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ORIGINAL ARTICLE

A Novel Study on Anionic Surfactant Degradation Potential of Psychrophillic and Psychrotolerant *Pseudomonas* spp. Identified from Surfactant-contaminated River Water

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Abstract
The Yamuna River, a tributary of the holy Ganga, is heavily polluted in the Delhi-NCR region, India and has been gaining attention due to the excessive foaming of the river over the past few years. This can be directly or indirectly related to the overuse of surfactants and the discharge of untreated domestic and textile wastewater into the river. To determine the surfactant load and investigate potential surfactant-degrading bacteria in the region, 96 water samples from four sites in the Okhla Barrage stretch of the river were collected and analysed. The results showed that the selected sites have surfactant concentrations more than the permissible limit (1.00 mgL^{-1}). Also, at most of the sites, the concentration crossed the desirable limit of BIS (0.2 mgL^{-1}) during the period of analysis. The concentration of anionic surfactant reported in the region was found in the range of 0.29 mgL^{-1} and 2.83 mgL^{-1}. A total of 38 different bacteria were isolated using selective media from the same water samples, out of which 7 bacterial isolates were screened for sodium dodecyl sulphate (SDS) tolerance activity. Based on 16S rRNA gene sequencing, 2 species, namely *Pseudomonas koreensis* YRW-02 and *Pseudomonas songnenensis* YRW-05 have been identified and their degradation potential was assessed at different SDS concentrations. The results showed that our strains YRW-02 and YRW-05 degraded 78.29 and 69.24% of SDS respectively. Growth optimization was also performed at different substrate concentrations, pH, and temperature to investigate optimum degradation conditions. This study plays a significant role in assessing the surfactant load and also gives a promising background for future use in in-situ bioremediation experiments.

Keywords Surfactant · Sodium dodecyl sulphate · Bioremediation · 16S rRNA sequencing · Growth optimization · Characterization

Introduction

The river Yamuna is the second-largest tributary of the holy Ganga and is considered sacred in Hindu mythology. It originates from the glacier Yamunotri in Uttarakhand, India and travels a length of 1,376 km while passing through 5 Indian states, including the National

Extended author information available on the last page of the article

Capital Territory of Delhi. Approximately 57 million people rely on Yamuna waters and serve for over 70% of water supplies to the NCT of Delhi [24]. It is an important river in the northern Indian plains and accounts for more than 90% of irrigation practices [15]. In its entire 22 km stretch in Delhi, which is less than 2% of the total length of the river, 16 out of 23 drains are pouring their effluents into the Yamuna in between the Wazirabad downstream to Okhla upstream stretch, 4 drains join the river at Okhla Barrage downstream and 3 drains join further at Agra Canal and Gurgaon Canal [3, 11]. Yamuna in Delhi reports approximately 80% of the total pollution in the river "Brief on the Yamuna: WHAT AILS THE YAMUNA?" [9]. Monitoring of water quality parameters of the river over the past years has indicated high levels of heavy metals [6, 40], pesticides [45, 46], pharmaceuticals [7, 27], and various other organic and inorganic pollutants [43, 51]. The occurrence of these contaminants in the river water has been mainly attributed to industrial, municipal, and domestic sewage discharge of waste [5, 55, 56].

During the last decade, there have been several reports of excessive foaming in the Okhla Barrage stretch of the Yamuna River [65]. In fact, foaming in the river has been a regular phenomenon, which has recently gathered a lot of media attention. Unfortunately, no scientific study has been conducted yet to address this issue. Although some studies have suggested that the foam in the river is caused due to the presence of detergents [8, 34, 48, 57], no substantial work in the same field has been done so far.

A major constituent of detergents, surfactants have been categorised as 'contaminants of emerging concern' due to their excessive usage and recent studies suggesting their toxicity to various living organisms Kaczerewska et al. [23, 26, 66]. Multiple studies have been conducted where bacteria from surfactant-contaminated sites have been isolated and exploited for their surfactant-degrading capabilities [12, 17, 25, 58]. Extremophiles have exceptional adaptation capabilities that make them reservoirs of biocatalysts and enzymes that can be used remarkably in the bioremediation of contaminants. Psychrophiles are organisms exhibiting optimal growth below 15 °C and maximum growth at 20 °C [29, 30]. They have been largely studied for their role in the bioremediation of xenobiotics. Psychrotolerant microbes on the other hand show growth at much lower rates at low temperatures and exhibit optimum growth at temperatures ranging between 20–40 °C, similar to mesophilic organisms [29]. Marine psychrotolerant *Pseudomonas* sp. from the Mediterranean sea has been reported to show effective azo dye decolorization. The same species also showed heavy tolerance to lead and was able to resist and accumulate 700–800 ppm of the metal [39]. A psychrotolerant *Rahnella* sp. isolated from the soil samples of apple orchards in Western Himalayan regions effectively degraded organophosphate profenofos [61]. *Pseudomonas syringae* and *P. fluorescens* isolated from Sub-arctic river Glerá in Northern-Iceland were found to possess surfactant-degrading abilities. The species were psychrotropic in nature, associated with freshwater environments in the cold environment [41].

Bioremediation helps in naturally breaking down the pollutants, offering an eco-friendly approach to environmental cleanup. By harnessing the power of microorganisms and plants, bioremediation can degrade contaminants and restore contaminated ecosystems [31]. For an effective remediation approach, quantification of pollutants is the first step towards its mitigation. Therefore, in the current study, we aimed at analysing the average concentration of anionic surfactants in the Yamuna river. We isolated and characterized bacteria possessing surfactant-degrading capabilities from the same environment and investigated their growth potential in different environmental conditions so as to make it closer to the field conditions.

Materials and methods

Reagents, chemicals, and bacteriological media

Various bacteriological media and reagents used in the study include nutrient agar (NA), nutrient broth, starch agar, King's B media, buffered peptone water, Oxidase test disc, Kovac's reagent, nitrate broth, Methyl red- Vogues Proskauer broth (MR-VP agar), Simmon's citrate agar, Gram's staining kit, DNA isolation kit were obtained from Hi-Media, Mumbai, India. Hydrogen peroxide, Ethanol, Sulphanilic acid, Isopropyl alcohol (70%), Alpha-Napthylamine, Sodium chloride, α-Napthol, methyl red indicator, Sodium dodecyl sulphate (SDS) (purity ≥ 98.0%), were of analytical grade and procured Sigma Laboratories, India. Chloroform (purity ≥ 99.8%) was obtained from Fischer Scientific, India, and Methylene blue was purchased from Qualikems, India.

Study area and sample collection

Collection, preservation, and transportation of water samples to the laboratory and analysis were executed as per standard methods. Water samples were collected in 500 mL acid-washed containers with air-tight caps, after filtering with Whatman filter paper No. 1 (pore size 11 µm), as prescribed in the standard procedure [2]. The containers were soaked in 10% nitric acid and rinsed with deionized water 2–3 times. All the samples were immediately carried to the laboratory and stored at 4 °C until analysis. The surfactants were determined by the methylene blue active substances (MBAS) assay (APHA, 5440-C) using UV–vis spectrophotometer (Agilent Cary 60-G6860A). Bureau of Indian Standard (BIS), formerly known as Indian Standards Institution (ISI) is the National Standards Body of India which develops and publishes Indian Standards. As per BIS: 10,500 (1991), a concentration of 0.20 ppm is considered the desirable limit and 1.0 ppm is the permissible limit for surfactants in water (Table 1).

Analytical method (MBAS Assay) for quantification of anionic surfactants

The evaluation of anionic surfactant concentration was done by a standard method with methylene blue dye and chloroform as solvent (APHA, 5540 C, 2017). Anionic surfactants form

Table 1 Coordinates of the sampling site

S. No	Site Code	Latitude	Longitude
1	L1	77°18′42.8"E	28°32′43.0"N
2	L2	77°18′45.1"E	28°32′43.2"N
3	L3	77°18′46.9"E	28°32′42.9"N
4	L4	77°18′46.5"E	28°32′40.3"N

L1 was the area of most foaming which was just next to the opening of the barrage. L2 is 50 m away from L1 and L3 was 100 m away from L1. L4 was the area near the bank. A geographical representation of the sampling area is shown in Fig. 1

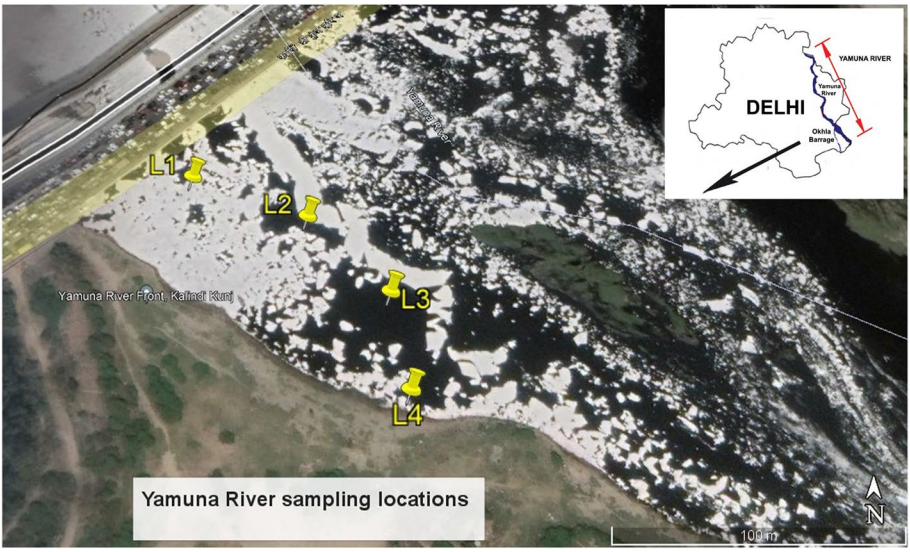

Fig. 1 Yamuna River sampling location depicting the four sampling sites L1-L4

a blue-coloured complex with methylene blue, which is extracted over chloroform and the absorbance is measured at 652 nm using a UV/Visible spectrophotometer (Cary 60, Agilent Technologies). This method is popularly known as methylene blue active substances assay (MBAS Assay).

Quantification and isolation

Quantification of bacteria in each water sample was performed by serially diluting (ratio 1:10; 25 mL sample in 225 mL of 0.85% saline) the sample and following the pour plate method using NA and incubating the samples at 37 °C. The total number of colonies on each media was calculated using the formula:

$$\mathrm{N} = \frac{\sum C}{(n1 + 0.1n2)d}$$

where, N is the sum of colony-forming units in one ml of sample, d stands for the dilution factor from which the primary counts were attained, n1 and n2 correspond to the number of plates considered in the first and second dilution respectively, and ΣC indicates the sum of all colonies counted on all the plates. The colonies were observed under a microscope, and identical colonies were re-streaked on NA plates to obtain pure cultures. These cultures were then subjected to SDS-tolerance tests [14].

Screening of SDS-tolerant bacterial strains

For screening of SDS-tolerant strains, the bacterial isolates were further grown in minimal media having the following composition (per liter): 3 g K_2HPO_4, 10 g KH_2PO_4, 2 g NH_4NO_3, 0.3 g $MgSO_4{\cdot}7H_2O$, 0.1 g NaCl, 0.01 g $CaCl_2{\cdot}2H_2O$, 0.01 g MoO_3, 0.001 g, $MnCl_2$, 0.001 g

$CuCl_2$, 0.001 g $FeSO_4$, 0.001 g $ZnSO_4$ [17]. SDS ($NaSO_4C_{12}H_{25}$; molecular weight 288.38 $gmol^{-1}$) at concentrations of 12.5, 25, 50, 100, 200, 300, and 500 mgL^{-1} respectively, was added using a 0.45 µm nylon syringe filter. This SDS acted as the only source of carbon for the bacteria. The cultures were cultivated at 20 °C temperatures and were observed for turbidity as an indicator of bacterial growth.

Optimization of growth conditions

Variations in bacterial growth at different pH, incubation temperatures, and substrate (SDS) concentrations were observed. Multiple experiments were performed to assess the tolerance of the bacterial isolate at SDS concentrations of 12.5, 25, 50, 100, 200, 300, and 500 mgL^{-1}, at varying pH (5.0, 7.0, and 8.0), and incubation temperatures of 4 °C, 20 °C or 37 °C. Bacterial growth was estimated by measuring optical density (OD) using a UV/visible spectrophotometer at 600 nm [28].

Biodegradation studies

Biodegradation tests were carried out in 250 mL conical flasks in sterile condition containing 100 ml of minimal media for strains that exhibited tolerance towards SDS. Based on the results obtained during growth optimization, the parameters selected for the biodegradation study were as follows: 50 mgL^{-1} concentration of SDS, 7 pH, and 4 °C, and 37 °C temperature for YRW-02 and YRW-05 strains respectively. A loopful of freshly grown (24 h old) bacterial isolates were dissolved in 0.85% saline for the preparation of inoculum for each strain. The OD of each inoculum was adjusted to 25% transmittance at 530 nm using a UV/Visible spectrophotometer [17].

To cross-verify the number of colonies present per ml of adjusted inoculum, validation was done using the serial dilution pour-plate method [13].

A 50 mgL^{-1} working solution of SDS was prepared from a stock solution of 100 mgL^{-1}. The flasks were incubated at 4 °C and 37 °C respectively, and stirred at 150 rpm for 216 h under aerobic conditions. At every 24-h interval, samples were withdrawn for assessing the SDS concentration using MBAS assay.

Morphological and biochemical characterization

The bacterial isolates exhibiting tolerance towards SDS were subjected to morphological, biochemical, and molecular identification. Colony morphology with respect to shape, size, margin, elevation, and colour was observed under a light microscope (Olympus BX41).

Biochemical tests viz., Catalase, Oxidase, Citrate, Starch, Nitrate, Indole, Fluorescence and Voges-Proskaauser [13] were performed.

16S gene sequencing

DNA was extracted using GenElute™ Bacterial Genomic DNA kit (Sigma-Aldrich, USA). The ~ 1.5 kbp, 16S-rDNA fragment was amplified using high–fidelity PCR polymerase, and the primers used were 16S forward (GGATGAGCCCGCGGCCTA) and 16S reverse (CGG

TGTGTACAAGGCCCGG). The PCR amplicon was purified for the removal of contaminants and was also resolved on agarose gel. The PCR product was sequenced bi-directionally and the sequence data were aligned and analyzed to identify the bacteria and its closest neighbors using NCBI's (National Center for Biotechnology) Blast software. Based on the maximum identity score, the first ten sequences were selected and aligned by importing them to multiple alignment software programs Clustal W. The phylogenetic tree was constructed using the neighbor-joining algorithm, by the software MEGA 10 [33]. The stability within the phylogenetic tree clades was determined with a 1000-replication bootstrap analysis.

Results

Quantification of anionic surfactants

The results of quantification of anionic surfactants at four sites in the Kalindi Kunj, Yamuna riverfront show a significantly higher concentration during all times of analysis. Site-wise results from November have been summarized in Table S1 (supplementary material). During the analysis, it was observed that site L1, which was the area of most foaming, reported the maximum concentration of anionic surfactants in all sampling months, followed by L2, L3, and then L4, which was the area near the river bank. The highest and lowest concentration of anionic surfactant in the study area was reported to be 2.83 mgL^{-1} (at L1) and 0.29 mgL^{-1} (at L4) respectively. This investigation also showed that there was no significant month-wise difference in anionic surfactant concentrations in the study area. The reduction in concentrations from L1 to L4 could be due to the dilution of surfactants in the river water. The results show a clear relation between the area of most foaming (L1) with significantly high anionic surfactant concentration (with a mean concentration of 1.98 mgL^{-1} for the study duration), establishing that high surfactant concentration is one of the major reasons for foaming in the region (Fig. 2).

Quantification and isolation of bacteria

At the four sampling sites across the Okhla Barrage stretch of river Yamuna, the total bacterial population was found in the range of 1.3×10^2 -2.6×10^9 CFU mL^{-1}. A detailed site-wise bacterial population is given in Table 2. The maximum bacterial population was found near the banks (L4) and was found to decrease on moving from the banks towards the area of most foaming (L1). The observed bacterial population is also indicative of the anti-bacterial property of surfactants as some samples with high surfactant concentration showed less bacterial count and vice-versa. However, a stable and constant trend has not been observed in this regard which also indicates interference of other chemical and biological contaminants.

On the basis of colony morphology observed on NA during the enumeration process, 38 isolates with identical colonies were selected for further screening of SDS-tolerant strains.

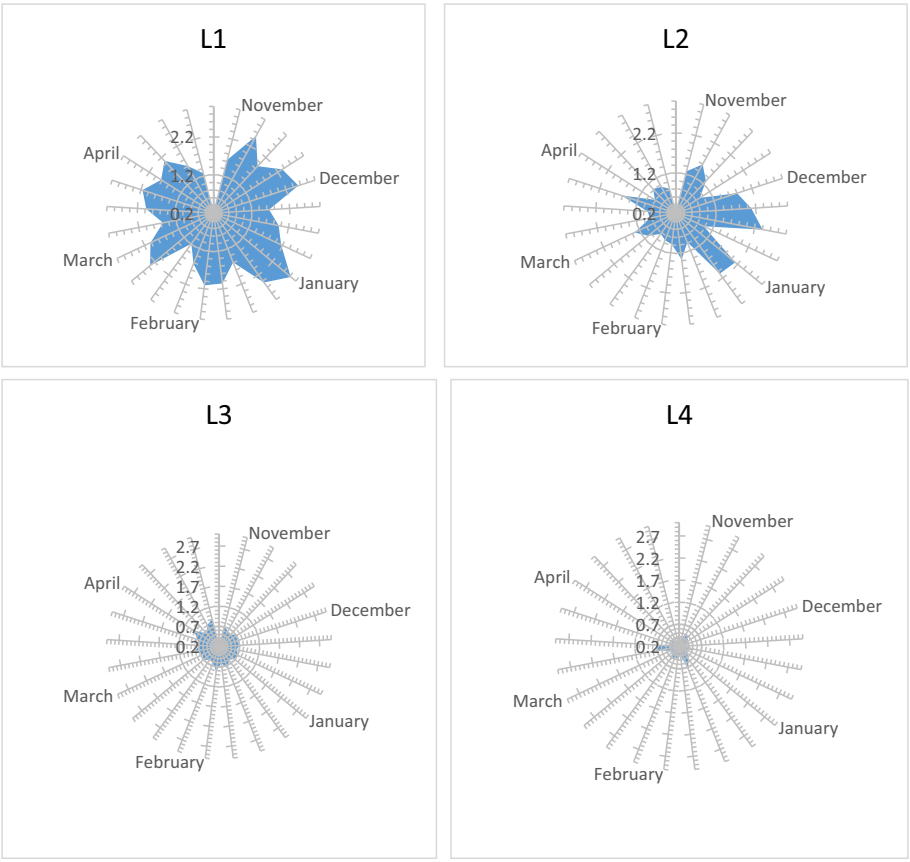

Fig. 2 Graphical representation of anionic surfactant concentration at sites L1, L2, L3, and L4 at Okhla Barrage region of Yamuna River during the sampling period starting from November 2021- April 2022

Screening of SDS-tolerant bacterial strains

All bacterial isolates were subjected to SDS tolerance at 12.5, 25, 50, 100, 200, 300, and 500 mgL^{-1} concentrations. On the basis of turbidity observed in the samples in 48–72 h, 26 isolates did not show any growth at any SDS concentration, even at increasing the incubation period to 96–120 h. For the remaining 12 samples, bacterial growth was observed in 2 isolates after 24 h and in 10 isolates after 96 h of incubation, respectively in 12.5, 25, and 50 mgL^{-1} SDS concentrations, one of which was also able to grow in 100 mgL^{-1} concentration. None of these 12 isolates show any turbidity in concentrations above 200 mgL^{-1}. Thus, the two isolates exhibiting growth in 24 h out of the total thirty-eight were found suitable for further studies as they were able to tolerate SDS and utilize it for their growth in the least incubation time.

Table 2 Quantification of bacteria at various sites in the Okhla Barrage region of Yamuna River

Sampling months	Total Bacterial Population ($CFUmL^{-1}$)							
	L1		L2		L3		L4	
November	1	3.4×10^3	1	4.1×10^4	1	7.3×10^6	1	8.0×10^6
	2	3.6×10^2	2	4.4×10^3	2	5.4×10^4	2	7.7×10^6
	3	6.4×10^3	3	4.1×10^4	3	3.2×10^5	3	8.1×10^8
	4	1.9×10^2	4	3.7×10^4	4	2.9×10^5	4	5.3×10^6
December	5	5.1×10^3	5	5.3×10^2	5	3.8×10^6	5	7.4×10^7
	6	7.1×10^4	6	5.9×10^3	6	7.3×10^4	6	3.8×10^7
	7	6.8×10^4	7	6.4×10^2	7	4.8×10^5	7	6.9×10^6
	8	5.3×10^3	8	3.8×10^4	8	4.4×10^4	8	5.6×10^8
January	9	1.3×10^2	9	4.7×10^3	9	3.8×10^6	9	7.6×10^7
	10	6.2×10^3	10	7.1×10^3	10	5.7×10^5	10	8.9×10^8
	11	3.2×10^2	11	4.9×10^4	11	6.1×10^4	11	3.1×10^8
	12	4.1×10^2	12	8.3×10^4	12	5.5×10^6	12	8.8×10^7
February	13	2.9×10^3	13	6.3×10^3	13	7.1×10^5	13	3.5×10^9
	14	8.4×10^2	14	5.7×10^4	14	6.5×10^6	14	4.6×10^8
	15	7.6×10^2	15	3.9×10^4	15	4.1×10^6	15	6.0×10^8
	16	5.1×10^3	16	7.3×10^3	16	8.1×10^5	16	7.1×10^8
March	17	4.7×10^3	17	6.4×10^4	17	6.6×10^5	17	5.2×10^7
	18	6.1×10^3	18	2.8×10^4	18	3.8×10^5	18	9.6×10^6
	19	8.1×10^3	19	4.6×10^4	19	9.6×10^5	19	4.4×10^8
	20	7.7×10^2	20	5.9×10^3	20	2.1×10^6	20	2.7×10^8
April	21	6.1×10^3	21	8.2×10^4	21	4.8×10^5	21	3.4×10^8
	22	4.9×10^2	22	3.1×10^4	22	8.4×10^5	22	4.0×10^8
	23	7.1×10^3	23	9.5×10^4	23	8.1×10^5	23	2.6×10^9
	24	5.6×10^3	24	3.8×10^4	24	1.8×10^6	24	7.1×10^8

Optimization of growth conditions

The most suitable temperature and pH for the growth of YRW-02 were found to be 4 °C, followed by 20 °C and 7 pH. A slight difference was observed between pH 5 and 8, with 8 pH exhibiting better growth conditions. YRW-02 showed the highest growth at 50 mgL^{-1} (OD = 0.4269), relatively lower growth at 100 (OD = 0.2966) and 200 mgL^{-1} (OD = 0.1371), followed by complete inhibition of growth at 300 and 500 mgL^{-1} (OD = 0.0093, 0.0051) concentration of SDS. No bacterial growth was seen at 37 °C. Thus, the best growth conditions for YRW-02 were observed at a temperature of 4 °C and 7 pH, at 500 mgL^{-1} substrate concentration with SDS as a sole carbon source. Figure 3. gives a graphical representation of different growth parameters and their results for the strain YRW-02.

As compared to YRW-02, the strain YRW-05 showed the highest growth at 100 mgL^{-1} at 37 °C (OD = 0.358) and at 20 °C (OD = 0.3213) at the same substrate concentration,

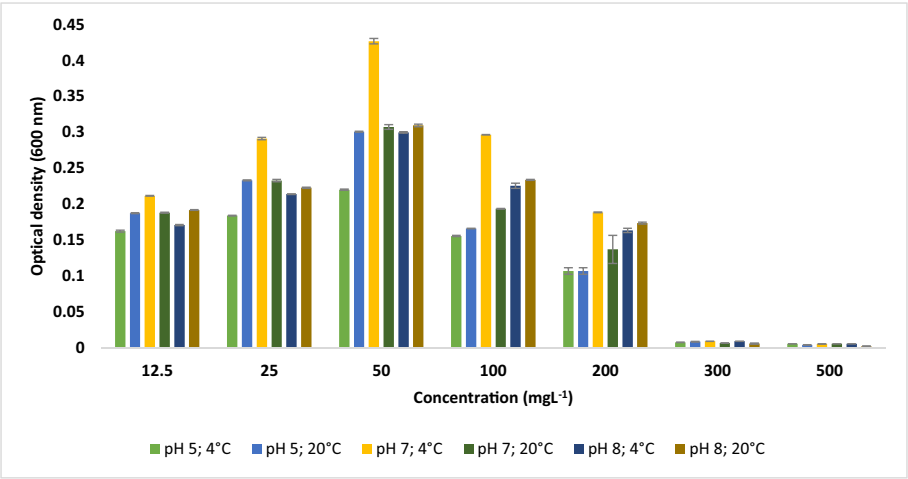

Fig. 3 Optimization of growth of YRW-02 with changing parameters, viz., concentration, temperature, and pH

followed by growth at 50 mgL^{-1}(OD = 0.311) at 37 °C, which further decreased from 200 mgL^{-1} (OD = 0.148) to negligible growth at 500 mgL^{-1} (OD = 0.0091), indicating the limit of tolerance for the strain. Contrary to YRW-02, YRW-05 did not show any growth at 4 °C, however, neutral pH was found to favour optimum growth in both strains. Hence, a neutral pH, 37 °C temperature, and 100 mgL^{-1} SDS concentration were found to be the optimum growth conditions for YRW-05. Figure 4. gives an illustration of changing growth parameters on the growth of strain YRW-05 in graphical form.

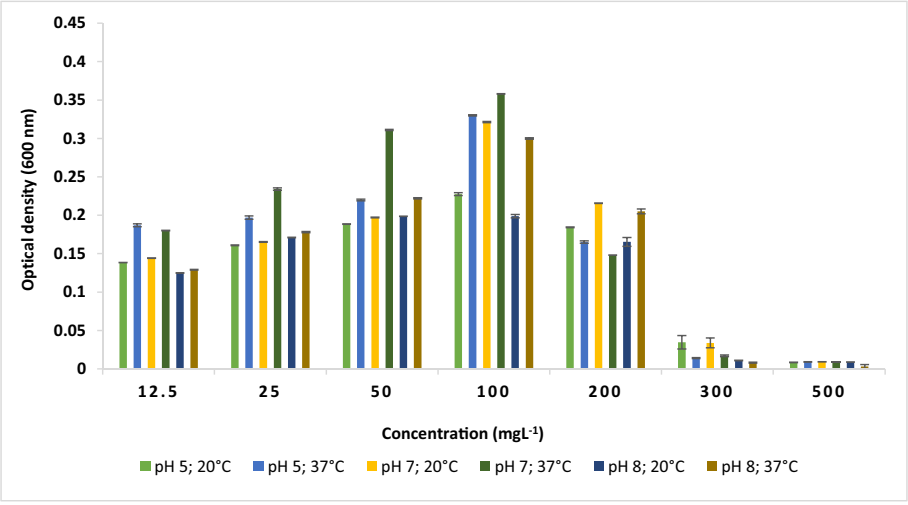

Fig. 4 Optimization of growth of YRW-05 with changing parameters, viz., concentration, temperature, and pH

Biodegradation studies

The MBAS assay was used for the calculation of the concentration of SDS in the flasks under observation. As per the observations made during the growth optimization experiments, flasks with YRW-02 strain as inoculum were incubated at 4 °C, and flasks with YRW-05 strain as inoculum were incubated at 37 °C. Both sets of flasks at their respective incubation temperatures showed OD values in decreasing order, starting from 0 h, suggesting the degradation of SDS by the bacterial strains. For YRW-02, OD values continued to decrease till 96 h and did not show any further reduction in SDS concentration. Whereas the strain YRW-05 showed a decreasing trend in SDS concentration till 120 h and stabilized thereafter. YRW-02 and YRW-05 degraded 50 mgL^{-1} of SDS to 10.124 and 15.157 mgL^{-1} respectively. Figure 4 gives a combined graphical representation of SDS degradation with the two strains. Table S2 (supplementary material) and Fig. 5 shows an hour-wise change in the concentration and percentage degradation of SDS with YRW-02 and YRW-05. Thus, a maximum degradation of 78.29 and 69.24% was achieved with these two strains.

Morphological and biochemical characterization of potential SDS-degrading bacterial strains

On microscopic analysis, both YRW-02 and YRW-05 were found to be Gram's negative strains. Both were motile with one or more flagellum, rod-shaped, and non-spore-forming. Colony characteristics showed YRW-02 to possess yellow, circular, and convex colonies with smooth margins, while YRW-05 had radial, yellow-orange flat colonies with wrinkled margins.

Biochemical tests revealed that YRW-02 showed fluorescence on King's B medium, was negative for catalase, starch, and nitrate but positive for oxidase and citrate test. YRW-05 was found to be negative for Indole and positive for Voges-Proskauer, nitrate,

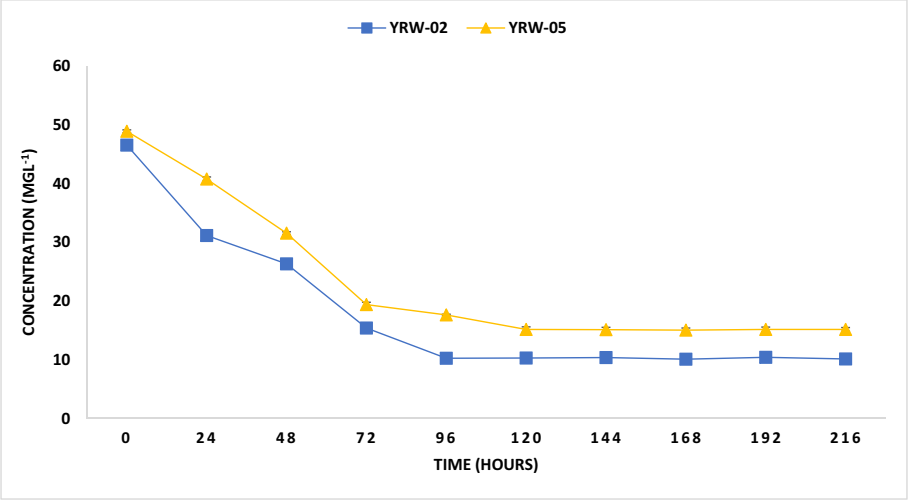

Fig. 5 Graphical representation of degradation of SDS using YRW-02 and YRW-05 with respect to time

and starch tests. Table 3. describes all the microscopic, morphological, and biochemical tests performed on the strains YRW-02 and YRW-05.

16S rRNA sequencing

A total of 188 and 172 ng of DNA was extracted from YRW-02 and YRW-05 strains respectively. On performing 16S rRNA sequencing, YRW-02 was found to bear 99.74% similarity with *Pseudomonas koreensis* strain Ps 9–14 and YRW-05 was found to be 98.96% similar to *Pseudomonas songnenensis* strain NEAU-ST5-5. The 16S rRNA phylogenetic tree confirmed that YRW-02 and YRW-05 corresponded to *Pseudomonas koreensis* (Fig. 6) and *Pseudomonas songnenensis* (Fig. 7) respectively.

Discussion

Apart from providing the basic necessities of life, rivers in India have shaped history, culture, and faith and are regarded as mythological entities. However, the condition of a few Indian rivers is deteriorating due to increasing load of pollution. The presence of toxic substances viz. pharmaceutical products, household chemicals, agrochemicals, microplastics are emerging pollutants in wastewater [35, 37]. The generation of immense sewage discharge load on the treatment plants leads to inefficient water treatment and it has been a major reason for the ever-increasing pollution of rivers and other water bodies

Table 3 Microscopic, morphological, and biochemical characterization of SDS-degrading isolates

Cellular characteristics		
	YRW-02	YRW-05
Size	0.65 µm in diameter and 2.8 µm long	0.58 µm in diameter and 1.8 µm long
Shape	Rod-shaped	Rod-shaped
Motility	Motile by more than one polar flagellum	Motile by several polar flagella
Spore-forming	Non-spore forming	Non-spore forming
Gram's staining	Negative	Negative
Colony morphology		
Shape	Circular	Radial
Colour	Yellow	Yellow-orange
Elevation	Convex	Flat
Margin	Smooth	Wrinkled
Biochemical tests		
Fluorescence	Positive (on King's B medium)	-
Catalase	Negative	Positive
Oxidase	Positive	Positive
Citrate	Positive	Positive
Starch	Negative	Positive
Nitrate	Negative	Positive
Indole	Negative	Negative
Voges-Proskauser	Negative	Positive

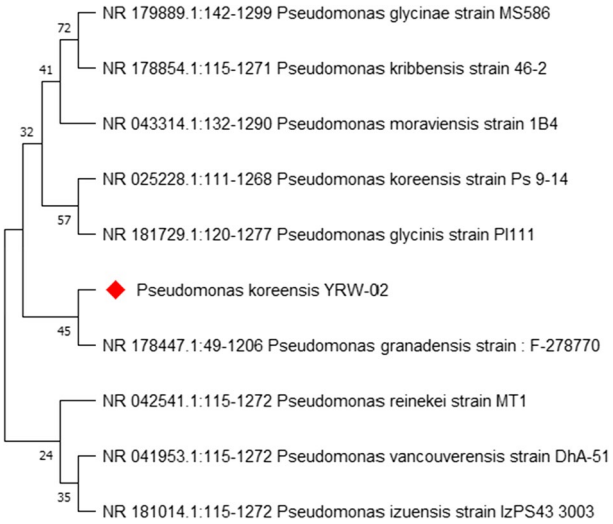

Fig. 6 Phylogenetic tree of the selected isolate YRW-02 with its nearest neighbours

[59]. Surfactants are one of the major groups of contaminants which exist in almost all the urban, rural, and industrial wastewater [16]. The adverse effects of surfactants on different environmental components have been well-studied and thus, surfactants have been rightly classified as 'contaminants of emerging concern' [4]. Surfactants not only reduce the quality of water but are also responsible for the excessive foaming of the water bodies. Among all types of surfactants, anionic surfactants are most widely used in household detergents and personal care products, and industries [16].

Limited research has been done in terms of the quantification of anionic surfactants in water bodies. Patel and Patel, [47] aimed at determining the levels of anionic surfactants in municipal wastewater wherein the concentration of sodium lauryl sulphate was found in the range of 1.0—42.2 mgL^{-1}. A study conducted on Hugli river, Kolkata, India, reveals

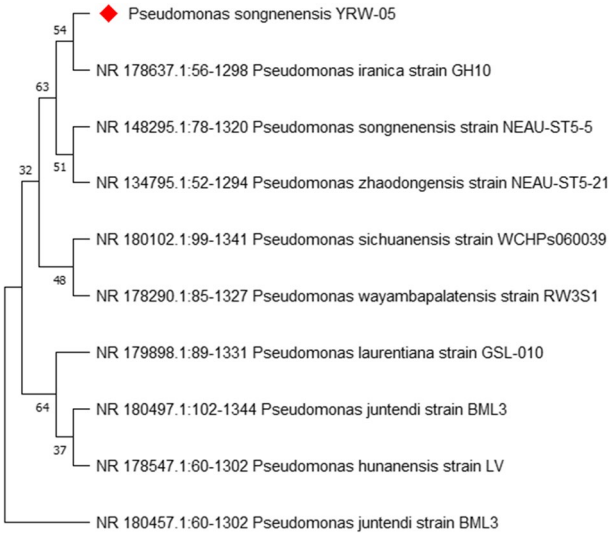

Fig. 7 Phylogenetic tree of the selected isolate YRW-05 with its nearest neighbours

the presence of anionic surfactants in surface and groundwater samples, where a concentration ranging between 0.013–0.425 mgL^{-1} was reported [19]. Assessment of surfactants in the Ganga Canal was carried out by a group of researchers wherein a maximum of 2.5 mgL^{-1} of anionic surfactants were reported [53]. Another study on the assessment of water systems in and around Tirupati, India, showed the presence of anionic surfactants in the range of 0.01–0.092 mgL^{-1} [49]. Water samples from 10 different locations were procured to assess the concentration of surfactants in the flowing water of Gomti river, India. The results obtained were suggestive of significantly higher concentrations of surfactants in the river ranging between 0.1005 and 0.2758 mgL^{-1} [36]. Considering the alarming effects, the present study was conducted on a small stretch of the Yamuna river, which is the region reported to exhibit most foaming throughout the year, was investigated for anionic surfactant concentrations. Over a span of six months, our results show anionic surfactants concentration in the range of 0.29–2.83 mgL^{-1}. These results suggest that high concentrations of surfactants in the region are one of the major reasons for the foaming of the river in this region, which can be due to untreated discharge of effluents from the industries and households.

Several studies have reported the isolation of surfactant-degrading bacteria from surfactant-contaminated wastewater and water bodies. River-water sample from New Calabar river, Nigeria were evaluated for the presence of detergent-tolerating bacteria. The study concluded that microbial isolates of *Vibrio, Flavobacterium, Klebsiella, Pseudomonas, Enterobacter, Bacillus, Escherichia, Shigella, Citrobacter, Proteus, Anabaena* and an actinomycete was readily utilizing liquid detergents and shampoos present in the river water [44]. In a study undertaken in Turkey, water samples from a detergent-polluted river were screened for SDS-degrading bacteria where a *Delftia acidovorans* was found to optimally grow at 1 gL^{-1} of SDS as a sole carbon source and degraded 87% of the substrate in 11 days of incubation [64]. In a study where SDS-degrading bacteria was isolated from a detergent-contaminated pond, *Pseudomonas putida* was found to be a potential strain as it showed complete degradation of 0.3% SDS in 16 h of incubation [12]. Multiple studies have reported a variety of SDS-degrading bacteria comprising many strains of *Pseudomonas* sp. with different variables and degradation efficiencies [12, 54, 63]. In this study, we observed that the YRW-02 strain showed the highest growth at a concentration of 50 mgL^{-1} and pH 7. Increasing the concentration from 300–500 mgL^{-1} was seen to be unfavourable for the growth of strains.

Extremophilic microbes are a great reservoir of enzymes and thus their role in bioremediation has been significant. Psychrophilic and psychrotolerant bacteria have also been extensively studied for their roles in the degradation of various contaminants, signifying their biotechnological importance. Studies of degradation of chlorpyrifos by psychrophilic *Shewanella* sp. [20], hydrocarbons by psychrophilic *Oleispira Antarctica* [18], pyrene by *Shewanella* sp. [50], malathion by psychrotolerant *Ochrobactrum* sp. [62], naphthalene and chrysene by psychrotolerant *Bacillus* sp. [32] have been reported in the past. In the current study, a psychrophilic *Pseudomonas koreensis* and a psychrotolerant *P. songnenensis*, isolated from the surfactant-contaminated stretch of Yamuna River, have been found to utilize SDS as a sole carbon source. Other psychrophilic and psychrotolerant species from the *Pseudomonas* genus have also been utilised for the degradation of various contaminants. In this study, we observed that YRW-02 and YRW-05 showed maximum degradation of 78.29 and 69.24% respectively. 16S RNA gene sequencing revealed the two species to be *P. koreensis* and *P. songnenensis*. Comparing the results obtained during this study with previous literature from the recent past, it was observed that *Pseudomonas* sp. has been associated with SDS degrading potentiality. For example, *P. frederiksbergensis* have been reported for

the degradation of long-chain alkanes [1], *P. putida*, *P. synxantha*, and *P. azotoformans* for the degradation of p-xylene [42], another *Pseudomonas* sp. for degrading phenol [52], *P. palleroniana* for the degradation of bisphenol [60], and *P. putida* for the degradation of nitrobenzene [38]. A study conducted in Antarctica identified an SDS-degrading psychrophilic *Pseudomonas* sp. that utilized 90% of SDS in 8 days of incubation. This strain optimally grew at 10 °C and pH of 7.25 [21]. These results were in agreement with the results obtained in the current study wherein cold-adapted *Pseudomonas* sp. was found to degrade SDS.

Conclusion

The quantification of anionic surfactants in the Okhla Barrage region of the Yamuna river, Delhi was carried out in this study. This stretch of the river has been reported for maximum foaming in the past few years, thus our study establishes the presence of anionic surfactants as one of the major reasons for foaming in the water. Anionic surfactants in the range of 0.29–2.83 mgL^{-1} were found in the river water samples. The same water samples were used for the isolation and identification of native SDS-degrading bacteria that can be utilised for the bioremediation of surfactant pollution in the river. Two potential strains, *P. koreensis* YRW-02 and *P. songnenensis* YRW-05, with SDS-degrading capabilities, were successfully isolated in the study. Psychrophilic *P. koreensis* YRW-02 was found to degrade 78.29% of SDS in 96 h at 4 °C while, psychrotolerant *P. songnenensis* YRW-05 degraded 69.24% SDS in 120 h of incubation at a temperature of 37 °C. Several studies have reported surfactants to cause blood toxicity, eyes, and skin irritation and thus their determination in river water as raw water supply and drinking water are important since surfactants have become a threat to our water supply network. Previously, both *P. koreensis* and *P. songnenensis* have been utilised for bioremediation purposes, however, their applicability in the degradation of surfactants has not been much explored. The variation in the mechanism of degradation by both species can be further studied with respect to varying environmental parameters such as temperature, pH, etc. These initial results suggest the possibility of *P. koreensis* and *P. songnenensis* being further used in environmental remediation studies.

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Data Availability All the data pertaining to this manuscript has been included in the manuscript.

Declarations

Ethical approval This article does not contain any studies with human participants or animals.

Consent to Participate All authors agree mutually with the participation and publication of this work and declare that this is original research.

Consent to publish All authors agree mutually to publication of this work.

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