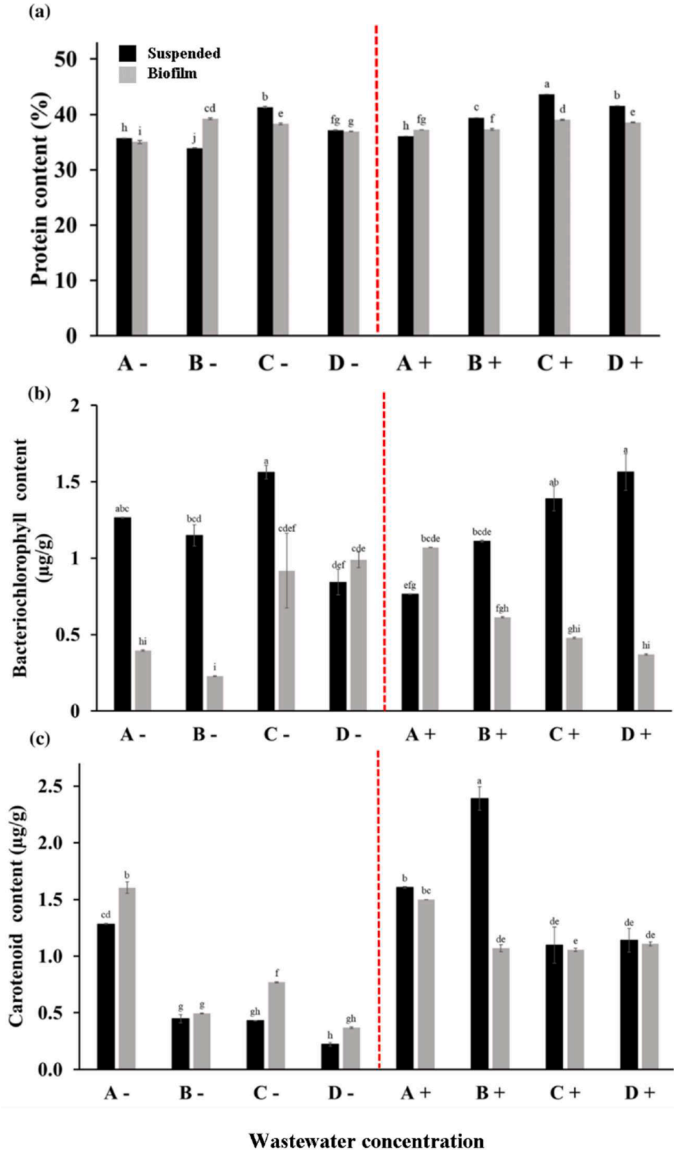

Fig. 6. (a) Protein (b) BChls and (c) Crts content in suspended and biofilm growth of all BPBRs in N^- and N^+ condition (A: 100 %, B: 75 %, C:50 %, D:25 % wastewater concentration, − indicates N^-, and + indicates N^+ condition). Alphabetical letters are used to highlight significant differences ($p < 0.05$) as determined by a Bonferroni post-hoc test conducted after a significant Welch's ANOVA test.

Protein contents in this range are comparable to those reported by Cao et al. [57], who observed 41 % and 40 % values when examining C/N ratios of 20 and 50 on protein synthesis in light anaerobic conditions. This study found a protein content of 36.0 %, 39.4 %, 43.6 %, and 41.5 % at C/N ratios of 76, 56, 40, and 21, respectively. The protein contents found in the current study are similar to our previous study [31], where the protein content was between 40 and 44 % using undiluted FSW in both N^+ and N^- conditions. Higher values of protein content have been recorded in other research using photosynthetic bacteria, including 90 % by Yang et al. [58], which is seen as an outlier. The discrepancies can be attributed to various factors, including the organisms present (e.g., Yang et al. used a pure strain of *Rhodopseudomonas*) and carbon substrate (Yang et al. used biogas slurry). Other than the author's study [31], only Hülsen et al. [25] reported protein content in both biofilm and suspended growth, with values higher than this study (62.1 % and 64.3 % for suspended and biofilm, respectively). This discrepancy is probably a result of the crude protein quantification method, which we have found overestimates the amount of protein in PNSB augmented biomass when standard conversion factors are used [59]. Nonetheless, they also observed that the protein contents of biofilm and suspended biomass were very similar.

Interestingly, all BPBRs operated with N^- condition still show respectable SCP content since nitrogen is an essential amino acid element that comprises proteins [60]. This study did not add a nitrogen source under N^- conditions. Thus, nitrogen gas, initially in the liquid or introduced during daily sampling, likely supplied adequate nitrogen to facilitate amino acid synthesis. This indicates that these bacteria exhibit robust nitrogen fixation capabilities. A deficiency in nitrogen does not significantly alter their cellular composition but merely decelerates their growth rate. The effects of nitrogen limitation on PNSB in the context of SCP remain under-researched. Hence, further investigations into temporal protein fluctuations and amino acid profiling are recommended. However, from a practical viewpoint, this capability presents a significant advantage. Traditional protein sources necessitate nitrogen inputs, typically derived from ammonium/urea, synthesized via the energy-intensive Haber-Bosch method. However, nitrogen inputs are essential from a rate perspective, with the N^+ conditions producing at least 4.4 times that of the equivalent N^- reactor (Table A4).

3.5. Effect of nitrogen and wastewater concentration on bacteriochlorophyll and carotenoids

The PNSB generates energy using photopigments such as BChls and Crts. However, these pigments cannot directly predict the abundance or activity of PNSB, as they can vary depending on the light and culture conditions [61]. Nevertheless, the concentrations of these pigments can provide an approximate indication of the relative amount of PNSB in a mixed culture. Fig. 6b shows the BChls content in all BPBRs in suspended and biofilm growths. BChls content was higher in suspended biomass than biofilm, except for conditions A^+ and D^-.

The study did not observe any trends between BChls content and wastewater concentration in the N^- condition, which showed limited growth. However, in the N^+ condition, the BChls content increased in the suspended growth and decreased in the biofilm with decreasing wastewater concentration. Higher VSS had a more significant impact on absorbing and decreasing the light passing into the reactor. The taxonomic composition of the biomass, light intensity, and culture conditions can also influence BChls content. For instance, *Rhodopseudomonas* was found to increase its mass fraction of BChls six times when the light intensity was changed from 33 to 190 W/m^2 [62]. In contrast, the low light intensity of infrared light increased BChls content in another study [63]. Hülsen et al. [25] reported higher BChls content in the biofilm than in suspended biomass, but in their BPBR, the biofilm was grown directly

on the surface closest to the light source, which may have blocked the light from reaching the cells in the suspended growth biomass. In a repeated experiment considering only BPBR A and D, results were similar in the N^- condition between Run 1 and Run 2. For the N^+ conditions, BChls differed, particularly for A^+, due to differences in biomass growth. However, the trend of decreasing BChls in the biofilm compared to suspended growth with lower concentration was confirmed (Fig. A10).

Crts are natural pigments that absorb light in the 450–550 nm spectral range and transfer the energy to BChls. As a result, Crts serve as essential energy providers in photosynthesis [64]. Additionally, Crts contribute to the structure of light-harvesting (antenna) complexes and serve a photoprotective function by dissipating undesirable excited states in antenna complexes [65]. The Crts content in all BPBRs in suspended and biofilm growths is shown in Fig. 6c. In general, Crts showed little difference between suspended and biofilm growth. Considering the data shown in Fig. 6c and the repeat test of A and D conditions (Figure A11), no trends were observed for Crts. The Crts observed in this study were significantly less than those reported in some pure culture studies [[66],[15]], implying that community composition may be a factor. For instance, Zhou et al. [15] reported the Crts content in the range of 276–4889 μg/g from artificial sugar wastewater utilizing a pure culture of PNSB. This indicates that alternative culturing approaches are desirable for resource recovery of pigments to receive highly enriched cultures.

3.6. Effect of nitrogen and wastewater concentration on coenzyme Q_{10}

The CoQ10 content in all BPBRs in suspended and biofilm growth is shown in Fig. 8. Apart from the A^+ biofilm, the CoQ10 content showed a clear trend of increase with decreasing wastewater concentration. The maximum CoQ10 content (suspended and biofilm) was obtained from 25 % wastewater concentration under N^- condition. No apparent differences were observed between biofilm and suspended growth modes. Fig. 7

The maximum CoQ10 observed in this study was 13.5 μg/g. Several other studies that used photosynthetic bacteria have found higher values. For instance, Lu et al. [67] reported 30,000–45,200 μg/g, and Lu et al. [68] reported 38,600 μg/g. Based on these differences, FSW is not a feasible substrate for CoQ10 production. Several factors can be identified as responsible for the significant disparities, such as PSB seed, light sources, redox conditions, and substrate, which in the first case was brewery wastewater and glucose and yeast extract in the second case. The PSB seeds used in both studies were commercial PSB seeds containing a mixture of purple phototrophic bacteria species. The CoQ10 content is inversely proportional to that of Crts content, which indicates that a higher Crts accumulation led to a low CoQ10 accumulation because competition exists in the synthesis pathway of CoQ10, with the formation of pigments [8].

3.7. Interplay and optimization of bioproduct pathways in mixed microbial cultures of PNSB

In our study, the production of PHB was notably enhanced under nitrogen-deficient conditions, indicating a metabolic shift towards carbon storage in response to nutrient stress, as has been observed in other studies [52]. This redirection of carbon flux from biomass and protein synthesis to PHB accumulation suggests a competitive substrate utilization between PHB and SCP production, with SCP synthesis being optimized in nutrient-rich environments. Conversely, the synthesis of Crts and BChls is largely influenced by light intensity and quality rather than carbon or nitrogen availability, aligning with findings that highlight their synergistic production under specific light conditions [64]. However, both Crts and BChls share metabolic precursors with coenzyme Q10, potentially leading to competitive interactions under conditions that favor cellular respiration over photosynthesis [8].

To intensify PHB production, adjusting the carbon to nitrogen (C/N) ratio to promote carbon accumulation while limiting nitrogen can be effective, leveraging the metabolic flexibility of purple non-sulfur bacteria under stress conditions. For SCP enhancement, maintaining a balanced C/N ratio that supports maximal biomass production without inducing stress responses conducive to PHB accumulation is recommended, alongside optimizing light conditions to support growth rather than pigment production. Strategies to increase Crts and BChls production include manipulating light intensity and wavelength to optimize photosynthetic efficiency and pigment protection roles, respectively. For CoQ10, shifting metabolic conditions to favor respiratory pathways, possibly through controlled oxygenation or substrate selection, might improve its biosynthesis due to its role in cellular energy mechanisms.

These insights into the metabolic interactions and competitive substrate utilization among PHB, SCP, Crts, BChls, and CoQ10 underscore the complexity of optimizing bioproduct recovery from wastewater using mixed microbial cultures. Future efforts to enhance the production of specific bioproducts can benefit from a detailed understanding of these metabolic pathways and their regulation, as well as the environmental conditions that influence them.

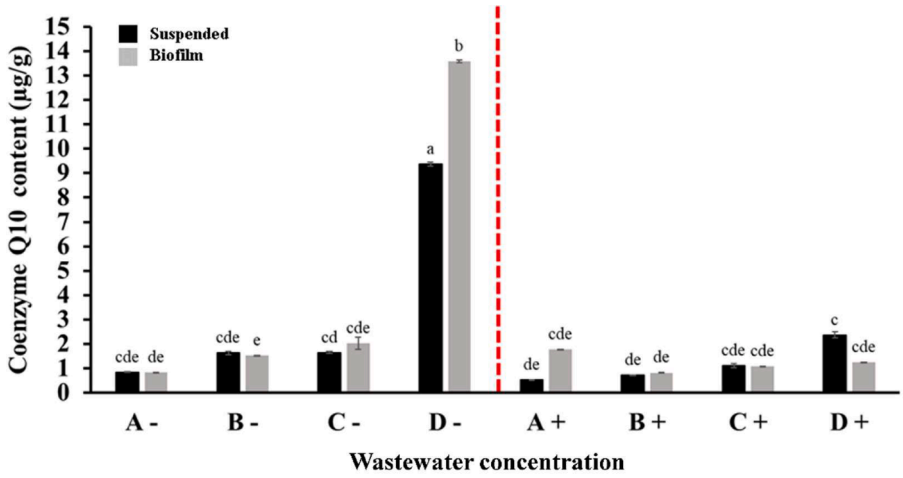

Fig. 7. Coenzyme Q10 production in suspended and biofilm growth of all BPBRs in N^- and N^+ conditions (A: 100 %, B: 75 %, C:50 %, D:25 % wastewater concentration, - indicates N^-, and + indicates N^+ condition). Alphabetical letters highlight significant differences ($p < 0.05$) as determined by a Bonferroni post-hoc test conducted after a significant Welch's ANOVA test.

4. Conclusions

This study demonstrates the potential of using fuel-synthesis wastewater (FSW) for the production of value-added bioproducts through the cultivation of purple non-sulfur bacteria, contributing to the circular economy. The investigation revealed that varying concentrations of FSW and nitrogen conditions (N^- and N^+) influenced the recovery of resources such as polyhydroxybutyrate (PHB), single-cell protein (SCP), bacteriochlorophylls, carotenoids, and coenzyme Q10. The N^- media with 100 % and 75 % wastewater concentration removed more COD than the corresponding N^+ condition by the stationary phase, but after a large lag phase, resulting in slower kinetics. The highest PHB concentration was obtained from 100 % FSW under both nitrogen conditions. The FSW concentration and nitrogen availability did not affect the protein content significantly, and it was at a satisfactory level as an SCP source. The pigments and coenzyme Q10 production did not present viable recovery routes due to lower yields than pure-culture methods. Overall, SCP provides the best comparative results to typical bioresource production methods and is relatively unaffected by wastewater concentration and growth mode, making it a robust resource recovery route. While PHB content is notably lower than what could be achieved in some cultures, there are significant opportunities to optimize conditions to improve on the best cellular concentration of 22.6 % already achieved, and this recovery route should also be investigated further.

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CRediT authorship contribution statement

Sultan Shaikh: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Naim Rashid:** Writing – review & editing. **Gordon McKay:** Writing – review & editing, Supervision. **Hamish Robert Mackey:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The authors do not have permission to share data.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.ceja.2024.100614.

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