Water Science & Technology

IWA PUBLISHING

Water Science & Technology Vol 84 No 9, 2406 doi: 10.2166/wst.2021.433

Phycoremediation of pollutants from secondary treated coke-oven wastewater using poultry litter as nutrient source: a cost-effective polishing technique

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ABSTRACT

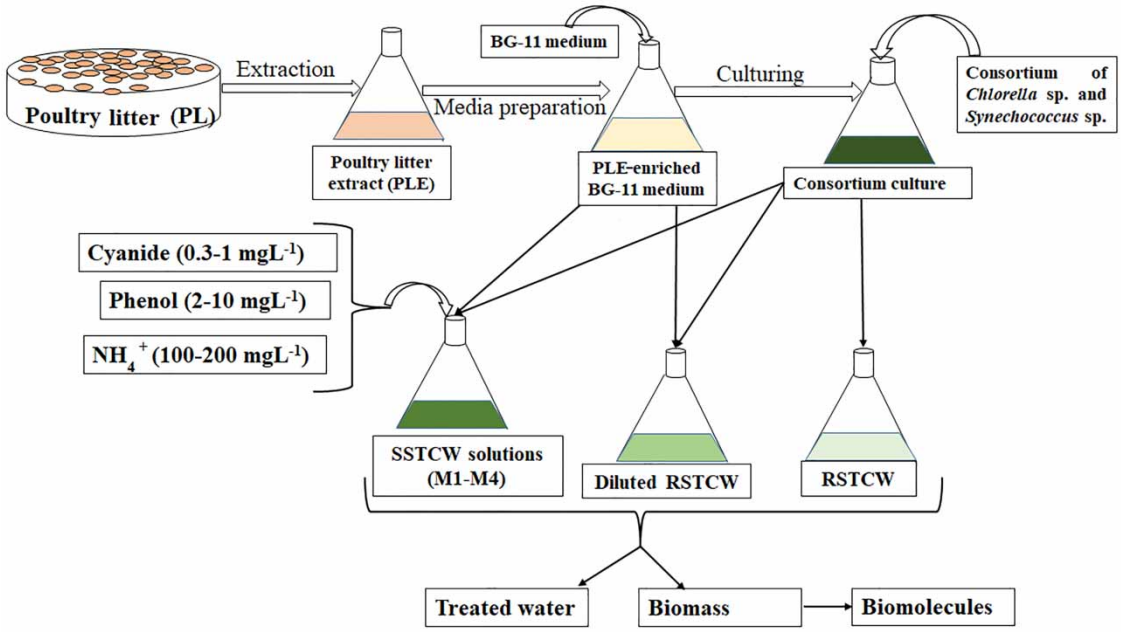
This article focuses on the phycoremediation of pollutants from secondary treated coke-oven effluent through a green and economical route. A microalgal sample was collected and identified as a consortium of *Chlorella* sp. and *Synechococcus* sp. The culture cost was reduced by using poultry litter extract as supplementary material to BG-11 medium. Since the major pollutants present in real secondary treated coke-oven wastewater are phenol, ammoniacal-N (NH_4^+) and cyanide, several matrices were designed with these three major pollutants by varying their initial concentrations such as phenol (2–10 mg/L), cyanide (0.3–1 mg/L) and NH_4^+ (100–200 mg/L), termed as simulated secondary treated coke-oven wastewater. Maximum removal was observed with individual solutions of phenol (4 mg/L), cyanide (0.6 mg/L) and NH_4^+ (175 mg/L), while maximum removal in simulated secondary treated coke-oven wastewater was observed at higher concentrations of phenol (8 mg/L) and cyanide (0.8 mg/L) and the same concentration of NH_4^+ (175 mg/L). A consortium was found effective to meet statutory limits of pollutants. Kinetic model was developed for predicting growth of consortium and observed that the poultry litter extract-enriched BG-11 medium showed higher values of maximum specific growth rate (0.56 per day) and carrying capacity (1,330 mg/L) than that in BG-11 medium only.

Key words: biomolecules, coke-oven wastewater, consortium, phycoremediation, poultry litter, tertiary treatment

HIGHLIGHTS

- A consortium was identified for tertiary treatment of coke-oven wastewater.
- Poultry litter extract was found effective as a growth medium for consortium.
- Phycoremediation of NH_4^+, phenol and cyanide was examined with the present consortium.
- Biomass and biomolecules were extracted and estimated.
- A consortium was found effective for real wastewater treatment.

GRAPHICAL ABSTRACT



INTRODUCTION

The coke-oven plant is the integrated part of the steel industry where the coal pyrolysis process occurs at a higher temperature (1,000–1,200 °C) to produce coke (Kwiecińska-Mydlak *et al.* 2019). Wastewater (approximately 1–1.5 m^3/tonnes coke production) is generated during the byproducts recovery processes and is known as coke-oven wastewater (Kwiecińska-Mydlak *et al.* 2019). Generally, raw coke-oven wastewater is characterized by high contents of chemical oxygen demand (COD), biochemical oxygen demand (BOD), phenol, ammoniacal-N (NH_4^+), cyanide, sulfide and thiocyanate (Kwiecińska-Mydlak *et al.* 2019). While the physico-chemical methods such as pH neutralization, flow equalization, coagulation and chemical oxidation were used as primary treatment for reduction of COD to BOD ratio of coke-oven wastewater, a membrane-based technique, ion exchange separation, electro-coagulation, photocatalysis and adsorption were used as a tertiary treatment method or as a polishing method in the effluent treatment plant (Maiti *et al.* 2019). Coagulation and precipitation methods and chemical oxidation methods have disadvantages of high chemical cost, generation of sludge and are inefficient at removing pollutants at the parts per million (ppm) level (Waghmare & Arfin 2015). The generation of secondary pollutants is another characteristic problem of chemical oxidation methods (Waghmare & Arfin 2015). Bacterial treatment of wastewater is a widely accepted secondary treatment method due to its economical and ecofriendly nature. However, it has several limitations such as generation of secondary sludge and maintenance of proper nutrient condition (Waghmare & Arfin 2015). Biological treatment of physico-chemical treated coke-oven wastewater is also found inefficient sometimes due to the presence of refractory compounds and toxic chemicals in the influent of the biochemical oxidation and dephenolisation (BOPD) plants (Sharma & Philip 2016). Usage of non-specific bacterial strain and poor operation conditions are the major challenges faced by bacterial treatment processes. Even after primary and secondary treatment processes, the wastewater may contain an array of pollutants above permissible limits as set by authorized bodies (Central Pollution Control Board (CPCB), India). For wastewater, the permissible limits of phenol (1 mg/L), cyanide (0.2 mg/L) and NH_4^+ (50 mg/L) have been fixed by the CPCB. Scientists reported the presence of pollutants even in biologically treated coke-oven wastewater as phenol (2–4 mg/L) (Das *et al.* 2020), cyanide (0.4–0.5 mg/L, 0.8 mg/L) (Biswas *et al.* 2010; Das *et al.* 2020) and NH_4^+ (210 mg/L) (Biswas *et al.* 2010). These toxic chemicals have adverse effects on terrestrial and aquatic ecosystems. Therefore, tertiary treatment of coke-oven wastewater is essentially required for the attainment of stipulated water quality before its discharge to the environment. Adsorption and membrane-based separation are commonly used tertiary treatment methods. Although adsorption is a technically simple method, it has the problem of disposal of spent adsorbent (Waghmare &

Arfin 2015). Membrane-based separation requires an extensive pretreatment process to prevent membrane fouling. In addition, the operational cost is also high (Waghmare & Arfin 2015). Therefore, an apposite ecofriendly and economic tertiary end-of-pipe treatment method of coke-oven wastewater is required. More information related to primary and secondary methods for treatment of raw coke-oven wastewater and the degradation potential are shown in Table S1, attached as Annexure I in the Supplementary Material.

The application of cyanobacteria and microalgae for nutrient recovery such as nitrate and phosphate from different sources has been well explored. Cyanobacteria and microalgae are capable of growing in agricultural, industrial and municipal wastewater. Tertiary treatment of wastewater using cyanobacteria and microalgae is a unique method and the production of value-added products from produced biomass makes this process economically feasible. Phycoremediation has several advantages such as (i) it can remove the pollutants in an environmentally and ecofriendly manner to ppm levels and (ii) generated biomass can be used for the production of value-added products which makes the process profitable one. Gurbuz *et al.* (2004) tested the three microalgal cultures *Chlorella* sp. *Arthrospira maxima* and *Scenedesmus obliqus* for the biological detoxification of cyanide from the mining process wastewater. Sen *et al.* (2018b) used the cyanobacterial consortium for phycoremediation of cyanide from coke-oven wastewater. Thakurta *et al.* (2018) used *Leptolyngbya* sp. for the bioremoval of phenol from the synthetic and real wastewater. Thus, it can be stated that while a few studies were carried out for removal of individual pollutants such as phenol, cyanide and NH_4^+ using microalgae/cyanobacteria, the studies on the application of microalgae/cyanobacteria for the removal of multipollutant from coke-oven wastewater as tertiary treatment is very limited. Again, the main objective of all these studies was to establish the applicability of microalgae/cyanobacteria in the removal of pollutants and to ascertain proper operating conditions, and no discussion was made to curtail the cost of operation by supplementing any nutrient-rich waste material to BG-11 for growth of algae. Therefore, in the current study, an attempt has been made to fulfil the present knowledge gap by using a phycoremediation method for tertiary treatment of coke-oven wastewater in an economic and ecofriendly way.

In this study, the phycoremediation technique has been applied to remediate pollutants (NH_4^+, phenol and cyanide) from both simulated secondary treated coke-oven wastewater (SSTCW) and from real secondary treated coke-oven wastewater (RSTCW). Several researchers reported that microalgal cultivation requires macronutrients (nitrogen and phosphorus) from different organic and inorganic nutrient sources, which increases the cost of microalgal production (Bhatnagar *et al.* 2011; Singh & Das 2014). Poultry litter is a hazardous waste of poultry farms and is a rich source of nitrogen and phosphorus (Lynch *et al.* 2013). In this work, such waste has been utilized as a food supplement to grow the consortium of cyanobacteria and microalgae. Furthermore, kinetic modelling is an essential part of designing a bioreactor and for the development of a proper control strategy. In this article, a kinetic model was developed to predict growth of a consortium of cyanobacteria and microalgae during the phycoremediation process. Therefore, a comprehensive study of the simultaneous removal of the three pollutants, and an assessment of biomolecule production and kinetic modelling is the strength of this study.

MATERIALS AND METHODS

All experiments were done in three sets and obtained results were shown as mean ± standard deviation (SD).

Collection, identification and cultivation of microalgal sample

Since the natural adaptation of the microalgal sample in the contaminated site is the key point for the phycoremediation process, a microbial sample was collected from the runoff of secondary treated coke-oven wastewater from a nearby coke-oven plant. The microalgal sample was incubated in collected native wastewater (100 mL) mixed with BG-11 (blue-green) medium (100 mL) (HIMEDIA, India) in a 500 mL of Erlenmeyer flask (Borosil, Mumbai, India). The flask was placed in an algal incubator at specified culture conditions as described by Sen *et al.* (2018a). The composition of the BG-11 medium is shown in Annexure II in the Supplementary Material. The isolation and identification of the microalgal sample were performed following basic spreading and streaking techniques, and the grown cultures were observed under a microscope (Premium, Dewinter Technologies, Italy) for assessing microalgal morphology.

Media preparation with poultry litter extract

Poultry litter was collected from a broiler farm of Naihati, North 24 Parganas (22.8895° N, 88.4220° E) West Bengal, India, and nutrients were extracted in liquid form following the protocol as suggested by Bhatnagar *et al.* (2011). Collected poultry litter was dried in a hot air oven (Universal Hot Air Oven, India) for 16 h at 60 ± 1 °C. Dried poultry litter (5 g) was added to

1,000 mL of distilled water, and the mixture was stirred using a magnetic stirrer for 2 h. The solution was then filtered with Whatman filter paper, and the filtrate was autoclaved. Finally, the solution was stored at 4 °C. The stored solution was termed as poultry litter extract (PLE). The characterization of PLE is shown in Table S2 attached as Annexure III in the Supplementary Material. PLE was diluted with sterile BG-11 medium in three ratios (v/v) so that the PLE extract was BG-11–1:5, 1:10, 1:15. The isolate was cultured in these three solutions individually. Culture broths were collected after 14 days of incubation and centrifuged at 5,000 rpm for 15 min. Pellets were washed twice with deionized water, and finally, placed in a hot air oven at a temperature (65 $\pm$ 1 °C) for 18 h. The weight of dried biomass was taken, and the growth of the culture was measured in terms of dry biomass concentration (DBC). Maximum growth was seen in the 1:10 dilution, and to compare the isolated sample growth in pure BG-11 medium and PLE-enriched medium, the PLE which was diluted 10 times with BG-11 medium was used for the next stage of the study.

To perform growth studies, the isolated sample was inoculated in BG-11 medium and in PLE-enriched BG-11 medium (10 times diluted with BG-11 medium) separately. In both media, inoculum size (IS) and initial pH were kept constant at 10%, and pH 9, respectively. The high alkaline pH helps to prevent the growth of other microorganisms. Both cultures were then placed in an algal incubator at specific conditions (Sen *et al.* 2018a) and incubated for 18 days. Samples were collected from both cultures at 2-day intervals and centrifuged. The growth of the culture was measured for DBC, and the best one was selected. Biomolecules such as chlorophyll, carbohydrate and protein were extracted from the produced biomass following the methods described by Biswas *et al.* (2018). A detailed description of standard protocols for biomolecules estimation are described in the Supplementary Material as Annexure IV.

Design of experiments and preparation of SSTCW solutions

Since the major pollutants present in RSTCW are phenol, ammoniacal-N (NH_4^+) and cyanide, several matrices were designed with these three pollutants by varying their initial concentrations, i.e. phenol (2–10 mg/L), cyanide (0.3–1 mg/L) and NH_4^+ (100–200 mg/L), to mimic the real wastewater. The concentrations of these three pollutants are shown in Table 1, where rows describe the concentrations of a single pollutant and columns represent the concentrations of mixed pollutants.

An aqueous solution of a mixture of these pollutants was prepared by adding all the pollutants phenol, cyanide and NH_4^+ as per Table 1. The solution, thus prepared, was named as SSTCW labelled as M1–M5 in Table 1. The ranges of model pollutants were selected based on the previous studies.

Standard solutions of these model pollutants were prepared following the protocol as described by Rai *et al.* (2021b). Required concentrations of pollutants were made by adding the required amount of PLE-enriched BG-11 medium.

Tolerance level study of isolated sample in simulated solutions

To determine the susceptibility of isolated sample in diverse concentrations of pollutants, a tolerance level study (TLS) was performed by growing the isolated strains in simulated solutions, as prepared row-wise (aqueous solution of individual pollutants) and column-wise (SSTCW) of Table 1. The isolated sample was cultivated in all prepared solutions at initial pH 9 and IS 10%, and placed in the algal incubator. Samples were collected after 10 days of incubation period and centrifuged. The supernatant was examined for analysis of residual concentrations of pollutants (NH_4^+, phenol and cyanide) following the methods described by Rai *et al.* (2021b). Pellets were dried at 65 $\pm$ 1 °C in a hot air oven for 18 h. The DBC was measured for each study. For every set of experiments, the following controls were kept as (i) biotic control – PLE-enriched BG-11 medium with the isolated sample, and (ii) abiotic control – pollutant(s) containing PLE-enriched BG-11 medium without isolated sample. The controls were incubated under the same condition as for the experimental ones. To see the effect of pH on the growth and removal of multipollutants, the test consortium was grown in M1–M5 solutions where the initial pH was varied from 8 to 10.

Table 1 | Matrix of pollutants for experiments

Name of pollutants	M1	M2	M3	M4	M5
Phenol (mg/L)	2	4	6	8	10
Ammoniacal-N (NH_4^+) (mg/L)	100	125	150	175	200
Cyanide (mg/L)	0.3	0.4	0.6	0.8	1

Time variation studies

Time variation studies were performed to analyze the trend in bioremoval of NH_4^+, phenol and cyanide from simulated solution with time. The isolated sample inoculate (14 days old and IS: 10%) was added to SSTCW (M4) at pH 9, and thereafter, the culture was incubated for 14 days in the microalgal growth chamber. Samples were collected after at 2-day intervals. The biomass was harvested by centrifugation, and the supernatant was analyzed for remaining concentrations of pollutants. Finally, chlorophyll, carbohydrate and protein were estimated using standard protocols.

Application of isolated sample for treatment of RSTCW

RSTCW was collected from the runoff of the BOPD plant of the coke-oven plant in West Bengal, India. RSTCW was filtered with Whatman filter paper (grade no. 42). Generally, RSTCW was found to be polluted with three major pollutants – phenol, NH_4^+ and cyanide (Singh & Mishra 2018) – and the secondary clarifier effluent was reported to be loaded mainly with phenol, cyanide and NH_4^+ (Biswas *et al.* 2010; Das *et al.* 2020). Therefore, the RSTCW was characterized in terms of pH, phenol, NH_4^+, cyanide, total suspended solids and total dissolved solids as shown in Table 2.

The growth of isolate was examined in both diluted and non-diluted RSTCW. Bioremoval of NH_4^+, phenol and cyanide from RSTCW using microalgae isolate was investigated. The filtrate of wastewater (RSTCW) was diluted 50% with a requisite amount of PLE-enriched BG-11 medium. Inoculum (size: 10%) was added to wastewater and kept in the microalgal growth chamber for 14 days. After that, samples were collected and centrifuged at 5,000 rpm for 15 min. The supernatant was analyzed for all residual concentrations of pollutants. The growth of the isolated sample was determined by DBC and chlorophyll content.

Theoretical analysis

Kinetic model for biomass growth

Kinetic modelling is imperative in understanding the mechanisms of growth of biomass. Determination of kinetic parameters is important for (i) suitable bioreactor designing, (ii) scaling-up of process, (iii) developing adequate control strategies and (iv) cost optimization. While the kinetics of growth of indigenous novel strains in elemental growth medium (like BG-11) can be explained by conventional kinetic equations, the behaviour of strains in wastewater containing a number of pollutants is somewhat complex. An insightful, experienced and pragmatic approach is therefore required to obtain a unified model to explain the growth of microbial strains in complex systems.

Kinetic models for predicting the growth of the isolated sample in different systems, including (i) different medium composition, (ii) different pollutant systems like phenol, NH_4^+ and cyanide in their individual solutions and (iii) in their mixed conditions (M1–M5), were developed. To avoid the limitations of the Monod model, a logistic model was used to represent the kinetics of biomass growth. The main characteristic of this model is that it provides the maximum sustainable carrying capacity of a biomass population under constant environmental conditions as a parameter. The integral form of the logistics model (Sen *et al.* 2018a) is:

$$X_t = \frac{X_0 e^{\mu_{\max} t}}{1 - \left(\frac{X_0}{X_{\max}}\right)(1 - e^{\mu_{\max} t})} \tag{1}$$

Table 2 | Characterization of RSTCW

Sl. no.	Parameter	Value	Standard value (Kwiecińska *et al.* 2017; Maiti *et al.* 2019)
1	pH	6.9 ± 0.127	5.5–9.0
2	Cyanide (mg/L)	0.65 ± 0.042	0.2
3	Phenol (mg/L)	8.05 ± 0.057	1.0
4	NH_4^+ (mg/L)	157.3 ± 3.05	50.0
5	Total suspended solids (mg/L)	98 ± 11.2	15–50
6	Total dissolved solids (mg/L)	3,645 ± 19.55	1,000–2,000

where X_t = biomass concentration at time t (mg/L); μ_{max} = maximum specific growth rate (per day); X_{max} = carrying capacity (mg/L); X_0 = initial concentration of biomass (mg/L), and t = time of incubation (days).

Maximum specific growth rate and carrying capacity of the microorganism are evaluated by fitting the logistic model with experimental biomass growth data obtained during the exponential growth phase in different systems using the generalized reduced gradient optimization technique. A novel approach was explored to define the maximum specific growth rate for mixed pollutant systems.

For analyzing the growth kinetics of the isolated sample in SSTCW solutions (mixed pollutant systems), a modified logistic model was used. In this model, the maximum specific growth rate for the mixed pollutant system is assumed to be a function of the maximum specific growth rate for the individual pollutant systems as shown below.

$$\mu'_{max} = \alpha_1 \mu_{max_1} + \alpha_2 \mu_{max_2} + \alpha_3 \mu_{max_3} \tag{2}$$

The coefficients (α_1, α_2, α_3) of the above equations are regressed along with carrying capacity (X_{max}) using the generalized reduced gradient optimization technique, where μ'_{max} = maximum specific growth rate (per day) for mixed pollutant systems (M1–M5); μ_{max_1} = maximum specific growth rate for NH_4^+ pollutant systems (per day); μ_{max_2} = maximum specific growth rate for phenol pollutant systems (per day); and μ_{max_3} = maximum specific growth rate for cyanide pollutant systems (per day).

The logistic kinetic model was used to estimate the maximum specific growth rate and carrying capacity for the biomass production in the log phase by fitting the experimental biomass growth data. For regressing the model parameters (μ_{max}, X_{max}), the generalized reduced gradient optimization technique was used to minimize the average absolute relative deviation (AARD) between experimental (N being the number of data points) and model-predicted data. The objective function used for this method is:

$$AARD = \frac{1}{N}\sum_{n=1}^{n=N}\left|\frac{(\text{Experimental data}) - (\text{Model}-\text{predicted data})}{(\text{Experimental data})}\right| \times 100\% \tag{3}$$

Statistical analysis

Graph Pad Prism 5.01 (software) was used for statistical analysis. Nonparametric t-test, and unpaired t-test were performed to obtain the level of significance for the production of biomass between PLE-enriched BG-11 medium and BG-11 medium only. One-way analysis of variance (ANOVA) test was used for the (i) bioremoval of pollutants and the production of biomass during the time variation study and (ii) produced biomass and biomolecules during time variation study.

RESULTS AND DISCUSSION

Microalgal isolate

Microscopic observation shows that the isolated sample is a consortium consisting of a cyanobacterium *Synechococcus* sp. (rod-shaped non-motile, unicellular) and green algae *Chlorella* sp. (round-shaped, unicellular) as shown in Figure S1 which is attached as Annexure V in the Supplementary Material. A consortium culture is a robust candidate for treatment methods compared to a uniculture (Gonçalves *et al.* 2017). In the present consortium, the dominant strain is cyanobacteria over green algae. Generally, cyanobacterial samples have more tolerance and resistance for different wastewater such as agricultural, municipal and industrial than green algae (Renuka *et al.* 2015), and hence, it can be stated that the present consortium is suitable for the treatment of wastewater. Furthermore, *Synechococcus* sp. is a unicellular rod-shaped cyanobacterium and its unicellular structure is effective for the treatment of wastewater (Thangavel *et al.* 2018). In the case of green algae, *Chlorella* sp. is also effective for the treatment of different wastewater. Thus, the present consortium can be considered as an viable bioremediant for the treatment of wastewater.

Comparative growth studies

The consortium was cultured in two different media, BG-11 and PLE-enriched BG-11 media (PLE: BG-11 medium = 1:10). PLE-enriched BG-11 medium was found to be the best alternative for the growth of consortium (Figure 1(a)). Extracted biomolecules such as chlorophyll, carbohydrate and protein are shown in Figure 1(b). Throughout the experiment, the

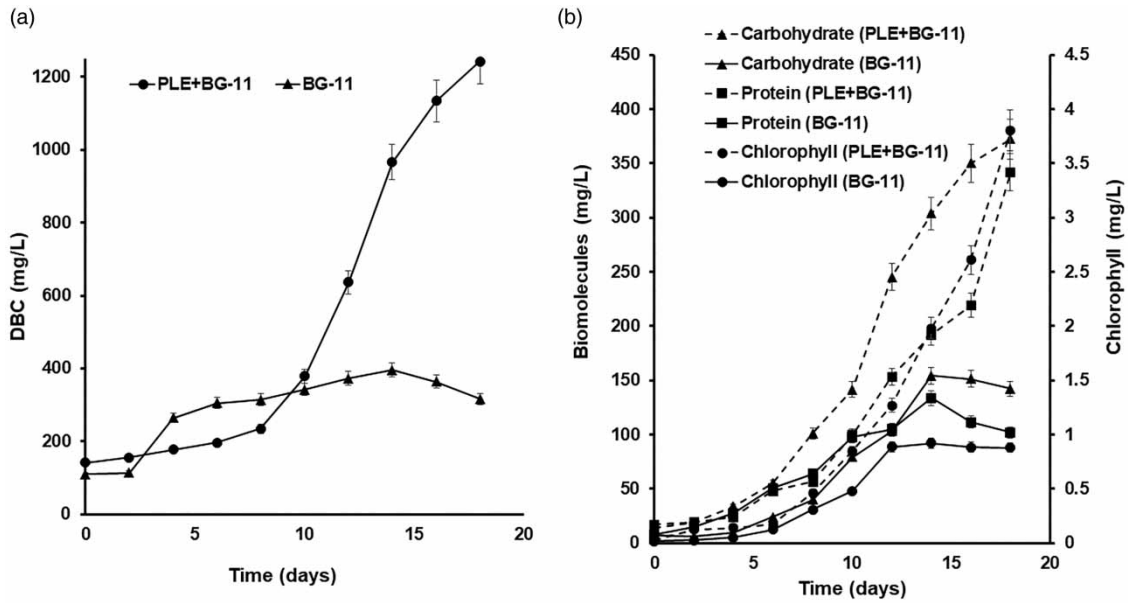


Figure 1 | (a) Comparative growth study on PLE-enriched media BG-11 and BG-11 media, (b) comparative study of extracted biomolecules in PLE-enriched BG-11 media and BG-11 media.

production of biomass from PLE-enriched BG-11 compared to only BG-11 medium was found to be significant with time ($P < 0.0001$). In BG-11, the consortium showed a regular growth pattern with four consecutive phases: (i) preparatory phase – where culture was acclimatized to the surrounding environment, (ii) exponential phase – in this phase microorganism synthesize primary metabolites for their exponential growth, thus the growth rate of cells become high, (iii) plateau phase – here the culture growth rate is equal to the death rate due to nutrient deficiency and (iv) decline phase – cells die due to lack of nutrients (Sen *et al.* 2018a). Figure 1(a) shows that the growth of the consortium in PLE-enriched BG-11 medium increased 8.75-fold from the initial DBC, while in the BG-11 medium, growth increased 2.9-fold under identical conditions after 18 days of incubation. A similar increment was obtained for biomolecules chlorophyll (0.3 ± 0.001 mg/L to 3.8 ± 0.012 mg/L and 0.015 ± .002 mg/L to 0.88 ± .031 mg/L), carbohydrate (13.4 ± 1.1 mg/L to 374 ± 11.5 mg/L and 7.2 ± 1.0 mg/L to 142 ± 18.4 mg/L) and protein (17.0 ± 1.7 mg/L to 341.5 ± 10.9 mg/L and 7.5 ± 2.9 mg/L to 102 ± 2.3 mg/L) in PLE-enriched BG-11 medium and in only BG-11 medium, respectively (Figure 1(b)). Maximum growth (390 ± 6.0 mg/L) was seen on the 14th day of incubation in the BG-11 medium while in PLE-enriched BG-11 medium the biomass increased to 1,240 ± 17.0 mg/L on the 18th day. Bhatnagar *et al.* (2011) showed higher growth of microalgae (180%) than that obtained in the BG-11 medium, and total chlorophyll was obtained as 2.7 mg/L in the PLE-enriched BG-11 medium. This might be due to the availability of more nutrients in the case of the PLE-enriched medium, which favoured the growth. Due to its rich source of macronutrients, PLE supports the growth of the consortium compared to the BG-11 medium only. Therefore, it can be stated that the PLE-enriched BG-11 media is an effective alternative nutrient source for microalgal growth.

Tolerance level study

Growth of the consortium was observed in both the simulated individual solutions and in SSTCW (M1–M5 solutions) at a varying range of initial concentrations of the pollutants (Figure 2(a)–2(d)).

Bioremoval of NH_4^+ from simulated NH_4^+ solution

NH_4^+ is the preferred inorganic source of nitrogen for microalgal growth; cyanobacteria and microalgae can assimilate it directly (Rai *et al.* 2020). The growth of the consortium was examined in simulated solutions of NH_4^+, which is shown in Figure 2(a). Maximum growth (0.365 ± 0.001 g/L) of test strain was observed at 175 mg/L NH_4^+ while maximum removal (75.464 ± 0.581%) occurred at 100 mg/L. The test consortium grew well in all concentrations of NH_4^+. This might be due to the utilization of NH_4^+ (pollutant) as the nitrogen source by the strain. When NH_4^+ concentration increased from 100 mg/L to 175 mg/L, DBC increased from 0.329 ± 0.003 to 0.365 ± 0.001 g/L. Beyond this concentration, DBC of the

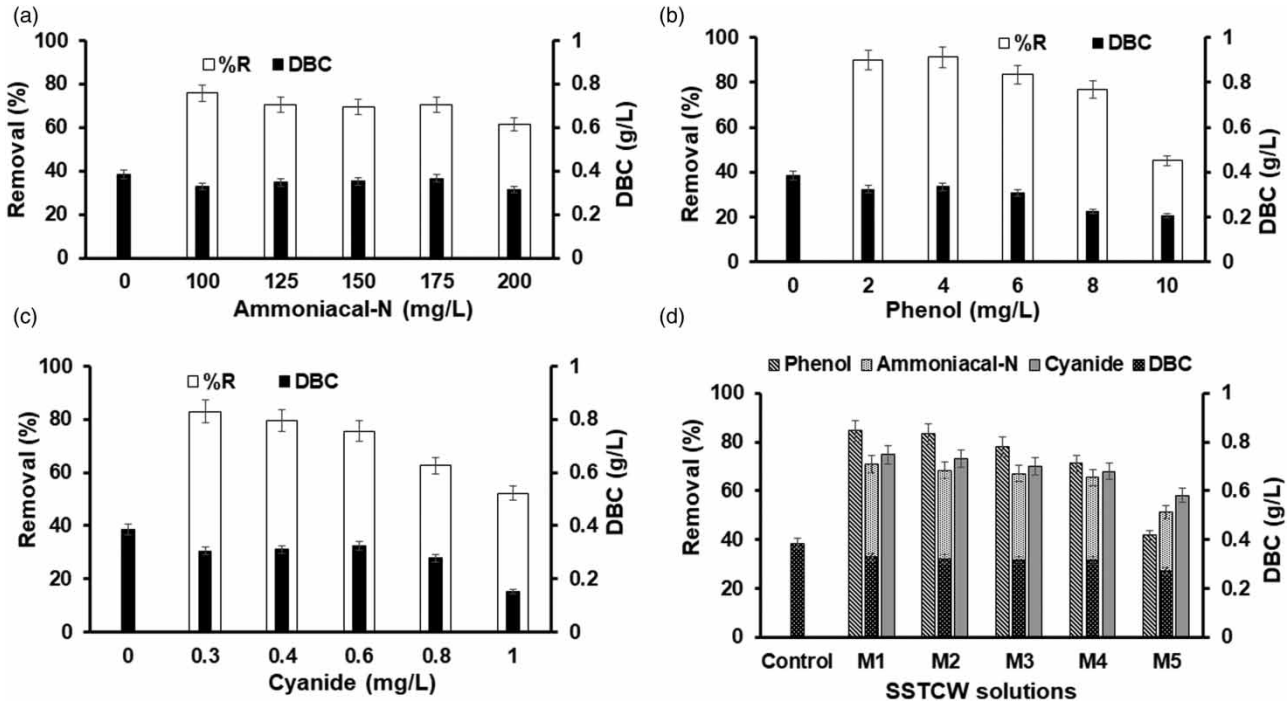


Figure 2 | (a) TLS of consortium in simulated ammoniacal-N solutions, (b) TLS of consortium in simulated phenol solutions, (c) TLS of consortium in simulated cyanide solutions, (d) TLS of consortium in SSTCW solutions.

consortium decreased to 0.315 ± 0.025 g/L (at 200 mg/L NH_4^+). Collos & Harrison (2014) reported that a concentration of ammoniacal-N <30.54 mg/L would not generally affect the growth rate of microalgal cells, and chlorophytes were found the most tolerant toward the ammoniacal-N. Therefore, it can be stated that the growth of consortium was restricted at a higher concentration of NH_4^+, and this value varies from species to species of cyanobacteria and microalgae (Collos & Harrison 2014).

Bioremoval of phenol from simulated phenol solutions

The growth of test strain in different concentrations of phenol is shown in Figure 2(b). Maximum DBC (0.335 ± 0.75 g/L) and maximum removal (91.072 ± 0.471%) were observed at 4 mg/L of phenol concentration. The consortium was found to grow in all concentrations of phenol (2–10 mg/L), but limited growth was observed beyond 6.0 mg/L phenol concentration. Aerobic biodegradation of phenol by cyanobacteria and microalgae provides CO_2 and other small organic molecules which help in the growth of strains (Stephen & Ayalur 2017). Similarly, Duan *et al.* (2017) performed a growth inhibition test in phenolic solutions using different microalgal cells and stated that the tolerance level of pollutants for microalgal samples varies from species to species (Duan *et al.* 2017). The lowering of biomass production at a higher concentration of phenol might be due to the generation of reactive oxygen (Duan *et al.* 2017).

Bioremoval of cyanide from simulated cyanide solutions

The microalgal consortium was grown in simulated cyanide solutions with different initial cyanide concentrations (0.3–1.0 mg/L) (Figure 2(c)). Growth of the test strain was observed in all cyanide concentrations; however, DBC of the test strain increased up to 0.6 mg/L cyanide concentration; beyond this, growth was restricted. Hammed *et al.* (2016) reviewed cyanide removal using microalgae, and stated that some microalgal cells used cyanide as a carbon and nitrogen source. In the present study, while maximum biomass (0.255 ± 0.046 g/L) was obtained at 0.6 mg/L, maximum removal (72.814 ± 1.192%) was observed at 0.3 mg/L cyanide concentration. Lower growth at higher cyanide concentrations might be due to its inhibitory effect. Cyanide is highly toxic due to its high affinity towards the metals, which block the metabolic activity at higher concentrations (Gupta *et al.* 2010). Furthermore, Choi *et al.* (2012) analyzed the toxic effect of cyanide on the chlorophyll of five different microalgal species and observed the threshold concentration of cyanide below 0.305 mg/L.

Effects of initial pH on the growth, and bioremoval of multipollutant from all SSTCW (M1–M5 solutions) are shown in Figure S2(a–c), attached as Annexure VI in the Supplementary Material. pH 9 was found suitable for both removal and growth perspectives.

Simultaneous bioremoval of NH_4^+, phenol and cyanide from SSTCW solutions

The microalgal consortium was grown in all SSTCW (M1–M5) solutions, and their DBC was measured as shown in Figure 2(d). It is noteworthy that the consortium can grow in all SSTCW (M1–M5) solutions with varying initial concentrations of pollutants. Therefore, it can be stated that the present consortium might be a suitable option for the treatment of RSTCW. Maximum biomass (0.328 ± 0.78 g/L) was observed at SSTCW with initial concentrations of pollutants: NH_4^+-100 mg/L, cyanide 0.3 mg/L and phenol 2 mg/L (M1). Maximum removal of all pollutants such as phenol ($94.551 \pm 1.213\%$), NH_4^+ ($70.832 \pm 1.076\%$) and cyanide ($74.702 \pm 2.014\%$) was also observed in M1 solutions. However, for further studies, the selection of a suitable set of SSTCW was done based on maximum consumption of pollutants, and this was achieved with M4 solution with the following composition: phenol 8 mg/L, NH_4^+ 175 mg/L and cyanide 0.8 mg/L. Maximum consumption in such case (M4 solution) was: phenol 0.284 ± 0.023 mg, NH_4^+ 5.71 ± 2.7 mg and cyanide 0.27 ± 0.01 mg. Therefore, the M4 set of SSTCW was chosen for further studies. At the highest concentrations (M5 solution), the removal of all pollutants was found least. In M1–M4 solutions, biomass was found to increase with an increase in the concentrations of all pollutants. The pollutants present in the mixed solutions might be helping the growth of the test consortium due to the increased biodegradation of pollutants through their positive interaction and, thereby, increased bioavailability of carbon and nitrogen (Rai *et al.* 2020).

The DBC obtained for biotic control (PLE-enriched BG-11 medium) is shown in Figure 2(a)–2(d). From the data, it can be stated that although the enhanced biomass was not obtained during the treatment of pollutant-loaded simulated solutions, the values were nearly the same as that obtained for growth in the PLE-enriched BG-11 medium without pollutant. Therefore, the robustness of the isolated consortium in the treatment of such wastewater was preliminarily established.

Time variation studies on the treatment of SSTCW solution in batch mode

Time variation studies are important from a chemical kinetics point of view. Therefore, variation of simultaneous removal of all three pollutants NH_4^+, phenol and cyanide and variation of pH of medium with time were investigated (Figure 3(a)). The final concentrations of all three pollutants (NH_4^+: 48.19 ± 0.28 mg/L, phenol: 0.33 ± 0.08 mg/L and cyanide: 0.2 ± 0.001 mg/L) met the permissible levels within 12 days of incubation of the test strain with M4 solution (SSTCW). Maximum removal was observed for NH_4^+ ($72.804 \pm 0.275\%$), phenol ($95.852 \pm 0.075\%$) and cyanide ($74.449 \pm 0.001\%$) after 12 days of incubation.

Carbohydrate (167.67 ± 6.1 mg/L), protein (129.6 ± 2.45 mg/L) and chlorophyll (1.15 ± 0.021 mg/L) were estimated from the produced biomass (356 ± 0.25 mg/L) after the treatment of SSTCW (Figure 3(b)). Among all biomolecules, the concentration of carbohydrates was highest. Therefore, produced biomass can be used for the production of carbohydrate or protein-based value-added products like biofuels, cattle feed or biochar. The increase in biomass and biomolecules concentrations of the microalgal consortium with time after treatment of SSTCW has demonstrated that the microalgal consortium is utilizing the pollutants as a nutrient source.

The pH of the medium was also tracked with time for 12 days of incubation period, as shown in Figure 3(a). Initially, the pH was constant for 4 days, after which there was a pH drop. A pH of 6.3 was observed at the end of the experiment. The change in the pH during the phycoremediation process might be due to the formation of intermediate compounds. pH plays a key role in the availability of pollutants for their removal in the solution. High alkaline solution prefers the conversion of NH_4^+ to free NH_3. Free ammonia directly affects the photosynthesis process. Rossi *et al.* (2020) studied thoroughly the inhibitory effect of free ammonia on the microalgae/cyanobacteria. Phenol and cyanide were previously removed when the pH was more than 8, and after the 6th day, NH_4^+ removal was observed. Uptake of NH_4^+ causes efflux of the H^+ (proton) from the cells, which shifts the pH from alkaline to slightly acidic (pH 6.3). In the early days (up to the 6th day), alkaline pH causes some ammonia formation, which is assimilated in the cell by the alleviation of the α-Keto-Glutarate (αKG), an intermediate of the Krebs cycle (Lu *et al.* 2018). Phenol and products obtained by cyanide degradation are directly or indirectly associated with the Krebs cycle (Figure 3(c)), which might be helpful for the growth of the present strain at a higher pH. Since the growth of the test strain was observed instead of the inhibitory effect of NH_3, it can be said that the consortium might have developed defence or tolerance mechanisms to overcome the NH_3 inhibition during the growth of cells. NH_3 inhibition

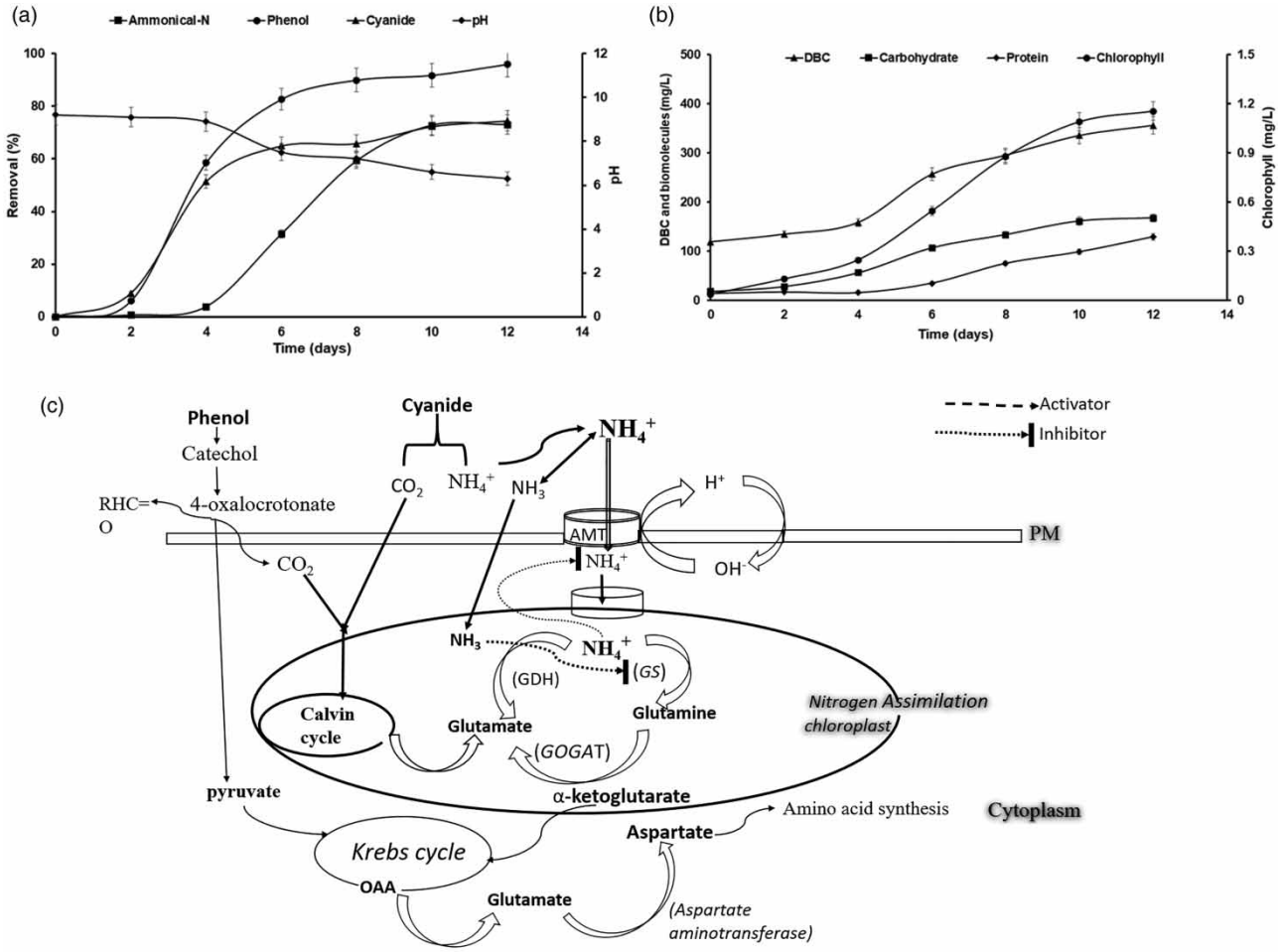


Figure 3 | Time variation studies on: (a) the removal of pollutants from SSTCW solution (M4) and variation of pH of the SSTCW and (b) the production of biomass and biomolecules during treatment of SSTCW solution (M4). (c) Proposed pathways for pollutants removal from SSTCW (Gurbuz *et al.* 2004; Wang *et al.* 2019; Rai *et al.* 2020).

towards the consortium can be overcome by either (i) regulating the pH or (ii) utilizing other nitrogen sources with or without NH_4^+ (Gutierrez *et al.* 2016). The uptake of NH_4^+ from the solution and its assimilation followed the GDH (glutamate dehydrogenase) and GS-GOGAT (glutamine synthetase-glutamine:2-oxoglutarate aminotransferase) pathways, as described by Rai *et al.* (2021a). Generally, a high concentration of NH_3 has a negative effect on the GS-GOGAT pathway, while another GDH pathway will continue and NH_3 is assimilated (Perez-Garcia *et al.* 2011). Phenol was utilized for the growth of cyanobacteria and microalgae through the catabolism process (Rai *et al.* 2020). The aerobic phenol biodegradation pathway is shown in Figure 3(c) (Wang *et al.* 2019). Phenol monooxygenase enzyme produces catechol from phenol. Catechol is converted into 2-hydroxymuconic semi-aldehyde in the presence of the enzyme catechol 2,3-dioxygenase (cyclic breakdown). The 2-hydroxymuconic semi-aldehyde is oxidized by dehydrogenase (NAD^+ dependent) enzyme and generates 4-oxalocrotonate. A cascade of reactions is followed for the conversion of 4-oxalocrotonate into pyruvate, CO_2 and alkyl aldehyde. This pyruvate is utilized in the Krebs cycle for energy generation (Figure 3(c)).

Cyanide degradation pathways in cyanobacteria and microalgae are not well explored compared to bacterial and fungal strains (Hammed *et al.* 2016). Scientists proposed that cyanide is converted into NH_4^+ and CO_2 after the cascade of enzymatic reactions (Gupta *et al.* 2010). Therefore, this NH_4^+ is again assimilated into the cells. The nitrogen assimilation process is regulated with C/N ratios (Collos & Harrison 2014). In this work, NH_4^+ assimilation might probably be regulated with carbon compounds in the form of CO_2, as well as with Krebs cycle intermediates (αKG) (Lu *et al.* 2018) by the degradation of phenol and cyanide in the SSTCW solution.

One-way ANOVA was performed for the analysis of removal of pollutants and production of biomass and biomolecules with time variation. Both the removal of NH_4^+–cyanide and NH_4^+–phenol were found significant while phenol–cyanide was not significant during the study (0–12th day). Only NH_4^+ was observed to be significant for biomass production during the phycoremediation, while phenol and cyanide were not significant. In the case of biomass and biomolecules, only carbohydrate was found to be significant.

Phycotreatment of RSTCW for removal of NH_4^+, phenol and cyanide using a consortium of *Synechococcus* sp. and *Chlorella* sp.

Analysis of collected RSTCW showed it contained NH_4^+ (157.2 mg/L) phenol (8.05 mg/L), and cyanide (0.65 mg/L). Removal of these three pollutants from undiluted (RSTCW) and diluted (RSTCW + PLE-enriched BG-11 medium) using a consortium of *Synechococcus* sp. and *Chlorella* sp. is shown in Figure 4(a). The test strain growth was measured for DBC (0.183 ± 1.01 g/L, 0.754 ± 1.17 g/L) and chlorophyll content (0.53 ± 1.1 mg/L, 1.0 ± 1.32 mg/L) during the treatment of undiluted and diluted RSTCW, respectively (Figure 4(b)). The removal of NH_4^+ (66.121± 1.665%, 96.127 ± 1.354%), phenol (62.152 ± 0.788%, 90.176 ± 2.341%) and cyanide (87.553 ± 1.335%, 95.972 ± 1.757%) was observed from undiluted and diluted RSTCW, respectively. Sen *et al.* (2018b) used cyanobacterial consortium for treatment of RSTCW where cyanide (1.2 mg/L) was found to be higher than the permissible limit. The cyanobacterial consortium was able to remove 60–80% of cyanide (Sen *et al.* 2018b). The present consortium was found effective for lowering the concentrations of NH_4^+, phenol and cyanide below the permissible limit from diluted RSTCW. In the case of undiluted RSTCW, the growth of the consortium and bioremoval of all pollutants except cyanide (87.553 ± 1.335%) were found to be less than in the SSTCW. The lower growth of the test strain in the undiluted RSTCW might be due to (i) the dark colour of wastewater, which prevents the entry of light into the solution and (ii) the pH (6.9) of the wastewater.

Liu *et al.* (2018) observed that by increasing the cyanide concentration (0.1–10 mg/L), the removal of cyanide increased (38–61%) while the cell count decreased below 0.1 mg/L cyanide concentration (Liu *et al.* 2018). However, in the current study, the maximum removal (72.814 ± 1.192%) and maximum DBC (0.365 ± .001 g/L) were obtained when the consortium was used to treat an aqueous solution of cyanide with initial concentrations of 0.3 mg/L and 0.6 mg/L, respectively. In SSTCW (M4 solution), the maximum removal of pollutants was observed as 74.449 ± 0.001%, 72.804 ± 0.275% and 95.852 ± 0.0751% for cyanide, NH_4^+ and phenol, respectively. Thakurta *et al.* (2018) used *Leptolyngbya* sp. for the removal of phenol (98.5%) from the synthetic phenolic (50 mg/L) wastewater. Limited studies were carried out in the removal of multipollutants from secondary treated coke-oven wastewater. In this study, solutions of mixed pollutants (SSTCW) and RSTCW (both diluted and undiluted forms) were treated with a test consortium to investigate its efficacy in the simultaneous removal of multipollutant. The pollutants concentrations reaching statutory limits for both SSTCW (M4 solution) and diluted RSTCW has preliminarily established the

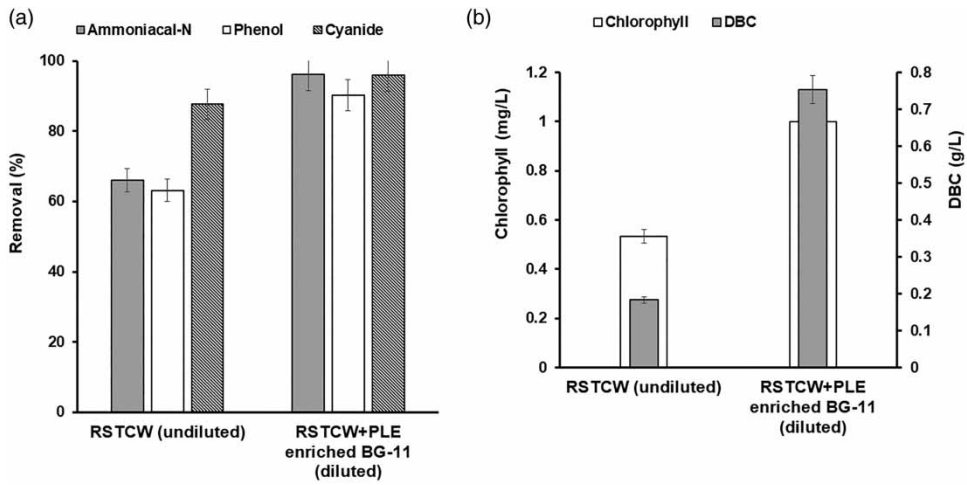


Figure 4 | (a) Bioremoval of NH_4^+ (ammoniacal-N), phenol and cyanide using a consortium of *Synechococcus* sp. and *Chlorella* sp. from RSTCW and (b) production of DBC and chlorophyll content during the treatment of RSTCW.

efficacy of the strain and opens up a new avenue for tertiary treatment of coke-oven wastewater in an environmentally friendly manner.

Comparative study of removal of NH_4^+, phenol and cyanide from SSTCW and RSTCW using the consortium of *Synechococcus* sp. and *Chlorella* sp.

The consortium was applied to remove major pollutants such as NH_4^+, phenol and cyanide from SSTCW and from RSTCW, both diluted and undiluted forms. The study showed that the consortium was able to remove all the pollutants below permissible limits within 12 days and 14 days when it was grown in SSTCW (M4 solution) and RSTCW (diluted), respectively. However, in the case of undiluted RSTCW, the residual concentrations of NH_4^+, phenol and cyanide were obtained as 53.2 mg/L, 3.04 mg/L and 0.081 mg/L, respectively. From this result, only cyanide could achieve below the permissible limit, while the concentrations of the other two pollutants could not meet the stipulated values. However, the concentrations of the other two pollutants are very close to the stipulated ones. Although the initial concentrations of model pollutants in SSTCW were higher than RSTCW, the removal was higher in SSTCW (M4 solution) NH_4^+ ($72.804 \pm 0.275\%$), phenol ($95.852 \pm 0.0751\%$) and cyanide ($74.449 \pm 0.001\%$) than in the undiluted RSTCW (NH_4^+ ($66.121 \pm 1.665\%$), phenol ($62.152 \pm 0.788\%$) and cyanide ($87.553 \pm 1.335\%$)). The lower removal of pollutants in the case of undiluted RSTCW might be due to less nutrient availability of real wastewater (Rai *et al.* 2021a).

Kinetics of growth of consortium in BG-11 and PLE enriched BG-11 medium

The model-predicted data are compared with the experimental ones as shown in Figure 5(a). From the figure, it is clear that the model holds good to represent experimental data. Regressed model parameters obtained in this work is presented in Table 3. As shown in the table; maximum specific growth rates and carrying capacity are estimated to be 0.56 per day and 1,330 mg/L, 0.45 per day and 351 mg/L for PLE + BG-11 and BG-11 medium, respectively. The higher values of maximum specific growth rate and carrying capacity indicate the vigorous growth of the present consortium in PLE enriched BG-11 medium.

Kinetic modelling for growth of the consortium in individual aqueous solutions of NH_4^+, phenol and cyanide

For simulated NH_4^+ solutions

Experimental log phase data were used to find the kinetic parameters of the logistic model for predicting the growth of microalgal consortium in NH_4^+ solutions. The maximum specific growth rate was estimated to be 0.133 per day in presence of NH_4^+ pollutants. Silva *et al.* (2015) studied the growth kinetics for microalgal biomass at different N:P molar ratios, and observed the specific growth rate (0.8 per day) of *C. vulgaris* at 8:1 (N:P) ratio where nitrogen source was only NH_4^+. The higher specific growth rate might be due to the higher N:P ratio (Silva *et al.* 2015). The maximum carrying capacity was estimated as 1,009 mg/L for 150 mg/L NH_4^+ solutions and AARD of 8.472% was obtained. The model-predicted data have been found to match reasonably well with experimental ones (Figure 5(b)). It is observed that the carrying capacity increases with an increase in initial concentration from 100 mg/L to 150 mg/L. Further increase in the pollutant concentration (175 mg/L) results in a very small decrease (920 mg/L) of carrying capacity. The values of maximum specific growth rate and carrying capacity are shown in Table S3(a), which is attached as Annexure VII in the Supplementary Material.

For simulated phenol solutions

A kinetic model for predicting the growth of the consortium in aqueous phenol solutions was developed. The maximum specific growth rate was found to be 0.167 per day and the maximum carrying capacity (699 mg/L) was obtained for 4.0 mg/L of phenol solution with 7.774% AARD. Lee *et al.* (2015) used *Spirulina maxima* for phenol removal, and a maximum specific growth rate of 0.088 per day at 50 mg/L phenol concentration was obtained. The lower specific growth rate as observed by Lee *et al.* (2015) might be due to a higher concentration of phenol. The model-predicted growth data in this study are compared with the experimental data (Figure 5(c)). From Figure 5 it can be stated that the developed model is good enough to predict the kinetic data during the log phase. The values of kinetic parameters are shown in Table S3(b), which is attached as Annexure VII in the Supplementary Material. The carrying capacity increased with an increase in initial concentration from 2 to 4.0 mg/L of phenol. Beyond that a sudden decrease was observed (Table S3(b)).

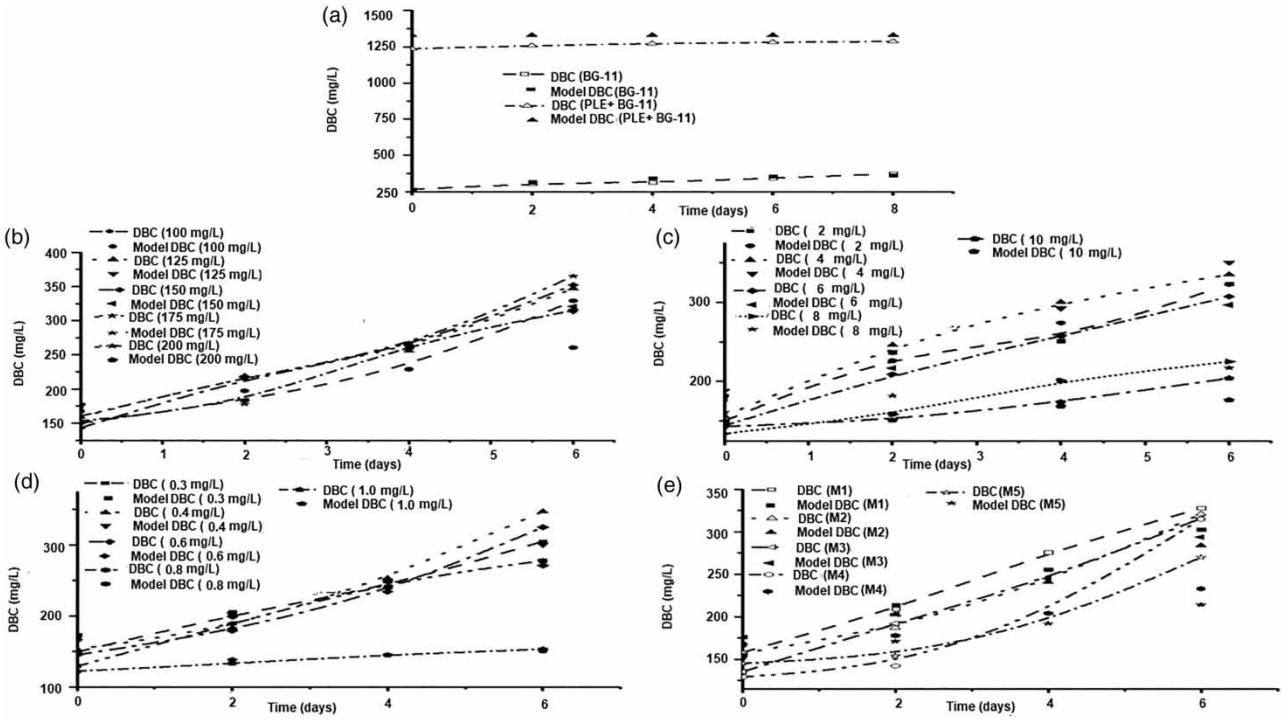


Figure 5 | Comparison of experimental and model-predicted DBC during exponential phase at different systems: (a) in different media (BG-11 and PLE-enriched BG-11 media), (b) in simulated solutions of NH_4^+, (c) in simulated solutions of phenol, (d) in simulated solutions of cyanide and (e) in SSTCW solutions.

Table 3 | Kinetic parameters for algal growth in BG-11 and BG-11 + PLE

	Biomass (mg/L)	
Parameters	**BG-11**	**BG-11 + PLE**
Maximum specific growth rate (per day)	0.446	0.56
Carrying capacity (mg/L)	350.7	1,330
AARD (%)	5.204	7.913

For simulated cyanide solutions

A growth kinetic model was developed to predict the growth of microalgal consortium in cyanide solution during the log phase. The maximum specific growth rate of 0.13 per day in cyanide solutions was observed. Mekuto & Musingadi (2019) obtained a maximum specific growth rate of 0.16 per day at 150 mg/L cyanide solution using *Scenedesmus obliqus*. The growth rate of microalgae depends on microenvironmental conditions and also varies from species to species (Mekuto & Musingadi 2019). The maximum carrying capacity of 990 mg/L for 0.4 mg/L of cyanide solution with 8.541% of AARD was obtained in this study. The values of kinetic parameters are shown in Table S3(c), which is attached as Annexure VII in the Supplementary Material. Carrying capacity varied with an increase in initial concentrations of cyanide (Table S3(c)). The model-predicted DBC data match quite well with experimental ones (Figure 5(d)).

Kinetic modelling for growth of consortium in SSTCW solutions

The values of maximum specific growth rate (μ'_{max}) and corresponding carrying capacity (X_{max}) for the mixed pollutant system with varying pollutant compositions (M1–M5) are estimated by fitting the experimental data obtained during the log phase in the modified logistic model. The values are shown in Table S3(d), which is attached as Annexure VII in the Supplementary Material. Maximum value of μ'_{max} (0.12 per day) (Equation (3)) is obtained for the mixed pollutant system with

composition M1: $NH_4^+ = 100$ mg/L, phenol = 2.0 mg/L and cyanide = 0.3 mg/L. Model predictions are compared with the experimental data for all these systems, as shown in Figure 5(e). From this figure, it can be stated the developed model has superior prediction capability with average absolute relative deviations of 7.232%, 6.872%, 9.103%, 17.70% and 9.031%.

The obtained maximum specific growth rate was also compared with other studies from the literature, as shown in Table S4 attached as Annexure VIII in the Supplementary Material. It is clear from Table S4 that the maximum specific growth rate of the present strain in both BG-11 and in PLE-enriched BG-11 are much more than those reported in the literature. Further, the values of maximum specific growth rates in polluted systems are comparable with others.

CONCLUSION

A consortium of *Synechococcus* sp. and *Chlorella* sp. was isolated from a contaminated site and was used for tertiary treatment of coke-oven wastewater. PLE obtained from poultry litter, a nitrogen and phosphorus-rich waste material, was mixed with BG-11 medium to curtail the cost of the growth medium. The consortium showed an enormous increase in growth (8.75-fold) in the PLE-enriched BG-11 medium after 18 days, while in the BG-11 medium the growth was observed to increase only 2.9-fold for the same time period. The consortium was found effective for the removal of multipollutants such as NH_4^+, phenol and cyanide from both the synthetic and RSTCW. The maximum removal of pollutants obtained from SSTCW (M4 solution) was NH_4^+ (72.804 $\pm$ 0.275%), phenol (95.852 $\pm$ 0.0751%) and cyanide (74.449 $\pm$ 0.001%). Bioremoval of NH_4^+ (66.121 $\pm$ 1.665%, 96.127 $\pm$ 1.354%), phenol (62.152 $\pm$ 0.788%, 90.176 $\pm$ 2.341%) and cyanide (87.553 $\pm$ 1.335%, 95.972 $\pm$ 1.757%) was achieved from undiluted and diluted RSTCW, respectively. The consortium was found efficient to reduce the concentrations of all the said pollutants below the permissible limits from both SSTCW (M4 solution) and the diluted RSTCW within the 12 days and 14 days, respectively. The consortium was also found suitable for the removal of a single pollutant from their aqueous solution. Therefore, it can be stated that the present consortium can be used for tertiary treatment of such pollutant-laden industrial effluent emitted from industries.

Other advantages of the phycoremediation technique include the fact that the produced biomass can further be used for the production of value-added products such as biodiesel, cattle feed, biofertilizer and others, depending on its macromolecule content. Moreover, the self-adjusting behaviour of pH during phycoremediation process makes it cost-effective. Hence, it can be stated that this study opens up a new avenue for tertiary treatment of industrial wastewater in an ecofriendly and economic manner. However, this work is a preliminary work performed under laboratory conditions. Therefore, a comprehensive field study encompassing all real-time parameters such as ambient temperature and light intensity using real wastewater is needed before its commercial application. Furthermore, efficient handling of other operational problems during continuous operation such as contamination with other microbes and frothing is a challenging job for process engineers for real field application of such a technique.

ACKNOWLEDGEMENTS

The authors are thankful to the IMPRINT, Government of India (Sanction order no: F.No.3-18/2015-T.S.-I (Vol. III) dated 24 April 2017) and National Institute of Technology Durgapur (Sanction order no: NITD/Regis/702/17 dated 15 February 2017) for providing the project funds. Authors are also thankful to DST-FIST for providing the equipment for carrying out the research work.

CONFLICTS OF INTEREST

The authors declare that they do not have any conflict of interest.

DATA AVAILABILITY STATEMENT

Data cannot be made publicly available; readers should contact the corresponding author for details.

REFERENCES

Bhatnagar, A., Chinnasamy, S., Singh, M. & Das, K. C. 2011 Renewable biomass production by mixotrophic algae in the presence of various carbon sources and wastewaters. *Applied Energy* **88**, 3425–3431. https://doi.org/10.1016/j.apenergy.2010.12.064.

Biswas, R., Bagchi, S., Urewar, C., Gupta, D. & Nandy, T. 2010 Treatment of wastewater from a low-temperature carbonization process industry through biological and chemical oxidation processes for recycle/reuse: a case study. *Water Science & Technology* **61**, 2563–2573. https://doi.org/10.2166/wst.2010.181.

Biswas, G., Thakurta, S. G., Chakrabarty, J., Adhikari, K. & Dutta, S. 2018 Evaluation of fluoride bioremediation and production of biomolecules by living cyanobacteria under fluoride stress condition. *Ecotoxicology and Environmental Safety* **148**, 26–36. https://doi.org/10.1016/j.ecoenv.2017.10.019.

Choi, C. J., Berges, J. A. & Young, E. B. 2012 Rapid effects of diverse toxic water pollutants on chlorophyll a fluorescence: variable responses among freshwater microalgae. *Water Research* **46** (8), 2615–2626. https://doi.org/10.1016/j.watres.2012.02.027.

Collos, Y. & Harrison, P. J. 2014 Acclimation and toxicity of high ammonium concentrations to unicellular algae. *Marine Pollution Bulletin* **80**, 8–23. https://doi.org/10.1016/j.marpolbul.2014.01.006.

Das, S., Biswas, P. & Sarkar, S. 2020 Tertiary treatment of coke plant effluent by indigenous material from an integrated steel plant : a sustainable approach. *Environmental Science and Pollution Research* **27** (7), 7379–7387. https://doi.org/10.1007/s11356-019-07309-x.

Duan, W., Meng, F., Lin, Y. & Wang, G. 2017 Toxicological effects of phenol on four marine microalgae. *Environmental Toxicology and Pharmacology* **52**, 170–176. https://doi.org/10.1016/j.etap.2017.04.006.

Gonçalves, A. L., Pires, J. C. M. & Simões, M. 2017 A review on the use of microalgal consortia for wastewater treatment. *Algal Research* **24**, 403–415. https://doi.org/10.1016/j.algal.2016.11.008.

Gupta, N., Balomajumder, C. & Agarwal, V. K. 2010 Enzymatic mechanism and biochemistry for cyanide degradation: a review. *Journal of Hazardous Materials* **176**, 1–13. https://doi.org/10.1016/j.jhazmat.2009.11.038.

Gurbuz, F., Ciftci, H., Akcil, A. & Karahan, A. G. 2004 Microbial detoxification of cyanide solutions: a new biotechnological approach using algae. *Hydrometallurgy* **72** (1–2), 167–176. https://doi.org/10.1016/j.hydromet.2003.10.004.

Gutierrez, J., Kwan, T. A., Zimmerman, J. B. & Peccia, J. 2016 Ammonia inhibition in oleaginous microalgae. *Algal Research* **19**, 123–127. https://doi.org/10.1016/j.algal.2016.07.016.

Hammed, A. M., Prajapati, S. K., Simsek, S. & Simsek, H. 2016 Growth regime and environmental remediation of microalgae. *Algae* **31** (3), 189–204. https://doi.org/10.4490/algae.2016.31.8.28.

Kwiecińska, A., Lajnert, R. & Bigda, R. 2017 Review on formation, treatment and utilization methods of coke oven wastewater. *Ecological Chemistry and Engineering. A* **24** (2). http://dx.doi.org/10.2428/ecea.2017.24%282%2918.

Kwiecińska-Mydlak, A., Sajdak, M., Rychlewska, K. & Figa, J. 2019 The role of a chemical loop in removal of hazardous contaminants from coke oven wastewater during its treatment. *Open Chemistry* **17** (1), 1288–1300. https://doi.org/10.1515/chem-2019-0142.

Lee, H. C., Lee, M. & Den, W. 2015 *Spirulina maxima* for phenol removal: study on its tolerance, biodegradability and phenol-carbon assimilability. *Water, Air, & Soil Pollution* **226** (12), 1–11. https://doi.org/10.1007/s11270-015-2664-3.

Liu, Q., Zhang, G., Ding, J., Zou, H., Shi, H. & Huang, C. 2018 Evaluation of the removal of potassium cyanide and its toxicity in green algae (*Chlorella vulgaris*). *Bulletin of Environmental Contamination and Toxicology* **100** (2), 228–233. https://doi.org/10.1007/s00128-017-2208-1.

Lu, Q., Chen, P., Addy, M., Zhang, R., Deng, X., Ma, Y., Cheng, Y., Hussain, F., Chen, C., Liu, Y. & Ruan, R. 2018 Carbon-dependent alleviation of ammonia toxicity for algae cultivation and associated mechanisms exploration. *Bioresource Technology* **249**, 99–107. https://doi.org/10.1016/j.biortech.2017.09.175.

Lynch, D., Henihan, A. M., Bowen, B., Lynch, D., McDonnell, K., Kwapinski, W. & Leahy, J. J. 2013 Utilisation of poultry litter as an energy feedstock. *Biomass and Bioenergy* **49**, 197–204. https://doi.org/10.1016/j.biombioe.2012.12.009.

Maiti, D., Ansari, I., Rather, M. A. & Deepa, A. 2019 Comprehensive review on wastewater discharged from the coal-related industries–characteristics and treatment strategies. *Water Science and Technology* **79** (11), 2023–2035. https://doi.org/10.2166/wst.2019.195.

Mekuto, L. & Musingadi, D. 2019 A dataset representing the impact of cyanide on the fatty acid profile of *Scenedesmus obliquus*. *Data in Brief* **24**, 103900. https://doi.org/10.1016/j.dib.2019.103900.

Perez-Garcia, O., Escalante, F. M. E., de-Bashan, L. E. & Bashan, Y. 2011 Heterotrophic cultures of microalgae: metabolism and potential products. *Water Research* **45**, 11–36. https://doi.org/10.1016/j.watres.2010.08.037.

Rai, A., Wadhwa, G. K., Chakrabarty, J. & Dutta, S. 2020 Application of cyanobacterial consortium to remove ammoniacal-N, phenol, and nitrate from synthetic coke-oven wastewater as tertiary treatment. *Journal of Environmental Engineering* **146**, 04020062. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001731.

Rai, A., Chakrabarty, J. & Dutta, S. 2021a Phycoremediation of pollutants from coke-oven wastewater using *Tetraspora* sp. NITD 18 and estimation of macromolecules from spent biomass. *Journal of Water Process Engineering* **39**, 101746. https://doi.org/10.1016/j.jwpe.2020.101746.

Rai, A., Gowrishetty, K. K., Singh, S., Chakrabarty, J., Bhattacharya, P. & Dutta, S. 2021b Simultaneous bioremediation of cyanide, phenol, and ammoniacal-N from synthetic coke-oven wastewater using *Bacillus* sp. NITD 19. *Journal of Environmental Engineering* **147** (1), 04020143. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001835.

Renuka, N., Sood, A., Prasanna, R. & Ahluwalia, A. S. 2015 Phycoremediation of wastewaters: a synergistic approach using microalgae for bioremediation and biomass generation. *International Journal of Environmental Science and Technology* **12**, 1443–1460. https://doi.org/10.1007/s13762-014-0700-2.

Rossi, S., Díez-Montero, R., Rueda, E., Cascino, F. C., Parati, K., García, J. & Ficara, E. 2020 Free ammonia inhibition in microalgae and cyanobacteria grown in wastewaters: Photo-respirometric evaluation and modelling. *Bioresource technology* **305**, 123046–1460. https://doi.org/10.1016/j.biortech.2020.123046.

Sen, G., Sen, S., Thakurta, S. G., Chakrabarty, J. & Dutta, S. 2018a Bioremediation of Cr(VI) using live cyanobacteria: experimentation and kinetic modeling. *Journal of Environmental Engineering* **144**, 1–12. https://doi.org/10.1061/(ASCE)EE.1943-7870.0001425.

Sen, S., Bhardwaj, K., Thakurta, S. G., Chakrabarty, J., Ghanta, K. C. & Dutta, S. 2018b Phycoremediation of cyanide from coke–oven wastewater using cyanobacterial consortium. *International Journal of Environmental Science and Technology* **15** (10), 2151–2164. https://doi.org/10.1007/s13762-017-1568-8.

Sharma, N. K. & Philip, L. 2016 Combined biological and photocatalytic treatment of real coke oven wastewater. *Chemical Engineering Journal* **295**, 20–28. https://doi.org/10.1016/j.cej.2016.03.031.

Silva, A. G., Carter, R., Merss, F. L., Corrêa, D. O., Vargas, J. V., Mariano, A. B. & Scherer, M. D. 2015 Life cycle assessment of biomass production in microalgae compact photobioreactors. *GCB Bioenergy* **7** (2), 184–194. https://doi.org/10.1111/gcbb.12120.

Singh, M. & Das, K. C. 2014 Low cost nutrients for algae cultivation. In: *Algal Biorefineries*. Springer, Dordrecht, pp. 69–82. https://doi.org/10.1007/978-94-007-7494-0_3.

Singh, H. & Mishra, B. K. 2018 Degradation of cyanide, aniline and phenol in pre-treated coke oven wastewater by peroxide assisted electro-oxidation process. *Water Science and Technology* **78** (10), 2214–2227. https://doi.org/10.2166/wst.2018.503.

Stephen, D. P. & Ayalur, K. B. 2017 Phycoremediation of phenolic effluent of a coal gasification plant by *Chlorella pyrenoidosa*. *Process Safety and Environmental Protection* **111**, 31–39. https://doi.org/10.1016/j.psep.2017.06.006.

Thakurta, S. G., Aakula, M., Chakrabarty, J. & Dutta, S. 2018 Bioremediation of phenol from synthetic and real wastewater using *Leptolyngbya* sp.: a comparison and assessment of lipid production. *3 Biotech* **8** (4), 1–10. https://doi.org/10.1007/s13205-018-1229-8.

Thangavel, K., Radha Krishnan, P., Nagaiah, S., Kuppusamy, S., Chinnasamy, S., Rajadorai, J. S., Olaganathan, G. N. & Dananjeyan, B. 2018 Growth and metabolic characteristics of oleaginous microalgal isolates from Nilgiri biosphere Reserve of India. *BMC Microbiology* **18**, 1–17. https://doi.org/10.1186/s12866-017-1144-x.

Waghmare, S. S. & Arfin, T. 2015 Fluoride removal from water by various techniques. *International Journal of Innovative Science, Engineering and Technology* **2** (3), 560–571.

Wang, Y., Meng, F., Li, H., Zhao, S., Liu, Q., Lin, Y., Wang, G. & Wu, J. 2019 Biodegradation of phenol by *Isochrysis galbana* screened from eight species of marine microalgae: growth kinetic models, enzyme analysis and biodegradation pathway. *Journal of Applied Phycology* **31** (1), 445–455. https://doi.org/10.1007/s10811-018-1517-z.

First received 3 June 2021; accepted in revised form 16 September 2021. Available online 30 September 2021