REVIEW

Biodegradation of azo dye‑containing wastewater by activated sludge: a critical review

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Abstract

The effluent from the textile industry is a complex mixture of recalcitrant molecules that can harm the environment and human health. Biological treatments are usually applied for this wastewater, particularly activated sludge, due to its high efficiency, and low implementation and operation costs. However, the activated sludge microbiome is rarely well-known. In general, activated sludges are composed of *Acidobacteria, Bacillus*, *Clostridium*, *Pseudomonas*, *Proteobacteria*, and *Streptococcus*, in which *Bacillus* and *Pseudomonas* are highlighted for bacterial dye degradation. Consequently, the process is not carried out under optimum conditions (treatment yield). Therefore, this review aims to contextualize the potential environmental impacts of azo dye-containing wastewater from the textile industry, including toxicity, activated sludge microbiome identifcation, in particular using the matrix-assisted laser desorption/ionization time-of-fight mass spectrometry (MALDI-TOF MS) as a novel, rapid and accurate strategy for the identifcation of activated sludge microbiome (potential to enhance treatment yield).

Keywords Activated sludge · Textile industry · Microbiome · Biodegradation · Azo dye

Introduction

The high fuctuations in composition and other parameters such as biological oxygen demand (BOD), chemical oxygen demand (COD), colour, pH, and salinity during textile processing contribute to the complexity of textile wastewater treatment (Senthilkumar et al. [2011](#page-10-0); Farias et al. [2017](#page-8-0)). The textile industry generates, inherently, a high volume of toxic effluent, mainly due to the chemical baths and rinsing series (Harane and Adivarekar [2017\)](#page-9-0). According to Leão ([2002](#page-9-1)), 150 L of water are necessary to produce 1 kg of fabric, in which 132 L (88%) are wastewaters.

The dyeing stage leads to a large amount of wastewater with high levels of COD (from the chemical used in the process), BOD (due to the direct discharge of wastewater in water sources which promotes a rapid depletion of dissolved oxygen), pH, and dyes (Holkar et al. [2016;](#page-9-2) Swati and Faruqui [2018\)](#page-10-1). The incorrect disposal of textile industry

effluents into water bodies interferes with the penetration of light. Hence, it harms the photosynthetic activity of water body communities (Elisangela et al. [2009\)](#page-8-1). Thus, the textile industry effluent has to be properly treated before disposal into water bodies. In this sense, conventional textile industry treatments include coagulation/focculation, biological, membrane, and advanced oxidation processes (Vajnhandl and Valh [2014;](#page-10-2) Yukseler et al. [2017\)](#page-11-0). Among these techniques, the dye biodegradation by the activated sludge process is drawing attention due to its low cost (implementa-tion and operation) and high efficiency (Haddad et al. [2018](#page-9-3)). Regarding activated sludge, the efectiveness of COD and BOD reduction can reach up to 90% (Pereira et al. [2010](#page-10-3); Waghmode et al. [2019\)](#page-11-1).

Activated sludges are composed of microbial communities. However, there are few reports on their characterization (El et al. [2016;](#page-8-2) Zhu et al. [2018;](#page-11-2) Cao et al. [2019](#page-8-3)). The evaluation of activated sludges as microbial consortia is essential to comprehend the interactions among the microbial community and optimize biodegradation (Köchling et al. [2017](#page-9-4)). Nevertheless, experiments of dye-biodegradation by isolated strains from activated sludges makes easier the elucidation of biodegradation mechanisms (Khehra et al. [2005](#page-9-5)).

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Therefore, this review describes the main biodegradation aspects of textile effluent. Then, it correlates the MALDI-TOF MS as a promising methodology for identifying activated sludge microbiome and, consequently, improving the treatment yield.

Potential impacts of azo dyes

Azo dyes are chemically composed of aromatic groups and azo chromophore (-N=N-). They are highly water-soluble. It is worth noting that azo dyes are widely applied by textile industry corresponding≥50% out of the worldwide dye production (Brüschweiler and Merlot [2017](#page-8-4)). However, they hamper conventional textile wastewater treatment plants due to their recalcitrance.

It is usually observed a very low degradation rate of azo dyes at primary and secondary treatment stages due to their recalcitrant behaviour, which is related to their synthetic origin and chemical structure, providing them high resistance to photo-oxidation, biological activity, and other environmental conditions. The molecular arrangement and size of these substances contribute negatively to their biodegradation since a steric efect hinders the enzymatic access necessary to start the degradation (Rittmann [2018](#page-10-4)). Therefore, the accumulation of azo dyes can occur in sediments, soils, and contaminate the drinking water supply system (Salter-Blanc et al. [2016;](#page-10-5) Xiang et al. [2016;](#page-11-3) Mullai et al. [2017\)](#page-10-6). The colour reduction \approx 50% (azo dyes) from coloured wastewater is considered adequate. However, the toxicity factor must also be considered, particularly due to the aromatic amines (Xiang et al. [2016;](#page-11-3) Brüschweiler and Merlot [2017](#page-8-4)). Heavy metals, salts, and sulfdes are potentially microbial inhibitors of biological treatment system. Thus, it must be also evaluated (Sarayu and Sandhya [2012\)](#page-10-7).

It is known that the chromophore azo groups present in anionic and nonionic dyes sufer reductive cleavage produc-ing highly toxic aromatic amines (Table [1](#page-2-0)): $R-N=N-R'+4\bar{e}$ $+4H^+ \rightarrow R-NH_2+R'-NH_2$ (Xiang et al. [2016\)](#page-11-3). The chemically reduced form of dyes was already found in sediments of aquatic bodies. These molecules are carcinogenic, since they can be oxidized to *N*-hydroxylamines. Thus, the nitrenium ion generated can bind with cellular macromolecules as DNA, proteins, and RNA (Ford and Griffin [1992](#page-8-5); Sari and Simarani [2019\)](#page-10-8). Nevertheless, an activating metabolism varies according to the balance between numerous competing steps, the bioavailability of the reactive metabolite, and nutritional habits. Moreover, possible diferences in individual susceptibility infuence the higher complexity of the metabolic pathway, polymorphisms of enzymes associated with the metabolism of aromatic amines, and equilibrium between activating and inactivating steps (Gregory [2007](#page-9-6); Neumann [2010\)](#page-10-9).

Kumar et al. ([2019\)](#page-9-7) carried out the optimal process of decolourization of Acid Black 24 azo dye by *Bacillus pseudomycoides*. The authors also evaluated the genotoxicity and phytotoxicity of the degraded dye. Genotoxicity assay was carried out using *Allium cepa* (onion), evaluating the DNA damage in cells treated with dye solution performed by single-cell gel electrophoresis method. The results were obtained by comparing before and after decolourization. The untreated dye sample presented a genotoxic efect on the root cells of *Allium cepa*. Phytotoxicity experiments were also performed on seeds of *Vigna radiata* and *Sorghum vulgare* at 25 °C. After 48 h was observed the number of full seeds germinated, and with fve days of incubation was calculate the length of plumule (cm) and radicle of seedlings (cm) and germination percentage. The control and treated sample results presented similar values, which indicate the production of non-toxic metabolites correlated to high degradation yields.

On the other hand, according to Waghmode et al. ([2019\)](#page-11-1) higher phytotoxicity was observed with *Phaseolus mungo* and *Sorghum vulgare* after a sequential photocatalytic and biological treatment consisting of ZnO as the photocatalyst and a microbial consortium of *Brevibaccilus laterosporus* and *Galactomyces geotrichum*. Through HR-MS and GC–MS analysis, degradation products were analyzed after bacterial treatment. Many products with a fragmentation pattern inferring asymmetrical/symmetrical cleavage in azo bonds were found, probably due to the demethylation and desulfonation mechanisms. It was also observed that the produced metabolites in the activated sludge can be correlated to microbial species (Tabasum et al. [2019\)](#page-10-10).

The toxicity of a treated textile effluent was analyzed by Carvalho et al. ([2020](#page-8-6)), using *Vibrio fscheri* as a toxicity indicator, monitoring cell luminescence inhibition (30 min). The dilution factor (effluent) required to not affect bacteria metabolism was also used as a comparison parameter, in which the acute toxicity is correlated to 50% reduction in the bacteria bioluminescence. In the conventional up-fow anaerobic sludge blanket (UASB) effluent, it was obtained a dilution factor between 14 and 16, which can be associated with the presence of aromatic amines, (strong toxicity levels). Whereas the results of UASB effluent micro aerated on top indicated that aromatic amines were converted into nontoxic compounds.

Aromatic amines from the azo-dye degradation have mutagenic efects in *Salmonella* and *Mammalian*. Methyl Red, which is naturally mutagenic, has already been associated with *N*,*N*-dimethylphenylenediamine (DMPD) formation, a toxic and mutagenic aromatic amine (Wong and Yu [1999\)](#page-11-4). Consequently, methodical optimization studies simultaneously with metabolite toxicity testing should be implemented for each system, considering dye, microbe, enzyme, or mediator (Sen et al. [2016\)](#page-10-11).

Table 1 Reports about the decolorization capability of bacteria from textile-activated sludge **Table 1** Reports about the decolorization capability of bacteria from textile-activated sludge

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Daily large amounts of residual water containing a high concentration of azo dyes, between 100 and 250 mg L^{-1} , are incorrectly discharged into water bodies (Garcia-Segura and Brillas [2016](#page-8-10)). To mitigate the impact of azo dyes (colour), their chemical bonds must be broken within the chromophores group structures (Ghosh et al. [2017\)](#page-9-9). Aerobic biodegradation of dyes through the activated sludge process is recognized as an economical and efficient technology. Nevertheless, some drawbacks can cause serious environmental impact, in particular, due to the large amounts of sludge that are inherently generated (Lv et al. [2013\)](#page-9-10). In addition, most dyes and chemicals from the textile process have a low rate of biodegradability. Deraniyagala ([2017](#page-8-11)) reported that the treatment of textile industrial wastewater by activated sludge produces 2000 tons of dangerous sludge, considering a daily flow rate of 4000 m^3 of wastewater. Thus, the activated sludge treatment should be improved, constantly.

Regarding biodegradation investigations of textile efuents, usually they are outlined to enhance the activated sludge microbiome degradation, biosorption (sequestration of dyes from solution by chelation, complexation, precipitation, or ionic interactions), and mineralization (complete oxidation of dyes to H_2O , CO_2 and other inorganic compounds) by varying physicochemical parameters such as pH, temperature, carbon and nitrogen sources, dye concentration, inoculum size, among others (Chen et al. [2003;](#page-8-12) Moosvi et al. [2005](#page-9-11); Kapdan and Erten [2007](#page-9-12); Pandey et al. [2007;](#page-10-14) Khalid et al. [2008;](#page-9-13) Dhanve et al. [2008;](#page-8-13) Mullai et al. [2017\)](#page-10-6). However, there are new technologies as MALDI-TOF MS that can assist to reach higher treatment yields.

Dye adsorption process and textile sludge

Adsorption is a wide used wastewater treatment technology, also applied to the textile industry (Ho and McKay [2003](#page-9-14); Jain et al. [2003\)](#page-9-15). Intermolecular attraction forces between adsorbate and adsorbent lead to mass transfer, in which the accumulation of contaminants occurs at the interface between phases: gas–liquid, gas–solid, liquid–liquid, or liquid–solid interface (Reisch [1996](#page-10-15); Dąbrowski [2001\)](#page-8-14). In this sense, the molecular structure of the adsorbent, medium pH, solute solubility, and temperature signifcantly afect the adsorption process (Foust et al. [1980](#page-8-15)).

Regarding adsorption of dyes, there are four main steps: (i) the dye movement from the crude solution to the liquid flm or the interface of the adsorbent solid; (ii) its difusion through the liquid flm to the external sites of adsorption; (iii) its inner difusion through the adsorbent solid pores or capillaries, and; (iv) its adsorption at the available places of the capillary surfaces or walls (Reynolds and Richards [1996](#page-10-16)).

The most usually used adsorbent for textile wastewater treatment and colour removal is the activated carbon. However, its high cost is promoting the development of lowcost alternative adsorbents (Aksu [2001;](#page-8-16) Calvo [2001](#page-8-17); Wang and Hu [2007](#page-11-5); Ju et al. [2008](#page-9-16); Smith et al. [2009](#page-10-17); Rafatullah et al. [2010\)](#page-10-18). In this sense, waste materials, such as activated sludge, are an interesting alternative since large quantities of sludge are inherently produced (Smith et al. [2009\)](#page-10-17).

The sludge-based adsorbent can be produced by the carbonization of sludge, centrifugation, H_2SO_4 treatment, NaOH treatment, pyrolysis, steam activation. It is worth noting that microbial membranes of sludge afect the adsorbent properties since microbial membranes are negatively charged surfaces (Pavithra et al. [2019\)](#page-10-19). Thus, the control mechanisms of dye adsorption include chelation, complexation, ion exchange, and surface adsorption (Crini [2006](#page-8-18); Wang and Hu [2007;](#page-11-5) Sadhasivam et al. [2007](#page-10-20)).

The decolourization efficiency of an alternative adsorbent from textile effluent sludge was tested by Vasques et al. ([2011\)](#page-10-21) on Reactive Orange 16 (RO16), Reactive Red 2 (RR2), and Reactive Red 141 (RR141) dyes. The absorbent was submitted to a thermal activation at 500 °C followed by the chemical activation with acetic acid. At 25 ºC—equilibrium—it was observed the complete removal of RO16 and RR2. Regarding RR141 and RO16, the adsorption capacity was enhanced with NaCl and $Na₂SO₄$, respectively.

Sludge from the textile industry was also used as a lowcost adsorbent for Reactive Red 2 dye. A sequential thermal (500 °C for 70 min) and chemical (H₂SO₄, 25 °C for 3 h) treatment were evaluated. The kinetic experiments, the pseudo-second-order model, were performed in batch mode. The adsorption isotherm model was evaluated under diferent temperature and pH conditions. Maximum adsorption 213.9 mg g⁻¹ was obtained with pH 2 and 25 °C (Sonai et al. [2016](#page-10-22)).

Autoclaved bio-sludge was tested for disperse dye adsorption in sequencing batch reactor (SBR) systems with and without granular activated carbon (GAC–SBR) using textile wastewater (TWW) collected from a central wastewater treatment plant in a textile factory in Thailand; and synthetic textile wastewater (STWW). The GAC–SBR system presented more efectiveness compared with SBR in treating TWW, resulting in a dye decolourization rate of $93.0 \pm 1.1\%$, under the organic loading of 0.18 kg BOD5 m⁻³ (Sirianuntapiboon and Srisornsak [2007\)](#page-10-23)*.*

Haddad et al. ([2018](#page-9-3)) highlighted the optimization of aerobic biodegradation efficiency to reduce the residual adsorbed dye in the fnal waste sludge. Laboratory and pilot-scale investigations were carried out. The process at pilot-scale was tested under diferent hydraulic retention times (HRT) of 2–5 days and sludge recycling rates (SRR) of 220–680 m³ day−1, which achieved the optimal result at HRT of 5 days and a SRR of 0.22 with dye biodegradation efficiency of 95%. These best conditions applied at full-scale reduced the amount of the discharged dyes (89%) signifcantly.

Water treatment residuals (WTR) in the dried form were used as adsorbents in fltration column tests for the colour removal from a real textile dye wastewater. The process presented a maximum colour removal of 36% in the adsorption process and a decolourization rate in the range of 60–70% in column operation, which generally shows a greater removal. The authors defended the use of WTR as a primary treatment for textile wastewater decolourization (Gadekar and Ahammed [2020\)](#page-8-19)*.*

There are some limitations to applying biomass at an industrial scale, including the accessibility of adsorbents, adsorption sites, adsorbent stability, low adsorption, and desorption rates at specifc pH and other environmental factors such as ions and salts. These factors should be improved to make this technology competitive (Li et al. [2019a](#page-9-17); Zhou et al. [2019](#page-11-6)). Regarding emerging advanced technologies used for dye adsorption, the most promising are: magnetic nanoparticles; metal/nonmetal-doped nanostructures; ceramic and modifed nanoclays; and carbonaceous nanomaterials such as single and multiwalled carbon nanotubes, carbon quantum dots, and expanded graphite and graphene nanosheets (Fraga et al. [2021](#page-8-20)). These technologies also present an environmental clean-up perspective and have been attempted to achieve high rates of colour removal efficiency and low cost (implementation and operation) (Anand et al. [2020](#page-8-21); Nayak et al. [2020\)](#page-10-24).

Therefore, regarding the dye biodegradation by bacteria culture, the biological adsorption phenomenon interferes on decolourization results, making it difficult to understand biological degradation in details, since adsorption occurs at the same time (Ghosh et al. [2017;](#page-9-9) Wang et al. [2020\)](#page-11-7). In this sense, Kiayi et al. [\(2019\)](#page-9-18) investigated this factor through a biosorption test, based on spectrophotometric visualization of the solution from the suspension of bacterial pellets in methanol and water. However, no adsorption interference was detected. Corso and Maganha De Almeida ([2009](#page-8-22)) evaluated the adsorption contribution using diferent concentrations of isolated biomass (autoclaved and non-autoclaved) to inoculate on dye solution. After 120 h, it was measured absorbances of the supernatants, which revealed high levels of decolourization index. The identifcation of adsorption in biodecolourization processes was also pointed by Asad et al. ([2007](#page-8-23)) through the gradual decrease of adsorption peaks identifed in a decolourization. Besides this verifcation, the authors made an association between live and inactivated cells, inferring that inactivated cells cannot decolourize an aqueous system by the adsorption process.

The adsorption process is possible due to the cell surface composition from active functional groups (amine, carboxyl, hydroxyl, phosphate, and sulfhydryl) for dye binding (Kapoor et al. [1999](#page-9-19); Corso and Maganha De Almeida [2009](#page-8-22)).

Nevertheless, it also depends on the concentration of dye (Ghosh et al. [2017\)](#page-9-9). The presence of these functional groups on the cellular wall provides a negative charge that attracts positively charged molecules as cationic azo dyes or with positively charged groups (e.g. basic red 29, and basic blue 41) (Srivastava and Thakur [2006;](#page-10-25) Congeevaram et al. [2007](#page-8-24)). Therefore, the adsorpition must be considered, carefully, in biological treatments.

Activated sludge microbiome

Activated sludge is an association among many organisms in a community, mostly composed of aerobic and anaerobic bacteria. Some bacterial species can focculate, which favours sedimentation (Paździor et al. [2019](#page-10-26)). In addition, they can reach high rates of decolourization and mineralization, which leads to low toxic sludge generation and a cost-efective process. Species belonging to the genera *Aeromonas, Bacillus, Proteus,* and *Pseudomonas* are some of the widely investigated bacteria for dye degradation (Mullai et al. [2017](#page-10-6)).

The azo dyes biodegradation by bacteria generally requires a combination of two stages. First, an anaerobic step responsible for discoloration when azo bonds are broken in the presence of redox mediators through the azo reductase enzyme (Klepacz-Smółka et al. [2010](#page-9-20)). Then, an aerobic phase promotes the efficient removal of organic compounds. Since the decolourization by pure cultures is associated with the development of aromatic amines (toxic compounds), mixed cultures (Table [1\)](#page-2-0) are often used due to their synergistic metabolisms. This synergy promotes the conversion of toxic intermediates into nontoxic by-products (Yang et al. [2012;](#page-11-8) Patel [2013](#page-10-27); Lotito et al. [2014](#page-9-21); Manekar et al. [2014](#page-9-22); Ali et al. [2016;](#page-8-25) Mullai et al. [2017](#page-10-6)).

Carvalho et al. [\(2020\)](#page-8-6) evaluated two up-fow anaerobic sludge blanket (UASB) reactors R1 and R2 (with aeration in the upper part), as a comparative system to remove tetra-azo dye Direct Black 22 (DB22). The discoloration and COD removal efficiencies for both reactors were similar (67 e 72% for R1 and 59 e 78% for R2), pointing to no considerable infuence of oxygen in R2. DNA extraction (Power Kit Soil®—MO Bio laboratories, Carlsbad-CA, USA), quantifcation (NanoDrop2000 spectrophotometer—Thermo Scientifc, USA), storage at − 20 °C, and Illumina MiSeq with the universal primer 515 F paired with 907R for *Archaea* and *Bacteria* domains, with 20,000 reads and 2×300 bp. Microbiome identifcation of the sludge bed of both reactors was carried out (sequencing), which were similar to each other, that is, *Methanosaeta*, *Syntrophus*, and *Trichococcus* genera. Sequences with less than 150 bp and ambiguous base calls were not considered, and the Operational Taxonomic Units (OTUs) were defned by clustering at 97% similarity. The authors proved that higher salinity in some zones of the reactors promoted some alterations on the microbial community and the association between putative genera *Brevundimonas* and *Ornatilinea* and aromatic amine microaerobic removal.

An investigation of the metagenomic of activated sludge from the common effluent plant of Chennai (India), used in the textile effluent treatment process with mixed azo dyes, was conducted by (Krishnaswamy et al. [2020](#page-9-23)). The nanopore sequencing was carried out with PCR amplifcation and barcoding of the sample from the acclimatized sludge used to treat synthetic textile wastewater treatment. After the obtaining and purifcation of the activated sludge sample, it was amplifed with PCR and barcoded. Then, adapters were connected to the amplifed fragments constructed a library, which was sequenced using nanopore sequences. The fragments of 16 s rRNA genes were computed, and in the diversity of the organisms was found Actinobacteria, Proteobacteria (abundantly), and Terrabacteria. The Proteobacteria phylum were represented by the *Acidithiobacilia*, *Burkholderiales*, *Betaproteobacteria*, *Neiseriales*, *Nitrosomonadales*, and *Rhodocyclales* genera.

Cao et al. [\(2019\)](#page-8-3) isolated and developed an indigenous bacteria consortium from a sludge sample of a dying factory for characterizing the active functional microbial communities involved in the degradation of a sulfonated azo dye, Direct Blue 2B (DB2), in a simple batch reactor. The decolourization potential of isolated and combined cultures was analyzed under diferent temperatures, pHs, dye, and NaCl concentrations, operation modes (static, and agitated). The study obtained 90.74% of maximum decolourization of 100 mg L⁻¹ DB2 at 48 h, static condition, with 38.7 °C of culture temperature; initial pH was 7.57, and initial NaCl concentration was 20.10 g L^{-1} predicted by the quadratic model.

To identify the main microorganisms from activated sludge responsible for the degradation of Congo red (CR) and Amino Black (AB) dyes, Zhu et al. [\(2018\)](#page-11-2) proposed a combined model. Besides identifying the species directly involved in azo dye degradation, the study aimed to reveal the relationship between azo dye degradation and microorganisms through DNA extraction, polymerase chain reaction (PCR), and Illumina Sequencing Analysis, with a multiple linear regression model. The reactions of transformation in each of the six reactor compartments were investigated, and it was verifed that degradation intermediates present in each compartment were afecting the microbial communities diferently. Concerning the functional species and decolourization process, *Bacteroides* and *Lactococcus* exhibited signifcant correlations with the azo bond with the t-value of the corresponding regression coefficient larger than 2.0. The study highlighted the occurrence of the decolourization process by anaerobic condition and mineralization under an aerobic environment. The microbial community

was signifcantly afected by the structures of azo dyes and, consequently, their intermediates.

Zhang et al. [\(2018](#page-11-9)) investigated activated sludge samples from three typical Chinese municipal wastewater treatment plants: domestic sewage, fne chemical industry, and textile dyeing wastewater. Microbial DNA was extracted by the liquid-nitrogen grinding pretreatment method; metagenomic sequencing and bioinformatic analysis were executed to understand their metabolic potentials. The dominant phyla in every sample included Proteobacteria (12.3–58.5%), Acidobacteria (1.8–35.1%), Chlorofexi (2.8–37.7%). However, were also found in all samples Bacteroidia (0.7–19.2%), Actinobacteria (0.7–6.8%), TM7 (0.1–5.2%), Synergistetes $(0.02 - 5.6\%)$ and Thermi $(0.03 - 7.89\%)$. In the textile dyeing industry wastewater, Nitrospirae (48.68%) and Acidobacteria (34.82%) were prevailing in the oxidation ditch.

A synergic efect of activated sludge treatment and partial Fenton's oxidation for decolourization of azo dye Reactive Violet 5 (RV5) was observed. Pretreatment with Fenton's reagent in 500 mg L^{-1} RV5 aqueous solutions promoted 52.9, 83.9, and 91.3% of colour removal within 60 min to H_2O_2 concentrations of 1.0, 1.5, and 2.0 mM, respectively. Then biological treatment removed 70.2%, on average, of the residual RV5 concentration. An activated sludge microbial community analysis was realized through Genomic DNA using the Power Soil DNA isolation kit (MoBio Laboratories Inc., Solana Beach, CA, USA). Several of the most abundant bacteria were *Acidithiobacillus, Acidocella*, and *Streptococcus*, that presented azo dye reducing abilities. The study also revealed that exposure to RV5 modifed a highly-specialized community with degrading activity to azo dye, including *Aspergillus, Clostridium,* and *Trichosporon* species (Meerbergen et al. [2017\)](#page-9-8).

Thus, the wide variety of activated sludge microbiomes mentioned above is directly associated with the complexity of each textile effluent, the structures of the azo dyes, their intermediates, and treatment conditions. However, the microbial community afects directly the decolourisation yield. Thus, it should be investigated deeply.

Screening of bacteria from activated sludge; rapid identifcation by MALDI‑ToF

Usually, microbial identifcation is carried out using multiple experiments and analytical procedures, for instance, extraction, purifcation, separation (e.g. through 16S rRNA and 18S rRNA gene sequencing), complex phenotypic, molecular, and morphological characteristics. These methods are costly and often do not provide information on microbial physiology (Padliya and Wood [2004](#page-10-28); Kemptner et al. [2009](#page-9-24); Kim et al. [2010;](#page-9-25) Singhal et al. [2015](#page-10-29)).

In this sense, matrix-assisted laser desorption/ionization time-of-fight mass spectrometry (MALDI-TOF MS) is a technique for a wide range of chemical identifcation. It can also be used for rapid microbial identifcation (protein pattern overlay) (Murugaiyan et al. [2012\)](#page-10-30). Generally speaking, a reference library (e.g. MALDI Biotyper) composed of proteomic signals (spectrum) of known microorganisms is used. The spectrum of an unknown sample is instantly matched against the reference library to identify microorganisms by their molecular fngerprint. Its comprehensive database of pathogenic microorganisms, rapid process, relatively higher accuracy, sensitivity, and economy in terms of labour and costs involved lead to advances over other microbial identifcation methods prevalent in clinical diagnosis. To date, there are some limits to the applicability of MALDI-TOF MS in the area of microbial ecology research due to the defciency of data on non-clinical microorganisms. In other words, the reference library should be expanded to all microbial species as soon as possible (Singhal et al. [2015;](#page-10-29) Rahi et al. [2016\)](#page-10-31)*.*

Nevertheless, this technique is becoming increasingly fundamental for microbial characterization and identifcation, describing new species due to its ability to distinguish at the species level (Lang et al. [2015;](#page-9-26) Patil et al. [2015](#page-10-32); Tong et al. [2015](#page-10-33)). MALDI-TOF MS technique was already used in a wide range of application, for instance, to distinguish bacterial species of the *Rhizobiaceae* family (Ferreira et al. [2011\)](#page-8-26), to identify bacterial species from the human gut (Lagier et al. [2012](#page-9-27)), to detect pathogenic bacteria (food security assessment) (Bier et al. [2017](#page-8-27); Fröhling et al. [2018\)](#page-8-28), to make faster the urinary tract infection identifcation (Li et al. [2019b](#page-9-28)), to identify marine bacterial symbionts (Dieckmann et al. [2005](#page-8-29); Vidal et al. [2020](#page-11-10)).

In the context of the textile industry, the MALDI-TOF MS analysis was already used to obtain mass spectra of bacterial proteins from cotton cloth samples contaminated with *Shigella fexneri*, *Escherichia coli*, and *Aeromonas hydrophila*, which are species that could cause illness through the faecal-oral routes. The authors confrmed the technique as a rapid method with a high potential for detecting biomarker proteins recovered directly from clothing samples (Holland et al. [2000](#page-9-29)).

A *Bacillus* sp. isolated from sediments of distillery unit was found to overproduce laccase with enormous potential for decolourization of various recalcitrant dyes. The enzyme peptide sequences were obtained with MALDI–TOF MS, the spectra were analyzed using MASCOT software (Matrix Science) and compared with the NCBI database for placement of enzyme with known sequences. About decolourization tests, after enzymatic action, there was around 73% decolourization of dye (trypan blue) and 62% of BBR along with the precipitation of dye contents (Kaushik and Thakur [2013\)](#page-9-30). A study developed by Afreen et al. ([2017](#page-8-30)) showed the use of MALDI-TOF MS to bacterial enzyme identifcation by peptide mass fngerprinting. The enzyme was obtained from *Spirulina platensis* CFTRI, purifed, and used in the decolourization of anthraquinone dye Reactive blue 4 (96%) within 4 h.

In this context, MALDI-TOF MS analysis could bring promising results when used to identify activated sludge microbiome. As subtly explored by Mulinari et al. [\(2020](#page-10-34)), who used MALDI-TOF MS analysis for species identifcation of activated sludge, which showed the presence of both types of microorganisms: aerobics (e.g. *Lysinibaciullus fusiformis*) and facultative anaerobic (e.g. *Escherichia coli* and *Kosakonia cowanii*). The MALDI-TOF MS-based biotyping is a remarkable resource. Due to the speed, accuracy, and sensitivity, the wastewater treatment plant can operate with fne adjustments to enhance the biodegradation, for instance, correlates specifc dyes with microbial changes. In addition, the MALDI-TOF MS data can be used for more drastic changes, for example, after microbial isolation and identifcation, the best azo-degrading species could be immobilized and then added into the treatment plant, or it could be growth (ex-situ), then periodically inoculated into the treatment plant.

State‑of‑the‑art and perspectives

The textile industry produces large volumes of recalcitrant effluents, including azo dyes that negatively affect water bodies and their biological activity. The biodegradation of textile industry effluent, in particular azo dyes, by activated sludge stands out due to its high yields of decolourization. The genera *Pseudomonas*, *Bacillus*, *Proteobacteria*, *Clostridium*, *Acidobacteria,* and *Streptococcus* are usually found in activated sludges. However, there are unknown microbial species that should be investigated, besides the seasonality and complexity of textile wastewater composition change activated sludge microbiome, inherently. Thus, the evaluation of isolated cultures from activated sludge can provide insights and signifcantly enhance biodegradation yield. In this sense, MALDI-TOF MS, a rapid with high accuracy and sensibility technique for microbial identifcation, is a potential strategy to enhance the biodegradation of azo dye-containing wastewater from the textile industry, in particular identifying microbial species that degrade azo dyes.

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Declarations

Conflict of interest The authors have no confict of interest to declare.

Research involving human and/or animal rights This article does not contain any studies with human participants or animals performed by any of the authors.

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