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SHORT COMMUNICATION

Characterization and microalgal toxicity screening of diagnostic fixer solution toward bioremediation

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Abstract
The present study involves the characterization and microalgal toxicity screening of diagnostic X-ray fixer solution toward utilization as a probable full-scale bioremediation study. The characterization results showed a BOD value of the waste X-ray fixer solution 11,833 ± 485.62 mg/l and 506,733 ± 251.66 ppm COD. The *Scendesmus abundans* was well grown using BBM, 1500 lx light intensity, 12 h:12 h light and dark conditions with 100 rpm shaking at 25 ± 1 °C on 1-month cultivation. The executed toxicity screening results of the diagnostic fixer solution on *S. abundans* with different dilutions have shown a promisable growth between 15 and 21 days with the 3 BBM:1 X-ray fixer solution dilution with a maximum cell count of 370×10^4 cells/ml on the 21st day of microalgal cultivation. The present study puts forth the physical and chemical parameters of X-ray fixer solution with proven toxicity tolerance limits of *S. abundans* toward the probable logical step of algal-based bioremediation of fixer solution.

Keywords Characterization · Diagnostic fixer solution · *Scendesmus abundans* · Toxicity assessment

Introduction

The hospital and other medical clinics generate various waste; radiographic waste is one of the hazardous waste that has fixer solution, developer solution, lead foils, and other chemicals. Silver and heavy metals make the radiographic waste as one of the hazardous effluents from hospitals (Molinuevo-Salces et al. 2019). The fixer solution is considered more toxic (sliver content > 3000 mg/l) compared to other solutions used in the processing of the radiographs along with the sulfites, thiosulfates, and other minute amounts of heavy metals (Muzio et al. 2005; Lorenzo et al. 1982). The waste fixer solution has higher levels of biological oxygen demand (BOD) and chemical oxygen demand (COD) (Bas et al. 2012). The diagnostic lab effluents discharging into the public sewage system consist of above the permissible limits of inorganic components (such as silver), physical (color, pH, turbidity, sulfates, chlorides, and total dissolved solids), and chemical (BOD and COD) components. Generally, the radiographic effluents (with less than 5 ppm silver) from hospitals release into a sewer system. The fixer solution has an immense amount of silver 3000–8000 ppm, which is more than the allowed limits. Thus, there is a need for an appropriate silver removal approach for possible discharge into the sewage system (Madhavan et al. 2015). The adopting existing silver recovery approaches (electrolysis, adsorption, metallic replacement, ion exchange, enzymatic method, and precipitation) suffer from the high cost and toxic chemicals usage that is prone to different environmental and economic issues (Koneru et al. 2014). From the past, many years' use of microalgae is one of the enormous approaches for removing the contaminants from the waste (Sevda et al. 2019). The microalgae are easily cultivated in the nutrient medium enriched with the nitrogen, phosphorus, trace elements, and other salts, which help in microalgae growth (Ilavarasi et al. 2011).

The advantages of using a microalgal-based system over conventional treatment technologies include the easy growth with the utilization of available inorganic constituents of industrial effluents and atmospheric CO_2 and light, uptake of metal ions and possible biocommodities production along

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the bioremediation in a sustainable way (Gour et al. 2018, 2020; Jha et al. 2017). The most permissive microalgal genera in wastewater treatment were *Chlorella*, *Oscillatoria*, *Chlamydomonas*, *Navicula*, *Scendesmus*, *Nitzschia*, *Stigeoclonium,* and *Euglena* (Álvarez-Díaz et al. 2017). The determination of metal toxicity tolerance of microalgae in wastewater effluent is a prerequisite for algal-based bioremediation's possible success (Essa et al. 2018). The green microalgae *Scendesmus sp.,* with easy cultivation and fast growth rate has been taken as the microalgae of interest for the present study (Tripathi et al. 2019). The research domain of characterization and toxicity screening studies of fixer solution is less attempted one. Hence, the present study puts forth the characterization of the diagnostic fixer solution along with the toxicity tolerance limits of *S. abundans,* which helps toward the next logical step of probable algal-based bioremediation.

Materials and methods

Fixer solution collection

The diagnostic fixer solution was collected from the private lab in Shimla. The waste solution was stored at 4 °C for forthcoming studies.

Physicochemical properties determination of diagnostic fixer solution

The diagnostic fixer solution's physical (pH, total solids, total suspended solids, total dissolved solids, total volatile solids, total fixed solids), and chemical (biological and chemical oxygen demands) properties were determined based on the APHA (2005) protocols.

Density and conductivity

The density and conductivity of the diagnostic fixer solution were analyzed by density meter and conductometer, respectively.

Estimation of total Kjeldahl nitrogen and phosphorus

The total Kjeldahl nitrogen and phosphorus contents in the fixer solution were estimated based on the protocols of Jackson (1958) and Fiske et al. (1925), respectively.

Silver analysis

The diagnostic fixer solution was analyzed in inductive coupled plasma–mass spectrometry (ICP-MS). The waste solution was diluted with distilled water and filtered by a 0.2-μm filter paper followed by silver analysis in the diluted sample.

Microalgae growth conditions and growth monitoring studies

The microalgae *S. abundans* obtained from the NCIM Pune was subcultured in a 250-ml flask using standard Bold basal medium (BBM) (Nichols et al. 1965). The microalgal flask was incubated in an incubator shaker equipped with photosynthetic light (12 h light and dark periods) and 100 rpm shaking conditions at 25 ± 1 °C. The 10 ml of subcultured *S. abundans* (809×10^4 cells/ml) was inoculated in the 100 ml of BBM medium in 250 ml of the flask. The microalgal growth was monitored through optical density (after every 3 days at 680 nm in UV–VIS spectrophotometer) and cell count ($\times 10^4$ cells/ml, using improved Neubauer chamber under a light microscope) studies for 1 month.

Toxicity assessment of *S. abundans* in diluted diagnostic fixer solution

The diagnostic fixer solution is diluted in various concentrations 1 BBM:3 waste fixer solution, 2 BBM:2 waste fixer solution, 3 BBM:1 waste fixer solution using 100 C (BBM medium/radiographic solution as controls, without dilution, as controls) and inoculated with *S. abundans*. The inoculated *S. abundans* samples were incubated under above- mentioned growth conditions, and cell count was taken every three days to monitor the microalgal growth for one month.

Table 1 Physicochemical parameters of the waste X-ray fixer solution

Parameters	Results
pH	10.13 ± 0.005
Total solids (mg/l)	139,733.3 ± 28.86
Total dissolved solids (mg/l)	128,750 ± 390.51
Total suspended solids (mg/l)	10,983.33 ± 419.32
Total volatile solids (mg/l)	36,900 ± 350
Total fixed solids (mg/l)	102,833.3 ± 375.27
Conductivity (mS)	68.56 ± 0.05
Density (g/cm^3)	1.09 ± 0.0
BOD (mg/l)	11,833.33 ± 485.62
COD (ppm)	506,733.3 ± 251.66
TKN (%)	10.67 ± 0.69
TP (µg/ml)	37.33 ± 3.05
Silver (mg/L)	3497 ± 12.76

All the values are represented as ± s.d of three replicates

Results and discussion

Physicochemical analysis of diagnostic fixer solution

The release of the fixer solution and other toxic material directly into the wastewater treatment plant or other sewerage system is not accepted worldwide. The wastewater parameter analysis is mandatory to know the toxicity values and their impact on the environment. The fixer solution contains many organic and inorganic compounds, which make the solution alkaline and acidic. The pH of the waste fixer solution is 10.13 ± 0.005 (Table 1). The total dissolved solids and total suspended solids in waste fixer solutions are 128,750 ± 390.51 mg/l and 10,983.33 ± 419.32 mg/l, respectively (Table 1). The fixer solution's conductivity is 68.56 ± 0.05 mS, and the density is 1.09 ± 0.0 g/cm^3 (Table 1). Various types of hardeners, preservatives, chemicals, and buffers make them more hazardous in the fixing process. The BOD and COD values of waste fixer solution were found to be 11,833.33 ± 485.62 mg/l and 33.3 ± 251.66 ppm, respectively (Table 1), which were very high compared with the usual standards. The nitrogen and phosphorus corresponding amount in fixer solution is 10.67 ± 0.69% and 37.33 ± 3.05 µg/ml (Table 1).

The radiography process generates a high amount of silver in the form of silver thiosulfate complexes in the fixer solution. The fixer solution contains a high amount of silver, around 3497 ± 12.76 mg/l (Table 1). The alarming situation will be generated when the fixer solution is waste with 3000–8000 ppm silver in it. The management, disposal, and recovery of silver from waste solutions are crucial for the environment safety movement (Madhavan et al. 2015). The harmful effects of the fixer solution toward the environment are due to silver 2000–6000 mg/l. The toxic compounds from the waste precisely splash into the surroundings (Khunprasert et al. 2008). The analysis of physical and chemical parameters reveals the information about the toxicity values of a fixer solution to the environment (Table 1). The fixer solution has surpassed the maximum allowed limits set by the Indian government to dispose of the fixer solution by utilizing a sustainable remediation approach.

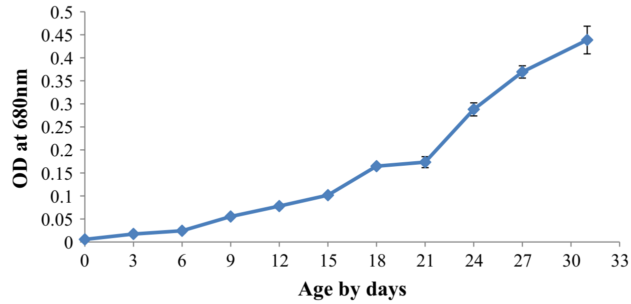

Fig. 1 Cell growth of *S. abundans* in BBM. All the values are represented as ± s.d of three replicates

Growth conditions of *S. abundans* in BBM

The microalgae cultivation is achieving the new demands with full application in food, feeding, pharmaceuticals, biofuels, and bioremediation of wastewater (Sevda et al. 2020;

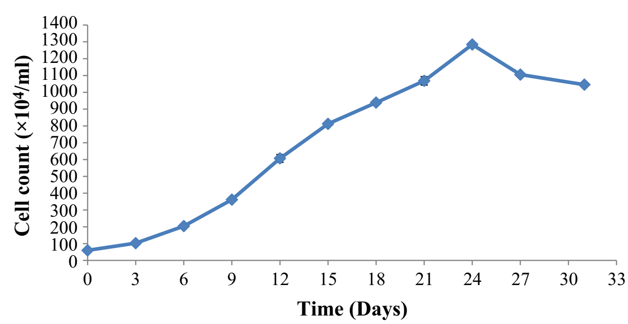

Fig. 2 Cell count (× 10^4/ml) of *S. abundans* in BBM. All the values are represented as ± s.d of three replicates

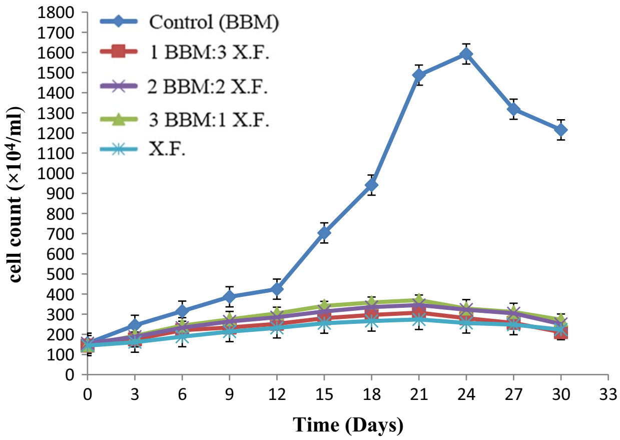

Fig. 3 Toxicity tolerance of *S. abundans* (in terms of cell count, $\times 10^4$/ml) in different dilutions of fixer solution and BBM. All the values are represented as ± s.d of three replicates

Lizzul et al. 2014). The growth phase (days) and the optical density of different microalgae diverged with microalgal species (Gojkovic et al. 2019). In the present study, *S. abundans* grew in the BBM medium for 1 month, and the results are depicted in Figs. 1 and 2. The utmost cell count 1284×10^4 cells/ml was retrieved on the 24th day of cultivation. The OD measured at the end of the microalgal growth cycle was 0.438 ± 0.03 (Fig. 2). The exponential growth phase of microalgae is 6–24 days from the cell count and optical density studies (Fig. 2).

Toxicity assessment of *S. abundans* in different concentrations of fixer solution and BBM

The fixer solution has high values of physical–chemical parameters (Table 1); removing these contaminants is crucial before discharge to the environment. Microalgal-based bioremediation is a sustainable approach to bioremediate the diagnostic effluents that can uptake the heavy metals (such as silver) and utilize the excess nitrogen and phosphorus for growth and available organic and biological components for different macromolecules synthesis (Kshirsagar 2013). The *S. abundans* grew in the different fixer solution dilutions, and the BBM medium for 1 month has shown different growth patterns. The cell count in the initial day is not that steep, but as days progressed, the cell count also increased until 21 days. Among different tested dilutions, the highest cell count (370×10^4 cells/ml) has been observed with the 3 BBM:1 fixer solution (Fig. 3). The tolerance limits of microalgae in cultivation media, i.e., *Scendesmus* sp. ISTGA1 and BG11 medium (Tripathi et al. 2019) and *N. oculata* and F/2 medium (Essa et al. 2018) with different dilutions of industrial effluents have been shown to be promising before the full-scale study of bioremediation (Patil et al. 2019). The present research showcases the importance of tackling the x-ray waste fixer solution and has proven that the 3 (BBM):1 (Fixer) dilution is the best tolerance limit of *S. abundans* with promising growth which will be ideal for taking carry forward the full-scale investigation of algal-based bioremediation toward silver removal and reduction of environ-threat physicochemical properties for proper effluent disposal.

Conclusion

The fixer solution discharged from the radiographic processing is significantly more dangerous and has a high amount of silver content 3497 mg/l. The physicochemical parameters investigation of the waste fixer solution concedes the vast amount of BOD 11,833 mg/l and COD 506,733 ppm. The higher toxicity of the waste fixer solution leads to its hazardous effects on the atmosphere. A maximal microalgal growth was observed in the 3 BBM:1 fixer solution dilution as compared with other dilutions. The cell count 370×10^4 cells/ml was noticed in a highly diluted waste fixer solution at 21 days of the growth curve compared with other dilutions.

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Author contributions SS: Methodology, Resources, Formal analysis and Investigation, Writing—Original Draft. VKG: Supervision, Conceptualization, Writing—review and editing, Validation.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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