

RESEARCH ARTICLE

Composite hydrogels based on calcium alginate and polyethyleneimine for wastewater treatment

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Abstract

Water shortage has risen severely in recent years, confronting mankind with a worldwide challenge, especially as the accessible water resources are further limited by diverse contamination. The most widespread industrial process for water treatment is the activated sludge process, in which, however, excessive sludge production has become an enormous environmental problem worldwide. To overcome this problem, hydrogels possess outstanding potential in view of adsorptive removal of contaminants like heavy metal ions, fertilizers, and dyes. In this paper, we report about the synthesis of biobased alginate hydrogel beads along with polyethyleneimine-modified composite hydrogels for water treatment. The adsorption of methylene blue as a positively charged and of congo red as a negatively charged model dye is quantitatively investigated, both separately and in combination. In addition, the pH-dependent adsorption of the dyes is determined. The use of alginate-based hydrogel systems combines several prospects: they are bio-based, inexpensive, easily available to a sufficient extent, sustainable, and are applicable in a broad range of wastewater treatment by its charged groups.

KEYWORDS

calcium alginate, composite hydrogel, dye adsorption, polyethyleneimine, wastewater treatment

1 | INTRODUCTION

Much of the earth's water resources are inaccessible to humans, as they are present in the form of salt water or in polar ice.¹ At the same time, global water shortage arises from resource overexploitation, anthropogenic interventions in the hydrological cycle, the climate crisis, human made water pollution, and a geological redistribution of water resources.² As a result, it is necessary to maximize the availability of sufficient quantities of drinking water. This can be achieved by either desalination of sea water and the purification of wastewater. The most

common technical process for wastewater treatment is the activated sludge process.³ However, excessive sludge production has become an enormous environmental problem worldwide, mainly due to a steadily growing demand for drinking water.⁴ Hence, attempts for optimization of existing technologies and replacement by alternative biochemical or physicochemical methods are in the focus of researchers and plant operators. For this purpose, hydrogels are a promising material platform.

One of the key features of hydrogels is their ability to swell in water instead of dissolving. In combination with their strong hydrophilic properties given by their ionic

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moieties, this offers great potential in utilizing this class of materials for wastewater treatment through adsorption mechanisms. In this context, the fast swelling kinetics and capacities, modifiable surface characteristics, high surface areas, controllable pore structures and permeabilities, and pH dependencies lead to a wide range of wastewater treatment applications.⁴ Due to this characteristics, various hydrogels are able to bind pollutants, heavy metal ions, nutrients, and toxic organic molecules such as dyes by adsorption.⁵

To truly benefit from the favorable features of hydrogels, though, they must be optimally adapted to the requirements of the application, so they will work effectively, sustainably, and resource saving. To replace the activated sludge process and improve the process of wastewater purification, it is necessary to fulfill some requirements: First, the adsorption system has to be adjusted specifically to the targeted impurities. Second, the used material should be on a reusable or at least biodegradable basis. Third, the system should be non-toxic and compatible with microorganisms and multicellular organisms. Fourth, it is essential that the system is available in almost unlimited amount and based on renewable resources. On top of these necessities, another desirable property would be a long durability to keep the material and cost expenditure low.

To fulfill these various requirements, the following strategical corners appear suitable: First, to tailor the adsorption system, a polymer-based hydrogel system in which the properties can be properly adjusted by the specific synthesis of the chemical structure of the polymer chains should be used. On top of that, the molecular network of the hydrogel should be capable of being engineered to incorporate functional groups that have the ability to bind with targeted contaminants. This molecular engineering can significantly improve the efficiency of hydrogels in wastewater treatment. Second, to obtain a reusable or degradable system, polymer strands that build up the network should be crosslinked reversibly. This can be achieved by non-covalent chain interconnection, such as hydrogen bonding, hydrophobic interactions, crystallite formation, or complexation.⁶ Third, to obtain a non-toxic system compatible with microorganisms and multicellular organisms, the network should be based on biopolymers (or synthetic, bio-compatible polymers). Fourth, to design such a polymer system, the biopolymer or at least the monomer itself should be accessible out of regrowing raw materials.

One of the potential candidates that fulfill these diverse criteria is alginic acid. It is a polysaccharide consisting of a copolymer of two 1,4-glycosidically linked uronic acids: β -D-mannuronic acid and α -L-guluronic acid. This linear polymer is highly hydrophilic with a

great extent of biodegradability and can be easily cross-linked. In the presence of divalent cations, it forms a three dimensional physically crosslinked polymer network in which the cations are coordinated in an egg-nest structure by carboxyl and hydroxyl groups of the guluronic acid blocks. According to the HSAB concept, the affinity and strength of these physical crosslinks decreases in the sequence of the cations cadmium, barium, copper, calcium, nickel, cobalt, and manganese. A particularly favorable feature of alginic acid is that it is derived as a by-product of the extraction of iodine out of brown algae⁷ and is extensively available in the market, with relatively low price (sodium alginate costs 23.16 € per kilogram⁸ compared to PEG 4000 45.53 € per kilogram⁹).

From the perspective of water purification, a key feature of alginate hydrogels (ALG) is that they consist of a negatively charged backbone that can interact with positively charged contaminants from wastewater due to electrostatic interactions. However, a similar treatment of wastewater containing anionic contaminants is not efficient with alginate hydrogels. To overcome this limitation, sodium alginate can be mixed with cationic polymers like polyethyleneimine (PEI) before introducing Ca^{2+} to form a composite gel (ALG/PEI). With that, the network of the resulting hydrogels contains both negatively- and positively charged functional groups, and thereby, can form electrostatic interactions with both cationic and anionic contaminants.

So far, a number of polymer systems and hydrogels have yet been studied for wastewater treatment, since the purification by means of adsorption is a quick, cheap, and environmentally friendly process. For example, Sha et al. and Sinha et al. reviewed several hydrogel systems capable to remove organic dyes, toxic chemical drugs, pharmaceuticals or inorganic (heavy) metal ions due to adsorption.^{10,11} Further literature is focusing on alginate hydrogels. In this context, various organic dyes are often chosen as a model system of water contamination, since they represent a major contribution to industrial water pollution themselves, and in addition, can be well detected analytically.¹² Jeon et al. described the investigation of methylene blue adsorption on alginate and polyaspartate composite hydrogels.¹³ Hassan et al. studied the methylene blue adsorption on composites of activated carbon and calcium alginate.¹⁴ Benhouria et al. used composite hydrogels of betonite, activated carbon, and calcium alginate to probe methylene blue adsorption for the treatment of wastewater.¹⁵ Zhu et al. studied the adsorption of congo red on composite hydrogels of polyethyleneimine and cellulose.¹⁶ He et al. described the preparation of composite hydrogels of calcium alginate and polyethyleneimine for use in biochemical and biomedical applications, whereby the polyethyleneimine adheres to the backbone of the calcium alginate system.^{17,18}

In further work, composites of polymer systems of different compositions and morphologies were in focus, resulting in altered chemical and physical properties, such as enhanced mechanical and thermal stability and pronounced swelling.^{19,20}

Despite of all that effort, however, the adsorption of congo red and methylene blue on composite hydrogels of calcium alginate and polyethyleneimine have not been studied. This is detrimental, because the organic dye methylene blue makes a major contribution to industrial water pollution and is also easy to trace analytically. Furthermore, methylene blue has already been used several times in comparable studies in the literature with other hydrogel systems, so that a comparison of the results is possible.¹² Congo red, in turn, is an oppositely charged dye to methylene blue, so that the adsorption of both positively and negatively charged water pollutants on the hydrogel systems can be investigated. With the alginate system and the composite system out of alginate and polyethyleneimine, a negatively and a positively charged hydrogel species is used respectively, which is both sustainable and biocompatible. To close this gap, this work aims to investigate the preparation and properties of a composite hydrogel of calcium alginate and polyethyleneimine, with a particular focus on the adsorption of the organic dyes methylene blue and congo red as an attractive alternative to the currently used activated sludge process for water treatment.

To realize that goal, we prepare hydrogel beads based on calcium alginate and form a composite hydrogel with polyethyleneimine through the use of a millifluidic method to investigate and compare the adsorption of the above-mentioned organic dyes into the hydrogel beads. In particular, the swelling and degradation of the hydrogels is investigated in different solutions. Additionally, the capacity of dye uptake of methylene blue and congo red is determined. Furthermore, the influence of electrostatic interactions is investigated with regard to the adsorption selectivity; we find that the adsorption process is based on an electrostatic interaction of opposite charged molecules.

2 | RESULTS AND DISCUSSION

2.1 | Preparation of alginate based and polyethyleneimine-modified hydrogel beads

The goal of this work is to develop and investigate a hydrogel system based on alginate and polyethyleneimine and its adsorptive properties for a more effective and sustainable process for water treatment compared to the activated sludge method. For this purpose, a simple and well-defined sample geometry in the form of

hydrogel spheres is used. These specimens can be produced and observed easily, and can most efficiently be rinsed by sample solution while forming a dense packing.

We prepare hydrogel spheres of calcium alginate using a millifluidic method. For this purpose, an aqueous solution of sodium alginate (Figure 1A) consisting of blocks of the uronic acids β -D-mannuronic acid (m-blocks, gray) and α -L-guluronic acid (g-blocks, red) as polymer substrate is dropped into a calcium chloride solution that acts as a gelation bath by a syringe pump. The calcium cations are complexed in an egg-box geometry by the g-blocks of alginic acid, forming a three-dimensionally crosslinked calcium alginate network (Figure 1B). With this method, colorless hydrogel spheres are obtained with an average diameter of $d = (2.8 \pm 0.2)$ mm.

Analogously a composite hydrogel consisting of polyethyleneimine and alginate, ALG + PEI, is prepared to study the adsorptive properties on a modified hydrogel matrix (Figure 1C). The corresponding hydrogel beads exhibit an average diameter of $d = (2.7 \pm 0.2)$ mm.²¹ In both cases, a low dispersity and uniform appearance of the hydrogel species are observed. Concerning the role of the polycationic additive, polyethyleneimine is resistant to biological influences, but degradable by chemical processes. PEI has antimicrobial activity and can be used for disinfection, as it acts against microorganisms by either destroying them or inhibiting their growth.²² Due to its polycationic structure, it adheres to the polyanionic polysaccharide alginate. Both polyelectrolytes, alginate and polyethyleneimine, do not form a simple layered structure, but instead the charged polymer chains mutually interpenetrate and are electrostatically crosslinked.¹⁸

2.2 | Characterization of the gel system

2.2.1 | Molar mass determination of sodium alginate and polyethyleneimine

The molar mass distribution (MMD) of sodium alginate is determined by size exclusion chromatography (SEC). The elugramm is transferred into a molar mass distribution using a calibration curve of narrowly distributed pullulan standards and PSS WinGPC UniChrom software, yielding a number average molar mass of $M_n = 1.81 \times 10^5$ g·mol⁻¹, a weight average molar mass of $M_w = 6.00 \times 10^5$ g·mol⁻¹, and a polydispersity index of $\mathcal{D} = 3.31$. The molar mass distribution for sodium alginate is shown in Figure 2.

The molar mass of polyethyleneimine is taken from the supplier with a weight-average molar mass of $M_w =$

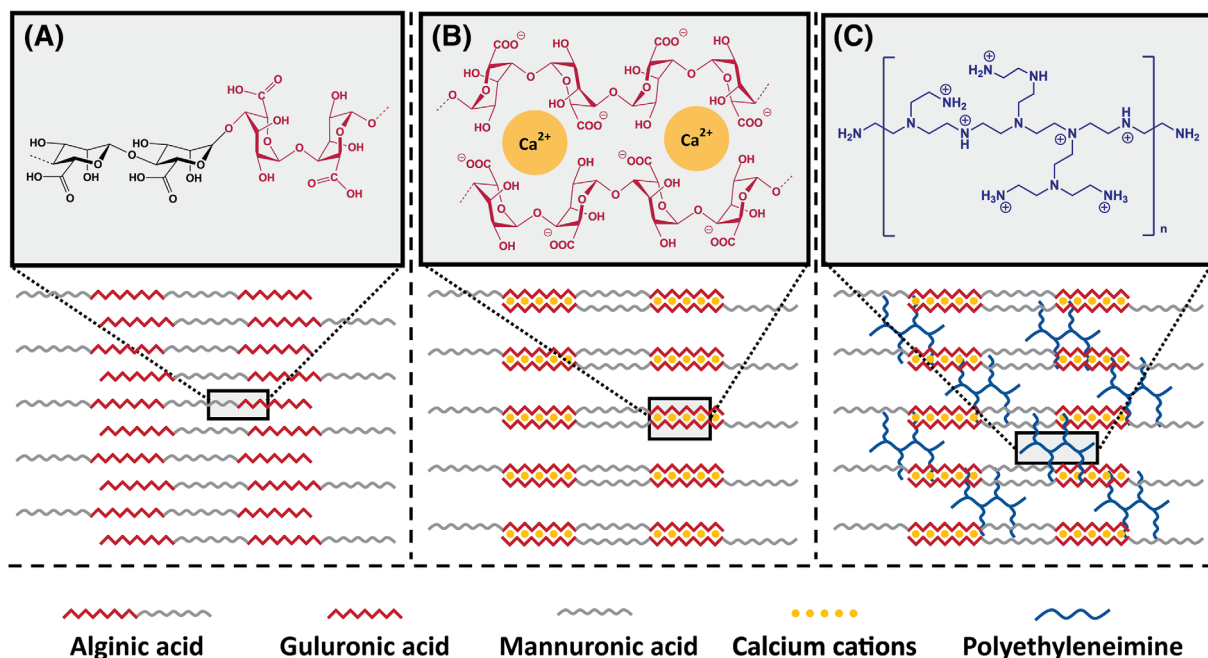


FIGURE 1 Schematic representation of the polymer systems used in this work. (A) Alginate polymer strands based on blocks of the uronic acids β -D-mannuronic acid (m-blocks, gray) and α -L-guluronic acid (g-blocks, red). (B) Crosslinking of linear alginate polymer strands to form a three-dimensional polymer network based on complexation of divalent cations in an egg-box coordination. (C) Incorporation of the polycationic polyethyleneimine (blue) into the alginate network. The polyelectrolytes penetrate each other and adhere due to intermolecular electrostatic interactions.

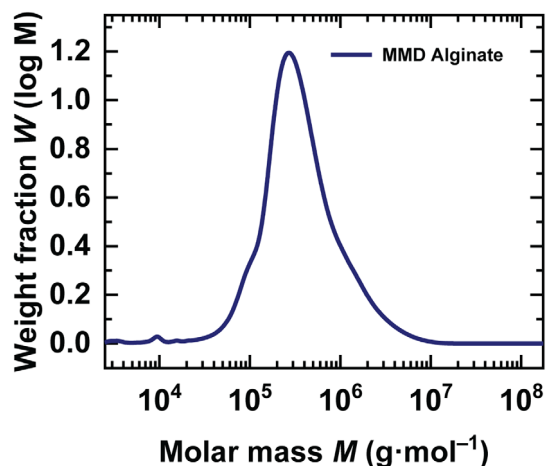


FIGURE 2 Molar mass distribution (MMD) of the sodium alginate investigated by size exclusion chromatography.

$2.5 \times 10^4 \text{ g}\cdot\text{mol}^{-1}$ and a number-average molar mass of $M_n = 1.0 \times 10^4 \text{ g}\cdot\text{mol}^{-1}$ with a polydispersity index of $\mathcal{D} = 2.5$.

2.2.2 | Investigation of viscometric and mechanical properties by oscillatory shear rheology

Rheological experiments are performed on macrogels with a concentration of 4 wt.%. An amplitude sweep is

performed at various angular frequencies to determine the linear viscoelastic region. From this, a shear deformation of $\gamma = 0.03\%$ is selected at which subsequent frequency sweeps are performed. Figure 3A shows the frequency sweeps of the two hydrogel species. All four graphs represent an approximately linear plot. The alginate and composite polymer samples are physically crosslinked gels, which macroscopically behave like elastic solids. If these are macroscopically deformed, they return to their unperturbed state when the stress is released. Consequently, an elastic plateau is expected in the rheological spectrum. The small deviation from a constant modulus is due measuring artifacts. The gels could not be produced with uniform thickness, so that a positive force had to be used to press the samples onto the plate.

As already observed in the amplitude sweep, the linear viscoelastic range between angular frequencies of 0.02%–0.05% is only small. The reason for the observed slightly positive slope is the use of a plate-plate geometry and the deviation from linearity can be addressed to a shear force gradient.

The values of the storage and loss moduli of the calcium alginate hydrogel are higher than the measured values of the composite hydrogel modified with polyethyleneimine. Although a quantitative discussion of the absolute values of the measured data is not possible in detail due to the above-mentioned

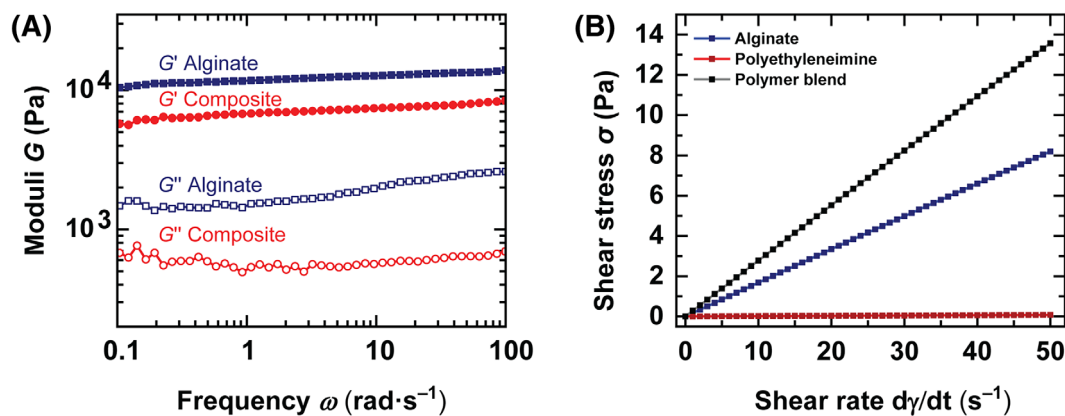


FIGURE 3 Investigation of viscometric and mechanical properties by oscillatory shear rheology. (A) Rheological investigation of the storage (closed symbols) and loss moduli (open symbols) of the calcium alginate and calcium composite gel. The moduli of the alginate gel exceed the moduli of the composite hydrogels. (B) Viscometric investigation of a 4 wt.% alginate solution, a polyethyleneimine solution (4 wt.%), and a polymer blend solution consisting of 4 wt.% alginate and 4 wt.% polyethyleneimine each. The polymer blend exhibits the highest viscosity. The alginate solution is also fairly viscous. Only the polyethyleneimine solution largely approximates the viscosity of water.

sources of error, the data sets for the alginate macrogel and the composite hydrogel are in good agreement with expectation. As depicted in Figure 3A, the ALG/PEI composite gels possess lower G -moduli, which suggests a stiffer mechanical elasticity of the ALG gel compared to the composite. Modification with PEI leads to a reduction in mechanical stability compared to the pure alginate gel. This observation can be interpreted by a reduction of the crosslinking density due to replacement of the Ca^{2+} ions by the positive PEI chains, which are only monovalent compared to Ca^{2+} .

Figure 3B shows the results of viscosity determination of a 4 wt.% aqueous alginate solution, polyethyleneimine solution, and a mixture of these two polymers. Shear stress is plotted against shear rate, and the viscosity is given by the slope of a linear regression, showing a pure Newtonian response. It can be observed that the aqueous solution of polyethyleneimine has the lowest viscosity of $\eta = 1.53$ mPa·s. Due to its tenfold higher molar mass, the aqueous solution of the sodium alginate has a much higher viscosity of $\eta = 169$ mPa·s. The composite gel possesses the highest viscosity of $\eta = 274$ mPa·s. This finding can be explained by entanglement of oppositely charged polymer strands.

The main difference in the characteristic of the Ca^{2+} -Alginate and the PEI/Alginate system is the charge/size ratio of the (poly)cation according to the HSAB concept. Divalent cations are able to complex the carboxylic groups of the alginate polymer strand, while polycations intercalate between the negatively charged alginate chains.

2.2.3 | Swelling tests on the alginate matrix and alginate composite matrix modified with polyethyleneimine

Wastewater consists of a variety of (aqueous) solutions and organic compounds and does not have a constant pH value. In commercial industrial and wastewater treatment plants, immense costs and resources need to be spent to pre-treat the wastewater so that the activated sludge process can be applied. This is necessary because microorganisms and bacteria used for water treatment require a slightly alkaline environment, so that the pH value of the wastewater must be adjusted to a pH value between 6 and 8. It would therefore be useful to have a system in which such limitations can be omitted. As a result, the requirement for a new technology for wastewater treatment has to be resistant to all these factors, to be versatile, and at the same time highly efficient in the actual application. Hence, the behavior and the resistance of the filter material to different aqueous and organic waste compositions is of high interest. For this purpose, degradation and swelling tests in different media of the pure alginate-based hydrogels and of polyethyleneimine alginate composite hydrogels are performed. We place the hydrogels in three different liquid media: ultrapure water as well as aqueous solutions of methylene blue and congo red.

$$Q = \frac{m_{\text{swollen}}}{m_{\text{dried}}} \quad (1)$$

The degree of swelling is described in Equation (1). It is determined as mass-weighted degree of swelling in the

form of a ratio of the mass of the swollen hydrogels divided by the mass of the dried gels.²³

The mass-weighted degree of swelling Q is plotted against time t for the pure alginate hydrogels in Figure 4A. Hydrogels in congo red show an initial swelling followed by degradation. This is due to the capability of congo red to complex calcium ions in competition with the carboxylic groups of the alginate. Gels stored in water by contrast show an initial shrinking up to a state of equilibrium of constant mass of absorbed water. For the alginate spheres in methylene blue solution, the mass decreases continuously but less than with congo red.

The results of the swelling tests of the composite hydrogels (ALG + PEI) over a period of 28 days are displayed in Figure 4B. The composite beads retain their shape in all media and show a higher stability against swelling and decomposition compared to the pure alginate gels. The dyes methylene blue and congo red are absorbed by the hydrogels stored in the respective dye solutions. Furthermore, hydrogels in a congo red solution show an increased degree of swelling despite the competitive complexation of the calcium ions by congo red. The positively charged dye methylene blue is adsorbed to the polymer backbone, leading to a contraction of the network due to stronger electrostatic interaction indicating that not all negative charges on the alginate backbone are screened by PEI so far. No remarkable changes over 28 days are observed for the composite gels stored in deionized water as well as for the alginate gel, after a state of equilibrium of the swelling is reached. Finally, we observe a good solvent stability for the alginate–polyethyleneimine composite material. This result is of great importance in the sense that the composite systems offer better resistance of the material to various

contaminants, organic substances, solvents, and pH values, and therefore a long service life for the filter material.

2.2.4 | Degradation under UV irradiation

In addition to the previously investigated stability with respect to the components of the wastewater, the use of a method for wastewater treatment is not regionally limited, but must be applicable in a wide range of environmental conditions. UV-stability is therefore of high relevance, because filtration units are naturally exposed to ultraviolet radiation by daylight, and also because ultraviolet radiation (UVC) is commonly used for water disinfection. Hence, we also track the degradation by irradiation with ultraviolet radiation of wavelength $\lambda = 365$ nm. This wavelength of the ultraviolet spectrum is chosen because it is in a range that is not absorbed by the ozone layer and the oxygen in the atmosphere and therefore can reach the surface of the earth. By exposition with UV-light of wavelength $\lambda = 365$ nm, the alginate hydrogels initially show low mass losses. After 16 h, the loss of mass increases sharply until the hydrogels are completely degraded after 26 h (Figure 5A).

The composite hydrogels display a nearly linear loss in mass with time. The durability is 100 h longer than the alginate hydrogels, and full degradation finally occurs after 126 h (Figure 5B). Hence, the modification with PEI makes the system remarkably more stable. Because of this remarkably prolonged life time under UV irradiation, filter materials consisting of this composite material then need to be replaced less often and are thus more

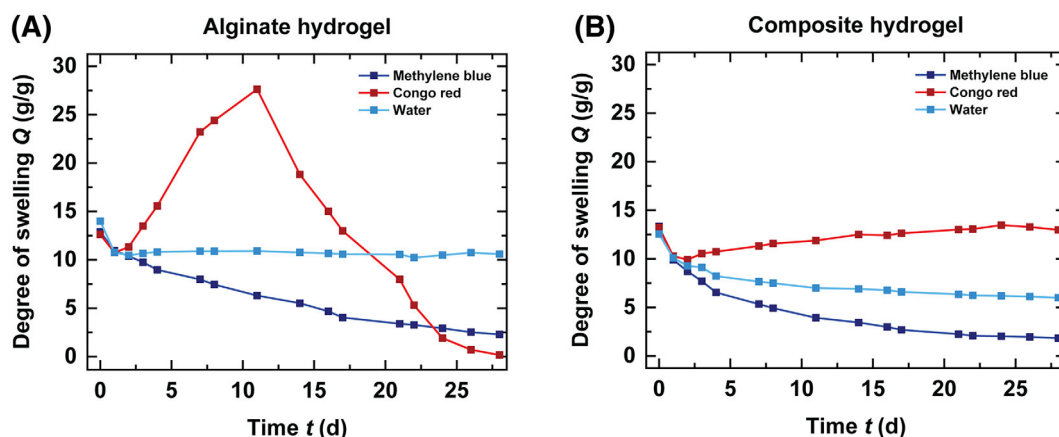


FIGURE 4 Plots of the mass-weighted degree of swelling Q against time t . The solvents ultrapure water and aqueous solution of methylene blue or congo red are used as liquid media. (A) shows the mass-weighted swelling degree for the ALG hydrogels. (B) displays the mass-weighted degree of swelling for ALG + PEI composite hydrogels.

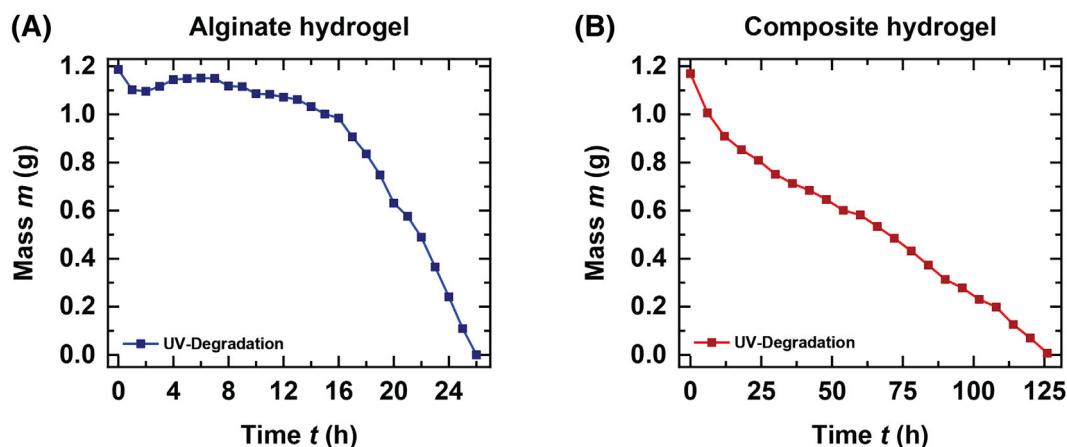


FIGURE 5 Time dependent mass loss of the hydrogels by degradation under UV-irradiation with wavelength $\lambda = 365$ nm. (A) The ALG hydrogels fully degraded after 26 h of irradiation. (B) Degradation of the composite hydrogels modified with polyethyleneimine, showing a linear loss of mass during 126 h.

sustainable in terms of environmental impact, material use, and costs. Nevertheless, the experiments with ultraviolet radiation show the need to protect the hydrogels from the influence of ultraviolet radiation. Yet, the average power of sunlight detectable at the surface of the earth can be estimated by the solar constant of $0.1366 \text{ W}\cdot\text{cm}^{-2}$. The applied irradiated power here of $6 \text{ W}\cdot\text{cm}^{-2}$ is larger by a factor of minimum 44, so a corresponding longer stability of the polymer system can be assumed.²⁴

2.3 | Dye adsorption experiments

While the swelling and stability tests against solvents, ultraviolet radiation, and mechanical effects of the hydrogels represent an essential part of the research in new technologies for wastewater treatment, the key step in treatment by means of hydrogel systems is the adsorption of impurities by the polymeric matrix. Without adequate adsorptive properties, the system would not offer a meaningful substitute for the activated sludge process. Hence, the investigation of the adsorptive properties of the alginate–polyethyleneimine system is a core part of this work. In general, such adsorption processes occur in three steps. In a first step, molecules diffuse from the solvent medium through the boundary layer into the hydrogels. In a second step, intra-hydrogel diffusion occurs. Finally, in a third step, molecules are adsorbed onto the polymer-network strands that constitute the hydrogel.²⁵ In our work, various dyes are used, since they represent a major contribution to industrial water pollution, and in addition can be well detected analytically.¹² Figure 6 shows the molecular structures of the six dyes used in this paper, three negatively charged

(A congo red, B methyl orange, C xylene orange) and three positively charged (D malachite green, E methylene blue, F crystal violet).

2.3.1 | Qualitative dye adsorption

For a first qualitative test of the adsorption properties of alginate and alginate-polyethyleneimine hydrogels, six different dyes with different molecular structure and electric charges are used. PEI-modified composite hydrogels have positive charges in their polymer backbone and therefore adsorb negatively charged dyes like congo red (A), methyl orange (B), and xylene orange (C). The ALG hydrogels are characterized by negative charges on the polymer backbone and therefore show good adsorptive properties for the positively charged dyes malachite green (D), methylene blue (E), and crystal violet (F). The adsorption can be tracked optically, as depicted in Figure 7.

Figure 8 represents the quantification of the dye uptake of the six dyes used on the respective hydrogel system. The three negative dyes congo red (A), methyl orange (B), and xylene orange (C) are absorbed by the composite hydrogel with an amount of 70%–80% from the solutions. The three positive dyes malachite green (D), methylene blue (E), and crystal violet (F) are removed from solution by the alginate hydrogel to an extent of 70%–75%.

The opposite combination of negatively charged congo red (A), methyl orange (B), and xylene orange (C) on the alginate hydrogel and of positively charged malachite green (D), methylene blue (E) and crystal violet (F) on the composite hydrogel show no adsorption at all due to repulsion of the equal charges between the dyes and the gel systems and are therefore not relevant.

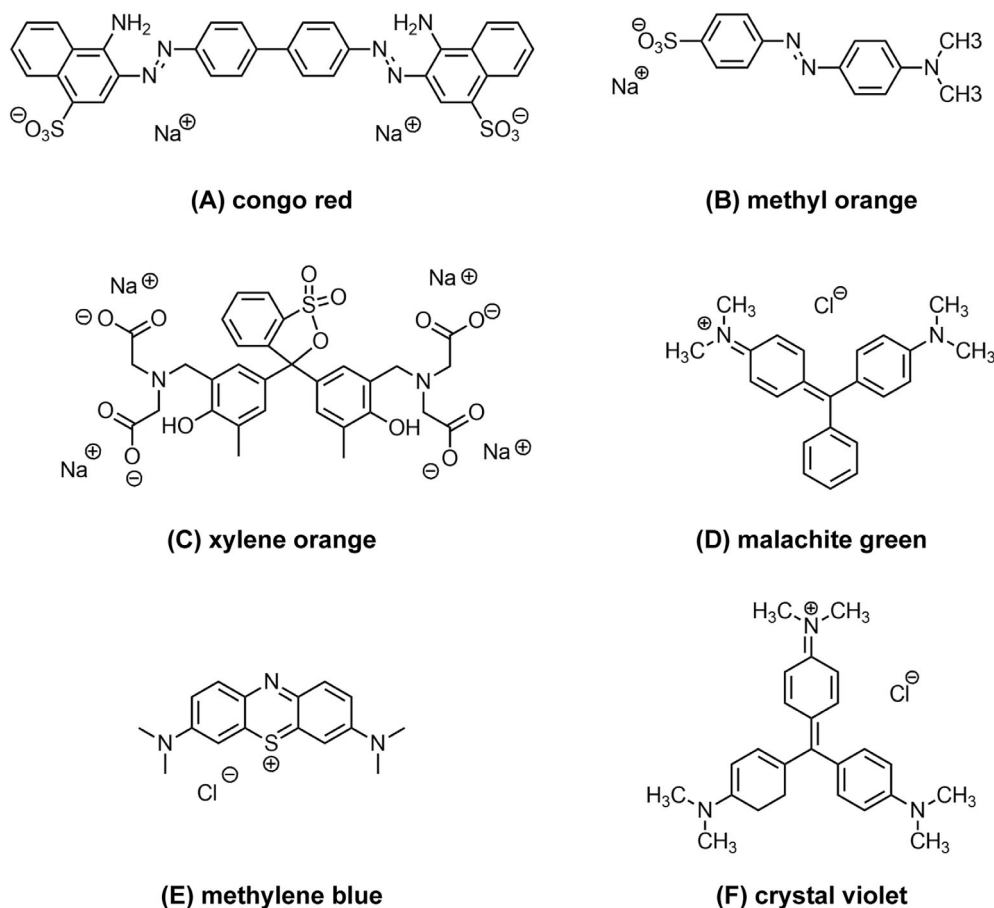


FIGURE 6 Molecular structures of the six different dyes (A) congo red (B) methyl orange (C) xylene orange (D) malachite green (E) methylene blue and (F) crystal violet.

In the following, the dyes congo red and methylene blue are selected on the basis of these measurements to be investigated as model systems because congo red is the most efficiently adsorbed dye by the composite gel and methylene blue that for the alginate gel.

2.3.2 | Determination of the extinction coefficients of the dyes

So far, we have been able to qualitatively investigate and confirm the adsorption of dyes based on the

charge balances. Our composite system differs from the previous alginate system with negative polymer backbone charge due to the positive charge introduced by the PEI. To study the effectiveness of water treatment by adsorption of pollutants into the hydrogels, it is necessary to quantify the dye uptake. To assign the measured values of the intensity of a spectrophotometer to a dye concentration and thus to be able to make a quantitative observation of the dye adsorption, the molar extinction coefficient of the dyes, ϵ , is determined first based on the Lambert-Beer law (Equation 2):

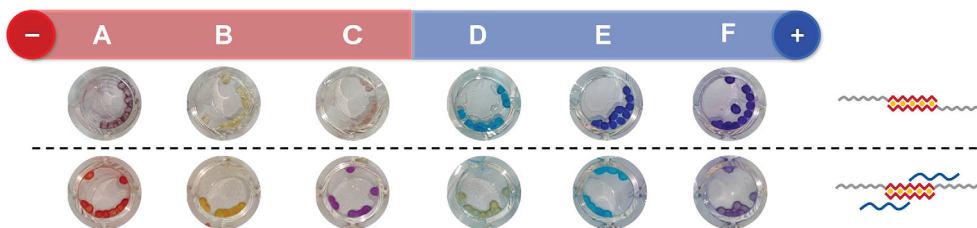


FIGURE 7 Qualitative adsorption experiment of some basic organic dyes. ALG hydrogels (top) and PEI-modified composite hydrogels (bottom) are used for the dye adsorption experiments. Positively charged dyes malachite green (E), celestine blue (F), methylene blue (G), and crystal violet (H) are well adsorbed by the negatively charged alginate hydrogel. Negatively charged dyes congo red (A), methyl orange (B), xylene orange (C), and thymol blue (D) are well adsorbed by positively charged PEI-modified composite hydrogel.

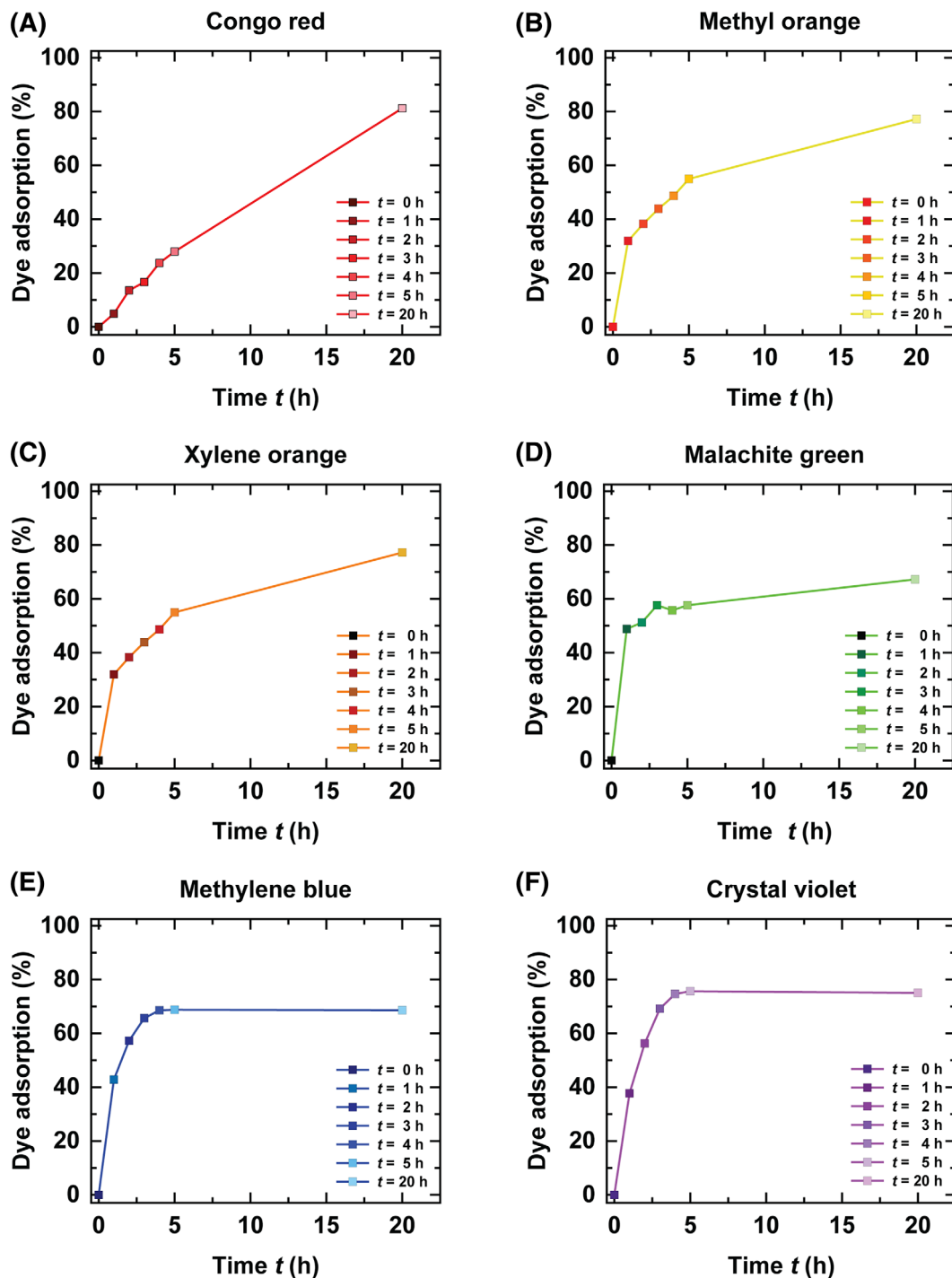


FIGURE 8 Quantification of the dye adsorption of congo red (A), methyl orange (B) and xylene orange (C) on the composite hydrogel and of malachite green (D), methylene blue (E) and crystal violet (F) on the alginate hydrogel. The adsorptions of the dyes A–C on alginate hydrogel and D–F on the composite hydrogel are not shown, since they are of very low magnitude and therefore not relevant for further investigations.

$$A_{\lambda} = \varepsilon_{\lambda} \cdot c \cdot d \quad (2)$$

Here, the absorbance A_{λ} is linearly related to the concentration c and the optical path length d . Dilution series of methylene blue (MB) and congo red (CR) are measured (Figure 9A,B) using a spectrophotometer and the

absorbance (MB: $\lambda = 664$ nm and CR: $\lambda = 497$ nm) is plotted versus concentration. One anionic and one cationic dye are selected from previously six candidates of dyes each.

The dyes methylene blue and congo red are chosen, which had already been used in literature as model

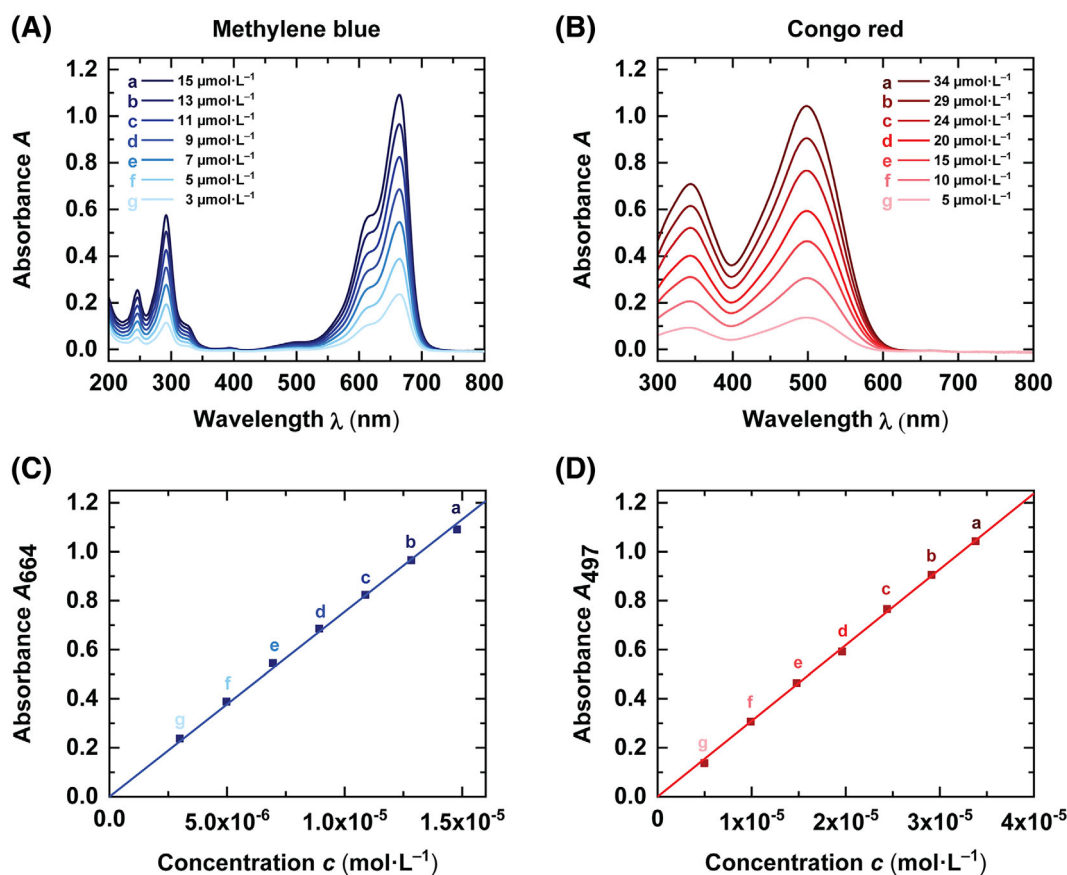


FIGURE 9 UV-Vis spectra and linear calibration fit of various methylene blue and congo red solutions to determine the molar extinction coefficients: (A) Absorption spectra of a concentration series of methylene blue solutions at concentrations between 3 and 15 $\mu\text{mol}\cdot\text{L}^{-1}$ and (B) congo red solutions at concentrations between 5 and 34 $\mu\text{mol}\cdot\text{L}^{-1}$. (C) Concentration-dependent plots of the absorbances at the absorption maxima for methylene blue at $\lambda = 664$ nm and (D) congo red at $\lambda = 497$ nm to determine the molar extinction coefficients ϵ of both dyes.

systems and at the same time showed the greatest optical adsorption. The extinction coefficients are calculated from the slope of the linear fit (Figure 9C,D). ϵ (congo red) is determined to be $30944.4\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$, whereas ϵ (methylene blue) possesses more than the double extinction capability of $75469.3\text{ L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$.

2.3.3 | Quantitative determination of the dye absorption

To monitor the efficiency of dye removal by the polymer gels, a time-dependent dye adsorption experiment is carried out to determine the kinetic course as well as the time-dependent efficiency of the water treatment. For this purpose, the dye adsorption is quantified by spectrophotometric analysis of methylene blue and congo red. In total, four permutations resulting from two different dyes and two hydrogel species are investigated.

Figure 10A,C illustrates the adsorption of methylene blue (MB) on the alginate system. Up to 68% of the positively charged MB molecules are adsorbed on the negatively charged alginate polymer matrix, as calculated from the intensity of the characteristic UV/Vis band at $\lambda = 664$ nm. Figure 10B,D shows the adsorption of MB on the ALG + PEI composite hydrogel system. Only 10% of the positively charged molecules are adsorbed on the positive composite hydrogel system, this might be due to the remaining, not compensated negatively charged groups in alginate.

Figure 11B,D shows no meaningful adsorption of congo red (CR) onto the ALG hydrogels. By contrast, Figure 11A,C display the adsorption experiments on the ALG + PEI composite hydrogel, where up to 82% of the CR molecules are adsorbed. The adsorption is determined by the characteristic congo red UV/Vis band at $\lambda = 497$ nm. In this case, the combination of opposite charges of the dye and polyethyleneimine results in good adsorption characteristics.

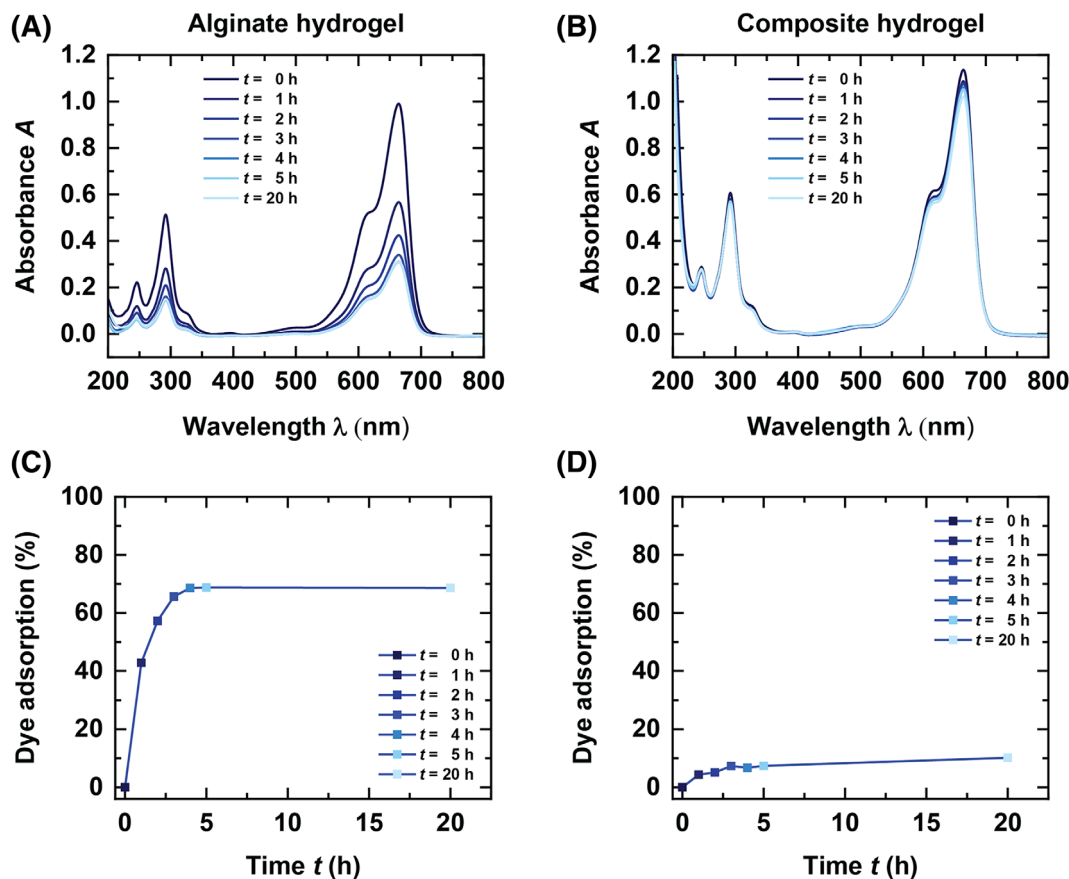


FIGURE 10 Studies on the adsorption of the dye methylene blue on the alginate hydrogels, as well as the composite hydrogels modified with polyethyleneimine over a period of 20 h: Panel (A) shows the adsorption spectra of methylene blue on alginate hydrogels at $t = 0, 1, 2, 3, 4, 5,$ and 20 h. Panel (B) plots the adsorption spectra of methylene blue on an alginate-based hydrogel system modified with polyethyleneimine at $t = 0, 1, 2, 3, 4, 5,$ and 20 h. Panel (C) represents a plot of the percentage dye adsorption of methylene blue by the alginate hydrogels over time during the adsorption experiment. A total adsorption of 68% of the methylene blue molecules is observed. Panel (D) shows a plot of the percentage dye adsorption of methylene blue by the composite hydrogels over time. Only 10% of the dye molecules could be adsorbed from the solution.

It can be deduced from the experiments that high adsorption capacities can be achieved by both the alginate hydrogel and the composite hydrogel for the respective oppositely charged dyes. The adsorption spectra of the alginate hydrogels show that the maximum of methylene blue adsorption is already reached after 4 h. The maximum adsorption of congo red is found after 20 h, whereby a linear course can be observed up to this time. Overall, the adsorption kinetics of methylene blue adsorption is faster than that of congo red adsorption. The maximum adsorption capacity of congo red on the composite hydrogel, however, exceeds that of methylene blue on the alginate hydrogel.

2.3.4 | Determination of adsorption capacities

Up to this point, the adsorption of different dyes was investigated both on a qualitative and on a kinetic level.

Yet, to assess the efficiency of the adsorption, further experiments are necessary. For this purpose, the extinction coefficients yielded in the previous section allow to determine the remaining dye concentrations after dye adsorption by the hydrogels. With this information, it is possible to determine dye adsorption capacities as quantitative values. For this purpose, the number of hydrogel beads is varied and the concentration of the dye solutions is kept constant. The dye adsorption versus the quantity of hydrogel spheres is plotted in Figure 12.

The adsorption of CR by the composite hydrogels shows an additive trend. A doubling of the hydrogel particle number doubles the adsorption capacity, which is determined as $6.69 \cdot 10^{-5} \text{ mol} \cdot \text{g}^{-1}$. During adsorption of MB onto ALG hydrogels, no linear course is observed. The adsorption asymptotically approaches a maximum. The capacity is determined to be $2.31 \cdot 10^{-4} \text{ mol} \cdot \text{g}^{-1}$ out of the first section and initial slope. In that calculation, the reference mass is defined

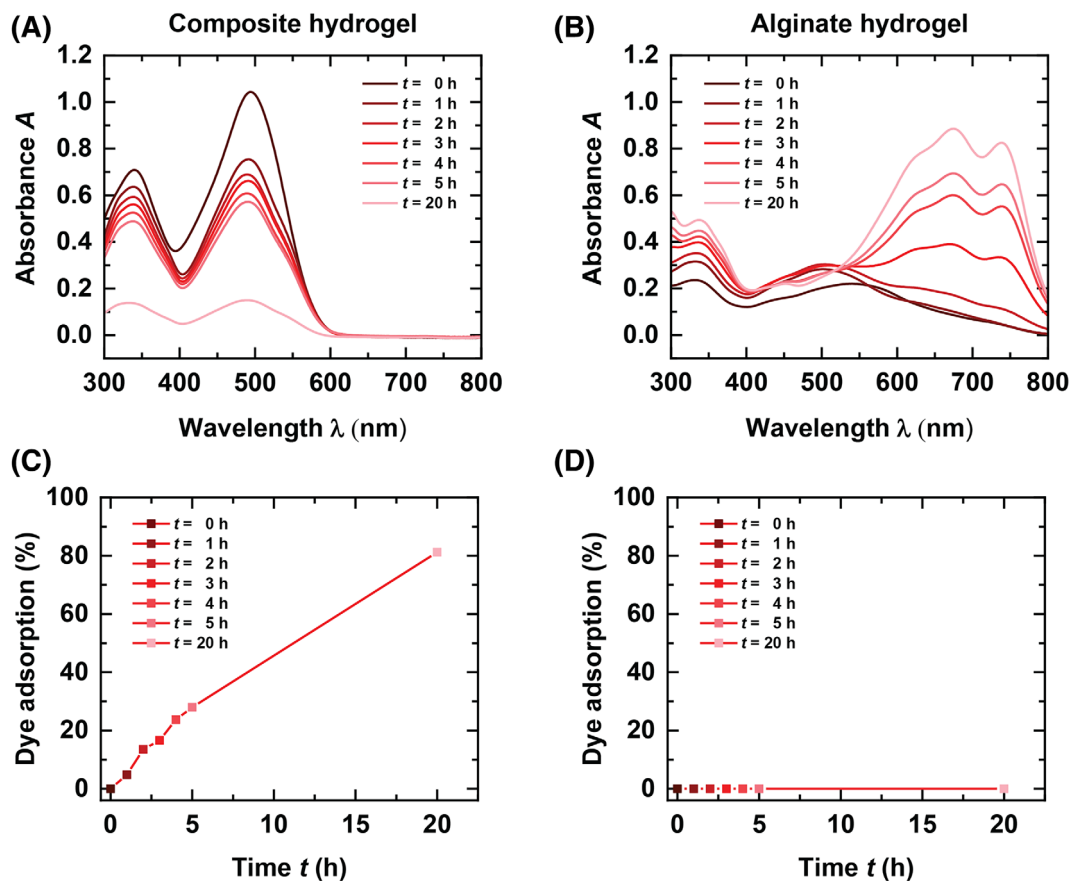


FIGURE 11 Studies on the adsorption of the dye congo red on the composite hydrogels modified with polyethyleneimine, as well as the alginate hydrogels over a period of 20 h: Panel (A) shows the adsorption spectra of congo red on an alginate hydrogel system modified with polyethyleneimine at $t = 0, 1, 2, 3, 4, 5,$ and 20 h. Panel (B) depicts the adsorption spectra of congo red on an alginate hydrogel system at $t = 0, 1, 2, 3, 4, 5,$ and 20 h. In this case, contrary to expectations, adsorption occurs due to a complexation reaction between congo red and the calcium cations. Panel (C) represents a plot of the percentage dye adsorption of congo red by the composite hydrogels over time. Overall, 82% adsorption of congo red molecules is observed. Panel (D) shows no dye adsorption of congo red by the alginate hydrogels.

as the dry mass of the hydrogels, which is first determined by freeze-drying a sample of the alginate or composite hydrogel.

2.3.5 | Variation of the pH value

So far, various experiments on qualitative, quantitative, and kinetic dye adsorption, a broad comparison of the alginate hydrogel system and the novel composite hydrogel with polyethyleneimine modification has been done. In addition to these investigations under standard laboratory conditions, however, the real conditions in an industrial water treatment plant are of great interest.

Waste water treated in such plants is not a homogeneous compound, so it is first necessary to investigate the resistance to different solutions, solvents, and pH values during the design of the test specimens. However, not

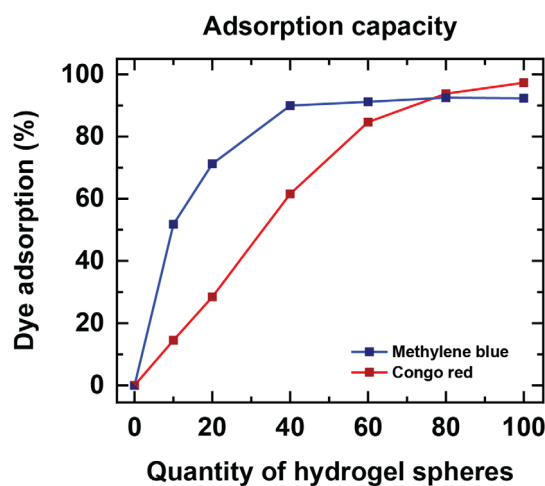


FIGURE 12 Relative dye adsorption as a function of the number of hydrogel beads, plotted to determine the adsorption capacities of the hydrogel systems.

only the resistance of the hydrogel spheres is affected by a changing pH value. An important starting point in those investigations is the question of the pH dependence of the dye adsorption with regard to the effectiveness of the purification of the dirty water samples.

Microorganisms and bacteria in current wastewater treatment plants require a neutral environment. For this purpose, the pH value needs to be adjusted between 6 and 8, and a large amount of resources is used for neutralization of the sewage.²⁶

The pH dependence of dye adsorption on hydrogels is investigated and plotted in Figure 13. For high and low pH values (2 and 12), dye adsorption is strongly reduced compared to the other samples. Between a pH value of 4 and 10, the dye adsorption increases slightly with increasing pH for CR, whereas it decreases for MB. This is because MB is protonated in acidic milieu, so there is a concentration of positive charges and therefore a better adsorption at lower pH values. By contrast, the sulphonate groups of CR are protonated with decreasing pH, so the negative charge is reduced and the adsorption decreases with lower pH values. Furthermore, just small reductions of dye adsorption up to 10% are observed while varying the pH value. Hence, alginate-based hydrogels are superior to commercial systems for water treatment in terms of pH dependence.

It is shown that the adsorption of contaminants by the hydrogels is much less pH dependent than the previously used activated sludge method in wastewater treatment plants. When using hydrogels, an effective adsorption range between pH values of 4–10 is found. The activated sludge method, on the other hand, is only possible in a pH range of 6–8, which is a significant disadvantage compared to our described method.

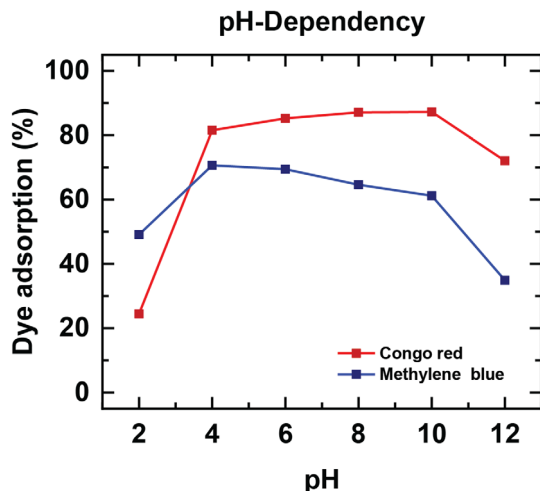


FIGURE 13 Dye adsorption against pH values from 2 to 12 for the dyes methylene blue and congo red.

2.3.6 | Microstructure control as an optimization test of adsorption kinetics

The hydrogel spheres investigated so far are simple monodisperse spheres with a smooth surface. Despite their simple synthesis procedure and their homogeneity, the spheres have a relatively small surface and pore area. To improve the diffusion processes into and in the gel matrix to improve dye uptake efficiency, we employ a templating method to introduce pores into the hydrogel matrix, which offers the possibility regulate the flow of liquids into the filtration material.^{27,28} In this method, calcium carbonate templates are decomposed, calcium cations are complexed by the G-blocks of alginic acids strands, and released carbon dioxide fills the sites and forms a porous structure.^{20,29,30} Following that strategy, porous gels are prepared with CaCO₃ contents of 5, 10, 15, 20, and 25 wt.%. Additionally, different grain sizes of 5 and 50 μm are added and then dissolved under acidic conditions.

The porous structure is investigated by confocal laser scanning microscopy (Figure 14A). Fluorescent pictures of the porous gels are recorded after staining the calcium cations with tetracycline by complexation (green fluorescence image). With this, the degree of gelation of the gels could be investigated. In addition, brightfield transmission-mode images of the pores in the hydrogel are taken to examine the porous structure (gray image). The pores are not completely uniform in size, but the gel matrix between these pores is of uniform thickness. The staining in the fluorescent images shows a uniform distribution of the calcium cations and thus a homogeneous cross-linking density of the polysaccharide strands.

A dependence of the volume flow on the calcium carbonate content is observed (Figure 14B). An increasing calcium carbonate addition increases the number of crosslinking points in the gel, so a higher calcium cation content yields a more stable gel and thus leads to higher flow rates. Decreasing calcium content causes a collapse of the spherical structures. As a consequence, dense packing in columns is not possible due to a lack in mechanical stability.

Dye adsorption on hydrogels without templating additive (MB00 & CR00) and porous hydrogels with 25 wt.% calcium carbonate with a particle size of 5 μm (MB05 & CR05) and 50 μm (MB50&CR50) using methylene blue and congo red as model dyes are discussed below. Methylene blue adsorption experiments on non-porous alginate hydrogels is inferior to porous hydrogels (Figure 15A). Experiments on hydrogels with templates of 5 and 50 μm grain size show a preferential dye adsorption of 74% and 80%, respectively, compared to the one of non-porous hydrogels around 72%. These findings could be explained

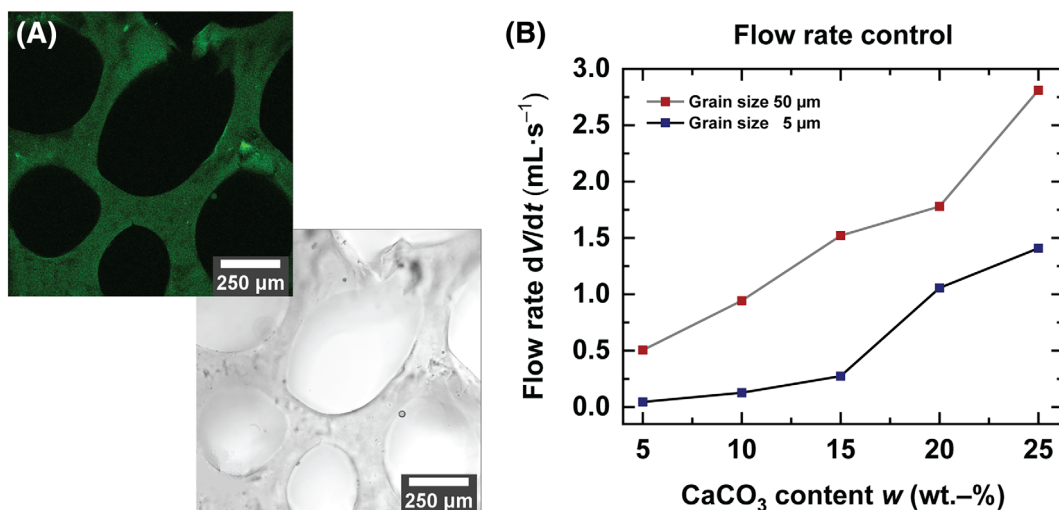


FIGURE 14 Confocal microscopy images as well as flow-through experiments recorded on porous hydrogels prepared by a templating method. (A) Fluorescence image taken after using tetracycline for staining the calcium cations. (B) Transmission image of the polymer matrix and pores by confocal microscopy. (C) Plot of the flow rate ($\text{dV}/\text{d}t$) of water as liquid medium against the CaCO_3 template content.

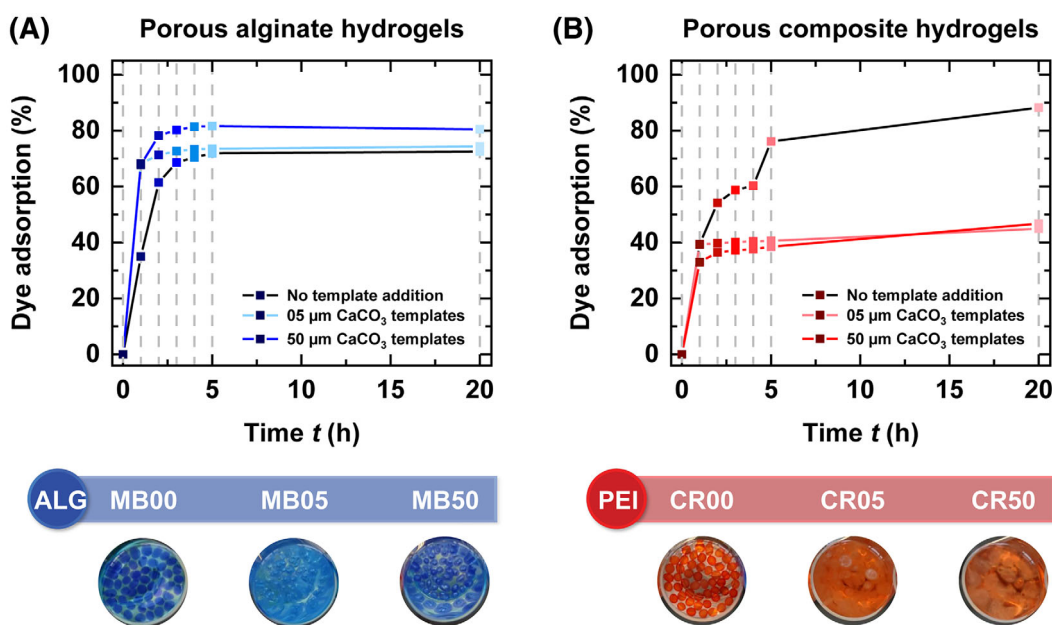


FIGURE 15 Dye adsorption of methylene blue (MB) and congo red (CR) studied on hydrogels without pores (00) and on porous hydrogels that are produced by a template method with calcium carbonate grain sizes of 5 μm (05) and 50 μm (50). (A) Methylene blue adsorption experiments on non-porous alginate hydrogels (72%) has inferior properties to porous hydrogels (74%–80%). (B) Adsorption on composite hydrogels showed the non-porous composite hydrogel (88%) exceeding the adsorption capacity of the porous species (44%–46%).

by an increasing number of pores resulting in a larger surface area and shorter diffusion pathways, resulting in rapid penetration of the matrix by dye molecules.

Adsorption on composite hydrogels reveal that the non-porous composite hydrogel outperforms the adsorption capacity of the porous species (Figure 15B). While very high absorbance of congo red (88%) is observed in

non-porous hydrogels, the adsorption efficiencies of porous hydrogels prepared by templating method are only half the size (44%–46%). The introduction of the templating method in the composite gel species results in more unstable and thus softer gels. Finally, the pores in the system collapses, so that the number of pathways available for diffusion is reduced. If, however, the pores

are collapsed, less dye adsorption is possible because the diffusion pathways are closed. Accompanying rheological studies reveal mechanically lower stability of the PEI-modified system in comparison to pure alginate hydrogels.

2.3.7 | Selective dye adsorption

So far, the dye adsorption of the dyes has only been studied isolated from each other. Dirty water, however, does not usually consist of isolated impurities, but generally contains many different contaminants at the same time. Therefore, it is necessary to investigate the removal of various impurities simultaneously.

Methylene blue and congo red form a salt with low solubility. Therefore, it is not possible to conduct these dye adsorption tests at the same time. A total amount of 40 beads, 20 of each species, are first suspended in a methylene blue solution and subsequently transferred to a congo red solution. The pure alginate adsorbs the positively charged methylene blue, whereas the modified

hydrogel beads only adsorb a small amount. In contrast to this, the modified hydrogel spheres adsorb a higher amount of the red dye. The selective adsorption on the respective system is due to electrostatic interactions between the oppositely charged polymer backbones and the charge of the dye molecules. Dye adsorptions is quantified spectrophotometrically. Figure 16A shows the respective spectra, whereby graph a and b contain the adsorption bands of methylene blue before the adsorption (a) and after the adsorption (b). The congo red adsorption bands are depicted by graph c and d. In total, a MB adsorption of 65% and a CR adsorption of 86% is achieved.

Furthermore, the experimental procedure is inverted for both types of hydrogel beads. A congo red solution is initially adsorbed by the composite hydrogels, and then the spheres are placed in a methylene blue solution. Contrary to our expectations, the methylene blue is not adsorbed by the alginate hydrogels. Only the composite hydrogels enriched with congo red absorb the methylene blue solution, the other beads stay colorless. A black salt is formed between the two dyes. Figure 16B shows the respective spectra of the dye adsorption experiments. Graph a and b

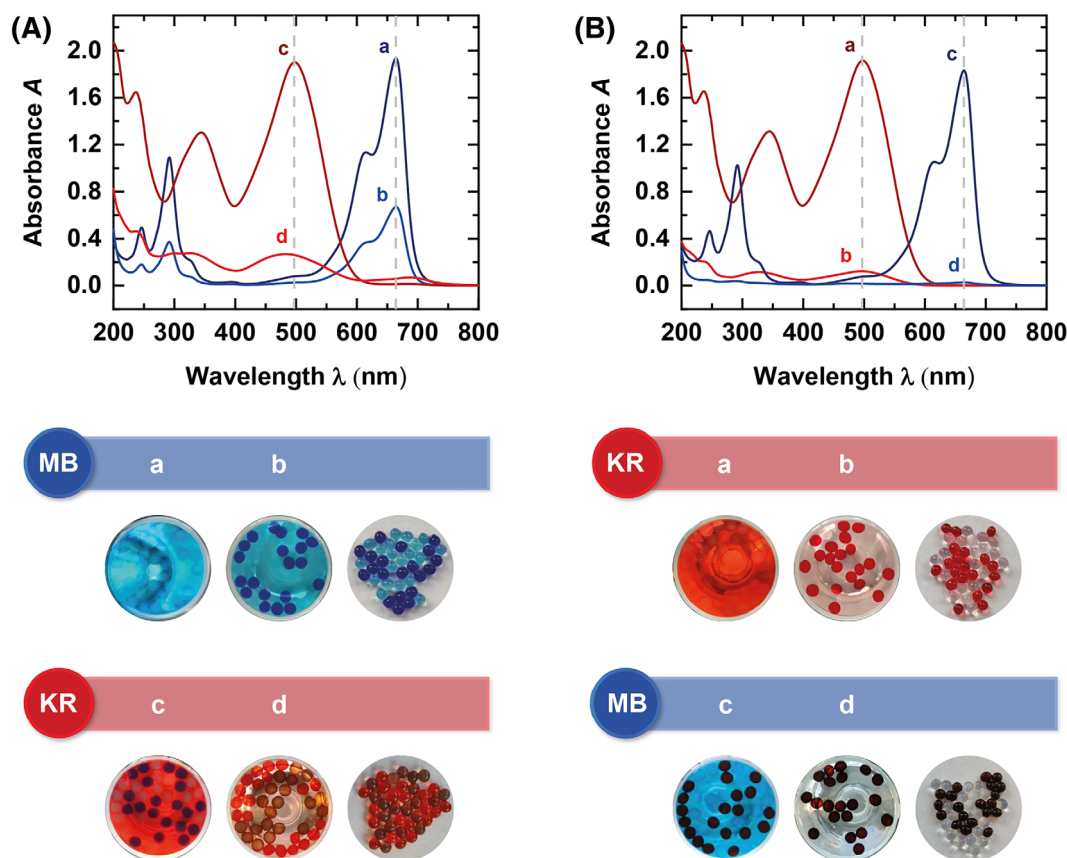


FIGURE 16 UV/Vis spectra of selective dye adsorption of methylene blue and congo red on a mixture of alginate hydrogels and modified composite hydrogels with different dye order. First, a methylene blue solution followed by a congo red solution is added to the hydrogel mixture and the adsorption is investigated spectrophotometrically (A). In a second experiment, first a congo red solution and then a methylene blue solution is added to the hydrogel mixture (B).

show that the congo red concentration in the solution is reduced by 94%. Graph c and d reveal that methylene blue is removed from the solution to an extent of 99%.

The adsorption of the dyes by the hydrogels is selective and not disturbed by the other dyes. In contrast, a simultaneous presence of the dye species leads to a better adsorption result, as seen in Figure 16B. Overall, the adsorption of congo red on the composite hydrogels exceeds the adsorption performance of methylene blue on the alginate hydrogels.

3 | CONCLUSION

In this article, we have described the synthesis of an alginate hydrogel modified with polyethyleneimine and studied its swelling and degradation in various aqueous and organic solutions at different conditions. Adsorption studies showed that selective and quantitative adsorption of a model dye system by the composite hydrogels occurred. While methylene blue was adsorbed by the alginate hydrogels at a rate of $2.31 \cdot 10^{-4} \text{ mol} \cdot \text{g}^{-1}$, congo red was adsorbed by the composite hydrogels at a rate of $6.69 \cdot 10^{-5} \text{ mol} \cdot \text{g}^{-1}$. Studies on the pH dependence of adsorption indicated a wide tolerance range to acidic and alkaline conditions, which far exceeded the application limits of a commercial wastewater treatment plant. To conclude, the polyethyleneimine-alginate composite system is prospectively suited as a treatment system for wastewater. Initial experiments on the reusability of the hydrogel system revealed a very broad and interesting field of research. These experiments will receive further attention in our further research.

4 | EXPERIMENTAL SECTION AND METHODS

4.1 | Chemicals

All chemicals and solvents used in this work were purchased from usual suppliers (Acros Organics, Alfa Aesar, Fisher Scientific, Merck, Roth, Sigma Aldrich, and VWR Chemicals) and used without further purification. Sodium alginate, calcium carbonate (powder $\leq 50 \mu\text{m}$ 98%), celestine blue (80%), thymol blue, polyethyleneimine (branched, 25 k) and tetracycline (98%) were obtained from Sigma Aldrich. Calcium carbonate (powder $5 \mu\text{m}$, 99.5%) and malachite green (oxalate) were purchased from Alfa Aesar. Calcium chloride (fused, granular) was received from Fisher Scientific. Congo red, crystal violet, methyl orange, xylene orange and the millipore ultrapure water system were acquired from Merck. Methylene blue (trihydrate) was used from Acros Organics.

4.2 | Preparation of hydrogel beads

4.2.1 | Alginate adsorbant beads

A 4 wt.% solution of the sodium salt of alginic acid in ultrapure water was prepared. For this purpose, sodium alginate (2 g) was dissolved in ultrapure water (48 g) under reflux and stirring at a temperature of 60°C overnight. A clear, yellowish solution was obtained. This solution was then drawn up into an injection syringe ($V = 50 \text{ mL}$, BD plastic) and an injection cannula (Braun Sterican $0.80 \times 120 \text{ mm}$) was attached. A 4 wt.% solution of calcium chloride (24 g) in ultrapure water (576 mL) was prepared, and a magnetic stirring core was added.

The injection syringe containing the substrate solution was clamped into the holder of a syringe pump (PhD Ultra - Harvard Apparatus), the needle was bent in a 90° arc, and the addition was started. We used a flow rate of $1000 \mu\text{L} \cdot \text{min}^{-1}$, and the substrate was dropped into the gelation solution. After addition was complete, the formed substrate droplets were left to complete gelation for an additional 4 h in the gelation solution. Subsequently, these now clear and colorless hydrogel beads were separated from the calcium chloride solution and rinsed three times with deionized water and then once with ultrapure water. The hydrogel spheres were then stored in ultrapure water, which was changed daily for the first 5 days to remove excess calcium chloride from the hydrogels.

4.2.2 | Polyethyleneimine-modified adsorbant beads

The preparation of the composite hydrogel spheres modified with polyethyleneimine was carried out analogously to the production of the alginate hydrogel spheres described before. After sodium alginate (2 g) was dissolved in ultrapure water (48 g), PEI (2 g) was added and this mixture was homogenized using a laboratory shaker. Subsequently, the resulting substrate was drawn up into an injection syringe and dropped into a 4 wt.% solution of calcium chloride (24 g) in ultrapure water (576 mL). At a flow rate of $1000 \mu\text{L} \cdot \text{min}^{-1}$, the substrate was added and the hydrogel spheres were left in the gelation solution for additional 4 h. These were separated from the calcium chloride solution and washed three times with fully demineralized water and then once with ultrapure water. The ALG/PEI composite hydrogel spheres were stored in ultrapure water, which was changed daily for the first 5 days after production to remove excess calcium chloride from the hydrogels.

4.3 | Characterization of the gel system

4.3.1 | Size exclusion chromatography

2 g·L⁻¹ sodium-alginate were dissolved in ultrapure water (Milli-Q Type 1), to which an amount of 0.5 g·L⁻¹ sodium azide was given to prevent algae formation. Additionally, a trace of ethyleneglycol was added as flowmarker to correct for flow rate inhomogeneities. Measurements were performed on an Agilent 1260 Infinity system, equipped with an isocratic pump (Agilent 1260 series), an refractive index detector RID (Agilent 1260), a column oven (Hitachi), and an analytical column set of three 8 × 300 mm Shodex OHPak SB-802.5-804 HQ columns. An amount of 100 µL of the solution was manually injected onto the system and chromatographed with 0.5 mL·min⁻¹ flowrate at 35°C. The elugramm was transferred into a molar mass distribution according to a pullulan calibration curve using PSS WinGPC UniChrom software.

4.3.2 | Viscometry and rheology

Rheological measurements were performed on a MCR 302 modular rheometer (Anton Paar, Graz, Austria) equipped with a PP25 (rheology) or CP50 (viscometry) geometry at a temperature of 25°C that was kept constant by use of a Peltier element. The mechanical spectrum was recorded at a shear deformation of 0.03% in a frequency range of 0.1–100 rad·s⁻¹. A gel disc consisting of 4 wt.% alginate or composite solution was gelled in a Teflon mold using 4 wt.% calcium chloride solution for 45 min. The sample was rinsed three times with ultrapure water and then applied to the rheometer. To determine the viscosity, the shear stress was measured as a function of shear rate in the range of 1–50 s⁻¹. The viscosity was obtained as the slope from linear regression. Each sample was measured three times and the viscosity is given as the mean value. For this purpose, aqueous solutions containing 4 wt.% alginate as well as 4 wt.% PEI and a mixture of 4 wt.% alginate with 4 wt.% PEI were prepared.

4.3.3 | Swelling study with solvent variation

The swelling of the hydrogel spheres was investigated in different solvents. In each case, 100 hydrogel specimens were weighed. These samples were then placed in plastic sample containers and seven different solutions were added: ultrapure water, methylene blue solution ($c = 1$ mM), congo red solution ($c = 1$ mM), sodium hydroxide solution ($c = 100$ mM), hydrochloric acid solution ($c = 100$ mM), Dulbecco's Phosphate Buffered

Saline (DPBS), and isopropanol. Samples were analyzed over a period of 28 days. For this purpose, the gel spheres were separated from the solutions. The hydrogel spheres were weighed and placed back into 20 mL of the sample solutions. Each was weighed three times to compensate for any error in the balance during measurement.

4.3.4 | UV degradation

The investigation of the resistance to ultraviolet radiation of the ALG hydrogel spheres and the ALG/PEI composite hydrogels was carried out using light-emitting diodes of wavelength $\lambda = 365$ nm. In each case, 100 of these hydrogel spheres were weighed to obtain the mass at the start of the experiment. In each case, 15 mL of ultrapure and a magnetic stirrer core were added to these samples and the irradiation with two light emitting diodes, each with a power of 3 W, was started. After 1 h of irradiation in each case, the alginate hydrogel spheres were separated from the solution and then weighed. For the hydrogels modified with PEI, irradiation was stopped after preliminary tests at intervals of 2 h each, followed by analogous weighing. After each weighing, 15 mL of ultrapure water were added and irradiation was continued. Weighing was performed three times in each case to compensate for any error in the balance during measurement.

4.4 | ADSORPTION EXPERIMENTS

4.4.1 | Qualitative tests

Six different dye solutions of concentration $c = 1 \cdot 10^{-3}$ mol·L⁻¹ were prepared. 100 µL of the dye solutions (1 mM) (crystal violet, methylene blue, malachite green, xylene orange, methyl orange and congo red) and 1000 µL of ultrapure water were added. To this solutions, ten hydrogel spheres of ALG or ten hydrogel spheres of the modified ALG + PEI composite gels were added. The samples were shaken for 20 h in the absence of light and then the dye adsorption was evaluated optically.

Additional quantitative experiments on the dye adsorption were performed according to the former described qualitative investigations. Dye solutions of concentration $c = 1 \cdot 10^{-3}$ mol·L⁻¹ were used. Twenty spheres of the respective hydrogel type were placed in snap-lid vials and ultrapure water (7 mL) and the dye solutions (congo red (1 mM; 358 µL), methyl orange (1 mM; 443 µL), xylene orange (1 mM; 375 µL), malachite green (1 mM; 155 µL), methylene blue (1 mM; 177 µL), or crystal violet (1 mM; 250 µL)) were added respectively. The samples were homogenized under exclusion of light for a period of 20 h on a

laboratory shaker. Aliquots were taken at time points $t = 0, 1, 2, 3, 4, 5,$ and 20 h and analyzed by spectrophotometry.

4.4.2 | Determination of the extinction coefficients

To determine the extinction coefficients of the two dye solutions used, the absorbance of the dye solutions was determined as a function of concentration by means of concentration series and the extinction coefficient was received from a linear regression as the slope of the linear fit. Ultrapure water (1 mL) was placed in each of seven micro-reaction vessels. 3, 5, 7, 9, 11, 13, and 15 μL of a methylene blue (1 mM) solution, or 5, 10, 15, 20, 25, 30, and 35 μL of a congo red solution (1 mM), respectively, were added. The solutions were then homogenized on a laboratory shaker and absorbance was determined on the spectrophotometer.

4.4.3 | Quantitative tests

The dyes methylene blue and congo red were used to quantify the dye adsorption. The dye solutions of concentration $c = 1 \cdot 10^{-3} \text{ mol} \cdot \text{L}^{-1}$ were used. Twenty spheres of each hydrogel types were placed in snap-lid vials and ultrapure water (7 mL) and methylene blue solution (1 mM; 105 μL) or congo red solution (1 mM; 245 μL), were added respectively. The samples were homogenized under exclusion of light for a period of 20 h on a laboratory shaker. Aliquots were taken at time points $t = 0, 1, 2, 3, 4, 5,$ and 20 h and analyzed by spectrophotometry.

4.4.4 | Determination of adsorption capacities

A congo red solution (1 mM) and a methylene blue solution (2 mM) were prepared to determine the adsorption capacity. 10 mL of the 2 mM methylene blue solution was added to 10, 20, 40, 60, 80, and 100 alginate hydrogel beads. 2.45 mL of the 1 mM congo red solution and 7 mL of ultrapure water were added to 10, 20, 40, 60, 80, and 100 PEI-modified composite hydrogel beads. Different amounts of the dye solutions were used to limit the maximum intensity and to be within the linear measuring range of absorption spectroscopy. An aliquot of each solution was taken before hydrogel addition, as well as after 20 h of adsorption time. During adsorption, the samples were homogenized in the absence of light. These samples were analyzed spectrophotometrically after the methylene blue samples were diluted to a 1:20 ratio, and the congo red solutions were diluted to a 1:10 ratio. This

was necessary to restore linearity of absorbance in spectrophotometry, which is only present below an absorbance of 1.

4.4.5 | Variation of the pH value

20 alginate hydrogel beads were placed in 14 mL ultrapure water. The pH was adjusted to 2, 4, 6, 8, 10, and 12 using sodium hydroxide and hydrochloric acid solutions ($c = 0.1 \text{ mol L}^{-1}$). 7 mL of these samples were removed and used as reference. To each of the fourteen samples, 210 μL of a methylene blue solution ($c = 1 \text{ mM}$) were added and the solutions homogenized. After 20 h under light exclusion on a laboratory shaking plate, the samples were determined spectrophotometrically.

Analogous procedures were followed with 20 hydrogel spheres each of the composite hydrogels modified with PEI to investigate the pH dependence of dye adsorption of congo red. In each case, 490 μL of a congo red solution ($c = 1 \text{ mM}$) were used.

4.4.6 | Porous hydrogel spheres by template method

Preparation

For the preparation of porous hydrogel spheres, calcium carbonate of grain sizes 5 and 50 μm were used. To a 4 wt.% sodium alginate solution or a substrate phase modified with 4 wt.% polyethyleneimine, 5, 10, 15, 20 or 25 wt.% of calcium carbonate were added and homogenized with a sonotrode over a period of 5 min under ice cooling at an amplitude of 20%. The substrate phase was then drawn into a BD Plastic injection syringe ($V = 50 \text{ mL}$). A Braun Sterican 0.80 \times 120 mm cannula was screwed on and bend to a 90° corner. An aqueous acetic acid solution (for 5–10 wt.% CaCO_3 50% acetic acid, at 15–25 wt.% CaCO_3 25% acetic acid was used) with a magnetic stirring core was placed in front of it. The syringe containing the substrate was clamped into a PHD ULTRA syringe pump manufactured by Harvard Instruments and dropped into the acid solution at a flow rate of 1000 $\mu\text{L} \cdot \text{min}^{-1}$.

As the CaCO_3 content increased, gas evolution was increasingly observed, which could be attributed to the dissolution of the calcium carbonate. After addition, the hydrogel spheres were left in the aqueous acetic acid solution for another 3 h. Subsequently, the hydrogels were washed twice with demineralized water and then once with ultrapure water. On the following 5 days, the ultrapure water was changed at least once a day to wash any remaining protons from the hydrogels.

Dye adsorption on porous hydrogels

Dye adsorption on porous hydrogels was investigated on six samples and two dyes. Three samples each for adsorption of methylene blue and three samples for adsorption of congo red were prepared. The dye solutions of concentration ($c = 1$ mM) of methylene blue and congo red were used. In addition to nonporous alginate hydrogels and composite hydrogels modified with PEI, 20 wt.% CaCO₃ of both 5 and 50 μm template sizes were added to both species.

As a reference for methylene blue, 15 mL ultrapure water and 225 μL methylene blue solution (1 mM) were mixed and this sample was spectrophotometrically analyzed. Then, 50 alginate hydrogel spheres each of porous and nonporous structure were added to this dye solution prepared in this way. Adsorption in the absence of light took place over a period of 20 h on a laboratory shaking plate. Aliquots were taken after 1, 2, 3, 4, 5, and 20 h and analyzed spectrophotometrically.

Samples for congo red were prepared by mixing 15 mL ultrapure water and 525 μL congo red solution (1 mM). To the resulting dye solution, 50 PEI-modified composite hydrogels each with and without calcium carbonate templating were added. Dye adsorption was examined spectrophotometrically by taking aliquots after 1, 2, 3, 4, 5, and 20 h under light exclusion on the laboratory shaker plate.

4.4.7 | Selective dye adsorption

20 ALG hydrogels and 20 composite ALG + PEI hydrogels were placed in 7 mL ultrapure water. Then, 210 μL of methylene blue solution ($c = 1$ mM) was added and the sample was homogenized. The concentration of the dye solution was determined spectrophotometrically checked before dye adsorption and after 20 h. After the spectrophotometric analysis on the hydrogels, the hydrogel spheres were separated from the solution. A solution of 7 mL ultrapure water and 490 μL congo red solution ($c = 1$ mM) were added. The spectrophotometric examination of the solution was also performed before addition of the dye solution and after 20 h of the dye adsorption.


The dye adsorption procedure was also performed in the opposite manner. First, adsorption was studied on a congo red solution consisting of 7 mL ultrapure water and 490 μL congo red solution ($c = 1$ mM) for a period of 20 h and then on a methylene blue solution of 7 mL ultrapure water and 210 μL methylene blue solution ($c = 1$ mM).

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