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Dyes in Aquatic Environments: Sources, Impacts, Removal Technologies and the Emerging Role of Microbial Biosorption

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Abstract

The textile, paper, leather, food, cosmetic, pharmaceutical, printing and related industries cannot do without synthetic and natural dyes, but their release to water is still a major environmental problem. The review is generic for dye pollution and not based on a single model dye. Evidence for dye chemistry, environmental behavior, treatment techniques, biosorbent selection, sorption mechanisms, process variables, characterization, kinetic and equilibrium modeling and thermodynamics are brought together. Dyes have a wide range of charge, chromophore, molecular size, hydrophobicity and reactivity. Therefore, treatment performance cannot be generalized for all dye-adsorbent pairs. Physical and chemical technologies can offer fast color removal, while biological processes are less demanding in terms of chemicals and energy, but may require longer treatment times or combined treatment trains. Microbial biomass is particularly stressed as a biosorbent because cell wall polymers provide carboxyl, phosphate, amino, hydroxyl and related binding sites which facilitate electrostatic attraction, ion exchange, hydrogen bonding, hydrophobic interactions and other sorption mechanisms. Also, the literature shows that the apparent capacity and removal efficiency are jointly controlled by pH, biomass dose, initial dye concentration, contact time, temperature and agitation. A new bibliometric re-examination of the given reference list reveals that the prevailing inter-related topics are adsorption/biosorption, biomass, kinetics, equilibrium, thermodynamics and recurring model dyes. Future work should extend beyond single-dye batch tests to mixed-dye matrices, saline real effluents, regeneration, continuous-flow operation, mass-transfer analysis, nonlinear fitting of models, life-cycle assessment, and techno-economic validation

INTRODUCTION

Dyes are colour-producing compounds containing chromophores and usually also auxochromic or ionizable groups which determine spectral properties, solubility and interactions with fibers and treatment materials [1–3]. The change in the industry from natural colorants to synthetic dyes increased the range of colors, the fastness and the efficiency of production, but introduced highly persistent aromatic structures into wastewater streams [2,4–6]. Dye-bearing effluents are most commonly associated with textile processing, although dyes are also used in paper, leather, plastics, printing, paints, cosmetics, food and pharmaceutical applications [2–4,7]. It is not possible to provide a single-dye narrative because dyes are anionic, cationic, nonionic and sparingly soluble compounds and belong to chromophoric classes like azo, anthraquinone, triphenylmethane,

indigoid, xanthene, nitro, nitroso and others [2,3,8]. A general framework for treatment is better. The reactive, direct, acid, basic, disperse, vat, sulfur, mordant and solvent dye application classes have different affinities to substrates, salts, pH conditions and auxiliary chemicals used in processing [2,3]. These differences influence environmental fate and treatment response. Color is the most immediate visual indicator of contamination but the ecological significance of dye wastewater is not limited to the visual effect. Strongly colored water reduces light penetration and may suppress photosynthetic activity, while high chemical oxygen demand, salinity, suspended matter and auxiliary chemicals may compound stress on aquatic systems [4,7,9–14]. Some dyes or their transformation products may be mutagenic, carcinogenic or otherwise toxic and reduction of azo-dyes can yield aromatic amines of concern [9,14–17]. Thus the risk is not only a matter of disappearance of color but also whether the

parent compound is removed, transformed or mineralized. This distinction is important for assessing the treatment performance. Decolorization may occur without the complete removal of total organic carbon or toxicity by either adsorption or cleavage of a chromophore. Thus, biological decolorization and degradation studies should combine spectrophotometric color loss with chemical identification, toxicity testing and mineralization indicators where possible [14–18].

The dye charge strongly influences the adsorption. Negatively charged surface of the biosorbent favors cationic dyes while anionic dyes may require protonated amino or other positively charged sites. Reactive and acid dyes are predominantly anionic, basic dyes are generally cationic and disperse dyes have low water solubility. Thus, the zero charge point of the sorbent and the acid-base chemistry of the dye should be considered together and not as an independent optimization variable [19–22].

Structure of the molecule

Molecular size, planarity, aromaticity, and substituent groups influence the accessibility of the pore and the balance of electrostatic, hydrogen bonding, hydrophobic, and π -related interactions. Even with favorable surface chemistry, large dye molecules may be excluded from narrow pores. Aromatic adsorbents, however, can have strong interactions with aromatic dye structures. These considerations justify why adsorption capacity cannot be predicted on the basis of surface area only [7,19,23–28].

Dye Removal Technologies: Advantages, Disadvantages and Challenges

There is no one treatment method that is best for all cases as industrial dye effluents show a great deal of variation in their dye composition, dye concentration, salt content, pH, organic load, suspended solids and other physicochemical characteristics [23–25,29–34]. Such variations may have a great impact on the efficiency of treatment and complicate the selection of a single technology. A treatment process that is efficient in removing one class of dye, may not be effective on waste water containing dyes of different molecular configurations or ionic properties. Likewise, high salinity, extreme pH and high concentrations of organic matter can influence the efficiency of biological, chemical or physical treatment processes [23–25,29–34].

Practical treatment strategies thus increasingly rely on complementary unit operations where two or more processes are integrated to overcome the limitations associated with individual methods. Such integrated systems can involve physical separation or adsorption as a first treatment step followed by chemical oxidation or biological degradation to eliminate residual pollutants. Biological processes can also be employed before membrane filtration or adsorption to reduce the contaminant load going to downstream treatment stages. So, the right choice on combination and sequence of these operations is to make it according to the characteristics of wastewater, desired effluent quality, operational cost, energy demand and possible generation of secondary waste [23–25,29–34].

Ion exchange and adsorption

Adsorption is widely studied process for the removal of dyes from aqueous systems because of the operational simplicity, flexible process design, relatively simple implementation and the ability to remove the dissolved color without the need to maintain biologically active cultures [7,23–25,29]. It is a process where dye molecules accumulate on the surface or in the accessible pores of an adsorbent due to physical and/or chemical

interactions. Hence, the effectiveness is affected by dye characteristics and adsorbent properties as well as operational conditions such as pH, temperature, contact time, initial dye concentration and adsorbent dosage [23–25,29]. Activated carbon continues to be a key benchmark adsorbent owing to its high surface area and strong affinity toward many organic contaminants. However, the cost of the material itself, mainly in terms of the regeneration and replacement, has triggered the search for alternative materials [12,13,24–26,28,29,35–37]. Therefore, agricultural residues, lignocellulosic materials, microbial biomass, industrial by-products and engineered adsorbents have gained considerable attention as potential low cost alternatives for dye removal [12,13,24–26,28,29,35–37]. Another adsorption-based approach is ion exchange, where charged functional groups on resins act as selective partners for the oppositely charged dye species. However, the use of ion-exchange resins for the removal of ionic dyes may be restricted due to the costs of the resins and the use of chemicals needed for regeneration [4,32,33].

Separation of dyes using membranes

Membrane separation is an important physical method for the treatment of dye-containing wastewater, because it can separate contaminants selectively based on the particle size, molecular size, membrane properties, etc. Microfiltration is primarily utilized for the removal of suspended particles and colloidal materials and is thus generally more appropriate as a pretreatment step than as a single process for dissolved dyes. Ultrafiltration is better at retaining macromolecules and larger organic compounds. However, many of the dissolved dye molecules can still pass through conventional ultrafiltration membranes.

Nanofiltration is particularly relevant to dye wastewater treatment due to its smaller pore dimensions that can effectively retain many dye molecules and its operation at pressures generally less than those required for reverse osmosis [30,32]. However, the accumulation of solutes near the membrane surface may result in concentration polarization, pore blockage and membrane fouling, which gradually reduce the permeate flux and treatment efficiency. Thus, proper pretreatment is often required prior to nanofiltration of highly contaminated dye effluents [30,32]. Reverse osmosis provides an even higher separation and can produce a high-quality permeate with low color and low salinity, suitable for potential water reuse. However, the higher operating pressure, energy consumption, membrane fouling and the related operational costs [30,32] can limit the application of this technology. Membrane processes are therefore especially useful in integrated treatment systems where upstream processes reduce color, suspended matter and organic loading prior to the membrane separation.

Oxidation and advanced oxidation Coagulation

Coagulation-flocculation is widely used in the treatment of dye-containing wastewater because it can quickly destabilize suspended and colloidal particles and promote their subsequent separation. The addition of appropriate coagulants neutralizes or decreases the surface charges, which promotes the aggregation of small particles and the attached dye molecules into larger flocs that can be later removed by sedimentation or filtration [4,25,32]. This approach can lead to considerable color removal, particularly for dyes bound to particulate matter, but may be less efficient for highly soluble dye molecules and other dissolved organic pollutants. Furthermore, coagulation-flocculation does not necessarily destroy the pollutants, but transfers them from the aqueous phase to the sludge, creating an additional waste stream that needs appropriate treatment and disposal [4,25,32]. Oxidative processes offer an alternative to simply separating dye

molecules from wastewater, by chemically transforming the dye molecules. Ozone, hydrogen peroxide, Fenton chemistry, ultraviolet-assisted oxidation, and related advanced oxidation processes can attack chromophoric groups and other recalcitrant molecular frameworks, causing decolorization and perhaps further degradation of organic pollutants [4,31,32,38]. However, their performance is strongly dependent on wastewater composition and operating conditions. Therefore these technologies should be evaluated in terms of high chemical consumption, interference of other constituents in complex wastewater matrices, treatment cost and the possible formation of intermediate or secondary oxidation products [4,31,32,38].

Biological treatment

Biological treatment is an environmentally attractive approach for removal or transformation of dyes in wastewater by the action of bacteria, fungi, algae, yeasts, enzymes or mixed microbial communities [14–16,18,34,36]. Depending on the biological system and properties of the dye, the removal could be achieved by several processes including biosorption onto cell surfaces, intracellular bioaccumulation, enzymatic transformation and biodegradation. Unlike physicochemical methods, which mainly transfer pollutants from one phase to another, biodegradation can break intricate dye molecules into simpler compounds and, under favorable conditions, may result in more complete degradation or mineralization [14–16,18,34,36]. Biological processes are especially attractive since they can lower the use of chemicals, production of sludge, and energy consumption compared to some traditional treatment technologies. However, their efficiency is highly dependent on the wastewater composition and the operating conditions.

Microbial activity can be inhibited by increased salinity, high dye levels, toxic textile auxiliaries, extreme pH and inappropriate oxygen or redox conditions, which decreases decolorization and degradation efficiency. The degradation of azo dyes is another challenge since the different steps of degradation may require opposite environmental conditions. Thus, sequential anaerobic-aerobic treatment is of particular relevance as reductive conditions can facilitate cleavage of azo bonds, while subsequent aerobic treatment can facilitate further transformation of the resulting aromatic intermediates [15–17]. Hence, well designed biological treatment systems can constitute an important component of integrated strategies for treatment of complex effluents containing dyes.

Biosorption as a separate treatment concept

Biosorption is the physicochemical binding and accumulation of dissolved substances by materials of biological origin and can occur by inactive, dead or non-growing biomass [39–41]. This feature sets biosorption apart from metabolism-dependent processes such as biodegradation and active bioaccumulation which depend on viable organisms and the presence of functioning cellular metabolism. The removal of pollutants by biosorption is closely associated with the interaction between the sorbate and the functional groups on the surface of biological materials. Thus, sorption capability of biomass can be retained even after cellular activity is stopped [39–41]. This distinction offers several key operational benefits. Dead biomass does not need nutrients or a constant maintenance of conditions needed for microbial growth and can often be stored for long periods of time before use. It also can tolerate concentrations of toxic dyes and other pollutants that might otherwise inhibit or kill metabolically active microorganisms [39,40,42,43]. Furthermore, the inactive biomass can be possibly re-formed, chemically modified, immobilized or incorporated into engineered treatment systems for easy handling and repeated use [39,40,42,43]. Another

advantage is the possibility of obtaining biosorbents from cheap biological wastes. In this way, waste microbial biomass produced during fermentation and other biotechnological processes may be recovered and re-used as a sorbent material rather than only disposed of as waste [42–45]. This approach simultaneously allows pollutant removal, waste valorization and more resource efficient wastewater treatment. The limitations are just as important. Biosorption does not destroy the dye necessarily, but transfers it from water to a solid phase. Before laboratory capacity values can be translated into treatment performance, regeneration, desorption, loss of capacity over cycles, mechanical stability, biomass separation and final disposal must be addressed [39,40,42].

Microbial biosorbents and their comparative performance

Cell surfaces of bacteria, fungi, yeasts and algae are chemically diverse. The bacterial peptidoglycan and associated polymers, fungal chitin/chitosan-rich structures, algal polysaccharides and extracellular polymeric substances contain functional groups such as carboxyl, phosphate, amino, hydroxyl and others at various densities [19,20,40,41,43]. Available dye systems include *Pseudomonas*, *Bacillus*, *Corynebacterium*, *Paenibacillus*, *Lysinibacillus*, *Rhodococcus*, *Streptomyces*, and mixed or anaerobic sludge biomass [21,44–74].

The presented literature shows capacities from about one order of magnitude to several hundred mg g⁻¹ depending on the dye, organism, pretreatment and model. These are 485 mg g⁻¹ for malachite green with dry *Bacillus cereus* M116 biomass [47], 419 mg g⁻¹ for Reactive Black 5 with *Corynebacterium glutamicum* fermentation waste [45], 289.8 mg g⁻¹ for crystal violet with *Rhodococcus erythropolis* AW3 [72] and lower capacities for several other bacterial or sludge systems [59,62,68,69]. Such values should not be ranked without considering the initial concentration range, pH, ionic strength, temperature, biomass basis, equilibrium criterion and whether nonlinear or linearized fitting was used.

Mechanisms of dye-biomass interactions

Dye biosorption is typically multimechanistic, not due to one type of bond. Electrostatic attraction is strongly pH-dependent, ion exchange involves replacement of counter-ions on charged sites, hydrogen bonding can involve hydroxyl, amino and carbonyl groups, hydrophobic and aromatic interactions become important for nonpolar dye moieties, and surface complexation can contribute where suitable donor groups and ions are present [19,20,27,39,40,46]. The speed at which these interactions are achieved is determined by diffusion in the liquid film and within pores or biomass aggregates [75–77]. Convergent evidence is needed to support mechanistic claims. A good fit to the pseudo-second-order model does not prove a chemisorption and a Langmuir fit does not prove a physically homogeneous monolayer surface. Kinetic modeling combined with pH dependence, ionic-strength effects, desorption behavior, shifts in FTIR, surface-charge measurements and microscopic or spectroscopic characterization provide a more robust mechanistic interpretation [19,20,28,77].

Variables of the biosorption process

pH is one of the most important parameters, which changes both dye ionization and the protonation state of biosorbent functional groups [21,22,41]. The direction of the effect is a function of the dye charge and biomass chemistry. For this reason, it is not providing any mechanistic insight to just report an optimum pH without describing the appropriate surface and dye speciation.

Dose and particle size of biosorbent

The biosorbent dosage is an important operational parameter due to its influence on the amount of biomass and consequently the number of available binding sites for dye removal. Generally, increasing the biomass dosage increases the total number of accessible functional groups and therefore may improve the percentage of dye removed from solution [22,43,48,78,79]. However, the increased percentage removal is not always accompanied by increased adsorption capacity expressed per unit mass of biosorbent. At higher dosages, the calculated uptake in mg g⁻¹ may be decreased because more binding sites are available for free and are not saturated.

Aggregation or overlapping of biomass particles at higher dosages may also decrease the effective surface area available for interaction with dye molecules [22,43,48,78,79]. Particle size is another important factor affecting the biosorption performance. Decreasing the particle size usually enhances the external surface area available for adsorption and reduces the distance to be diffused by the dye molecules to reach the internal binding sites. This is because smaller particles can increase the rate of biosorption as well as the access to sorption sites. However, reducing the particle size to a very small scale may introduce practical limitations, especially for separation and continuous-flow operation. Very fine biosorbent particles can be difficult to recover from treated water and may lead to excessive resistance to fluid flow, bed compaction or increased pressure drop when used in packed-column systems. Therefore, the biosorbent dosage and particle size should be optimized in terms of dye-removal performance and process practicality.

Initial dye concentration

The initial concentration of the dye is an important factor that controls the biosorption, as it influences the concentration gradient between the bulk solution and the surface of the biosorbent. The gradient is usually increased by raising the initial dye concentration, which leads to a larger mass transfer driving force, promoting the movement of dye molecules toward the available binding sites [37,41,49]. Hence, the equilibrium biosorption capacity, which is usually expressed as q or q_e in mg g⁻¹, may increase with the increase in the initial concentration until the available sorption sites become progressively saturated.

At low concentrations, the abundance of dye molecules is relatively low and thus many binding sites may not be occupied. At high concentrations, competition between dye molecules for available binding sites is enhanced [37,41,49]. Further increases in dye concentration may only produce a limited increase in uptake and a decrease in percentage removal when the majority of the most accessible sites have been occupied. Therefore, the performance of biosorbents should be always analyzed considering the percentage removal and biosorption capacity together. A very high percentage removal at a low initial dye concentration does not necessarily mean a better biosorption performance compared to a lower percentage removal at a much higher dye loading. Therefore, the comparison between biosorbents should be based on the initial concentration range, equilibrium concentration, dosage of biosorbents and the corresponding adsorption capacity rather than percentage removal alone.

Agitation and contact time

The contact time plays a significant role in dye biosorption since it determines the time available for mass transfer and interaction between dye molecules and binding sites on the biosorbent. The rapid uptake of many biosorption systems at the beginning of the treatment is related to the presence of many available surface

sites for binding [22,49,80]. With the progressive occupation of these sites, the rate of biosorption decreases and the system gradually reaches equilibrium. In this later stage, transport of dye molecules to less accessible sites and diffusion through pores or biomass structures may play a more important role [22,49,80]. Thus, it is necessary to determine the equilibrium contact time before conducting and interpreting the equilibrium isotherm experiments [22,80,81].

Agitation enhances biosorption by enhancing mixing and facilitating movement of dye molecules from the bulk solution toward the surface of the biosorbent. An increase in agitation can reduce the thickness of the external boundary layer surrounding the biosorbent particles, and hence, reduce the resistance to external mass transfer. However, when external mass-transfer limitations are lowered to a sufficient extent, further agitation increases may contribute only little to improvement. Further, over agitation may lead to an increase in energy utilization and, depending on the properties of the biomass, can lead to physical damage or breakage. Thus, contact time and agitation should be optimized simultaneously to achieve adequate mass transfer and equilibrium but without unnecessarily long treatment periods or excessive energy requirements [22,80,81].

Solution Temperature and Composition

Temperature can influence diffusivity, site affinity and biomass structure and can have a positive or negative effect depending on the system [41,49,82]. Salts and competing solutes are of special importance for real textile wastewater as they screen electrostatic interactions, and compete for sites. Therefore, the studies based on single-dye deionized-water systems only show an early screening stage on the industrial performance proof.

Biosorbents characterization

FTIR spectroscopy is commonly used to compare unloaded and dye-loaded biomass and to observe shifts associated with hydroxyl, amino, carboxyl, phosphate or other surface groups [50,83,84]. Peak assignments are most informative when they are conservative and supported by complementary data. Scanning electron microscopy provides morphological information on surface texture, aggregation and apparent porosity but images alone do not quantify accessible surface area. Where possible, surface area, pore-size distribution, zeta potential or point of zero charge, elemental analysis and thermal stability should also be included.

Kinetic Modeling: Uses and Misuses

Kinetic models are extensively used in dye adsorption and biosorption studies to determine the rate of dye uptake and to evaluate the time taken by the system to reach equilibrium. The most commonly used models are pseudo-first-order, pseudo-second-order and Elovich equations [77,85–87]. These models give a relatively simple mathematical description of the experimental uptake curves and enable the apparent kinetic parameters to be compared across different dyes, biosorbents, concentrations and operating conditions. However, a good fit with a specific kinetic equation does not necessarily mean a proof of a specific adsorption mechanism. In particular, fitting the experimental data to the pseudo-second-order model does not by itself prove that chemisorption is the dominant mechanisms [77]. One must also be very careful when kinetic equations are converted into linear forms. Linear transformations can change the basic error structure and overemphasize some parts of the experimental data and thus influence parameter estimation and model choice.

Nonlinear regression using the original qt-versus-time observations is therefore usually better. The performance of models should not be evaluated only by the coefficient of determination (R^2) [77] but also with residual distributions and adequate error or information criteria. Diffusion models may give further insight where the goal is to identify potential constraints on the rate of dye uptake. The transport process may involve movement of dye molecules through the bulk solution, diffusion through the external liquid film surrounding the biosorbent, intraparticle or pore diffusion, and binding to the available binding sites [27,77]. These processes can be sequential or simultaneous, with the relative importance of each of them varying at different stages of biosorption. Thus, it is rarely justifiable to assign a single "rate-controlling mechanism" just because experimental data fit one empirical kinetic model. A reliable interpretation must consider the kinetic modeling, diffusion analysis, experimental observations and independent evidence about the physicochemical interactions occurring between the dye and biosorbent [27,77].

Equilibrium isotherms and selection of model

In dye adsorption and biosorption researches, equilibrium isotherms are often used to express the relationship between the amount of dye adsorbed by a biosorbent and the concentration of residual dye in solution at equilibrium. The supplied literature is dominated by the Langmuir and Freundlich equations, although Temkin, Dubinin-Radushkevich, Sips, Toth, Redlich-Peterson and several other multi-parameter models have been employed [28,79,88-103]. The Langmuir model assumes a finite number of adsorption sites and the maximum adsorption capacity estimated by this model. The capacity parameter of the Langmuir model is specially useful to compare adsorption systems. The Freundlich model, however, is an empirical expression that accommodates heterogeneous adsorption behavior and does not constrain a finite saturation capacity. Three- or multi-parameter models are more flexible and can describe adsorption in wider concentration ranges and may account better for surface heterogeneity. However, more adjustable parameters can be added for improving the numerical fitting, without necessarily providing better mechanistic insight and can increase the danger of overfitting [28,79,88-103].

Thus, the selection of an appropriate model must extend beyond the search for the equation with the highest coefficient of determination. The parameters of the isotherm should be determined, whenever possible, by nonlinear regression of the original equilibrium data instead of by linear transformations. Model evaluation should include parameter uncertainty, residual distributions, appropriate error or information criteria, and model parsimony [28,77]. In addition, the estimated maximum capacities should be interpreted with caution when saturation has not been experimentally approached, since extrapolation far outside the investigated concentration range may result in unrealistic capacity estimates. Adsorption capacities of different biosorbents can only be compared to each other when the experimental conditions are sufficiently similar. Important factors are temperature, solution pH, ionic strength, initial and equilibrium concentration ranges, biosorbent dosage, and preparation or pretreatment of the sorbent [28,77]. So isotherm modeling should be considered more as a quantitative description of the equilibrium behavior under the given experimental conditions, rather than conclusive evidence of a specific adsorption mechanism.

Thermodynamics and activation energy

The thermodynamic analysis is often combined to the dye adsorption and biosorption studies in an effort to evaluate the influence of the temperature on the equilibrium and to describe the energetic behavior of the adsorption process. The most important parameters reported are the standard Gibbs free energy change (ΔG°), the standard enthalpy change (ΔH°) and the standard entropy change (ΔS°) [49,75,104-106]. With the standard-state treatment specified, a negative value of ΔG° is, in general, taken to indicate thermodynamic favourability. The variation of ΔG° with temperature gives information on the variation of favourability over the temperature range investigated.

The sign of ΔH° is often used to describe the overall thermal nature of the process. A positive sign indicates that the reaction is endothermic and a negative sign indicates that the reaction is exothermic. ΔS° is related to changes in the degree of disorder at the solid-solution interface. Dye-biosorption systems present a wide variation and the studies presented both positive and negative enthalpy changes, indicating that the effect of temperature is dependent on the specific dye-biosorbent combination and experimental conditions [45,53,60,61,64,67,72,107-110]. However, thermodynamic calculations should be treated with caution. In particular, the equilibrium constant that appears in logarithmic thermodynamic expressions should be dimensionless. Therefore, direct use of the dimensional Langmuir constants, distribution coefficients or other related adsorption parameters without proper conversion may lead to thermodynamically inconsistent estimates of ΔG° [75]. Likewise, an oversimplification is the classification of the adsorption as strictly "physisorption" or "chemisorption" on the basis of the magnitude of ΔH° .

The mechanisms of adsorption can be based on a combination of different interactions and therefore should be substantiated by independent experimental evidence [75]. The activation energy that characterizes the temperature dependence of the adsorption kinetics provides additional information and can be estimated using the rate constants obtained at different temperatures by applying the Arrhenius relation [49,104]. However, such an analysis only makes sense if the kinetic constant being compared relates to the same process and has a consistent mechanistic interpretation over all the temperatures studied. Thermodynamic and activation-energy parameters should thus complement rather than replace kinetic, equilibrium, spectroscopic and other mechanistic evidence.

Bibliometric re-analysis and research landscape

Bibliometric analysis provides a useful approach for examining the structure, dominant themes, and relationships within a body of scientific literature. The original document presented a VOSviewer analysis derived from a relatively narrow Scopus search that identified publications related to adsorption, dyes, and bacteria. In the present review, the scope has been broadened from an emphasis on a particular dye to the wider field of dye removal and biosorption. Accordingly, the bibliometric component was also reconsidered so that it more closely reflects the broader conceptual framework of the revised review (Figs. 1 to 3). Rather than implying that a new comprehensive Scopus dataset was retrieved, the present analysis uses terms occurring within the 145 references assembled for this review.

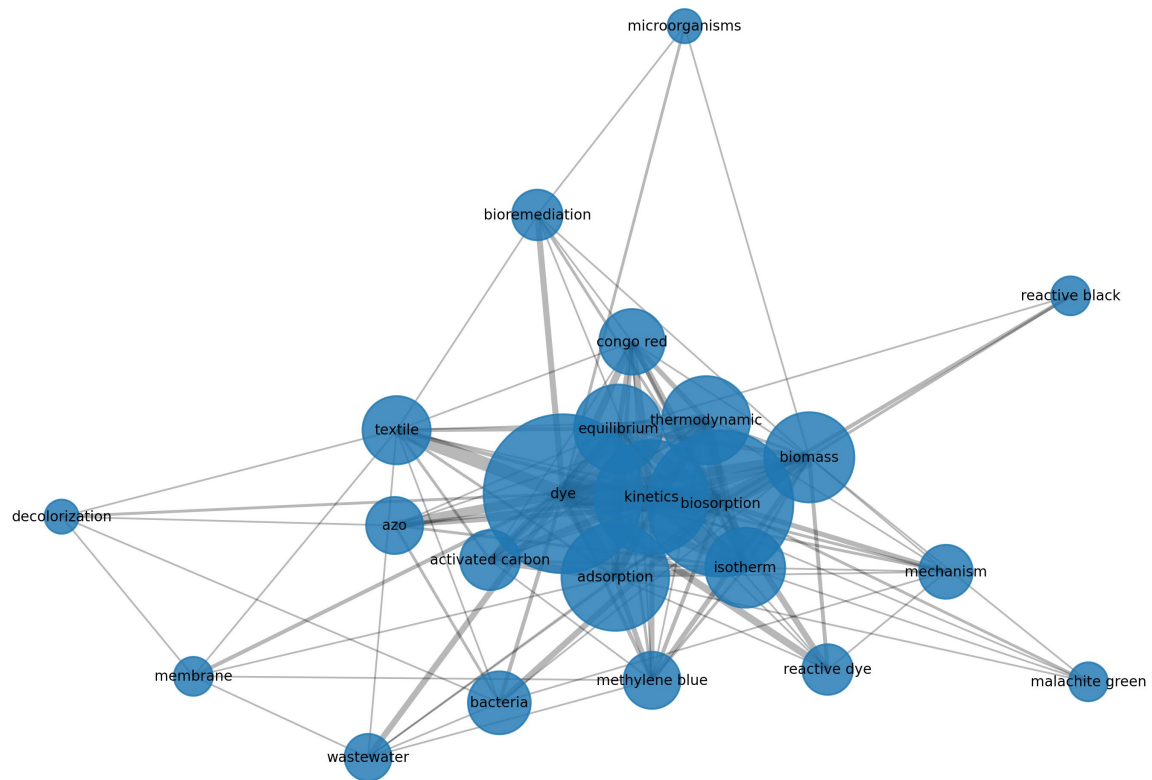


Figure 1. Reference-list-based keyword co-occurrence network of the literature included in this review on dye removal and biosorption. Node size represents the relative frequency with which a term occurs within the reference collection, while links indicate co-occurrence of terms within the same references. Larger and more strongly interconnected nodes represent themes that are more prominent within the supplied literature.

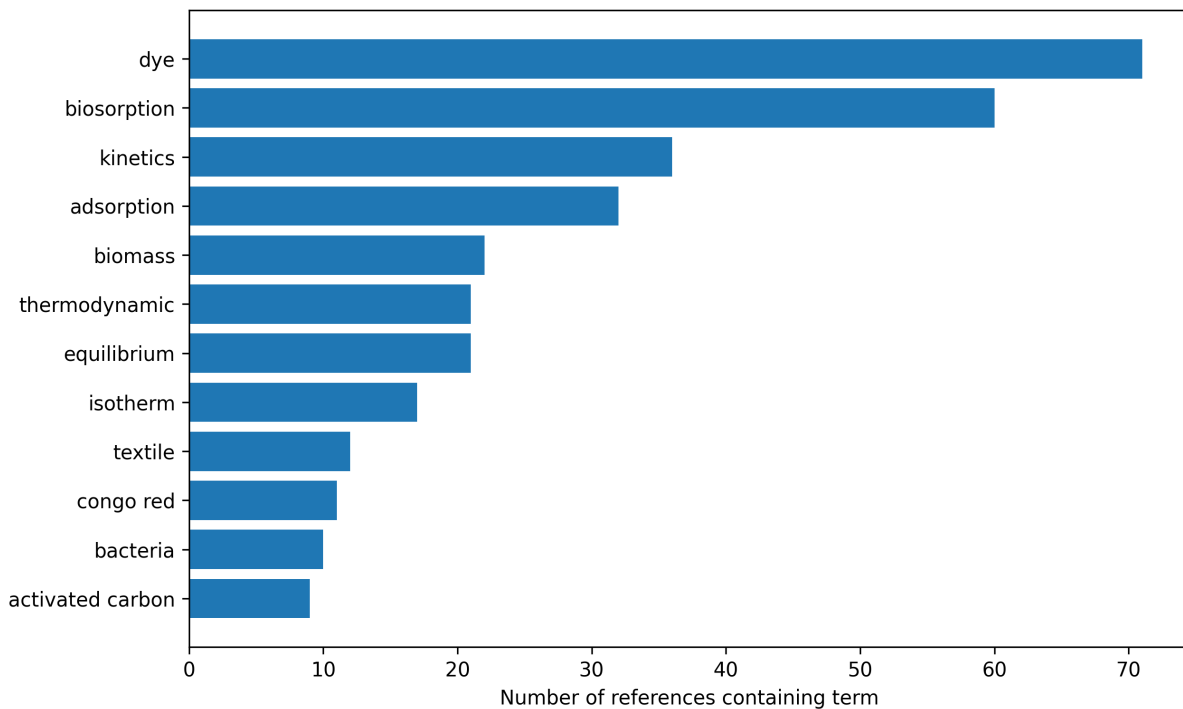


Figure 2. Most frequent thematic terms identified in the supplied reference list.

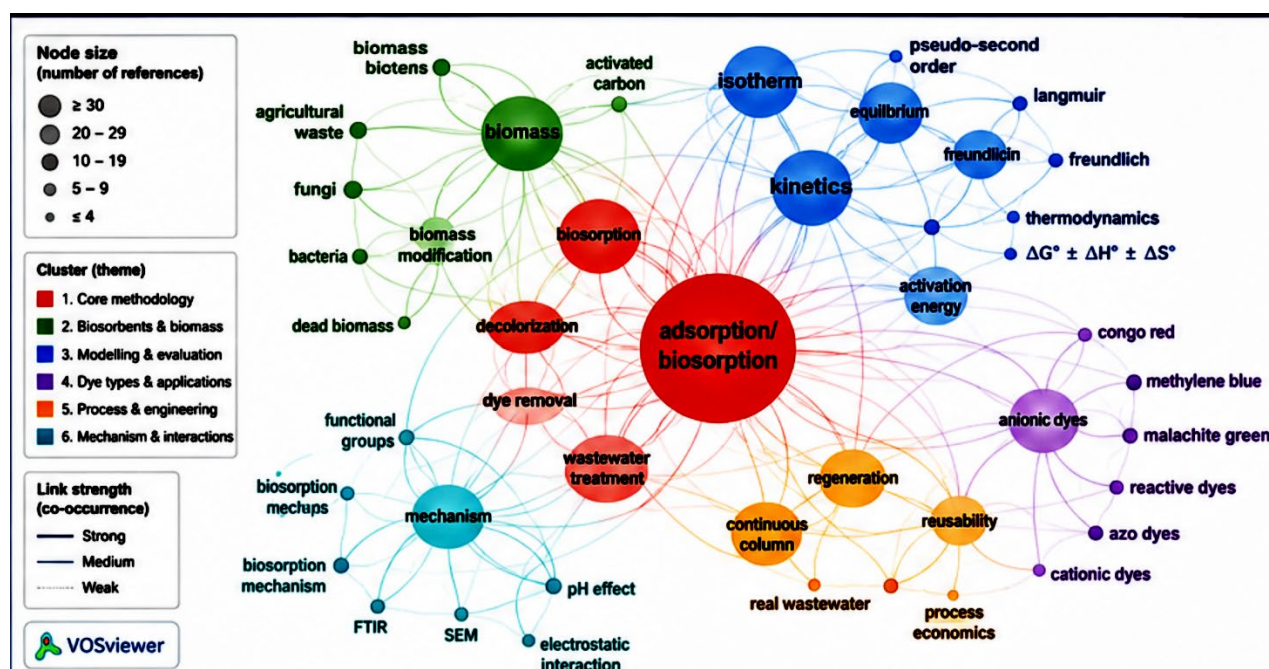


Figure 3. Frequency distribution of the major thematic terms identified within the reference list used for the present review. The relative frequency of individual terms indicates the major research topics represented within the assembled literature on dye removal, adsorption, and biosorption.

The resulting maps should therefore be regarded as a VOSviewer-style, reference-list-based co-occurrence analysis of the supplied literature rather than a comprehensive representation of the entire Scopus-indexed literature on dye remediation. The co-occurrence approach identifies terms that repeatedly appear within the supplied references and examines their relationships based on their occurrence within the same publications. Frequently occurring and highly connected terms consequently occupy more prominent positions within the network, whereas more specialized topics occur toward its periphery. This approach provides a reproducible means of visualizing the intellectual structure of the literature used to construct the present review. Nevertheless, the interpretation of the resulting network should remain restricted to this reference collection because its composition reflects the literature selected for inclusion in the review (Fig. 1).

The resulting research landscape is centred primarily on adsorption and biosorption, demonstrating that sorption-based approaches constitute a major methodological theme within the literature assembled for this review. These central concepts are strongly associated with microbial biomass and with investigations of adsorption kinetics, equilibrium behaviour, isotherm modelling, and thermodynamics. Their close association indicates that much of the literature has focused not only on demonstrating dye removal but also on mathematically describing the rate, capacity, and equilibrium characteristics of the process. This pattern is consistent with the extensive application of kinetic and equilibrium models discussed in the preceding sections (Figs. 2 and 3).

Individual model dyes, including Congo red, methylene blue, malachite green, and reactive dyes, appear as more application-specific components of the network. Their distribution supports the broader treatment of dyes as a heterogeneous group of contaminants rather than defining dye remediation according to the behaviour of a single model compound. Differences in ionic charge, molecular structure,

functional groups, and other physicochemical characteristics can consequently be considered explanatory variables influencing interactions between dyes and biosorbents. The bibliometric pattern also highlights an important imbalance within the supplied literature. Considerable attention has been directed toward batch adsorption experiments, kinetic modelling, equilibrium isotherms, and thermodynamic interpretation, whereas topics associated with practical implementation appear less prominent. In particular, continuous-flow treatment, biosorbent regeneration and reuse, treatment of complex real wastewater, process economics, scale-up, and full-scale validation are comparatively underrepresented. This difference suggests that the field has developed a substantial experimental and modelling foundation but that further effort is required to translate laboratory-scale biosorption studies into practical treatment technologies. Future investigations should therefore increasingly connect fundamental adsorption studies with regeneration, continuous reactor operation, realistic wastewater matrices, process sustainability, and techno-economic assessment.

Research gaps and priorities

Although promising advances have been made in the development of adsorption and biosorption techniques for removal of dyes, some relevant research gaps still impede the transfer of these methods from laboratory experiments to practical applications for wastewater treatment [28,39–41,111]. This is widespread, and a major limitation is the reliance on single model dyes under highly controlled experimental conditions. Such systems are useful for elucidating adsorption mechanisms and comparing biosorbent performance, but industrial effluents generally contain chemically diverse mixtures of dyes together with salts, surfactants, auxiliaries, organic compounds, metals and other contaminants [20,41,112,113]. Future works should then move from single dyes to multicomponent systems able to demonstrate competitive adsorption, synergistic or antagonistic effects, and biosorbent selectivity changes [19,20]. Closely related to this limitation is the need to validate promising biosorbents with real wastewater

rather than predominantly synthetic dye solutions. The pH, ionic strength, dissolved organic matter, suspended solids and competing colorants in real wastewater can be very different from those in synthetic wastewater and these factors may impact on the biosorption capacity and kinetics [41,112,113]. The importance of solution pH, biosorbent dosage, initial dye concentration, temperature, contact time and agitation has been extensively demonstrated, indicating that performance obtained under optimized laboratory conditions cannot necessarily be extrapolated directly to complex wastewater matrices [22,37,41,48,49,79,81,82].

Another important research area is the regeneration of biosorbents and the calculation of a complete mass balance of the pollutants. High dye-removal percentages alone do not reflect the sustainability of the process since biosorption mainly transfers contaminants from the aqueous phase to a solid phase and does not necessarily destroy the contaminants [39,40,42,111]. Hence, future research should quantify the desorption efficiency, regeneration performance, retention of adsorption capacity over repeated cycles, the subsequent fate of recovered dyes and safe disposal or beneficial reuse of exhausted biomass [39,42,79]. More attention should be paid to continuous-flow systems. Most of the experimental investigations still focus on batch adsorption while more realistic representation of treatment of industrial wastewater is continuous or fixed bed configurations. Hence, breakthrough behavior studies, bed-depth effects, mass-transfer-zone development, column regeneration and mathematical modeling are of particular importance for scale-up as demonstrated by fixed-bed investigations of dye biosorption [83]. Mathematical modeling also needs to improve. Preferably, instead of using only linearized forms of adsorption models [28,77,85–87,114], the kinetic and equilibrium data sets should be analyzed by statistically sound nonlinear regression and suitable goodness-of-fit criteria. Confidence intervals on parameters, reporting of parameter uncertainty, residual behavior and model-selection statistics would allow more reliable comparison between kinetic and isotherm models and less unwarranted mechanistic interpretation of empirical fittings [28,77,86,114].

Future treatment strategies should also integrate biosorption with processes capable of destroying the trapped dye molecules. Biosorption offers an efficient mechanism for concentrating pollutants, which can subsequently be altered or mineralized by microbial enzymes, biodegradation, photocatalysis or similar oxidative processes [17,112,115]. These hybrid approaches could overcome the fundamental limitation of dye transfer from wastewater to another material and may be particularly useful for chemically complex or recalcitrant dye mixtures [17,28]. Finally, adsorption capacity, percentage removal as well as environmental and economic performance should be evaluated in future studies. Technology assessment should include parameters such as availability, preparation and regeneration costs of biosorbents, chemical and energy requirements, waste generation, process scalability, and overall environmental impacts [39,42,111,116]. It is especially important to consider techno-economic and environmental assessments because an adsorbent that shows good capacity in the laboratory might not be a sustainable treatment material when considering its preparation, regeneration, disposal and operational requirements [42,116]. Addressing these research gaps in concert would help advance dye biosorption research from demonstrations of high removal percentages in idealized laboratory settings to **robust, regenerable, scalable, economically viable, and environmentally sustainable technologies for treating real industrial dye-containing wastewater [28,39–41,111].

CONCLUSION

Dye pollution should be thought of as a family of treatment problems rather than the behavior of one representative compound. The interaction of a colorant with water, treatment reagents and biosorbent surfaces is governed by dye charge, chromophore and molecular architecture and application chemistry. Adsorption and biosorption are still attractive due to their simplicity and adaptability. Microbial biomass offers a very diverse surface chemistry for possible low-cost valorization of biological residues. However, high batch adsorption capacity alone does not necessarily translate to practical applicability. The subsequent steps of dye-biosorption research should focus on mixed and real effluents, regeneration, continuous systems, rigorous non-linear modeling, mechanistic validation and sustainability analysis. Such a transition would bridge the vast equilibrium and kinetic literature to realistic applications in wastewater treatment and resource recovery.

DECLARATION OF COMPETING INTEREST

The authors declare no competing financial interests or personal relationships.

ETHICS APPROVAL

Not applicable

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AUTHORS' CONTRIBUTION

The conceptualization of the study was led by I.A.S. Data analysis was conducted by I.A.S. with the remaining authors contributing to the review and editing.

DATA AVAILABILITY STATEMENT

Not applicable

AI USAGE DECLARATION

During the preparation of this work, the authors used generative AI technologies solely for image generation, language editing and refinement. All of the authors reviewed and edited the content. The authors take full responsibility for the final content of the publication.

CONFLICTS OF INTEREST

The authors declare no competing interests exist.

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