



Valorization of purple non-sulfur bacteria biomass from anaerobic treatment of fuel synthesis process wastewater to microbial protein: a means of enhancing food security in arid climates

O.Z. Wada¹ · U. Onwusogh² · A.S. Vincent³ · G Mckay¹ · H.R. Mackey^{1,4} 

Received: 6 February 2023 / Revised: 6 June 2023 / Accepted: 15 June 2023 / Published online: 13 July 2023
© The Author(s) 2023

Abstract

The global shift from traditional fish farming to aquaculture has created an aquafeed production gap. Hence, the recovery of microbial protein from organic and nutrient-rich agro-industrial wastewaters has been identified as a suitable substitute. However, such waste streams are sparse in arid climates like the Middle East. Thus, this study explores the potential of single-cell protein recovery from a novel waste stream abundant in the region—fuel synthesis process water (FSPW), via anaerobic treatment with purple non-sulfur bacteria (PNSB). The feedstock (COD = 10.3 g/L) amended with essential nutrients was inoculated with a PNSB-dominated mixed culture in replicate 1-L batch fermenters. The wastewater characteristics and microbial biomass assays were performed using standard methods. Around two-thirds of the COD was degraded within 72 h at a rate of 2100 mg L⁻¹d⁻¹, which reduced to about 710 mg L⁻¹d⁻¹ by trial end. Also, total nitrogen levels (90 mg/L) were depleted within 72 h, indicating that nitrogen was a limiting nutrient. In addition, a peak biomass concentration of 1.11±0.037 g_{VSS}/L was obtained. Proximate analysis revealed that the biomass consisted of 35% protein, 32% lipid, 16% carbohydrate, 7% ash, 0.5% carotenoids, 0.6% bacteriochlorophylls, and 0.004% coenzyme Q10. Biomass protein's amino acid profile was comparable to soybean grain and meets dietary requirements for several aquatic livestock. Metal analysis of the biomass and wastewater indicated that nutritionally undesirable metals were undetected. Results show that PNSB not only efficiently degrade FSPW's organic load but also upcycles the waste to valuable feed constituents, potentially creating a regional circular economy.

Keywords Resource recovery · Purple phototrophic bacteria biomass · Single cell protein · Aquaculture feed · Industrial wastewater · Biomass valorization

1 Introduction

Arid climates that occur in the Middle East North Africa (MENA) region, and particularly the Gulf Cooperation Council states, have one of the highest food security risks globally [1]. The region is plagued with severe water stress, poor soil fertility, and increased irrigation requirements due to high ambient temperature, making agricultural practices expensive and challenging [2]. The agricultural challenges of the region have made fisheries a historical food source of current importance, which is reflected by the high per capita seafood consumption in countries like the UAE (51 kg/annum), Oman (36.7 kg/annum) and Qatar (16.5 kg/annum) [3, 4]. However, the gradual shift from fishing to aquaculture has created an increasing demand for aquaculture feed. Over the past three decades, there has been a 527% increment in aquaculture as opposed to a 14% increment in traditional

✉ H.R. Mackey
hamish.mackey@canterbury.ac.nz

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

² Qatar Shell Research and Technology Centre, Doha, Qatar

³ Biological Sciences Program, Carnegie Mellon University in Qatar, Doha, Qatar

⁴ Department of Civil and Natural Resources Engineering, University of Canterbury, Private Bag 4800, Christchurch 8140, New Zealand

fisheries [5]. Aquaculture was recently reported to account for 52% of global human seafood consumption [6]. The MENA region has also reported a similar trend [4]. Mainstream aquafeed protein sources like forage fish and soybean have been considered unsustainable due to the increasing overexploitation of the former and the scarce resources available, such as freshwater and arable land, to produce the latter [7]. Some alternative aquafeed protein sources explored are fish waste, food waste, insects, and microbial protein [7]. Of the substitute protein sources explored so far, microbial protein, also known as single-cell protein (SCP), has been widely appraised [8].

SCPs are microbial biomass mostly consisting of unicellular organisms like yeast, bacteria, and microalgae. They are broadly credited with having a high protein content and a high-quality amino acid profile [9, 10]. The comprehensive appraisal for SCP as a sustainable means of aquafeed production is chiefly because of its low energy inputs, high productivity, and potential circular economy approach using waste streams as feedstock. Protein-rich biomass has been generated by treating waste streams, such as lignocellulosic material, brewery wastewater, orange wastewater, fruit waste, and other forms of agro-waste [8, 11, 12]. Moreover, among the microbes examined for SCP production, purple non-sulphur bacteria (PNSB) have received recent attention. This is primarily due to the organism's metabolic versatility under diverse culture conditions and its ability to efficiently valorise nutrients by accumulating value products like high-quality protein, carotenoids, coenzyme-Q10, 5-aminolevulinic acid and polyhydroxyalkanoates [13, 14]. Also, PNSB reportedly has a comparatively higher cell protein content than SCP sources like fungi, microalgae, and yeast, with one study reporting over 90% protein content [15].

So far, most studies examining PNSB's resource recovery abilities have utilised agricultural waste streams because of their rich nutrient and organic carbon nature [8]. However, the paucity of such waste streams in arid climes creates the need to examine other more readily available alternatives. With the region being a hub for oil and natural gas, fuel synthesis process water (FSPW) from gas-to-liquid (GTL) plants stands to be a potential source for carbon substrate. The relative abundance, physical characteristics, and chemical composition of the FSPW enhances its suitability as an ideal feedstock for resource recovery in the MENA area, and being the result of a synthetic catalytic reaction, has a consistent composition with low toxicity [16]. The region houses 40% of GTL plants globally, including the world's largest plant in Qatar. With over a million cubic meters of FSPW generated on a monthly basis [17], there is an abundant feedstock for potential resource recovery processes. Such wastewater generation magnitude enhances large-scale SCP production's economic feasibility [18]. Using such readily available wastewater as the carbon substrate

significantly reduces the cost burden of the resource recovery process and guarantees continued operation, if a large-scale operation is achieved.

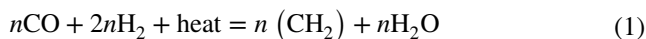
In addition, the physical properties of the wastewater make it highly favorable for SCP recovery compared to several other waste streams. This is in contrast to various SCP feedstocks that have been reported to require physical pretreatment (heating, milling, filtration, and ultrasonication), chemical pretreatment (acid, ionic liquid, and alkaline wet oxidation) and biological pretreatment (enzyme addition) [19]. Another study that employed animal manure substrate reported the need for extensive pretreatments like drying, crushing, reverse osmosis, incubation, and centrifugation [20]. These processes add significant cost, energy and time to the microbial protein production process. However, FSPW is clear, with negligible total dissolved solids and a total suspended solid of less than 30 mg/L, with no pretreatment requirements. Besides the reduced processing cost compared to other wastewater, large-scale production of SCP via liquid fermentation has also been reported to be more economically beneficial than SCP from solid-state fermentation [18]. FSPW also has an exceptionally high organic load, with chemical oxygen demand (COD) concentrations as high as 30 g/L reported [21]. The wastewater contains soluble organics like volatile fatty acids (VFAs), alcohols, and ketones that can be degraded directly [22, 23]. Hence this study examines the potential to upcycle biomass from the anaerobic treatment of FSPW with PNSB to microbial protein for aquaculture feed. A preliminary quality assessment was also performed to determine the safety of the wastewater and biomass.

2 Materials and methods

2.1 Raw wastewater

FSPW was obtained from a local GTL plant. The plant produces around 40,000 m³ of wastewater daily [17]. The process water, a byproduct of the natural gas liquefaction process, was collected from the plant in a 1 m³ intermediate bulk container totes, and later transferred to high-density polyethylene jerry cans without pretreatment. The wastewater was stored at 4 °C upon arriving at the laboratory, after which wastewater characterization was performed. The FSPW is shown in Fig. A1, demonstrating the transparent solid-free nature of this liquid, allowing for direct application without pretreatment requirements. FSPW's purity is owed to its means of generation, which is via the Fischer-Tropsch (FT) process. FSPW is a by-product of the FT process, which converts syngas into petroleum products via a systematic polymerization process at high pressure and temperature [24]. The reaction is usually accelerated

by applying catalysts like iron and cobalt [25]. The process can be summarised by Eq. (1) below as reported by Evans and Smith [25].



2.2 Inoculum

A non-axenic culture dominated by PNSB was utilized. The PNSB originated from microbial mats extracted from a local mangrove ecosystem as described by George et al. [26]. This culture was then developed on FSPW for over a year prior to the experiments. The dominant PNSB in the culture was *Rhodospseudomonas* sp. (>60%). The cells were incubated in a sub-culture of nutrient-enriched FSPW for 48 h before the experiment. A mixed culture is preferred due to the economic impracticability of utilizing an axenic culture at a large scale in an outdoor setting. The high selectivity of PNSB under light and limited oxygen conditions enhances the feasibility of their dominance in a culture solution.

2.3 Experimental design

Preliminary assessment of the PNSB-dominated mixed culture's (0.05 g/L) ability to thrive and sufficiently degrade FSPW was initially conducted via batch cultivation in a 2 L Duran glass bottle. The culture media consisted of diluted FSPW (1:5 dilution with ultrapure water), NH_4Cl (80 mg/L), KH_2PO_4 (70 mg/L), NaHCO_3 (500 mg/L), ATCC trace mineral supplements (which consists of EDTA, MgSO_4 , MnSO_4 , NaCl , FeSO_4 , $\text{Co}(\text{NO}_3)_2$, CaCl_2 , ZnSO_4 , CuSO_4 , $\text{AlK}(\text{SO}_4)_2$, H_3BO_3 , Na_2MoO_4 , Na_2SeO_3 , Na_2WO_4 , and NiCl_2), and ATCC vitamin supplements (which consists of folic acid, pyridoxine hydrochloride, riboflavin, biotin, thiamine, nicotinic acid, calcium pantothenate, vitamin B12, p-aminobenzoic acid, thioctic acid, and monopotassium phosphate). The culture bottle was subjected to continuous light via two LED strip lights (20 W) and continuous mixing via a magnetic stirring bar at 250 rpm using a magnetic stirrer (VELP Scientifica, CG-1994-V). The cultivation period lasted 16 days, with a pH between 7.45 and 7.84 and was conducted at ambient temperature (24.4 ± 0.1 °C). The LED strip lights had an intensity of 79 W/m^2 each, with most of the irradiance within the visible light spectrum (400–500 nm: 31.1%; 501–600 nm: 22.9%; 601–700 nm: 25.3%; 701–800 nm: 12.1%; 801–900 nm: 0.7%; 901–1100 nm: 1.9%).

Upon obtaining favorable results from the preliminary assessment, biological triplicates of a non-axenic culture dominated with PNSB (0.05 g/L) were prepared using 1-L cylindrical benchtop photobioreactors (Multifors, Infors), with a working volume of 0.7 L. By volume, the feedstock consisted mainly of undiluted FSPW (84%) as carbon

substrate. The other integral ingredients were in similar concentrations as the preliminary assessment, and their respective volumes were: ATCC vitamin supplement solution (10 mL/L), ATCC mineral supplement solution (10 mL/L), NH_4Cl (45 mL/L), KH_2PO_4 (45 mL/L), and NaHCO_3 (53 mL/L). The batch experiments were conducted for 11 days under continuous light (LED) at an intensity of 21.8 W/m^2 with most peaks within the visible light spectrum. The range of spectral irradiance were 400–500 nm: 15.9%; 501–600 nm: 37.5%; 601–700 nm: 40.5%; 701–800 nm: 4.8%; 801–900 nm: 0.4%; 901–1100 nm: 0.9%). The bioreactors were kept at a constant temperature of 30 °C. The fermenters were not sparged to remove oxygen before inoculation. The starting dissolved oxygen saturation was 100% but rapidly dropped below 2.0% after a couple of hours. A dissolved oxygen saturation level of $\leq 1.5\%$ was maintained for the rest of the batch culture period. The cultures' pH was automatically regulated between 7.4 and 7.7 using 0.1 N HCl and 0.1N NaOH. The fermenters also operated at a stirring speed of 300 rpm. Samples were periodically collected to monitor biomass growth and organic degradation.

2.4 Analytical methods

The cultures' pH, temperature, and dissolved oxygen levels were constantly monitored using Hamilton probes and sensors incorporated in the bioreactors' systems. In addition, the optical density of the samples at 420 nm and 620 nm (OD_{420} and OD_{620}) were measured using a UV-3600 plus UV-Vis-NIR spectrophotometer (Shimadzu). The specific growth rate was measured by determining the slope of the log plot of optical density vs time. Samples for COD, total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), VFAs, and anions were first centrifuged at 6,000 g (Sorvall Lynx 6000, ThermoScientific), after which the supernatant was sieved through 0.2 μm sterile syringe filters (polyethersulfone, VWR). COD was measured via the closed micro-digestion method, using Hach high-range COD vials (0 to 1500 mg/L). TOC, IC, and TN were determined using a TOC analyzer (TOC-L series with TNM-1, Shimadzu), which employs a 680 °C combustion catalytic oxidation method. Total carbon and IC were obtained directly, while TOC was determined by subtraction. Measurements of VFAs and anions were measured using ion chromatography (940 Professional IC Vario, Metrohm). Light intensity and spectrum were measured using the UV-vis-NIR spectroradiometer Black-Comet-HR-Series (StellarNet Inc.).

The biomass's total and volatile suspended solids (TSS, VSS) were measured using standard methods [27]. VSS for each day was estimated by absorbance measurements at 420 nm using Eq. (2), which was developed from measurements taken with R^2 of 0.999.

$$\text{VSS} = 14.343 \times \text{Abs}^2 + 91.88 \times \text{Abs} - 27.126 \quad (2)$$

Biomass morphological characteristics via imaging and elemental mapping were determined using a scanning electron microscope (SEM), model Quanta 650 FEG-ESEM (FEI) and using a transmission electron microscope (TEM), model Talos F200C TEM (Thermo Scientific), respectively. Suspended biomass samples were collected during the stationary phase. For SEM analysis, the liquid sample was directly placed in the sample holder using the environmental SEM feature. For TEM analysis, a 10 µL drop of suspended biomass was deposited onto a 400-mesh lacey carbon-Cu grid (CF400-CU, Electron Microscopy Sciences) and visualized directly. 16S metagenomics was performed by obtaining 5 ml culture on day 5 of the preliminary study, after which DNA extraction was performed as described by the DNeasy PowerBiofilm kit (Qiagen) protocol. Subsequently, DNA amplification and sequencing were performed as described by George et al. [26], while bioinformatics was performed using QIIME 2 via cross-referencing with the SILVA database. Biomass ash content was determined by accounting for biomass residual after oven drying at 550 °C. Biomass-based analysis was performed after harvesting the centrifuged biomass at 10,000 g and freeze-drying for 24 h. Freeze-dried biomass was then homogenized with a mortar and pestle, sealed with parafilm, and stored in a desiccator until further use. Following pretreatment via ultrasonic-assisted alkali extraction, the biomass protein was quantified using the Modified Lowry method. Subsequently, a 100 µg protein aliquot was hydrolyzed via exhaustive enzymatic hydrolysis under sterile oxygen-free condition using a PAL sample autoprocessor (CTC Analytics, Zwingen, Switzerland). The hydrolyzed protein was the utilized for amino acid characterization by LC-MS/MS using an AcquityTM UPLC system with a Quattro Premier tandem mass spectrometer as described by Rabbani and Thornlley [28]. In addition, biomass lipid and carbohydrate contents were determined using Bligh and Dyer and Anthrone methods, respectively [29, 30]. Coenzyme Q-10 was determined spectrophotometrically using the MyBioSource Human Coenzyme Q10 ELISA kit, with absorbance reading taken at 450 nm using a Tecan Spark plate reader. Pigment analysis was also performed spectrophotometrically as described by Lu et al. [12]. Trace elements and heavy metal concentration of the wastewater and microbial biomass were assessed using acid microwave digestion and inductively coupled plasma–optical emission spectrometry (ICP-OES; Agilent 5110). The limit of detection (LOD) for all characterized metals was 0.01 mg/L and 0.00001 mg/kg. Biomass digestion was performed using high-performance microwave digestion (Ethos Up, Milestone) by firstly treating 200 mg of sample biomass with 9 mL HNO₃ and 2 mL H₂O₂ and subsequently digesting the sample at 200 °C for a 30-min duration following a steady

ramp rate of 13 °C per min. After digestion, the sample was diluted and filtered through 0.2 µm sterile syringe filters (VWR) for metal content determination. The ICP-OES was operated with the radial view, and calibration for all metals of interest at their respective elemental wavelengths was performed at a range of 0 to 5 ppm. Moreover, extensive characterization for organic compounds in FSPW was conducted using gas chromatography/mass spectrometry via the headspace trap sampler (Agilent, 7820B GC system with 5977A MS) as described in the EPA methods 8260 B and 8260 C [31]. Over 130 volatile and semi-volatile organic compounds were characterized. Finally, freeze-dried biomass was qualitatively characterized using a Py-GC-MS setup (CDS 6200 pyroprobe, Shimadzu GCMS-QP2020 NX, Kyoto, Japan) according to the protocol described by Sabah et al. [32].

3 Results and discussion

3.1 Wastewater characteristics

Chemical characterization of FSPW revealed that the wastewater was acidic and rich in organic carbon, like short-chain volatile fatty acids, with a total COD of over 10,000 mg/L. Table 1 summarizes the physicochemical characteristic of the wastewater which show a varied VFA profile, PNSB has been reported to have a higher productivity when cultured in such a carbon substrate [33]. A study that determined the impact of VFAs on PNSB growth kinetics revealed that cultures with mixed VFAs had higher growth rates (up to 2.5 fold) than cultures with individual VFA [34]. A recent study utilizing a different PNSB strain also reported higher growth

Table 1 FSPW physicochemical characteristics prior to nutrient addition. ±values are standard deviation from duplicates

Characteristics	Concentration
pH	3.48±0.12
Electrical conductivity	191.6±0.14 µS/cm
Total dissolved solids	123 mg/L
COD	10,340±790 mg/L
Total organic carbon (TOC)	2794±214 mg/L
Total nitrogen	0 mg/L
Total phosphate	0 mg/L
Acetate	1420±46 mg/L
Butyrate	373±17 mg/L
Iso-butyrate	1493±127 mg/L
Propionate	318±21 mg/L
Valerate	283±13 mg/L
Formate	34±0.60 mg/L
Iso-valerate	46±0.01 mg/L
Characterized VFA fraction of COD	5,226.9 mg/L

rates with mixed VFA. However, the group with a sole VFA (acetate) had a similar growth rate [35]. In this study, the characterized VFAs accounted for about 51% of the total COD. In addition, further characterization revealed that alcohols like methanol and butanol account for the majority (>45%) of the outstanding COD. This is similar to the results obtained in another study that utilized process water from the same source [36]. Table A1 shows organic constituents of GTL process water from other studies, revealing similar organic profiles. The GC characterization of over 130 volatile and semi-volatile compounds revealed FSPW was void of toxic hydrocarbons like benzene, o-xylene, and a host of other benzene derivatives. In fact, the organics detected in the exhaustive screening accounted for less than 0.2% of the total COD. However, seven of the 16 US EPA priority polyaromatic hydrocarbons (PAHs)- acenaphthylene, chrysene, dibenz[a,h]anthracene, fluorene, fluoranthene, naphthalene, and pyrene were detected in low concentrations with a combined concentration of 218 µg/L. Based on the EPA classification, five of the priority PAHs detected are classified as “not classifiable as to human carcinogenicity” (Class D), while chrysene (0.008 µg/L) and dibenz[a,h]anthracene (0.0285 µg/L) are classified as “probable human carcinogenic” (Class B) [37]. An exhaustive list of the result is presented in Table A2. Even though in minute concentrations, the presence of potentially toxic aromatic compounds increases the need for scrutiny associated with possible microbial protein recovery from the feedstock. In this study, the characterized biomass was void of all priority PAHs, indicating that they were either degraded or released with the effluents. Organisms like *Rhodopseudomonas palustris* have been associated with the degradation of aromatic compounds [38], especially in low concentrations. Thus, extensively screening recovered biomass and effluent for toxicants of interest would be an integral consideration if the biotechnology materializes.

Heavy metal analysis of the wastewater revealed that heavy metals of public health concern were either absent or below the Water Environment Partnership Asia (WEPA) and the United States Environmental Protection Agency (USEPA) maximum permissible limits for both drinking water and industrial effluents, as shown in Table A3 [39, 40]. Only minute concentration of trace minerals like Co (0.2 mg/L), Fe (0.7 mg/L), Mn (0.9 mg/L), Zn (0.7 mg/L), and K (8.7 mg/L) was detected, which were likely related to reaction catalyst. This is an essential consideration when deliberating on potential feedstock for SCP production, as certain PNSB strains (*Rhodobium marinum* NW16 and *Rhodobacter sphaeroides* KMS24) have been reported to bioremediate heavy metals via biosorption and bioaccumulation [41]. Hence, using sources like domestic and non-agroindustrial wastewater is typically not recommended [42]. Another challenge with domestic wastewater and animal manure use

is the presence of faecal pathogens, which are not present in FSPW due to the pure chemical synthesis process that generates it. However, a notable drawback of FSPW's chemical characteristics is the paucity of essential nutrients like nitrogen and phosphorus, which is typically present in agro-based and domestic wastewater [20]. This challenge can be overcome by utilizing a relatively benign nutrient-rich waste stream as nitrogen and phosphorus sources. A study that examined hydrogen production from organic wastewater using PNSB successfully utilized monosodium glutamate crystallization (Aji-L) waste as a nitrogen source. Interestingly, the study found that Aji-L performed more favorably compared to the commercial nitrogen source (glutamate) as it contained some essential micronutrients [43].

3.2 Preliminary study with diluted FSPW

Results from preliminary treatment revealed that the FSPW was a suitable carbon substrate for the PNSB-rich mixed culture. After the 16-day culturing period, over 98% of the COD had been degraded. Around 70% of the organics were consumed by the fifth day, after which the COD removal efficiency decreased significantly over the next eight days until nitrogen was added to the culture. The reduced organic removal efficiency rate was most probably attributed to $\text{NH}_4\text{-N}$ depletion as nitrogen is a growth limiting nutrient, but is likely compounded by the complete VFA degradation within the period. Traditionally, it is expected that most PNSBs prefer to degrade only short-chain carbon substrate via a photoheterotrophic mode of metabolism; therefore, degrading more complex organics could be challenging [44]. A study that compared the feasibility of valorizing VFAs and sugar manufacturing wastewater (COD = 8 g/L) to hydrogen and lipids using *Rhodobacter sp.* reported maximum substrate degradation and higher productivity with VFAs compared to sugar manufacturing wastewater, the latter achieving only 65% degradation [43]. The reduced efficiency was attributed to the presence of more complex carbons like sugars. This is probably why COD degradation is fastest within the first five days in this study, as the easily degradable carbon substrates are expected to be consumed. Hence, studies have explored the possibility of degrading recalcitrant organics to VFAs via anaerobic fermentation before utilizing them as carbon substrates for photoheterotrophic biomass production [34, 45]. Nitrogen concentrations were closely monitored in the extensive experimentation to verify its growth limiting effect. Figure 1 shows the trends of COD removal. In addition, a peak $\text{OD}_{620\text{nm}}$ of 2.58 was obtained on day 13, while the average VSS obtained was 740 ± 14 mg/L. The biomass yield of the setup was about $0.5 \text{ gVSS}_{\text{produced}}/\text{gCOD}_{\text{consumed}}$, which is consistent with other studies that utilized non-axenic PNSB cultures for wastewater treatment [13, 46]. A biomass yield close to unity

Fig. 1 COD removal efficiency of the preliminary batch cultivation experiment of diluted FSPW

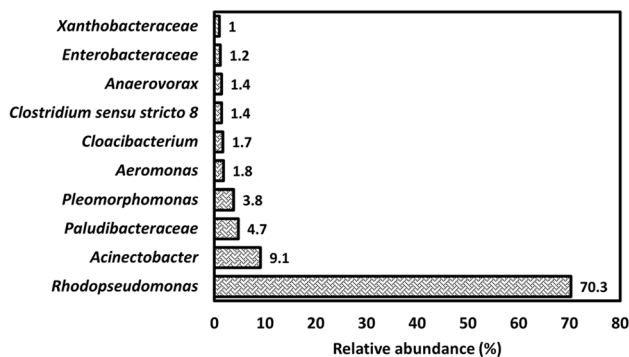
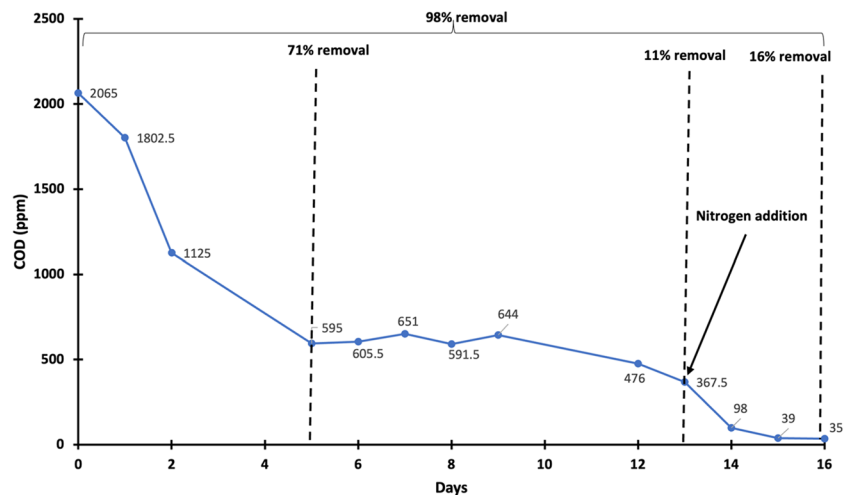


Fig. 2 Microbial community dynamics of preliminary assessment showing relative abundance

has been reported in studies that cultured PNSB in organic acid substrates, under continuous light-anaerobic conditions [34, 47]. However, in more complex carbon substrates, lower yields are obtained due to increased fermentation activity, PNSB H_2 production in nutrient deficient conditions, and competition with non-PNSBs [13]. In a similar study utilizing FSPW from the same source, high biomass yields were obtained when highly preferential VFAs like acetate, propionate and valerate were available [48]. However, upon complete degradation, the biomass yield reduced significantly. An additional factor identified was the reduced photon energy available at the trial end due to light attenuation [48].

Results from the preliminary assessment confirm PNSB growth and enrichment even when cultured under a light source mostly within the visible light spectrum. Metagenomics analysis revealed that *Rhodopseudomonas* sp was the most abundant organism detected (70.3%), followed by common chemoheterotrophs like *Acinetobacter* (9.1%) and *Paludibacteraceae* (4.7%) commonly found in mixed culture studies [49, 50]. Figure 2 shows the microbial community data for microbes with at least 1% abundance. High

PNSB growth and selectivity have also been reported in another study that examined PNSB's light-harvesting ability under several light sources within the visible light spectrum [51]. This is primarily because PNSB's carotenoids are typically present in the light-harvesting complex 2, which can absorb photons in a wide range of visible spectra (450 to 550 nm). Continuous light-anaerobic conditions favor the growth and selectivity of PNSB over other phototrophs [13], particularly over oxygenic phototrophs like algae. PNSB dominance was further supported by the cell morphological characteristics during the stationary phase via SEM and TEM analysis. Most of the cells captured had a distinctive cylindrical rod-like shape, as seen in several studies that examined axenic cultures of *Rhodopseudomonas* sp. [52–54]. This was anticipated as *Rhodopseudomonas* was the dominant microbe in the starting inoculum. Furthermore, the SEM and TEM images (Figs. 3 and 4) show that the cells accumulated granules, suspected to be polyphosphates, as seen in other wastewater treatment biomass characterization studies [55]. Moreover, PNSB has been reported to accumulate polyphosphates in the stationary phase [56]. Typically, excess light energy is stored as polyphosphate and released in energy-deficient conditions, while biopolymers are accumulated when carbon is available and depleted in carbon-deficient conditions. Further analysis via elemental mapping and annular dark-field imaging revealed that the percentage weight of phosphorus in the cells was 4.1% and that the granules were most likely comprised of potassium and magnesium polyphosphates. The annular dark-field imaging of carbon, nitrogen, oxygen, potassium, magnesium, and phosphorus can be found in Fig. 5. This shows that beyond the recovery of nutritionally desirable constituents, which is the primary objective of this study, PNSB can also be utilized for direct phosphorus recovery in other types of wastewaters containing significant phosphorus concentrations.

Fig. 3 **A** SEM analysis (secondary electrons); **B** SEM analysis (backscattered electrons) of the FSPW culture during stationary phase

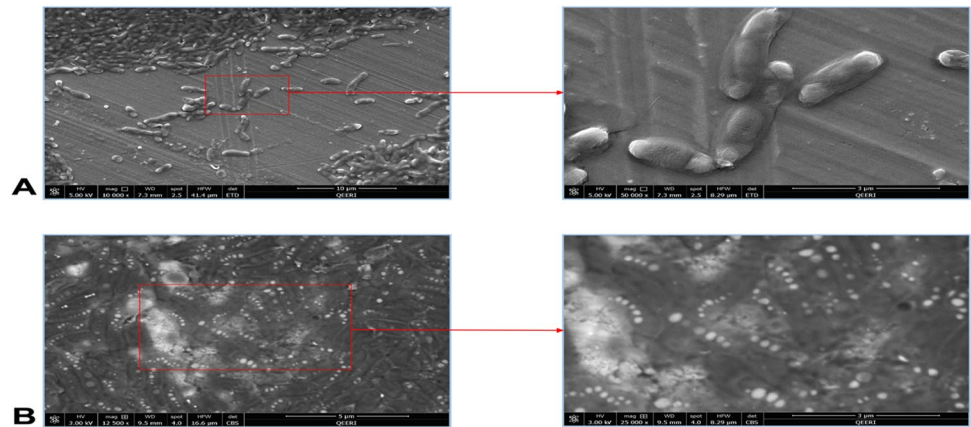
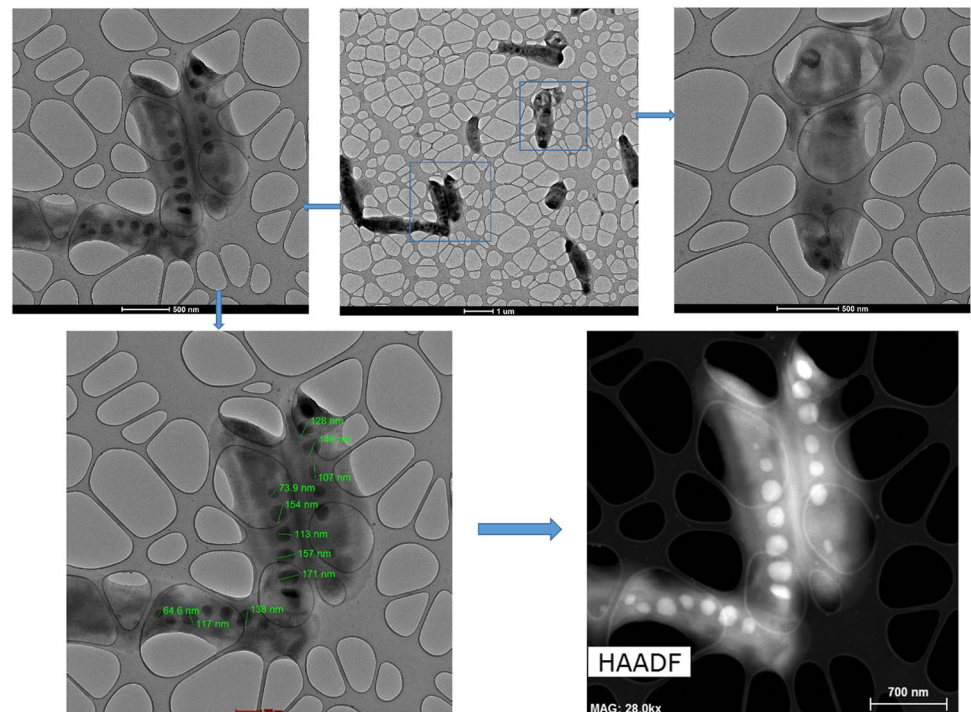


Fig. 4 TEM analysis of the FSPW culture during stationary phase



3.3 Extensive experimentation with undiluted FSPW

3.3.1 Pollutant degradation

The oxygen saturation of the culture was at its peak at the start of the trial ($93.9 \pm 5.5\%$); however, dissolved oxygen (DO) levels reduced significantly through the passage of time. By the 14th hour, DO levels reached $1.1 \pm 0.1\%$ and remained constant until the end of the trial. This created an illuminated-micro-aerobic environment beneficial for PNSB photoheterotrophic growth. The removal of oxygen in mixed cultures is typically facilitated by aerobic or/and facultative anaerobic microbes. However, once the oxygen level is depleted, PNSB outcompetes other microbes due to their

selectivity under light and oxygen-limited conditions [8, 13]. Confirmation of PNSB dominance (*Rhodospseudomonas* sp. >50%) was obtained from community diversity analysis (authors' unpublished data). Figure A2 shows the trend of DO removal.

The average COD removal efficiency over 11 days was $78 \pm 0.3\%$, of which around 63% was consumed within the first 72 h. On the 8th day, a peak average OD_{420nm} of 6.268 ± 0.096 was obtained, with average biomass concentration of 1.11 ± 0.037 g_{vss}/L and a specific growth rate (μ_{max}) of 0.589 d⁻¹. Figures 6 A and 7 B show the biomass growth at OD_{420nm} and biomass concentration through culture duration. A similar range of specific growth rate (0.48 – 0.71 d⁻¹) has been reported in a study that examined the impact of VFAs, light, and oxygen on *Rhodobacter sphaeroides*

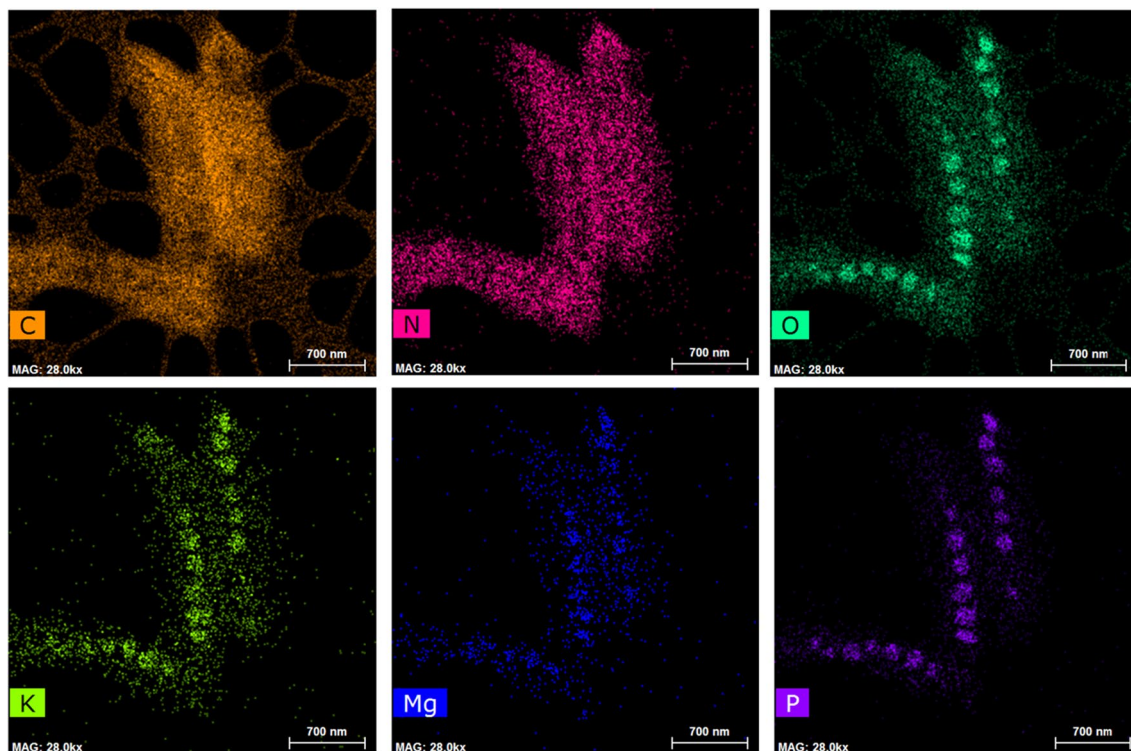
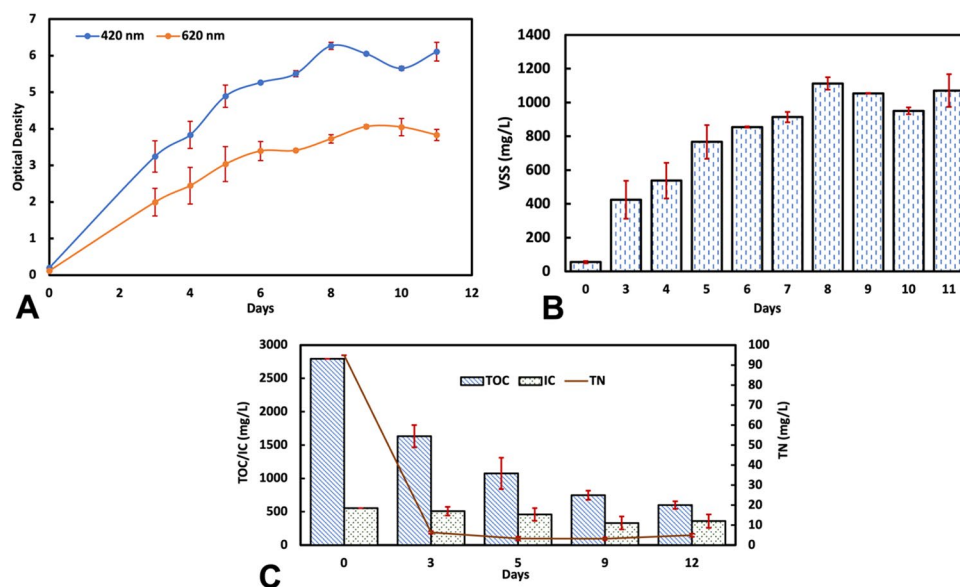


Fig. 5 TEM annular dark-field imaging of selected elements in PNSB showing the granules consistency with O, K, Mg, and P

Fig. 6 **A** Biomass growth at OD_{420nm} and OD_{620nm} ; **B** bio-mass concentration in $VSS_{mg/L}$; **C** trend of TOC, IC, and TN removal



[35]. Another study utilizing naturally occurring chemoheterotrophic bacteria in FSPW reported a significantly lower COD removal rate of around $260 \text{ mg L}^{-1} \text{d}^{-1}$ compared to the rate of about $710 \text{ mg L}^{-1} \text{d}^{-1}$ obtained in this study by the photoheterotroph-dominated community [36]. This demonstrates that PNSB's productivity and metabolic versatility have the potential to be a strong performer for the biological treatment of FSPW. The comparatively higher biomass

concentration obtained in the undiluted culture compared to the diluted culture is in line with another study that reported a positive correlation between PNSB biomass concentration and the organic strength of the wastewater [33] and is beneficial for industrial application. The average biomass yield was $0.14 \pm 0.005 \text{ gVSS}_{\text{produced}}/\text{gCOD}_{\text{consumed}}$, which was significantly lower than the yield obtained in the preliminary study. The substantially lower biomass yield obtained is

most probably attributed to the microaerobic condition of the reactors. Oxygen has been reported to suppress photoheterotrophic metabolism and favor chemoheterotrophy, resulting in lower biomass yields [47]. Besides, in non-sterile cultures, there is increased competition with aerobic heterotrophs. A similar level of biomass yield has been reported in mixed-culture studies under continuous light-microaerobic conditions [57].

The nitrogen concentration, measured by TN analysis, was depleted within 72 h, indicating that nitrogen was a limiting nutrient when considered along with the COD degradation profile. The complete organic degradation, aided by nitrogen replenishment in the preliminary study, supports this stance. The COD removal rate within the first 72 h prior to nitrogen depletion ($2100 \text{ mg L}^{-1}\text{d}^{-1}$) was significantly higher than the removal rate at the trial end ($710 \text{ mg L}^{-1}\text{d}^{-1}$). Similar trends have also been reported in past studies, where increased nitrogen concentrations significantly enhance biomass concentration, protein content, and COD removal [44]. Furthermore, the high nitrogen removal efficiency ($\text{NH}_4^+\text{-N}$) at a rate of 29.5 mg/L/day observed in this study outperforms the removal rates from similar studies. A recent study that examined nitrogen metabolism in photosynthetic bacteria reported an $\text{NH}_4^+\text{-N}$ removal efficiency of around 22 mg/L/d after three days under similar conditions [58]. This gives further credence to the suitability of FSPW for PNSB-based biomass production. Figure 6 C shows the trends of TOC, TN, and IC removal. TOC decreased throughout the test but at a lower rate after TN was nearly depleted. IC also decreased slightly throughout the test.

This study supports the ability of PNSB to thrive in a high-strength wastewater. PNSB's versatility in similar high-strength wastewater has been previously reported in several studies [48, 59, 60] and is one reason they are purported as an ideal organism for integration with wastewater treatment and resource recovery [8, 10]. Furthermore, in this study, PNSB's versatility was tested at an influent C/N ratio of 115:1, as opposed to the widely acceptable range of 2:1 to 20:1 for other activated sludge, microalgae, and purple phototrophic bacteria studies [61–63]. Results from this study provide further credence to PNSB's adaptability. In fact, in one study, *Rhodobacter sphaeroides*' versatility in pollutant removal and bioconversion was reported across C/N ratios ranging from 400 to 0.1, resulting in at least 60% COD removal and 40–60% microbial protein content [64]. This has been one of the drawbacks of microalgae-based wastewater treatment which has been more widely explored for potential resource recovery potential [65]. High COD concentrations ($\text{C:N}>20$) have been reported to inhibit algal growth, thereby making wastewater pretreatment an integral part of the process [66, 67]. However, in this instance, wastewater was directly applied to the biotechnology, resulting in respectable treatment efficiencies and biomass

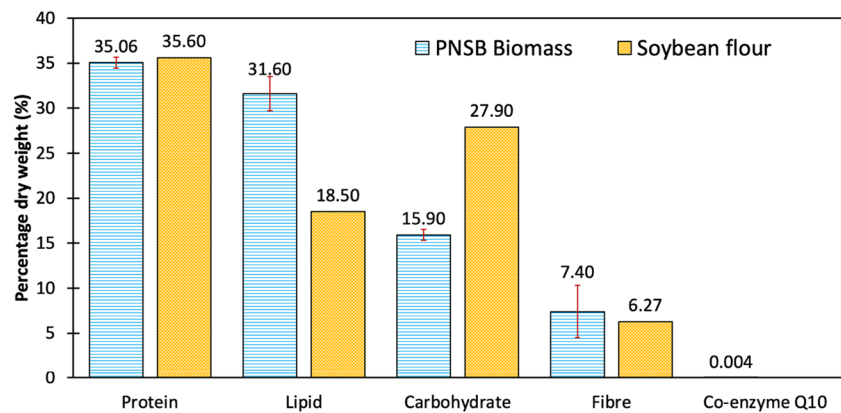
concentration. This is particularly advantageous for organic-rich wastewaters innately lacking essential nutrients like nitrogen. Industries involved in winery, textile processing, vinegar production, pineapple processing, and pharmaceuticals are among those that produce nutrient deficient wastewaters [68, 69]. Augmenting feedstock at recommended C/N ratios could potentially increase operating cost significantly. Therefore, being able to achieve pollutant removal rate and biomass concentration at high C/N ratios enhances the feasibility of utilizing such waste streams for integrated treatment and resource recovery purposes. Overall, the biomass concentration and organic and nitrogen removal rates obtained in this study exceed values reported in several algae-based wastewater treatment studies and are comparable to other purple phototrophic bacteria-based studies [8, 66]. For example, removal rates of around $1,000 \text{ mg}_{\text{COD}}\text{L}^{-1}\text{d}^{-1}$ and $38 \text{ mg}_{\text{NH}_4\text{-N}}\text{L}^{-1}\text{d}^{-1}$ were obtained in studies that cultured PNSB in pharmaceutical and brewery wastewater using cultures of *R. sphaeroides* and *R. capsulate* [12, 70].

3.3.2 Biomass characterization

Proximate analysis revealed that the biomass consisted of $35\pm0.6\%$ protein, $31.6\pm1.9\%$ lipid, $15.9\pm0.6\%$ carbohydrate and $7.4\pm2.9\%$ fiber. Similar protein content has been reported in aquafeed protein sources like soybean, squid liver paste, and commercial aquafeed [71, 72]. Figure 7 is a descriptive comparison between PNSB biomass in this study and soybean flour from another study. The detection of 35% dry cell protein content under baseline experimentation is promising, considering this result was achieved under nitrogen-limited conditions at trial end and that several studies have reported that PNSB protein content tends to increase significantly under optimized conditions [19, 20]. The COD:N:P uptake ratio was 408:5:1 as opposed to the widely reported 100:7:1 [13]. Comparative protein content was obtained in similar studies under nitrogen deficient conditions [60, 74]. This is promising for converting carbon-rich (nitrogen-limited) feedstocks to protein feed, such as FSPW, that may not usually be considered for such application. However, further studies are needed to enhance protein content and quality.

Amino acid characterization revealed the biomass protein consisted of all the nine essential amino acids for humans (tryptophan, threonine, phenylalanine, leucine, isoleucine, histidine, valine, methionine, and lysine). All other non-essential amino acids were also present. Other amino acids like glycine and arginine which specifically are considered as essential for aquaculture and poultry feed were also present in substantial quantities [75, 76]. This is highly desirable especially when compared to plant-based protein sources which have been reported to be deficient in essential amino acids like lysine and tryptophan and sulphur containing

Fig. 7 Proximate analysis of PNSB biomass (this study) and soybean flour (from Useh et al. [73])



amino acids like methionine and cysteine [77]. Thus, such amino acids are typically manufactured via chemical synthesis and often added as supplements to plant-based animal feeds [78]. For example, in a recent study that examined the amino acid profile of soybean grain, essential amino acids

like methionine and tryptophan were absent [79], thereby making feed mixing and supplementation important [76]. As seen in Table 2, the amino acid profile of the biomass protein was comparative to *R. faecalis* biomass and soyabean grains [20, 79]. The amino acid profile was also within the dietary

Table 2 Amino acid characterization of the PNSB-dominated mixed culture from this study compared with *R. faecalis*, soya bean grain and the requirements for penaeid shrimp, channel catfish and broiler chickens

Amino acids (g/g diet)	PNSB-dominated mixed culture	PNSB (<i>R. faecalis</i>) [20]	Soya bean grain [79, 80]	Requirement for penaeid shrimp [81]	Requirement for channel catfish [82]	Requirement for broilers [83]
Essential AA						
Phenylalanine	1.4	1.9	1.9	0.9	1.4**	—
Leucine	2.7	2.1	2.8	1.7	1.0	—
Isoleucine	1.1	1.0	1.7	0.8	0.7	0.6–0.8
Methionine	0.6	0.3	0.0	0.7*	0.6*	0.6–0.9*
Valine	2.2	1.2	1.7	1.0	0.8	0.7–0.9
Lysine	2.0	3.4	2.4	1.8	1.4	0.85–1.1
Threonine	2.4	1.4	1.4	1.8	0.6	0.7–0.8
Histidine	0.7	1.2	1.2	0.5	0.4	—
Tryptophan	0.4	—	0.0	—	0.14	0.2
Total	13.5	12.5	13.1			
Non-essential AA						
Cystine	0.3	—	0.8	—	—	—
Cysteine	0.03	—	—	—	—	—
Aspartate	2.4	3.5	4.4	—	—	—
Glutamate	2.2	2.1	8.1	—	—	—
Serine	3.4	0.5	2.5	—	—	—
Glycine	4.2	1.4	1.9	—	—	—
Alanine	2.7	1.4	1.8	—	—	—
Arginine	1.8	1.3	2.9	—	1.2	1–1.3
Tyrosine	0.9	0.8	1.4	—	—	—
Proline	2.4	1.5	2.2	—	—	—
Asparagine	0.6	—	—	—	—	—
Glutamine	0.6	—	—	—	—	—
Total	21.5	12.5	26.0			

*Total sulfur amino acids

**Phenylalanine + Tyrosine

requirement of feeds for penaeid shrimp, channel catfish, and broilers (poultry) [81–83]. Nevertheless, it is important for future studies to investigate the protein digestibility of the biomass, which has impacts on amino acid bioavailability in test organisms [84]. For instance, studies have suggested plant protein sources have a poorer digestibility than animal protein sources in humans [84].

In terms of biomolecules of interest, the biomass contained $0.55 \pm 0.03\%$ bacteriochlorophylls, $0.47 \pm 0.08\%$ carotenoids and $0.004 \pm (5.4 \text{ E}^{-6})\%$ coenzyme Q10. The presence of other valuable products of nutritional benefit to aquatic organisms and humans, such as lipids, carotenoids, and coenzyme Q10, can increase the monetary value of the SCP [85–87]. Compared to other studies, the biomass pigment and coenzyme Q10 concentrations are lower [12, 20]. This is probably due to the differences in culture conditions, such as increased light intensity and the use of pure PNSB strains, different feedstock, and light sources. However, when biomass pigment is compared with other mixed culture studies, similar pigment concentrations were obtained [13].

Metal analysis of the biomass also revealed that undesirable heavy metals like arsenic, cadmium, mercury, and lead were not detected. These metals have no nutritional value to livestock and are generally classified as heavy metals of public health concern with very low maximum permissible limits in animal feeds [88]. This supports the feasibility of SCP resource recovery from a relatively benign industrial process water rather than from other more hazardous waste streams. The bioaccumulation of toxic metals has been identified as a major hindrance to upscaling biomass protein recovery from other waste streams [8]. As seen in Table 3, recommended dietary concentrations of trace minerals essential for livestock growth, like zinc (152 mg/kg) and copper (86 mg/kg), were present in the biomass, thereby enhancing the value of the biomass. A deficiency of essential trace minerals like iron, copper, manganese, zinc, selenium, cobalt, and chromium in fish feed has been associated with high morbidity and mortality rate among several fish species [99].

On the other hand, metals like cobalt (33 mg/kg), manganese (519 mg/kg) and chromium (72 mg/kg) were above the limits recommended by the European Commission for animal feed [88]. The former two metals were present in both the FSPW and trace mineral solution, so there is a possibility to reduce them through optimization of the trace element solution. For instance, when estimating the total metal concentration from both FSPW and mineral supplements, under 50% of total feedstock Mn, Co, Cu, Mo, Ni, and Sr concentrations were from FSPW, indicating that these trace minerals can be significantly regulated. Chromium was not detected in the wastewater, trace mineral solution or vitamin solutions and is therefore expected to be a result of cross-contamination. Table A4 shows the metal concentrations of the trace mineral and vitamin supplements before adding

Table 3 Metal content of the dry PNSB biomass in relation to European Commission Standards

Metal	Concentration in dry biomass (mg/kg)	European Commission Standards (mg/kg)
Nutritionally undesirable metals		
As	Undetected	2 [89]
Cd	Undetected	0.5
Hg	Undetected	0.1–1.0 [89]
Pb	Undetected	10 [89]
Tl	Undetected	0 [90]
Nutritionally desirable metals		
Co	33 ± 0.0	0.3–2 [91]
Cr	71.5 ± 0.0	0.4 [92]
Cu	86.2 ± 0.05	25–170 [93]
Fe	$1,298 \pm 0.0$	450–1500 [94]
Mn	518.8 ± 0.5	100–150 [95]
Mo	2.75 ± 0.07	2.5 [88]
Ni	$49.5 \pm <0.01$	
Se	Undetected	0.5 [96]
Zn	152.2 ± 0.2	120–200 [97]
Other metals		
K	764.5 ± 0.6	6,000 – 12,000 [98]
Sr	Undetected	
Be	Undetected	

to the feedstock. Their relative concentrations signify that concentrations in the final biomass product can be controlled by regulating the nutrients added to the feedstock. Moreover, concentrations in the final feed product can also be controlled by blending biomass with commercial feed having reduced levels of the same metals.

4 Conclusion

Conventional industrial wastewater treatment plants are constructed to remove impurities and reuse treated water. However, incorporating a resource recovery process into wastewater treatment has recently been recognized as one of the hallmarks of environmental sustainability. Hence, this novel preliminary study has shown the potential of upcycling protein-rich biomass from anaerobic treatment of FSPW to biomass that could be considered for animal feed. While the study did not focus on overall treatment optimization, the results indicate that under non-axenic conditions, PNSB was able to efficiently treat high-strength industrial wastewater by achieving 78 to 100% COD removal and complete nitrogen removal at the end of the batch trials. The overall process rates were slow due to batch operation from a small seeding concentration (50 mg/L) and unconventional starting C:N:P ratio (429:4:1). However, the

upcycled biomass also had an amino acid profile comparative to soybean and meeting the dietary requirement of livestock like channel catfish, penaeid shrimp, and poultry birds. Considering the agricultural dynamics of the study region, developing this biobased circular economy could potentially strengthen food security. However, further experiments are required to optimize culturing conditions for high-quality protein/amino acid yield and ascertain the safety of the SCP with more extensive testing, including aquaculture studies.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s13399-023-04518-w>.

Acknowledgements The authors would like to acknowledge Shell Research and Technology Centre for their technical support. The authors also acknowledge the Qatar Biomedical Research Institute and HBKU Core-Labs for facilitating the amino acid quantification, GC-MS analysis, and SEM and TEM analysis. Special thanks to Prof Paul Thornalley, Dr Said Mansour, Dr Mohammad Wasim Aktar, Dr Patrick Wijten, Mr Arun K. K, Mr Mujaheed Pasha, Mr Abdulaziz Alemadi, and Mr Janarthanan Ponraj.

Author contribution OZW: conceptualization, methodology, formal analysis, investigation, data curation, writing – original draft. UO: resources, writing: review and editing, funding acquisition. ASV: supervision, writing: review and editing, funding acquisition. GM: supervision, writing: review and editing, project administration. HRM: conceptualization, methodology, writing: review and editing, supervision, funding acquisition, project administration.

Funding Open Access funding enabled and organized by CAUL and its Member Institutions. The authors would like to acknowledge the Qatar National Research Fund (grant MME01-0910-190029) for supporting this research.

Data availability Data available on request from the authors.

Declarations

Ethical approval Not applicable.

Competing interests Qatar Science and Technology Research Centre is an entity of Shell Global, a global producer of fuel synthesis products.

Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

References

- Lahlou F, Mackey HR, McKay G et al (2020) Water planning framework for alfalfa fields using treated wastewater fertigation in Qatar: an energy-water-food nexus approach. *Comput Chem Eng* 141:106999. <https://doi.org/10.1016/j.compchemeng.2020.106999>
- Mahmood F, Ghiat I, Govindan R, Al-Ansari T (2020) Reduced-order modelling (ROM) approach for optimal microclimate control in agricultural greenhouses. *Comput Aided Chem Eng* 48:1879–1884. <https://doi.org/10.1016/B978-0-12-823377-1.50314-1>
- FAO (2011) Markets in the Middle East: market trade and consumption. In: GLOBEFISH - Information and Analysis on World Fish Trade. <http://www.fao.org/in-action/globefish/fishery-information/resource-detail/en/c/338542/>. Accessed 10 Apr 2021
- Günay D, Tolon T, Emiroğlu D (2018) Current state of inland capture fisheries and aquaculture in Middle East countries and expectations for the future. *Journal of Limnology and Freshwater Fish Res*:122–129. <https://doi.org/10.17216/limnofish.363924>
- FAO (2020) The State of World Fisheries and Aquaculture 2020. FAO, Rome
- D'Abramo LR (2021) Sustainable aquafeed and aquaculture production systems as impacted by challenges of global food security and climate change. *J World Aquac Soc* 52:1162–1167. <https://doi.org/10.1111/jwas.12867>
- Hua K, Cobcroft JM, Cole A et al (2019) The future of aquatic protein: implications for protein sources in aquaculture diets. *One Earth* 1:316–329. <https://doi.org/10.1016/j.oneear.2019.10.018>
- Wada O, Vincent AS, Mackey HR (2022) Single-cell protein production from purple non-sulphur bacteria-based wastewater treatment. *Rev Environ Sci Biotechnol* 21:931–956. <https://doi.org/10.1007/s11157-022-09635-y>
- Wan AHL, Davies SJ, Soler-Vila A et al (2019) Macroalgae as a sustainable aquafeed ingredient. *Rev Aquac* 11:458–492. <https://doi.org/10.1111/raq.12241>
- Rashid N, Onwusogh U, Mackey HR (2022) Exploring the metabolic features of purple non-sulfur bacteria for waste carbon utilization and single-cell protein synthesis. *Biomass Convers Biorefin*. <https://doi.org/10.1007/s13399-022-03273-8>
- Øverland M, Skrede A (2017) Yeast derived from lignocellulosic biomass as a sustainable feed resource for use in aquaculture. *J Sci Food Agric* 97:733–742. <https://doi.org/10.1002/jsfa.8007>
- Lu H, Peng M, Zhang G et al (2019) Brewery wastewater treatment and resource recovery through long term continuous-mode operation in pilot photosynthetic bacteria-membrane bioreactor. *Sci Total Environ* 646:196–205. <https://doi.org/10.1016/j.scitotenv.2018.07.268>
- Capson-Tojo G, Batstone DJ, Grassino M et al (2020) Purple phototrophic bacteria for resource recovery: challenges and opportunities. *Biotechnol Adv* 43:107567. <https://doi.org/10.1016/j.biotechadv.2020.107567>
- Sali S, Mackey HR (2021) The application of purple non-sulfur bacteria for microbial mixed culture polyhydroxyalkanoates production. *Rev Environ Sci Biotechnol* 20:959–983. <https://doi.org/10.1007/s11157-021-09597-7>
- Yang A, Zhang G, Meng F et al (2017) Enhancing protein to extremely high content in photosynthetic bacteria during biogas slurry treatment. *Bioresour Technol* 245:1277–1281. <https://doi.org/10.1016/j.biortech.2017.08.109>
- Boogaard PJ, Carrillo J-C, Roberts LG, Whale GF (2017) Toxicological and ecotoxicological properties of gas-to-liquid (GTL) products. 1. Mammalian toxicology. *Crit Rev Toxicol* 47:121–144. <https://doi.org/10.1080/10408444.2016.1214676>
- Veolia Water Technologies (2015) Shell Pearl GTL. <https://www.veolia.com/en/our-customers/achievements/industries/oil-gas/qatar-shell-pearl-gtl>. Accessed 4 July 2023
- Puyol D, Bastone D, Hülsen T et al (2017) Resource recovery from wastewater by biological technologies: opportunities,

- challenges, and prospects. *Front Microbiol* 7. <https://doi.org/10.3389/fmicb.2016.02106>
19. Reihani SFS, Khosravi-Darani K (2019) Influencing factors on single-cell protein production by submerged fermentation: a review. *Electron J Biotechnol* 37:34–40. <https://doi.org/10.1016/j.ejbt.2018.11.005>
 20. Patthawaro S, Saejung C (2019) Production of single cell protein from manure as animal feed by using photosynthetic bacteria. *Microbiologyopen* 8. <https://doi.org/10.1002/mbo3.913>
 21. Surkatti R, El-Naas MH, Van Loosdrecht MCM et al (2020) Biotechnology for gas-to-liquid (GTL) wastewater treatment: a review. *Water (Basel)* 12:2126. <https://doi.org/10.3390/w12082126>
 22. Wang D, Ma W, Han H et al (2017) Enhanced treatment of Fischer–Tropsch (F-T) wastewater by novel anaerobic biofilm system with scrap zero valent iron (SZVI) assisted. *Biochem Eng J* 117:66–76. <https://doi.org/10.1016/j.bej.2016.09.012>
 23. Rahman NA, Jose Jol C, Linus AA et al (2021) Fischer Tropsch water composition study from distillation process in gas to liquid technology with ASPEN simulation. *Case Stud Chem Environ Eng* 3:100106. <https://doi.org/10.1016/j.csee.2021.100106>
 24. Martínez-Vargas DX, Sandoval-Rangel L, Campuzano-Calderon O et al (2019) Recent advances in bifunctional catalysts for the Fischer–Tropsch process: one-stage production of liquid hydrocarbons from syngas. *Ind Eng Chem Res* 58:15872–15901. <https://doi.org/10.1021/acs.iecr.9b01141>
 25. Evans G, Smith C (2012) Biomass to liquids technology. In: *Comprehensive Renewable Energy*. Elsevier, pp 155–204
 26. George DM, Ramadoss R, Mackey HR, Vincent AS (2022) Comparative computational study to augment UbiA prenyltransferases inherent in purple photosynthetic bacteria cultured from mangrove microbial mats in Qatar for coenzyme Q10 biosynthesis. *Biotechnol Rep* 36:e00775. <https://doi.org/10.1016/j.btre.2022.e00775>
 27. APHA (2012) Standard methods for the examination of water and wastewater, 22nd edn. Jointly produced by the American Public Health Association, American Water Works Association and Water Environment Federation. American Public Health Association, Washington DC
 28. Rabbani N, Thornalley PJ (2020) Reading patterns of proteome damage by glycation, oxidation and nitration: quantitation by stable isotopic dilution analysis LC-MS/MS. *Essays Biochem* 64:169–183. <https://doi.org/10.1042/EBC20190047>
 29. Sündermann A, Eggers LF, Schwudke D (2016) Liquid extraction: bligh and dyer. In: *Encyclopedia of Lipidomics*. Springer, Netherlands, Dordrecht, pp 1–4
 30. Katoch R (2011) Carbohydrate estimations. In: *Analytical Techniques in Biochemistry and Molecular Biology*. Springer, New York, New York, NY, pp 67–76
 31. USEPA (1996) Method 8260B: volatile organic compounds by gas chromatography/mass spectrometry (GC/MS). United States Environmental Protection Agency, Washington, DC. <https://19january2017snapshot.epa.gov/sites/production/files/2015-12/documents/8260b.pdf>. Accessed 24 Apr 2023
 32. Mariyam S, Alherbawi M, Rashid N et al (2022) Bio-oil production from multi-waste biomass co-pyrolysis using analytical Py–GC/MS. *Energies (Basel)* 15:7409. <https://doi.org/10.3390/en15197409>
 33. Okubo Y, Futamata H, Hiraishi A (2005) Distribution and capacity for utilization of lower fatty acids of phototrophic purple non-sulfur bacteria in wastewater environments. *Microbes Environ* 20:135–143. <https://doi.org/10.1264/jsme2.20.135>
 34. Alloul A, Wuyts S, Lebeer S, Vlaeminck SE (2019) Volatile fatty acids impacting phototrophic growth kinetics of purple bacteria: paving the way for protein production on fermented wastewater. *Water Res* 152:138–147. <https://doi.org/10.1016/j.watres.2018.12.025>
 35. Peng L, Lou W, Xu Y et al (2022) Regulating light, oxygen and volatile fatty acids to boost the productivity of purple bacteria biomass, protein and co-enzyme Q10. *Sci Total Environ* 822:153489. <https://doi.org/10.1016/j.scitotenv.2022.153489>
 36. Surkatti R, Al Disi ZA, El-Naas MH et al (2021) Isolation and identification of organics-degrading bacteria from gas-to-liquid process water. *Front Bioeng Biotechnol* 8. <https://doi.org/10.3389/fbioe.2020.603305>
 37. Ghosal D, Ghosh S, Dutta TK, Ahn Y (2016) Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. *Front Microbiol* 7:1369. <https://doi.org/10.3389/fmicb.2016.01369>
 38. Gibson J, Harwood CS (2004) Degradation of aromatic compounds by nonsulfur purple bacteria. In: *Anoxygenic Photosynthetic Bacteria*. Kluwer Academic Publishers, Dordrecht, pp 991–1003
 39. US EPA (2021) Effluent Limitations Guidelines and Standards (ELG) Database. In: United States Environmental Protection Agency. <https://owapps.epa.gov/elg/>. Accessed 26 Apr 2022
 40. WEPA (2013) Environmental quality standards for water and effluent standards. In: *Water Environmental Partnership Asia*. <http://www.wepa-db.net/policies/law/laos/standards.htm>. Accessed 26 Apr 2022
 41. Panwichian S, Kantachote D, Wittayaweerak B, Mallavarapu M (2010) Isolation of purple nonsulfur bacteria for the removal of heavy metals and sodium from contaminated shrimp ponds. *Electron J Biotechnol* 13. <https://doi.org/10.2225/vol13-issue4-fulltext-8>
 42. Lee JZ, Logan A, Terry S, Spear JR (2015) Microbial response to single-cell protein production and brewery wastewater treatment. *Microb Biotechnol* 8:65–76. <https://doi.org/10.1111/1751-7915.12128>
 43. Assawamongkholsiri T, Reungsang A, Sittijunda S (2019) Photo-hydrogen and lipid production from lactate, acetate, butyrate, and sugar manufacturing wastewater with an alternative nitrogen source by *Rhodobacter* sp. KKK-PS1. *PeerJ* 7:e6653. <https://doi.org/10.7717/peerj.6653>
 44. Chen J, Wei J, Ma C et al (2020) Photosynthetic bacteria-based technology is a potential alternative to meet sustainable wastewater treatment requirement? *Environ Int* 137:105417. <https://doi.org/10.1016/j.envint.2019.105417>
 45. Khatami K, Atasoy M, Ludtke M et al (2021) Bioconversion of food waste to volatile fatty acids: impact of microbial community, pH and retention time. *Chemosphere* 275:129981. <https://doi.org/10.1016/j.chemosphere.2021.129981>
 46. Cerruti M, Stevens B, Ebrahimi S et al (2020) Enrichment and aggregation of purple non-sulfur bacteria in a mixed-culture sequencing-batch photobioreactor for biological nutrient removal from wastewater. *Front Bioeng Biotechnol* 8:557234. <https://doi.org/10.3389/fbioe.2020.557234>
 47. Alloul A, Muys M, Hertoghs N et al (2021) Cocultivating aerobic heterotrophs and purple bacteria for microbial protein in sequential photo- and chemotrophic reactors. *Bioresour Technol* 319:124192. <https://doi.org/10.1016/j.biortech.2020.124192>
 48. Wada VA, McKay G, Mackey HR (2023) Converting fuel-synthesis process water to aquaculture feed by purple non-sulfur bacteria. *Chem Eng Technol*. <https://doi.org/10.1002/ceat.202200535>
 49. Hülsen T, Stegman S, Batstone DJ, Capson-Tojo G (2022) Naturally illuminated photobioreactors for resource recovery from pig-gery and chicken-processing wastewaters utilising purple phototrophic bacteria. *Water Res* 214:118194. <https://doi.org/10.1016/j.watres.2022.118194>
 50. Alloul A, Cerruti M, Adamczyk D et al (2021) Operational strategies to selectively produce purple bacteria for microbial protein

- in raceway reactors. *Environ Sci Technol* 55:8278–8286. <https://doi.org/10.1021/acs.est.0c08204>
51. Yu S, Peng L, Xu Y et al (2021) Optimizing light sources for selective growth of purple bacteria and efficient formation of value-added products. *J Clean Prod* 280:124493. <https://doi.org/10.1016/j.jclepro.2020.124493>
 52. Xie HG, Xia W, Chen M et al (2018) Isolation and characterization of the tellurite-reducing photosynthetic bacterium, *Rhodospseudomonas palustris* strain TX618. *Water Air Soil Pollut* 229:158. <https://doi.org/10.1007/s11270-018-3817-y>
 53. Li B, Liu N, Li Y et al (2014) Reduction of selenite to red elemental selenium by *Rhodospseudomonas palustris* Strain N. *PLoS One* 9:e95955. <https://doi.org/10.1371/journal.pone.0095955>
 54. Luo X-W, Zhang D-Y, Zhu T-H et al (2018) Adaptation mechanism and tolerance of *Rhodospseudomonas palustris* PSB-S under pyrazosulfuron-ethyl stress. *BMC Microbiol* 18:207. <https://doi.org/10.1186/s12866-018-1361-y>
 55. Streichan M, Golecki JR, SchÄ¶n G (1990) Polyphosphate-accumulating bacteria from sewage plants with different processes for biological phosphorus removal. *FEMS Microbiol Lett* 73:113–124. <https://doi.org/10.1111/j.1574-6968.1990.tb03931.x>
 56. Lai Y-C, Liang C-M, Hsu S-C et al (2017) Polyphosphate metabolism by purple non-sulfur bacteria and its possible application on photo-microbial fuel cell. *J Biosci Bioeng* 123:722–730. <https://doi.org/10.1016/j.jbiosc.2017.01.012>
 57. Capson-Tojo G, Lin S, Batstone DJ, Hülsen T (2021) Purple phototrophic bacteria are outcompeted by aerobic heterotrophs in the presence of oxygen. *Water Res* 194:116941. <https://doi.org/10.1016/j.watres.2021.116941>
 58. Yang A, Zhang G, Meng F et al (2019) Nitrogen metabolism in photosynthetic bacteria wastewater treatment: a novel nitrogen transformation pathway. *Bioresour Technol* 294:122162. <https://doi.org/10.1016/j.biortech.2019.122162>
 59. Wang H, Zhang G, Peng M et al (2016) Synthetic white spirit wastewater treatment and biomass recovery by photosynthetic bacteria: feasibility and process influence factors. *Int Biodeterior Biodegradation* 113:134–138. <https://doi.org/10.1016/j.ibiod.2016.01.001>
 60. Shaikh S, Rashid N, McKay G et al (2023) Nitrogen influence on suspended vs biofilm growth and resource recovery potential of purple non-sulfur bacteria treating fuel synthesis wastewater. *Biochem Eng J* 190:108754. <https://doi.org/10.1016/j.bej.2022.108754>
 61. Yadu A, Sahariah BP, Anandkumar J (2018) Influence of COD/ammonia ratio on simultaneous removal of $\text{NH}_4^+ - \text{N}$ and COD in surface water using moving bed batch reactor. *J Water Process Eng* 22:66–72. <https://doi.org/10.1016/j.jwpe.2018.01.007>
 62. Valchev D, Ribarova I (2022) A review on the reliability and the readiness level of microalgae-based nutrient recovery technologies for secondary treated effluent in municipal wastewater treatment plants. *Processes* 10:399. <https://doi.org/10.3390/pr10020399>
 63. Montiel-Corona V, Buitrón G (2022) Polyhydroxybutyrate production in one-stage by purple phototrophic bacteria: influence of alkaline pH, ethanol, and C/N ratios. *Biochem Eng J* 189:108715. <https://doi.org/10.1016/j.bej.2022.108715>
 64. Meng F, Yang A, Zhang G et al (2019) Effects of C/N ratio on pollution removal efficiency and cell proliferation during the bio-conversion of wastewater by photosynthetic bacteria. *Desalination Water Treat* 156:68–77. <https://doi.org/10.5004/dwt.2019.24093>
 65. George DM, Vincent AS, Mackey HR (2020) An overview of anoxygenic phototrophic bacteria and their applications in environmental biotechnology for sustainable Resource recovery. *Biotechnol Rep* 28:e00563. <https://doi.org/10.1016/j.btre.2020.e00563>
 66. Srimongkol P, Sangtanoo P, Songserm P et al (2022) Microalgae-based wastewater treatment for developing economic and environmental sustainability: current status and future prospects. *Front Bioeng Biotechnol* 10. <https://doi.org/10.3389/fbioe.2022.904046>
 67. Ma C, Wen H, Xing D et al (2017) Molasses wastewater treatment and lipid production at low temperature conditions by a microalgal mutant *Scenedesmus* sp. Z-4. *Biotechnol Biofuels* 10:111. <https://doi.org/10.1186/s13068-017-0797-x>
 68. Broderick TA, Joseph HS (1985) Treatment of Nutrient Deficient Wastewaters. *Water Pollut Control Fed* 57:1178–1182
 69. Sánchez M, Gonzalo OG, Yáñez S et al (2021) Influence of nutrients and pH on the efficiency of vertical flow constructed wetlands treating winery wastewater. *J Water Process Eng* 42:102103. <https://doi.org/10.1016/j.jwpe.2021.102103>
 70. Madukasi EI, Dai X, He C, Zhou J (2010) Potentials of phototrophic bacteria in treating pharmaceutical wastewater. *Int J Environ Sci Technol* 7:165–174. <https://doi.org/10.1007/BF03326128>
 71. Ayuba VO, Iorkohol EK (2012) Proximate composition of some commercial fish feeds sold in Nigeria. *J Fish Aquat Sci* 8:248–252. <https://doi.org/10.3923/jfas.2013.248.252>
 72. FAO (1997) *Aquaculture Feed and Fertilizer Resource Atlas of the Philippines*. FAO, Rome
 73. Mercy U, Adedayo Babafemi A, Mary Sunday D (2017) Proximate composition, phytoconstituents and mineral contents of soybean (glycine max) flour grown and processed in Northern Nigeria. *Adv Appl Sci* 2:48. <https://doi.org/10.11648/j.aas.20170204.12>
 74. Shaikh S, Rashid N, Onwusogh U et al (2023) Effect of nutrients deficiency on biofilm formation and single cell protein production with a purple non-sulphur bacteria enriched culture. *Biofilm* 5:100098. <https://doi.org/10.1016/j.biofilm.2022.100098>
 75. BATTERY PJ, D'Mello JPF (1994) Amino acid metabolism in farm animals: An overview. In: D'Mello JPF (ed) *Amino Acids in Farm Animal Nutrition*. CAB International, Wallingford, pp 1–10
 76. Monte Singer W, Zhang B, Rouf Mian MA, Huang H (2020) soybean amino acids in health, genetics, and evaluation. In: *Soybean for Human Consumption and Animal Feed*. IntechOpen
 77. Krajcovicova-Kudlackova M, Babinska K, Valachovicova M (2005) Health benefits and risks of plant proteins. *Bratisl Lek Listy* 106:231–234
 78. Karau A, Grayson I (2014) Amino acids in human and animal nutrition. *Adv Biochem Eng Biotechnol* 143:189–228. https://doi.org/10.1007/10_2014_269
 79. Kudelka W, Kowalska M, Popis M (2021) Quality of soybean products in terms of essential amino acids composition. *Molecules* 26. <https://doi.org/10.3390/molecules26165071>
 80. Carrera CS, Reynoso CM, Funes GJ et al (2011) Amino acid composition of soybean seeds as affected by climatic variables. *Pesqui Agropecu Bras* 46:1579–1587. <https://doi.org/10.1590/S0100-204X2011001200001>
 81. Oura E (1983) Biomass from carbohydrates. In: Reem H-J, Reed G (eds) *Biotechnology*, vol 3. Verlag Chemie GmbH, Weinheim, Germany
 82. National Research Council (1993) *Nutrient requirements of fish*. National Academy Press
 83. National Research Council (1994) *Nutrient requirements of poultry*. National Academy Press
 84. Mariotti F, Gardner CD (2019) Dietary Protein and amino acids in vegetarian diets-a review. *Nutrients* 11. <https://doi.org/10.3390/nut1112661>
 85. LaTurner ZW, Bennett GN, San K-Y, Stadler LB (2020) Single cell protein production from food waste using purple non-sulfur bacteria shows economically viable protein products have higher environmental impacts. *J Clean Prod* 276:123114. <https://doi.org/10.1016/j.jclepro.2020.123114>
 86. Saejung C, Salasook P (2020) Recycling of sugar industry wastewater for single-cell protein production with supplemental

- carotenoids. *Environ Technol* 41:59–70. <https://doi.org/10.1080/09593330.2018.1491633>
87. He S, Lu H, Zhang G, Ren Z (2021) Production of coenzyme Q10 by purple non-sulfur bacteria: current development and future prospect. *J Clean Prod* 307:127326. <https://doi.org/10.1016/j.jclepro.2021.127326>
 88. Hejna M, Gottardo D, Baldi A et al (2018) Review: nutritional ecology of heavy metals. *Animal* 12:2156–2170. <https://doi.org/10.1017/S175173111700355X>
 89. European Parliament (2019) Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed. OJ L 140, 30.5.2002. European Parliament, Brussels, pp 10–22. <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=celex%3A32019R1869>. Accessed 16 Oct 2022
 90. Doulgeridou A, Amlund H, Sloth JJ, Hansen M (2020) Review of potentially toxic rare earth elements, thallium and tellurium in plant-based foods. *EFSA J* 18. <https://doi.org/10.2903/j.efsa.2020.e181101>
 91. European Food Safety Authority (2016) Scientific Opinion on the use of cobalt compounds as additives in animal nutrition: Revision of the currently authorised maximum copper content in complete feed. *EFSA J* 14. <https://doi.org/10.2903/j.efsa.2016.4563>
 92. Bampidis V, Azimonti G, de L BM et al (2020) Safety and efficacy of Availa®Cr (chromium chelate of DL-methionine) as a feed additive for dairy cows. *EFSA J* 18. <https://doi.org/10.2903/j.efsa.2020.6026>
 93. European Commission (2016) Revision of the currently authorised maximum copper content in complete feed. *EFSA J* 14. <https://doi.org/10.2903/j.efsa.2016.4563>
 94. European Commission (2016) Safety and efficacy of iron compounds (E1) as feed additives for all animal species: ferrous carbonate; ferric chloride, hexahydrate; ferrous fumarate; ferrous sulphate, heptahydrate; ferrous sulphate, monohydrate; ferrous chelate of amino acids, hydrate; ferrous chelate of glycine, hydrate, based on a dossier submitted by FEFANA asbl. *EFSA J* 14. <https://doi.org/10.2903/j.efsa.2016.4396>
 95. European Food Safety Authority (2013) Scientific Opinion on the safety and efficacy of manganese compounds (E5) as feed additives for all animal species: manganous oxide, based on a dossier submitted by Poortershaven Industriële Mineralen B.V. *EFSA J* 11:3325. <https://doi.org/10.2903/j.efsa.2013.3325>
 96. European Commission (2016) Safety and efficacy of selenium compounds (E8) as feed additives for all animal species: sodium selenite, based on a dossier submitted by Todini and Co SpA. *EFSA Journal* 14. <https://doi.org/10.2903/j.efsa.2016.4442>
 97. Bampidis V, Azimonti G, M de L B et al (2021) Safety and efficacy of a feed additive consisting of zinc chelate of ethylenediamine for all animal species (Zinpro Animal Nutrition (Europe) Inc.). *EFSA J* 19. <https://doi.org/10.2903/j.efsa.2021.6467>
 98. Bampidis V, Azimonti G, M de L B et al (2022) Assessment of the feed additive consisting of potassium diformate for all animal species for the renewal of its authorisation (Addcon GmbH). *EFSA J* 20. <https://doi.org/10.2903/j.efsa.2022.7167>
 99. Chanda S, Paul BN, Ghosh K, Giri SS (2015) Dietary essentiality of trace minerals in aquaculture-a review. *Agric Rev* 36:100. <https://doi.org/10.5958/0976-0741.2015.00012.4>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.