

From functional groups to microbial communities: A portrait of adsorption hexavalent chromium by natural biofilm in the Manyar River, Gresik

Ion Tarsardo Sianturi¹, Agoes Soegianto², Lucia Tri Suwanti^{3,4*}

¹ Doctoral Program of Veterinary Science, Faculty of Veterinary Medicine, Airlangga University, Indonesia

² Department of Biology, Faculty of Science and Technology, Airlangga University, Surabaya, Indonesia

³ Department of Veterinary Science, Faculty of Veterinary Medicine, Airlangga University, Indonesia

⁴ One Health Research Group, Faculty of Veterinary Medicine, Airlangga University, Surabaya, Indonesia

* Corresponding author's e-mail: luca-t-s@fkh.unair.ac.id

ABSTRACT

Microbial biofilms play a crucial role in the adsorption and bioaccumulation of heavy metals in polluted aquatic environments, including toxic and persistent hexavalent chromium [Cr(VI)]. However, the information on the structural characteristics and accumulation capacity of natural biofilms in industrial river ecosystems, particularly in Indonesia, remains limited. This study aimed to investigate the structural characteristics, microbial community composition, and Cr(VI) adsorption capacity of natural biofilms in the Manyar River, Gresik. Biofilm morphology and structure were analyzed using scanning electron microscopy (SEM), functional groups were identified using Fourier transform infrared spectroscopy (FTIR), and microbial communities were characterized through 16S rRNA metagenomic analysis. The results showed that biofilm development follows three main stages: adhesion, maturation, and dispersion. FTIR analysis indicated the presence of functional groups (C–X, C–O, C=C, and O–H) involved in Cr(VI) binding. The microbial community was dominated by *Virgibacillus* spp., *Romboutsia* spp., and *Streptococcus* spp., reflecting adaptation to polluted conditions. The distribution coefficient (K_d) ranged from 0.99–1.05, while the bioaccumulation factor (BAF) reached 99.44–106.81, indicating strong accumulation capacity. These findings highlight the potential of natural biofilms as effective agents for Cr(VI) bioremediation and biomonitoring in industrial river ecosystems.

Keywords: adsorption, biofilms, chromium, functional groups, environment.

INTRODUCTION

Environmental pollution is one of the global issues that continues to be of concern to various parties. Growing industrial activities, especially in urban and coastal areas, have a major impact on aquatic ecosystems. Industrial waste contains a variety of harmful chemicals, including heavy metals, which are difficult to decompose naturally. The accumulation of heavy metals in the waters can cause ecosystem disturbances and endanger aquatic life (Aziz et al., 2023).

Heavy metals have high toxicity even at low concentrations. Among these metals, chromium, especially in hexavalent form, is known to be very dangerous, because it is carcinogenic, mutagenic,

and capable of causing organ damage in living things. The main source of hexavalent chromium generally comes from the waste of the metal coating, textile, dye, and leather industries. If not handled properly, chromium can accumulate in the environment and threaten the sustainability of aquatic ecosystems (Sharma et al., 2022).

The efforts to tackle heavy metal pollution have been widely made, ranging from physico-chemical methods to more environmentally friendly biological approaches. One of the biological approaches that is receiving more and more attention is the use of biofilm as a biosorption agent or natural bioremediation. Biofilms have a natural ability to absorb heavy metals due to their complex composition and surface structure,

making them potential agents in pollution mitigation (Mishra et al., 2022).

Biologically, biofilm is defined as a community of microorganisms that are attached to a surface (substrate) and surrounded by a matrix of extracellular polymeric substances (EPS). This matrix consists of polysaccharides, proteins, lipids, and nucleic acids that function to protect cells from external environmental stresses. In aquatic systems, biofilms can be found attached to the surface of rocks, wood, plant roots, and sediments. The complex structure and diversity of microorganisms within them make biofilm a highly dynamic microecological system (Yu and Lu, 2025).

The process of biofilm formation takes place through several stages: the initial attachment of planktonic cells to the surface, the formation of an organized layer of microorganisms, then the colony growth and intensive production of EPS. Over time, the biofilm layer thickens and forms a hollow structure that allows for the exchange of gases and nutrients. When the density reaches its maximum, some colonies will detach and move to a new substrate, so that the process of forming new biofilms continues in different locations (Peng et al., 2023).

In the context of river ecosystems, the existence of biofilms has an important ecological role. Biofilm serves as a habitat for various microorganisms that play a role in the decomposition of organic matter and nutrient cycling. In addition, biofilm also acts as a natural bioindicator that reflects water quality conditions. In other words, changes in the biochemical structure or composition of biofilms can indicate pollution or changes in aquatic environmental characteristics (Guerrieri et al., 2022). One of the factors that makes biofilms interesting to study is the ability of their extracellular matrix to interact with heavy metal ions. Chemical components in EPS, such as active groups, have the ability to bind metal ions through adsorption, ion exchange, or complexation mechanisms (Pagliaccia et al., 2022).

The Manyar River is located in an area that is densely populated with industrial activities, especially around the coastal area of Gresik. The industrial waste that enters the river carries a variety of harmful compounds, including heavy metals. This condition makes the Manyar River a representative location to research the natural ability of biofilms in dealing with pollution pressures. The rocks scattered on the riverbed serve as an ideal substrate for the growth of natural biofilms,

as well as an important point in environmental quality monitoring (Guerrieri et al., 2022).

However, the research on the potential of natural biofilms in Indonesian river ecosystems conducted so far is still relatively limited. Most biofilm studies focus more on artificial systems in the laboratory or in a specific industrial environment. In fact, natural biofilms formed in open waters have different biological and chemical complexities than under artificial conditions. Therefore, it is important to study the characteristics of natural biofilms in real environments, such as in the Manyar River, so that their use can be more optimal for environmental purposes (Jing et al., 2023).

In addition to its capacity to absorb heavy metals, biofilm also plays an important role as a biological indicator of environmental conditions. The efficiency of biofilms in interacting with heavy metals reflects the extent to which ecosystems are subjected to pollution stress. Through biofilm-based biomonitoring, changes in the concentration and distribution of heavy metals in aquatic ecosystems can be detected naturally and sustainably without always having to resort to expensive or invasive analytical instruments (Laderriere et al., 2021).

The use of biofilm in pollution monitoring and mitigation is in line with the principles of environmentally friendly technology (green technology). The approaches based on natural biological processes are more sustainable and economical than conventional methods. In addition, biofilm does not have an additional negative impact on the environment, so it has the potential to be developed as a marine biotechnology solution in the future. The combination of natural adsorption ability and its abundant presence makes biofilm an efficient and easy-to-apply ecological agent (Desiante et al., 2021).

To achieve optimal utilization, a deep understanding of the factors that affect biofilm efficiency is needed. Factors such as substrate type, physicochemical conditions of water (pH, temperature, salinity, DO), and composition of microorganisms that make up biofilms greatly affect the adsorption ability of heavy metals. Through a systematic study of biofilm characteristics and their environment, basic data can be obtained to develop more effective and specific aquatic bioremediation strategies for local conditions (Li et al., 2024).

Despite extensive studies on biofilm-mediated heavy metal removal, most investigations have focused on laboratory-scale or engineered

systems. There is a lack of field-based evidence describing the structural characteristics, microbial composition, and accumulation capacity of natural biofilms under real industrial pollution gradients, particularly in tropical river systems, such as those in Indonesia.

Therefore, this study aimed to (i) characterize the structural and morphological properties of natural biofilms, (ii) analyze their microbial community composition, and (iii) quantify their capacity to adsorb and accumulate Cr(VI) in the Manyar River ecosystem. This study hypothesized that the natural biofilms in industrially impacted rivers possess high metal-binding capacity driven by EPS functional groups and adapted microbial communities.

MATERIALS AND METHODS

Sampling area

The sampling location is on the Manyar River, Gresik Regency, East Java Province, which is located in the northern coastal area of Java and is known as an area with dense industrial activities (7° 6' 36.73" S; 112° 36' 21.8" E). This river receives various industrial wastes from the surrounding factories so the condition of its waters represents the anthropogenic pressure that leads to a polluted state. Three sampling stations were designated to illustrate the water quality gradient along the river flow, where the first station was located in an area that is within the direct reach of the industrial sewage inlet so that the influence of the discharge on the physico-chemical parameters of the water can be observed more realistically, the second station was in the middle of the river as a transition zone representing the natural process of dispersion and dilution of sewage compounds, while the third station was located near the estuary, which is the area where the river meets the sea (Figure 1).

Sampling was conducted during a single campaign in the dry season. At each station, samples were collected in duplicate ($n = 2$) to ensure representativeness. The study employed a spatial gradient design without temporal replication.

Aquatic quality measurement

The water quality parameters were measured in situ at each observation station in conjunction

with sampling, to ensure that the data obtained reflected the actual physical-chemical conditions of the waters. The main parameters observed were temperature, pH, dissolved oxygen (DO), depth, turbidity and current velocity to represent environmental factors that play an important role in regulating the solubility, speciation, and distribution of heavy metals, especially Cr(VI), in the water column. DO and temperature measurements were carried out simultaneously using a PDO-520 type DO meter equipped with a thermometer sensor. The pH value was measured with a Krisbow brand pH meter to determine the acidity condition of the water which also determines the chemical shape of the metal. Meanwhile, current velocity measurements using the Flowatch tool provided precise information about variations in water movement at observation points, and depth measurements with iron rulers help determine the vertical profile of the water column. All instruments were calibrated prior to measurement following manufacturer guidelines. The DO meter was calibrated using air-saturation method, and the pH meter was calibrated using standard buffer solutions (pH 4, 7, and 10).

Sample collection

Water sample

Water sampling was carried out by opening sterile bottles at the sampling location and then slowly filling them directly from the water body to avoid contamination by leaves or riverbed sediment. This activity was carried out at three observation stations (1, 2, and 3), each with one sampling session repeated twice to ensure data accuracy and representativeness. Once the bottles were full, the samples were immediately sealed, labeled according to the station code, and then stored in a refrigerated container at a temperature of approximately 4 °C to maintain the stability of physical and chemical parameters before laboratory analysis (Marino et al., 2023).

Sediment sample

Sediment sampling was carried out using a 1-inch diameter paralon pipe with the principle of pressure difference. The pipe was inserted slowly into the bottom until the sediment was sucked in, then the two ends were immediately closed to prevent material loss. The collected samples were then transferred to a labeled plastic bag according

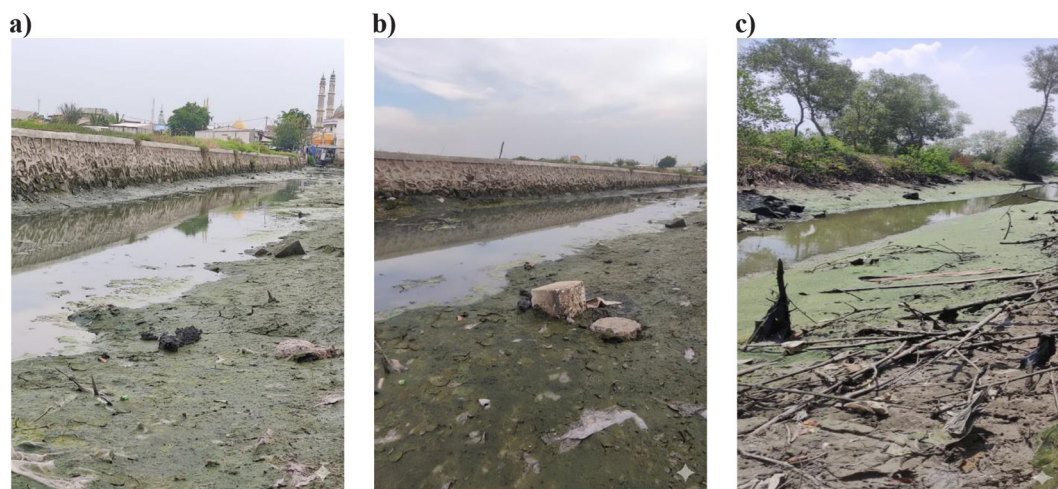


Figure 1. Research location (a) station 1; (b) station 2; and (c) station 3

to the collection location. To maintain the stability of the physical and chemical properties of the sediments, all samples were stored in a cool box at a temperature of about 4 °C until the analysis process was carried out (Razeghi et al., 2021).

Biofilm sample

Biofilms were obtained from the surface of rocks in rivers. Before retrieving, the stones were inspected to make sure there were no large organisms or sediments attached. Extraction was carried out by brushing the surface of the stone, then the brush was dipped and shaken in 80 mL of distilled water so that the biofilm was detached and mixed into the water. Any remaining dirt or small organisms that were still attached were cleaned manually using tweezers. Afterwards, the biofilm solution was stored in a sealed container at a temperature of about -4 °C and taken to the laboratory for further cleaning and analysis (Kurniawan and Fukuda, 2023).

Cr(VI) measurement using AAS

Water sample

The process of analyzing the content of Cr(VI) in river water began with a sample of 100 ml, which was then filtered using Whatman grade 40 fine filter paper to ensure that the filtrate was free of residual coarse solid particles, resulting in a clear solution that was ready for further testing, where the filtrate that has been placed in the reaction flask was then directly analyzed using an AAS. The next step was to light the fire first by pressing the PURGE and IGNITE (green

and blue colors) buttons on the tool together then click Start. Wait for the reading to finish and save the results (Rusiniak et al., 2024).

Sediment sample

A 1 g sediment sample was analytically weighed into an Erlenmeyer flask, then 10 mL of concentrated HNO₃ and 10 mL of concentrated H₂SO₄ were added. The mixture was then heated on a hotplate at a controlled temperature until the solution turns a pale yellow color. After the achievement of these conditions, the solution was cooled at room temperature for 24 hours. The solution was then transferred to a 100 mL volumetric measuring flask, diluted to a mark with distilled water (aquades), and filtered using Whatman Grade 40 filter paper to remove any insoluble solid residues. The obtained filtrate was then analyzed for Cr(VI) levels using an AAS at characteristic wavelengths (Perez et al., 2024).

Biofilm sample

A 1-gram biofilm sample was precisely weighed into an Erlenmeyer flask, then 10 mL of nitric acid (HNO₃) and 10 mL of sulfuric acid (H₂SO₄) were added for the decomposition of the organic matrix, followed by heating on the stirrer hotplate until a completely clear solution, which was then cooled for 24 hours at room temperature. The solution was transferred to a 100 mL volumetric measuring flask, diluted to a mark with aquades, and filtered using Whatman Grade 40 filter paper to remove solid residues, before the filtrate was finally analyzed with an atomic AAS at the characteristic wavelength of Cr(VI)

to accurately and sensitively determine the concentration of Cr(VI) accumulated in the biofilm (Nguyen et al., 2024). The detection limit of the AAS method for Cr(VI) was approximately 0.01 mg/L, with analytical precision maintained through triplicate readings.

SEM (scanning electron microscope) test

The SEM sample preparation and testing procedure began with sample preparation using carbon tape-coated metal SEM holders, where the powder sample was sprinkled and affixed with a flat surface and a maximum thickness of 1 cm, then fed into the sputter coater by turning on the START PUMP, minimizing LEAK and VENT (counterclockwise), vacuumed to 4×10^{-2} mbar, then coating was done at a current of 18 mA with SET PLASMA and rotated the LEAK clockwise until purple plasma appeared, the time needed was 15–180 seconds, after finishing turn off the pump, vent, and store. SEM testing involved turning the appliance on with Power ON SEM, turning the computer on, opening the XT Microscope software, logging in, then Stage Position OK until complete, filling the logbook in, entering up to 7 prepared samples, vacuuming with High Vacuum for mixed samples or Low Vacuum for non-metallic non-coating/slow solid samples until the yellow indicator turned green, BEAM ON, and cell morphology, density, as well as the three-dimensional structure of the biofilm matrix on the surface of the coupon were ready for observation (Relucenti et al., 2021).

FT-IR analysis

Preparation equipment such as mortar and marble pestle, sample holders and presses, and spatulas with acetone were prepared. The FTIR tool was turned on, supporting equipment was installed according to the type of sample, and background testing was carried out using powdered KBr for solid samples. The solid sample was mixed with a 1:10 KBr ratio, mashed, inserted into the holder, and pressed until solid. After preparation, the sample was entered into the tool, input the sample code and username in the software, recorded in the log book (sample name, sample code, date, and description), then tested. The results were processed by peak table manipulation to extract peak wavelengths (500–4000 cm^{-1}), stored as raw soft data files, txt, and pdf. The

test samples were discarded as they could not be reused, while the equipment was cleaned with acetone (Diaz-Jimenez et al., 2023).

Biofilm metagenomics

The metagenomic process at Genetika Science Indonesia was carried out to identify and analyze the communities of microorganisms contained in biofilms. The first stage included the extraction of total DNA from biofilm samples. The obtained DNA was then amplified using a 16S V3–V4 primer to obtain gene segments that are informative in bacterial identification. The next step was the creation of a gene library, which allows DNA to be read by a sequencing platform. This process was followed by sequencing using Illumina MiSeq technology to produce high-resolution double-sequence data. The raw data from the sequencing results were then processed using Cutadapt, then analyzed with DADA2 software to perform denoising, so that the original sequence data was obtained. Taxonomic identification was performed by matching ASV against the SILVA v138.1 database, which was a comprehensive reference for microbial rRNA. Further analysis and data visualization were carried out using R version 4.2.3 and RStudio (Tan et al., 2025). Sequencing generated approximately reads per sample. Quality filtering was performed using a minimum Phred score of 30. Alpha diversity indices, including Shannon and Simpson indices, were calculated to evaluate microbial diversity within biofilms.

Biofilm accumulation factor

According to Gómez-Regalado et al. (2023), biofilm accumulation factor (BAF) is a measure of bioaccumulation that describes the ratio between the concentration of a chemical in an organism and the concentration of that substance in its environmental medium (usually water). This factor covers the entire possible path of exposure.

$$BAF = \frac{CB}{CW} \quad (1)$$

where: CB – concentration of substances in biofilm (mg/kg), CW – concentration of substance in water medium (mg/L), if the BAF value is > 1 , it means that the concentration of substances in the biofilm is greater than in water.

Biota-sediment accumulation factor

According to Krivokapi (2021), biota-sediment accumulation factor (BSAF) is a comparison between the levels of contaminants in biota (e.g. biofilms) and the levels of contaminants in sediments.

$$BSAF = \frac{CB}{CS} \quad (2)$$

where: *CB* – concentration of substances in biota (biofilm) (mg/kg), *CS* – concentration of substance in sedimentary media (mg/kg), if *BSAF* > 10 high bioaccumulation and important vector contamination.

Distribution coefficient

According to Bugai et al. (2020), distribution coefficient (*Kd*) in the environment is generally the coefficient of adsorption distribution between the sediment phase and the water phase in equilibrium.

$$Kd = \frac{CS}{CW} \quad (3)$$

where: *CS* – concentration of substance in sedimentary media (mg/kg), *CW* – concentration of substance in water medium (mg/L), If *Kd* > 1 concentration in sediment is greater than in water, if *Kd* = 1 the concentration is balanced between the sediment and the water.

RESULTS AND DISCUSSION

Results of aquatic water quality measurement

The results of water quality measurements showed that the three stations had relatively uniform physical-chemical conditions of the waters (Table 1), with temperatures ranging from 27.8–28.2 °C, depths of 29–32 cm, and low current velocities between 0.057–0.073 m/s which indicated calm waters. The pH values at all stations were in the neutral–slightly alkaline range (7.61–7.65) so

they still supported aquatic organisms, while the relatively similar DO (1.51–1.54 ppm) showed low dissolved oxygen levels and had the potential to limit biota diversity. The observed DO levels (1.51–1.54 ppm) indicate hypoxic conditions, which are typically associated with high organic loading and microbial respiration. Such conditions are commonly found in industrially impacted rivers where organic waste stimulates oxygen consumption, thereby limiting aerobic aquatic organisms. Meanwhile, the turbidity value of 39.82 mg/L indicates that the waters are turbid.

Biofilm growth

The formation of biofilms observed with the scanning electron microscope (SEM) generally follows the initial stages of cell attachment, the formation of mature three-dimensional structures, and then the release (dispersion) of cells to colonize of new surfaces. At the initial adhesion stage (Figure 2a), planktonic cells that are free in the medium begin to attach reversibly to the substrate surface through weak physicochemical forces such as van der Waals force, hydrophobic, and electrostatic interactions, then proceed to irreversible adhesion when the cell expresses the adhesion structure and begins to produce a matrix of extracellular polymeric substances (EPS) (Cavitt and Pathak, 2021). The SEM image in this phase usually shows the attached cells proliferating and forming increasingly dense microcolonies, accompanied by increased EPS production, so that the cells appear as if they are embedded in an amorphous matrix that covers the surface of the SEM image. The cell clots that occur are still small in size and cavities are still rarely formed (Kher et al., 2023).

In the growth and maturation phase (Figure 2b), the biofilm structure develops into a complex three-dimensional architecture with towers, cell clumps, and water channels that appear as empty spaces between the biofilm masses in SEM observations. These cavities and channels serve to facilitate the diffusion of nutrients,

Table 1. Water quality parameters in Manyar River, Gresik

Station	Temperature (°C)	Depth (cm)	Current speed (m/s)	pH	DO (ppm)	Turbidity (mg/L)
1	28.2	29	0.066	7.61	1.51	39.82
2	27.9	32	0.057	7.65	1.54	
3	27.8	31	0.073	7.62	1.52	

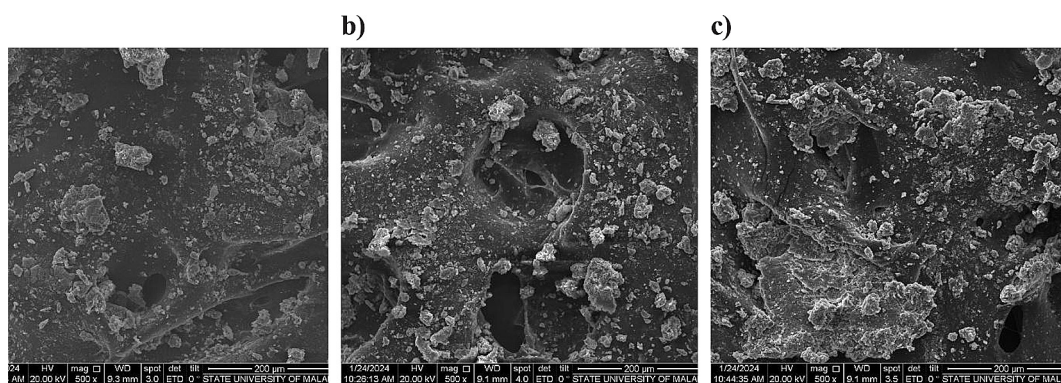


Figure 2. SEM images of biofilm development stages at different magnifications (a) adhesion phase, (b) maturation phase, and (c) dispersion phase

oxygen, and metabolite removal, thereby supporting heterogeneous growth and the formation of physiological gradients within biofilms, where the surface parts are more actively dividing while the deeper layers tend to be in dormant conditions (Quan et al., 2022).

The final stage of biofilm formation is release or dispersion (Figure 2c), when a portion of the cell population leaves the bound community to colonize a new substrate. This dispersion can be triggered by space and nutrient limitations on biofilm-saturated surfaces, changes in environmental conditions, or certain molecular signals that induce local degradation of the EPS matrix (Wang et al., 2023).

In SEM observations, the release phase can be indicated by an increasingly large aggregate clump that will break away from the consortium as well as a reduction in the thickness of the biofilm layer in a given area (Peng et al., 2023). The detached cells will return to the planktonic phase and seek a new substrate to start the next biofilm formation cycle, so that the adhesion–maturation–release dynamics seen from the SEM image series (Figure 2a–c) reflect the repeated life cycle of the biofilm and is the basis for the ability of biofilms to survive and spread in various environments (Fauzia et al., 2024).

FTIR spectra

On the basis of the FTIR spectrum analysis, the biofilm before treatment showed the presence of various functional groups such as C–H, C–C, O–H, and N–H which indicated the presence of hydrocarbon compounds, alcohols, and amines. After being treated with Cr(VI), new absorption bands appeared in several wavenumbers

indicating the presence of C–X, C–O, and C=C groups, as well as amplification in the O–H group (Figure 3). The changes and appearance of these bands suggest that the Cr(VI) ions interact with biofilm components, such as polysaccharides, proteins, and lipids, thereby altering the chemical structure of the surface and forming metal complexes in the biofilm.

The C–X functional group has a very important role in the process of adsorption of heavy metals in biofilms, because it can strengthen the interaction between the surface of the biofilm and metal ions through two main mechanisms, namely physical and chemical adsorption. Physical adsorption occurs due to weak van der Waals forces, while chemical adsorption involves the formation of coordination bonds or complexions between functional groups and metal ions (Qiongjie et al., 2022). In this case, the C–X group acts as a substitute or generalization of functional groups such as –Cl, –Br, or –OH which are able to form coordination bonds with heavy metals, such as Cr(VI). This complexity mechanism makes it difficult for heavy metals to come off because the bonds formed are strong and stable. This process is important in bioremediation systems, because it improves absorption efficiency and reduces heavy metal mobility in aquatic environments (Xing et al., 2022).

In addition to the C–X group, biofilms also contain a variety of other functional groups that contribute to the adsorption ability of heavy metals. The C–O and –OH groups play an active role in the process of ion exchange and complex formation, where oxygen atoms with free electron pairs can interact directly with metal ions, forming stable coordinate bonds (Tang et al., 2024). The C=C group found in the lipid part of the

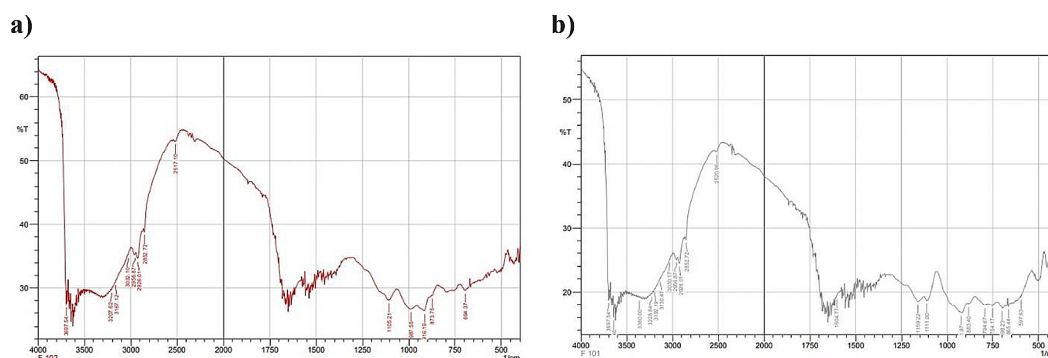


Figure 3. FTIR test results (a) before treatment and (b) Cr(VI) treatment

biofilm is responsible for maintaining the hydrophobic characteristics of the biofilm surface, facilitating the adhesion between the biofilm and metal particles in the aquatic environment. These hydrophobic properties are important, because they help biofilms maintain their structure and stability under a wide range of environmental conditions. The combination of these various functional groups creates an effective adsorption system, in which biofilms not only act as heavy metal binders, but also as biological filters that improve water quality through efficient and sustainable natural processes (Krsmanovic et al., 2021). The adsorption of Cr(VI) is primarily mediated by EPS functional groups, such as hydroxyl, carboxyl, and amine groups, which facilitate metal binding through electrostatic attraction, ion exchange, and complexation mechanisms. These interactions enhance metal immobilization and reduce its mobility in aquatic environments.

Composition of bacteria in biofilm

Analysis of the biofilm sequence data isolated from the Manyar River, Gresik, revealed the presence of more than 756 microbial species in the biofilm community. The taxonomic composition of biofilms is visualized through the Phylogenetic Tree (Figure 4), which shows the dominance of members of the *Bacillaceae* family, including *Virgibacillus* spp. and several other species of the same family. In addition, the genus *Alkaliphilus* and members of the *Peptostreptococcaceae* family such as *Romboutsia* spp. are also found in significant numbers. Relative Abundance analysis then conducted at the family level (Figure 5a) shows that *Bacillaceae* is the most dominant family with a score of 0.3611. Two other families with fairly high abundance are *Alkaliphilus*

(0.1667) and *Peptostreptococcaceae* (0.1495). At the genus level (Figure 5b), the results of the analysis indicated that *Virgibacillus* spp. had the highest abundance (0.5242), followed by *Romboutsia* spp. (0.2379) and *Streptococcus* spp. (0.0989). In general, these results show that the biofilm community formed in the Manyar River, Gresik, is dominated by three main genera, namely *Virgibacillus* spp., *Romboutsia* spp., and *Streptococcus* spp. The dominance of the three reflects the characteristics of the river environment that support the microbial growth of the *Bacillaceae* and *Peptostreptococcaceae* groups.

Virgibacillus is known as a biofilm-forming bacterium that plays an important role in providing the main skeleton of the biofilm structure. This species is able to adapt to the environments with high salt levels and becomes the foundation on which other microorganisms attach. In addition, *Virgibacillus* contributes to the process of biomineralization, which is the formation of inorganic mineral deposits that strengthen the biofilm and increase its stability against environmental stresses (Sharma et al., 2023). The dominance of *Virgibacillus* spp. is consistent with previous studies reporting its prevalence in saline and polluted environments, where it contributes to biofilm stability and metal tolerance through EPS production and mineralization processes (Sharma et al., 2023).

Romboutsia has the ability to produce extracellular polysaccharides, such as cellulose or polyglucuronates (PGa), which function to thicken the biofilm matrix. The production of these compounds not only strengthens cohesion between cells, but also creates a more protected environment from physical and chemical stress. Thus, *Romboutsia* acts as a biofilm structure

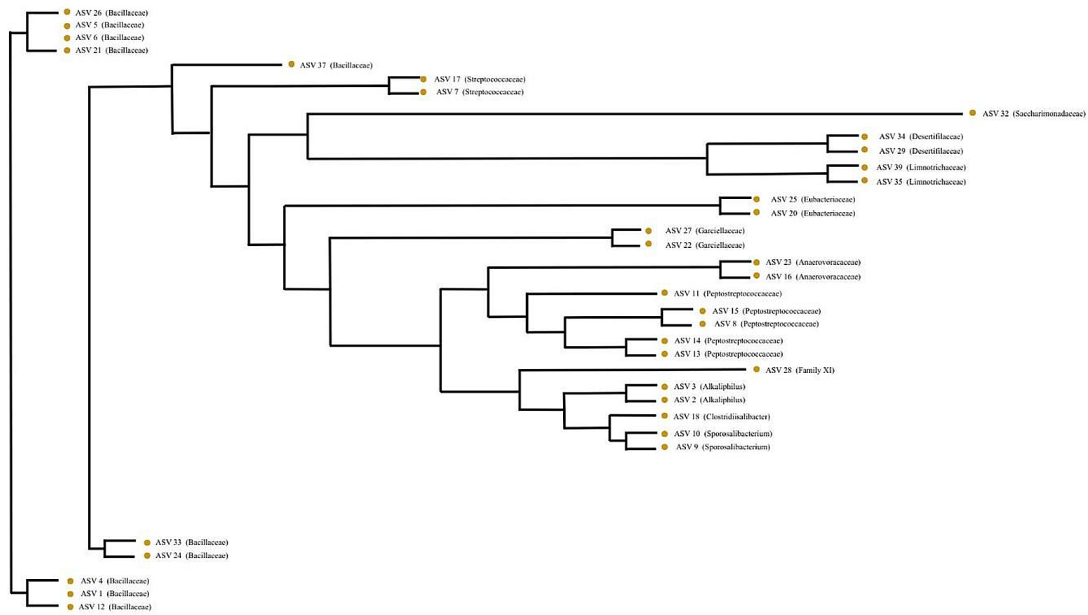


Figure 4. Phylogenetic tree biofilm from the Manyar River

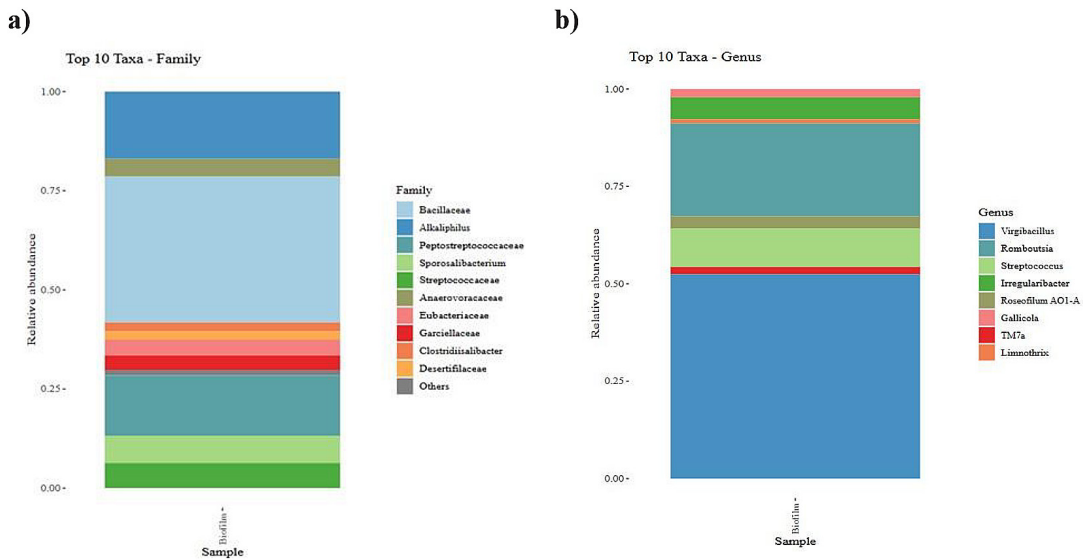


Figure 5. Relative abundance of the biofilm (a) family level; (b) genus level

reinforcer and a chemical provider that enriches the matrix composition (Chang et al., 2023).

Streptococcus solid biofilms that rely on adhesion as well as exopolysaccharide (EPS) production to adhere to surfaces and colonize. This process is important in the early stages of colonization until the formation of mature biofilms. In addition to structural function, *Streptococcus* biofilms are often associated with intercellular communication patterns (quorum sensing) that regulate gene expression related to virulence and resistance to environmental stress (Xie et al., 2024).

In terms of adsorption and immobilization of heavy metals, *Virgibacillus* exhibits strong bio-adsorption capabilities owing to its Gram-positive cell wall characteristics and EPS layer, allowing the deposition of the metal as a mineral (Qu et al., 2022). *Romboutsia* plays a role in supporting this process by increasing the amount and chemical properties of EPS, which results in polysaccharides rich in hydroxyl and carboxyl functional groups, thus providing more metal binding sites (Chang et al., 2023). Meanwhile, *Streptococcus* is able to bioaccumulate through metal homeostasis systems, such as transporters and binding

proteins, as well as utilize charged groups on the cell wall and EPS to effectively absorb metal ions (Goh et al., 2024).

Comparison of Cr(VI) concentrations in sediment with water

The difference in the Cr(VI) levels between water and sediment that is not too large indicates a partition balance between the two phases. This means that the Cr(VI) ions dissolved in water are constantly subjected to an adsorption process to the surface of the sediment particles as well as a desorption process that releases them back into the water phase. This dynamic balance keeps the concentration of Cr(VI) in both phases relatively stable, with neither phase dominating. The situation reflects the chemical stability of the aquatic system, where the transfer of heavy metals between water and sediment takes place continuously (Ao et al., 2025) (Table 2).

The data showed that the distribution coefficient (Kd) value ranged from 0.99–1.05, indicating that there was no significant difference between the concentration of Cr(VI) in water and in sediment. A Kd value close to 1 indicates that Cr(VI) has a relatively equal distribution between the two phases, so this heavy metal does not tend to accumulate in sediments or remain completely dissolved in water. This shows that the water conditions are in a dynamic state, where the process of adsorption and desorption of Cr(VI) takes place in a balanced manner. In addition, a turbidity value of 39.82 mg/L indicates a fairly high level of turbidity, which has the potential to affect the mobility of Cr(VI). Suspended particles in the water with high turbidity can be metal-ion binding media, but if the

stability is low, they can release Cr(VI) back into the water column, increasing the risk of pollution for aquatic organisms (Miranda et al., 2022; Kurniawan et al., 2024a).

Comparison of the Cr(VI) concentrations in biofilm with water

The concentration of Cr(VI) in the biofilm which reaches about 66.55–70.03 mg/kg, compared to only 0.62–0.70 mg/L in the water column, indicates that the biofilm matrix has a very high absorption and metal storage capacity that serves as the main accumulation compartment in the waters. This phenomenon is in line with the general concept that biofilms in aquatic environments are able to accumulate heavy metals in much higher levels than water media due to a combination of adsorption mechanisms in the extracellular matrix (EPS), adsorption on the cell surface, and intracellular uptake. Studies of the biofilms exposed to Cr(VI) have also shown that microbial communities can adapt and continue to grow at a specific range of Cr(VI) concentrations while continuing to accumulate and reduce Cr(VI), thus supporting the biofilm’s role as a sink (absorber) of metal pollutants in aquatic systems (Danouche et al., 2021; Kurniawan et al., 2024b) (Table 3).

The BAF value of biofilm to Cr(VI), which is in the range of 99.44–106.81 times, indicates a strong bioaccumulation process from the water phase to the biofilm compartment along the study site. This high range of BAF reflects the efficiency of the transfer of pollutants from the aquatic medium to the biota or biological matrix, as emphasized in the study of the concept of BAF and bioconcentration in aquatic organisms

Table 2. Distribution value between sediment and water

Station	Sediment (mg/kg)	Water (mg/L)	Kd
1	0.70	0.70	1.00
2	0.68	0.69	0.99
3	0.65	0.62	1.05

Table 3. Biofilm accumulation factor

Station	Biofilm (mg/kg)	Water (mg/L)	BAF
1	70.03	0.70	100.00
2	68.54	0.69	99.44
3	66.55	0.62	106.81

Table 4. Nilai biota-sediment accumulation factor

Station	Biofilm (mg/kg)	Sediment (mg/L)	BSAF
1	70.03	0.70	100.16
2	68.54	0.68	100.48
3	66.55	0.65	101.88

used to assess the potential accumulation and ecotoxicological risk of a chemical in the aquatic environment. In addition, biofilm is recognized in the literature as one of the important “sinks” of various contaminants in waters, which not only modulates the bioavailability of heavy metals, but also has the potential to be a pathway for the transfer of pollutants to higher trophic levels when the biofilm is consumed by other organisms (Gómez-Regalado et al., 2023). The BAF values obtained in this study (99–106) are considerably higher than those reported in previous studies of biofilm-mediated metal accumulation, which typically ranged between 10–80, depending on environmental conditions (Desiante et al., 2021; Laderriere et al., 2021). This suggests that the biofilms in the Manyar River possess enhanced accumulation capacity, likely due to prolonged exposure to industrial pollutants.

Comparison of Cr(VI) concentrations of biofilms with sediments

The BSAF value in the table is calculated as the ratio between the concentration of Cr(VI) in the biofilm (biota) and the concentration of Cr(VI) in the sediment, thus describing the ability of the organism to accumulate contaminants derived from the surrounding sediment. The greater the BSAF value, the higher the relative bioaccumulation rate; In general, $BSAF > 10$ shows the accumulation of sediment. Some literature classifies organisms with $BSAF > 10$ as high concentrators, so large BSAF values are seen as an important indicator (Yun et al., 2023; Kurniawan et al., 2025).

The results of Table 4 show that the BSAF value of 100.16–101.88 times indicates that the concentration of Cr(VI) in biofilms is much higher than in sediments, so that biofilms can be categorized as strong accumulators of Cr(VI) at all observation stations. This value is well above the range of BSAF 10 generally predicted by equilibrium partitioning theory for many nonionic organic compounds, thus confirming that bioaccumulation processes and biological interactions in biofilms play a very dominant

role in Cr(VI) enrichment, rather than merely physicochemical equilibrium between sediments and organism tissues. Thus, the biofilms at the study site have the potential to be key compartments in the dynamics of heavy metal pollution and play an important role as a vector for the transfer of contaminants from sediments to other components of aquatic ecosystems (Laderriere et al., 2021).

CONCLUSIONS

The natural biofilm isolated from the Manyar River, Gresik, was shown to have an extraordinary ability to effectively absorb and accumulate chromium hexavalent heavy metals [Cr(VI)]. This ability is closely related to its complex microbial structure and composition, where the dominance of the genera *Virgibacillus* spp., *Romboutsia* spp., and *Streptococcus* spp. shows a high degree of adaptation of microorganisms to environmental conditions of waters polluted by industrial waste. The biofilm serves as a biological matrix rich in extracellular polysaccharide compounds that play an important role in binding to metal ions through the mechanisms of cellular adsorption, reduction, and accumulation. The high values of distribution coefficient (Kd), BAF, and BSAF reinforce the indication that the microbial communities in this biofilm are able to actively interact with heavy metals in their environment, both through bioabsorption processes and biochemical transformations. With this capability, the natural biofilm from the Manyar River not only has great potential as a natural bioremediation agent to reduce the burden of heavy metal pollution in industrial aquatic ecosystems, but can also be further developed as a biological system model in sustainable and environmentally friendly liquid waste management. Future studies should integrate kinetic modeling and resistome analysis to evaluate the long-term safety and efficiency of biofilm-based bioremediation systems.

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REFERENCES

1. Ao, M., Zhou, R., Meng, R., Augustin, L. (2025). Chromium transformations and biological impacts in aquatic systems: From sediment-water interfaces to food web complexity. *Ecotoxicology and Environmental Safety*, 303, 1–17. <https://doi.org/10.1016/j.ecoenv.2025.118850>
2. Aziz, K. H. H., Mustafa, F. S., Omer, K. M. (2023). Heavy metal pollution in the aquatic environment: efficient and low-cost removal approaches to eliminate their toxicity: a review. *Royal Society of Chemistry*, 13, 17595–17610. <https://doi.org/10.1039/d3ra00723e>
3. Bugai, D., Smith, J., Hoque, M. A. (2020). Chemosphere Solid-liquid distribution coefficients (K_d-s) of geological deposits at the Chernobyl Nuclear Power Plant site with respect to Sr, Cs and Pu radionuclides: A short review. *Chemosphere*, 242, 1–12. <https://doi.org/10.1016/j.chemosphere.2019.125175>
4. Cavitt, T. B., Pathak, N. (2021). Modeling bacterial attachment mechanisms on superhydrophobic and superhydrophilic substrates. *Pharmaceuticals* 14(10), 977. <https://doi.org/10.3390/ph14100977>
5. Chang, S. C., Kao, M. R., Saldivar, R. K., Díaz-Moreno, S. M., Xing, X., Furlanetto, V., Yayo, J., Divne, C., Vilaplana, F., Abbott, D. W., Hsieh, Y. S. Y. (2023). The Gram-positive bacterium *Romboutsia ilealis* harbors a polysaccharide synthase that can produce (1,3;1,4)-β-d-glucans. *Nature Communications*, 14(1), 1–16. <https://doi.org/10.1038/s41467-023-40214-z>
6. Danouche, M., El, N., El, H. (2021). Phycoremediation mechanisms of heavy metals using living green microalgae: physicochemical and molecular approaches for enhancing selectivity and removal capacity. *Heliyon*, 7, 1–11. <https://doi.org/10.1016/j.heliyon.2021.e07609>
7. Desiante, W. L., Minas, N. S., Fenner, K. (2021). Micropollutant biotransformation and bioaccumulation in natural stream biofilms. *Water Research*, 193, 1–11. <https://doi.org/10.1016/j.watres.2021.116846>
8. Diaz-Jimenez, L., Garcia-torres, S., Carlos-herandez, S. (2023). High adsorption of hazardous Cr (VI) from water using a biofilter composed of native pseudomonas koreensis on alginate beads. *International Journal of Environmental Research and Public Health*, 20, 1–17. <https://doi.org/10.3390/ijerph20021385>
9. Fauzia, K. A., Effendi, W. I., Alfaray, R. I., Malaty, H. M., Yamaoka, Y., Mifhussurur, M. (2024). Molecular mechanisms of biofilm formation in *Helicobacter pylori*. *Antibiotics*, 13, 1–16. <https://doi.org/10.3390/antibiotics13100976>
10. Goh, K. G. K., Ulett, G. C., Thapa, R., Prince, D., Acharya, D., Sullivan, M. J., Ulett, G. C. (2024). An opportunistic pathogen under stress: how Group B Streptococcus responds to cytotoxic reactive species and conditions of metal ion imbalance to survive. *FEMS Microbiology Reviews*, 48, 1–20. <https://doi.org/10.1093/femsre/fuae009>
11. Gómez-regalado, C., Martín, J., Luis, J., Aparicio, I., Alonso, E., Zafra-gómez, A. (2023). Bioaccumulation / bioconcentration of pharmaceutical active compounds in aquatic organisms: Assessment and factors database. *Science of the Total Environment*, 861, 1–39. <https://doi.org/10.1016/j.scitotenv.2022.160638>
12. Guerrieri, N., Fantozzi, L., Lami, A., Musazzi, S., Austoni, M., Orr, A., Marziali, L., Borgonovo, G., Scaglioni, L. (2022). Biofilm and rivers: The natural association to reduce metals in waters. *Toxics*, 10, 1–12. <https://doi.org/10.3390/toxics10120791>
13. Jing, K., Li, Y., Yao, C., Jiang, C., Li, J. (2023). Towards the fate of antibiotics and the development of related resistance genes in stream biofilms. *Science of the Total Environment*, 898, 1–10. <https://doi.org/10.1016/j.scitotenv.2023.165554>
14. Kher, L., Kelley, K., Santoro, D. (2023). Ultrastructural analysis of differences in the growth and maturation of *Staphylococcus pseudintermedius* bio film on biotic and abiotic surfaces. *Microbiology Spectrum* 11, 1–12. <https://doi.org/10.1128/spectrum.03577-22>
15. Krivokapi, M. (2021). Study on the evaluation of (heavy) metals in water and sediment of Skadar Lake (Montenegro), with BCF assessment and translocation ability (TA) by *Trapa natans* and a review of SDGs. *Water*, 13, 1–23. <https://doi.org/10.3390/w13060876>
16. Krsmanovic, M., Biswas, D., Ali, H., Kumar, A., Ghosh, R., Dickerson, A. K. (2021). Hydrodynamics and surface properties in fluence bio film proliferation. *Advances in Colloid and Interface Science*, 288, 1–26. <https://doi.org/10.1016/j.cis.2020.102336>
17. Kurniawan, A., Dhea, L. A., Ulfa, S. M., Yanuar, A. T., Al Zamzami, I. M., Nurjannah. (2025). Assessment of water quality in the upper Brantas River through microplastic-associated biofilms and heavy metal accumulation. *International Journal of*

- Environmental Studies*, 82(4), 1707–1729. <https://doi.org/10.1080/00207233.2025.2516390>
18. Kurniawan, A., Fukuda, Y. (2023). Analysis of the electric charge properties of biofilm for the development of biofilm matrices as biosorbents for water pollutant. *Energy, Ecology and Environment*, 8(1), 62–68. <https://doi.org/10.1007/s40974-022-00253-6>
 19. Kurniawan, A., Salamah, L. N. M., Winarsih, W., Nurjannah, N., Al Zamzami, I. M. (2024a). Binary biosorption of Cu (II) and Cr (VI) by naturally formed biofilm matrices. *Environmental Research, Engineering and Management*, 80(3), 124–133. <https://doi.org/10.5755/j01.arem.80.3.35773>
 20. Kurniawan, A., Pramudia, Z., Susanti, Y. A. D., Al, Z. I. M., Yamamoto, T. (2024b). Comparative biosorption proficiency in intact and autoclaved biofilm matrices. *Journal of Ecological Engineering*, 25(4). <http://dx.doi.org/10.12911/22998993/183943>
 21. Laderriere, V., Faucheur, S. Le, Fortin, C. (2021). Exploring the role of water chemistry on metal accumulation in biofilms from streams in mining areas. *Science of the Total Environment*, 784, 1–12. <https://doi.org/10.1016/j.scitotenv.2021.146986>
 22. Li, S., Cao, L., Liu, Q., Sui, S., Bian, J., Zhao, X. (2024). Enhancing Pb adsorption on crushed microplastics: Insights into the environmental remediation, *Water*, 16(23), 354116, <https://doi.org/https://doi.org/10.3390/w16233541>
 23. Marino, F., Mazzotta, M., Pascale, M. R., Derelitto, C., Girolamini, L., Cristino, S. (2023). First water safety plan approach applied to a Dental Clinic complex: identification of new risk factors associated with *Legionella* and *P. aeruginosa* contamination, using a novel sampling, maintenance and management program First water safety plan appr. *Journal of Oral Microbiology*, 15, 1–15. <https://doi.org/10.1080/20002297.2023.2223477>
 24. Miranda, L. S., Ayoko, G. A., Egodawatta, P., Goonetilleke, A. (2022). Adsorption-desorption behavior of heavy metals in aquatic environments: Influence of sediment, water and metal ionic properties. *Journal of Hazardous Materials*, 421, 1–15. <https://doi.org/10.1016/j.jhazmat.2021.126743>
 25. Mishra, S., Huang, Y., Li, J., Wu, X., Zhou, Z., Lei, Q. (2022). Biofilm-mediated bioremediation is a powerful tool for the removal of environmental pollutants. *Chemosphere*, 294(133609), 1–15. <https://doi.org/10.1016/j.chemosphere.2022.133609>
 26. Nguyen, D., Dinh, V., Nguyen, T., Nguyen, P., Tuyen, K., Duong, B. (2024). RSC Advances Adsorption mechanism of aqueous Cr (VI) by Vietnamese corncob biochar: a spectroscopic study. *RSC Advances*, 14, 39205–39218. <https://doi.org/10.1039/d4ra07455f>
 27. Pagliaccia, B., Carreti, E., Severi, M., Berti, D., Lubello, C., Lotti, T. (2022). Heavy metal biosorption by extracellular polymeric substances (EPS) recovered from anammox granular sludge. *Journal of Hazardous Materials*, 424(7), 1–13. <https://doi.org/doi.org/10.1016/j.jhazmat.2021.126661>
 28. Peng, Q., Tang, X., Sun, N., Yuan, W. (2023). A review of biofilm formation of staphylococcus aureus and its regulation mechanism. *Antibiotics*, 12, 1–21. <https://doi.org/10.3390/antibiotics12010012>
 29. Perez, M., Parra, S., Ferrada, C., Bravo, M., Perez, P. A., Id, W. Q. (2024). Development of a new methodology for the determination of PET microplastics in sediment, based on microwave-assisted acid digestion. *Pols One*, 17, 1–17. <https://doi.org/10.1371/journal.pone.0314520>
 30. Qiongjie, W., Yong, Z., Yangyang, Z., Zhouqi, L., Jinxiaoxue, W., Huijuan, C. (2022). Effects of biofilm on metal adsorption behavior and microbial community of microplastics. *Journal of Hazardous Materials*, 424, 1–13. <https://doi.org/doi.org/10.1016/j.jhazmat.2021.127340>
 31. Qu, C., Yang, S., Mortimer, M., Zhang, M., Chen, J., Wu, Y., Chen, W., Cai, P., Huang, Q. (2022). Functional group diversity for the adsorption of lead (Pb) to bacterial cells and extracellular polymeric substances. *Environmental Pollution*, 295, 1–8. <https://doi.org/10.1016/j.envpol.2021.118651>
 32. Quan, K., Hou, J., Zhang, Z., Ren, Y., Peterson, B. W., Flemming, H., Mayer, C., Busscher, H. J., Der, H. C. Van, Quan, K., Hou, J., Zhang, Z., Ren, Y., Peterson, B. W., Flemming, H., Mayer, C., Busscher, H. J., Water, H. C. V. D. M. (2022). Critical reviews in microbiology water in bacterial biofilms: pores and channels, storage and transport functions. *Critical Reviews in Microbiology*, 1–21. <https://doi.org/10.1080/1040841X.2021.1962802>
 33. Razeghi, N., Hamidian, A. H., Wu, C., Zhang, Y., Yang, M. (2021). Microplastic sampling techniques in freshwaters and sediments: a review. *Environmental Chemistry Letters*, 19, 4225–4252. <https://doi.org/10.1007/s10311-021-01227-6>
 34. Relucenti, M., Familiari, G., Donfrancesco, O., Taurino, M., Li, X., Chen, R., Artini, M., Papa, R., Selan, L. (2021). Microscopy methods for biofilm imaging: Focus on SEM and VP-SEM pros and cons. *Biology*, 10, 1–17. <https://doi.org/doi.org/10.3390/biology10010051>
 35. Rusiniak, P., Wątor, K., Kmiecik, E., Vakanjac, V. R. (2024). Method validation and geochemical modelling of chromium speciation in natural waters. *Scientific Reports*, 14, 1–13. <https://doi.org/10.1038/s41598-024-77425-3>
 36. Sharma, A., Singh, R. N., Song, X. P., Singh, R. K., Guo, D. J., Singh, P., Verma, K. K., Li, Y. R. (2023). Genome analysis of a halophilic *Virgibacillus halodenitrificans* ASH15 revealed salt adaptation, plant growth promotion, and isoprenoid

- biosynthetic machinery. *Frontiers in Microbiology*, 14. <https://doi.org/10.3389/fmicb.2023.1229955>
37. Sharma, P., Singh, S. P., Parakh, S. K., Wah, Y. (2022). Health hazards of hexavalent chromium (Cr (VI)) and its microbial reduction. *Bioengineered*, 13, 1–17. <https://doi.org/10.1080/21655979.2022.2037273>
38. Tan, J. H., Liew, K. J., Sani, R. K., Samanta, D., Pointing, S. B., Chan, K., Goh, K. M. (2025). Microbial diversity and metabolic predictions of high-temperature streamer biofilms using metagenome-assembled genomes. *Scientific Reports*, 15, 1–16. <https://doi.org/10.1038/s41598-025-12132-1>
39. Tang, S., Ma, S., Lin, L., Ding, Y., Zhang, X., Wu, X., Zhang, Q., Pervez, N., Cao, C., Zhao, Y. (2024). Carrier effects of face mask-derived microplastics on metal ions: Enhanced adsorption by photoaging combined with biofilms, exemplified with Pb (II). *Journal of Hazardous Materials*, 477, 1–12. <https://doi.org/10.1016/j.jhazmat.2024.135311>
40. Wang, S., Zhao, Y., Breslawec, A. P., Liang, T., Deng, Z., Kuperman, L. L. (2023). Strategy to combat bio films: a focus on bio film dispersal enzymes. *NPJ Biofilms and Microbiomes* 63, 1–14. <https://doi.org/10.1038/s41522-023-00427-y>
41. Xie, H., Zhang, R., Guo, R., Zhang, Y., Zhang, J., Li, H., Fu, Q., Wang, X. (2024). Characterization of AI-2 / LuxS quorum sensing system in bio film formation, pathogenesis of *Streptococcus equi* subsp. *zoepidemicus*. *Frontiers in Cellular and Infection Microbiology*, 15, 1–12. <https://doi.org/10.3389/fcimb.2024.1339131>
42. Xing, Y., Tan, S., Liu, S., Xu, S., Wan, W., Huang, Q., Chen, W. (2022). Effective immobilization of heavy metals via reactive barrier by rhizosphere bacteria and their biofilms. *Environmental Research*, 207, 1–9. <https://doi.org/10.1016/j.envres.2021.112080>
43. Yu, S., Lu, X. (2025). Marine microbial bio films on diverse abiotic surfaces. *Frontiers in Marine Science*, 26, 1–18. <https://doi.org/10.3389/fmars.2025.1482946>
44. Yun, X., Lewis, A. J., Stevens-king, G., Sales, C. M., Spooner, D. E., Kurz, M. J., Suri, R., Mckenzie, E. R. (2023). Bioaccumulation of per - and poly fluoroalkyl substances by freshwater benthic macroinvertebrates: Impact of species and sediment organic carbon content. *Science of the Total Environment*, 866, 1–12. <https://doi.org/10.1016/j.scitotenv.2022.161208>