

REGULAR ARTICLE

Evaluation of the application of water kefir grains in the decolouration of solutions containing textile dye

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Regular Section

Academic Editor: Fernando Ferrari Putti

Statements and Declarations

Data availability

All data will be shared if requested.

Institutional Review Board Statement

Not applicable.

Conflicts of interest

The authors declare no conflict of interest.

Funding

Brazilian National Council for Scientific and technological development – CNPq.

Author contribution

ILPX and MA: experimental data collection, literature review, writing the manuscript; NSB: experimental data collection; MPB - conceptualization, data custody, data analysis, writing the manuscript, manuscript review, supervision and responsible for funding.

Abstract

One of the main environmental problems presented by the textile industry is the generation of effluents containing synthetic dyes. Because of that, studies have been carried out in order to remove or degrade the dyes present in wastewater and thus, the adsorption process in polymeric matrices has been highlighted due to its low cost and high efficiency in bioremediation. In this context, it was verified the potential of biodegradation of the anthraquinone C. I. Reactive Blue 4 HFG textile dye (Dianativo®) by water kefir grains, as well as the application of the insoluble exopolysaccharide produced by the grains as an adsorbent of the dye, evaluating the effect of pH and initial concentration of dyes in the reduction of staining in a model system. The grains and their insoluble polysaccharide matrix were presented as capable of decolourizing dye solutions under the evaluated conditions, reducing the dye concentration in the solution by up to 92%.

Keywords

Bioremediation; Adsorption; Kefir; Effluent; Textile Dye



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Introduction

The textile industry plays an important role in society, providing cloth for various applications, what is considered essential for human beings (Amaral et al., 2018). However, this sector causes severe environmental impacts, out of which, one of the main ones is the generation of effluents containing a significant concentration of textile dyes, which, even at concentrations close to 1ppm, are detectable with the naked eye, making it impossible to reuse water in the dyeing process (Guaratini & Zanoni, 2000).

There are now more than 100,000 compounds used as dyes. Out of these, about 2,000 types are available for application in the textile industry. It is estimated that about 20% of all dye used in the dyeing of fabrics and fibres are lost, being released in industrial effluents. Some of the molecules used as dyes, such as recalcitrant molecules, present risks to the environment, having toxic and carcinogenic effects in living beings (Guaratini & Zanoni, 2000; Jaikumar & Ramamurthi, 2009; Rani et al., 2014; Ardila-Leal et al., 2021).

With its application in the textile industry, synthetic dyes have different structures such as the chromophore (molecule region responsible for the compound colour) and, the most usual, the ligands (molecule region responsible for the interaction with the cloth) which divide the dyes by the way of

fixation to the cloth. Among the main classes are the reactive dyes, that contain an electrophilic group that can form covalent bonds with hydroxyls, amino groups, thiols, among others (Ardila-Leal et al., 2021). Another important class of dyes are acids, that have anionic characteristics due to the presence of sulfonic groups in the structure (Guaratini & Zanoni, 2000; Chaves, 2009).

The removal or degradation of textile dyes in effluents is conventionally carried out by photodegradation, coagulation, electrochemical oxidation, chemical oxidation, and adsorption processes, which is the most efficient treatment process for a wide variety of dyes (Suresh, 2016). Guaratini and Zanoni (2000) also report the use of aerobic and anaerobic microorganisms for biodegradation of dyes. The main modifications that the molecules undergo in this process are oxidation, hydrolysis, conjugation, and reduction.

Pereira et al. (2010) evaluated the ability of the fungus *Lentinula edodes* to degrade textile dyes and observed that it was able to degrade monoazo and anthraquinone dyes. Silva (2011) evaluated the degradation of dyes in textile effluents using crude extract of the peroxidase enzyme extracted from turnip, and it was observed that this enzyme was able to remove five textile dyes from wastewater. Barathi et al. (2020) showed that a strain of *Bacillus subtilis* was able to completely

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decolourize and reduce the toxicity of the reactive blue-160 textile dye after 48 h of incubation under agitation.

The use of biomaterials as adsorbent compounds has been a cheap and efficient alternative for removing dyes from effluents compared to conventional methods. Among the studied materials are sugarcane bagasse, ash, rice straw, and fungal biomass (Jaikumar & Ramamurthi, 2009). Honorato et al. (2015) evaluated the biosorption of methylene blue dye using corn straw and palm heart sheath as adsorbents, which showed, respectively, 102.8 mg.g⁻¹ and 50.9 mg.g⁻¹ of maximum adsorption capacity, being promising agro-industrial residues for effluent treatment.

Polymeric insoluble carbohydrates have been used to remove dyes from textile waste (Crini, 2005). Blackburn (2004) evaluated the use of cationic polysaccharides for the removal of dyes from textile effluents, and found that chitin, chitosan, guar gum, locust bean gum, and acacia gum showed satisfactory results, removing an average of 60% of the acid blue-193 textile dye.

The application of kefir grains in processes involving effluent bioremediation is rarely reported in the literature. Water kefir grains have a small and rigid structure, irregular shape, yellowish and transparent colour, being covered by a polysaccharide and protein matrix (Bergmann et al., 2010). This symbiosis of microorganisms (bacteria of the genera *Lactobacillus*, *Leuconostoc*, *Lactococcus*, *Acetobacter*, *Bifidobacterium* and *Streptococcus*, in addition to yeasts of the genera *Kluyveromyces* and *Saccharomyces*) presents lactic acid, ethanol, acetic acid, and carbon dioxide (Fiorda, 2017). These microorganisms multiply rapidly and survive extreme pH conditions for a long time. The application of this symbiotic complex of microorganisms to degrade and/or adsorb textile dyes can be an inexpensive option involving a simple process.

Lactic bacteria have been reported as microorganisms capable of completely mineralizing azo-type dyes, especially in anaerobic processes or in an anaerobic/aerobic sequential system (Pérez-Díaz & Mcfeeter, 2008; Elbana et al., 2010).

The exopolysaccharide (EPS) of the water kefir grain matrix is mainly composed of dextran-like structures, that is, glucose units joined by α -1,6 bonds (Paiva, 2013). Kefirane, the soluble fraction of the exopolysaccharide produced by kefir, has industrial applications such as increasing the viscosity and water retention of foods for the production of edible films with a high barrier to aromas (Paiva et al., 2016; Ghasemlou et al., 2011). It is also used in the production of microspheres for controlled drug release (Blandón et al., 2016). The insoluble fraction is still little explored, being reported to act as support and protection to microorganisms present in the grains. This fraction is mostly composed of insoluble dextran with a high amount of α (1 \rightarrow 3) bonds (Fels et al., 2018).

Hence, the present work investigated the application of the insoluble fraction of water kefir grains as adsorbents for synthetic dyes usually used in the textile industry, as well as the biodegradation of the same dyes incubated with water kefir grains, evaluating the effect of pH and initial concentration of dyes.

Materials and methods

Kefir grains

Water kefir grains were donated by the Bioprocesses laboratory of the Faculty of Animal Science and Food Engineering (FZEA) from the University of São Paulo (USP) and were kept at 5 °C in brown sugar solution (8%), changed weekly. Before carrying out the tests, the grains were kept at 27 °C for 24 hours, then removed from the solution and washed with distilled water before proceeding to the application.

Extraction of insoluble exopolysaccharides from kefir grains

The insoluble exopolysaccharide from kefir grains was extracted according to Pop et al. (2016), adapted for this study. Briefly, the kefir grains were added to distilled water at 80 °C in the proportion 1:10 (m:m). Extraction took place for 20 minutes under occasional stirring. After this period, the material was centrifuged (4 °C for 10 min at 6400 xg), the supernatant was discarded, and the precipitate washed with distilled water and centrifuged again under the same conditions. Then, the precipitate was lyophilized and stored at -18 °C until use. This material was named BioAdK.

Analysis of the zero-load point of the bioadk

The zero-load point (pHPCZ) of the BioAdK was evaluated as described by Cunha (2014), here adapted. For this purpose, a 1% solution of the bio-adsorbent was prepared in 0.1 M NaCl solution at different pH values (2 to 9), which was adjusted with 0.1 M NaOH, or 0.1 M HCl solution. The solutions were incubated on a rotary shaker at 30 °C and 50 rpm for 24 hours. After this period, the material was filtered through qualitative filter paper and the pH of the filtrate was measured. Then, the graph of Δ pH (pH_{initial} – pH_{final}) versus pH_{initial}, with the pHPCZ determined by the point where Δ pH equals zero.

Colorants

The anthraquinone C. I. Reactive Blue 4 HFG textile dye (Dianativo®) was donated by the company Danny Color Corantes and used in aqueous solution as a model system for the studied processes. Table 1 below presents the dye specifications.

Table 1. Anthraquinone C. I. Reactive Blue 4 HFG textile dye (Dianativo®) Information.

Name	C.I. Reactive Blue 4, C.I. n° 61205
Molecular formulae	C ₂₃ H ₁₂ Cl ₂ N ₆ Na ₂ O ₈ S ₂
Molecular structure	Anthraquinones
Molecular weight	681.39 g/mol
CAS	13324-20-4/ 4499-01-8

Evaluation of the maximum absorption wavelength and standard curve for concentration of the dye in solution

The wavelength of maximum absorption (λ _{max}) by the dye was determined through an absorption scan analysis at the wavelengths of 200 to 800 nm, performed in a Thermo Scientific model Genesys 10S spectrophotometer (Thermo Fisher Scientific, San Jose, USA).

To relate the dye concentration in aqueous solution with the absorbance at λ _{max}, standard curves were constructed, one

for each pH evaluated. The curves were constructed with 5 points each, all executed in triplicate.

Decolouration of textile dye solutions with kefir grains

Two treatments were evaluated, the first was executed with the direct incubation of viable kefir grains in a textile dye solution in order to observe the degradation of the dyes by the microorganisms present. In the second, the adsorption capacity of the dye by BioAdK was evaluated.

Decolouration of dye solution by direct incubation of viable kefir grains

Dye degradation was evaluated as batches in 150 ml Erlenmeyer flasks consisting of 50 ml of dye solution containing 2% (m:v) sucrose and 2% (m:v) kefir grains. The flasks were incubated at 30 °C, under agitation of 50 rpm, for 7 days. The pH and intensity of the dye in the solution were monitored at 0 h, 24 h, and 48 h, in aliquots taken from the incubation medium, which were previously centrifuged (4 °C for 10 min at 6400 x g) and evaluated in terms of absorbance at λ_{max} . Grain incubation was evaluated at two dye concentrations (5 ppm and 20 ppm) at an initial pH of 7.0. It is important to point out that part of the dye may have been adsorbed on the grains and this effect was quantified together with the possible degradation of the dyes. The percentage of dye removal (%CR) was calculated by equation 1.

$$\%RC = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

Where C_i and C_f are, respectively, the initial and final concentrations of the dye solution (mg mL^{-1}).

Decolouration of dye solution by adsorption on BioAdK

Dye adsorption by BioAdK was evaluated in batch form, in 50 ml conical flasks containing 5 ml of aqueous dye solution and 0.05 g of BioAdK. The flasks were kept at 30 °C and 50 rpm in an agitated bath, and aliquots were withdrawn after 0 h, 24 h, 48 h, and 118 h of incubation. The aliquots were centrifuged (4 °C for 10 min at 6400 x g) and the pH and absorbance were determined at λ_{max} . BioAdK incubation was evaluated at two dye concentrations (5 ppm and 20 ppm) at initial pH at zero-load point, below, and above it. The percentage of dye removal (%RC) by BioAdK was calculated by the aforementioned equation 1.

The amount of dye adsorbed at equilibrium (q_e) was calculated by equation 2, with the equilibrium point being the moment when no further changes in concentration of the dye in solution were observed.

$$q_e = (C_i - C_f) \times \frac{V}{m} \quad (2)$$

Where C_i and C_f are, respectively, the initial and final concentrations of the dye solution (mg mL^{-1}); V is the volume of the dye solution (mL), m is the mass of BioAdK (mg) used in the experiments.

Statistical analysis

All experiments were performed in triplicate and reported as mean \pm standard deviation. Statistical analyses were performed using one-way ANOVA, with the aid of the R software (R Foundation for Statistical Computing, Vienna, Austria) version 4.1.0, comparing the means using the Tukey test with 95% confidence.

Results and discussion

Wavelength of maximum absorption

A scanning analysis of the absorbances of the dye solutions was performed between the wavelengths of 200 nm and 800 nm, with the wavelength of maximum absorption (λ_{max}) determined at 625 nm.

Bioadk zero-load point analysis

The pHPCZ was determined to be 7.0. The experiments of dye adsorption by BioADK were carried out at the point of zero-load, above, and below it (pHs 7.0, 9.0, and 5.0, in that order), in which the matrix would be with, respectively, neutralized charges, negatively charged, and positively charged.

Follow-up of decolouration of the dye solution by direct incubation of viable kefir grains or by adsorption into bioadk

Figure 1 indicates that there was a significant reduction in the absorbances of the dye solutions at 5 ppm and 20 ppm after 24 h incubation with viable water kefir grains. The pH of the solutions was reduced from 7.0 to values below 3.0 after 24 h of incubation, with no significant differences between the values obtained for the pH after 48 h, which stabilized at 2.33 ± 0.09 . This pH value is consistent with the type of process, since kefir grains are mostly composed of lactic acid bacteria, which acidify the medium in which they are growing (Laureys & Vuyst, 2014).

Figure 2 indicates that there was a reduction in the absorbance of the dye solutions at 5 ppm and 20 ppm after 48 h of incubation with BioAdK. However, this drop was less expressive when compared to the tests carried out with kefir grains containing active microorganisms.

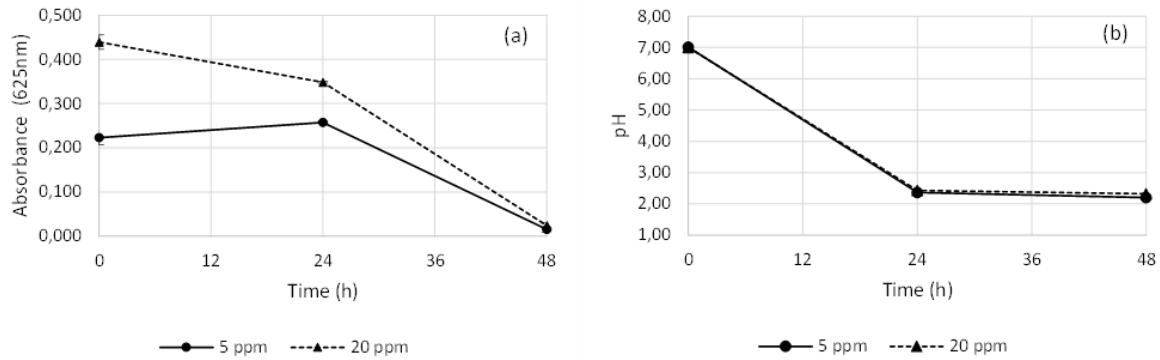


Figure 1. (a) Variation of absorbance of solutions at 625 nm, and (b) pH of dye solutions incubated with viable water kefir grains.

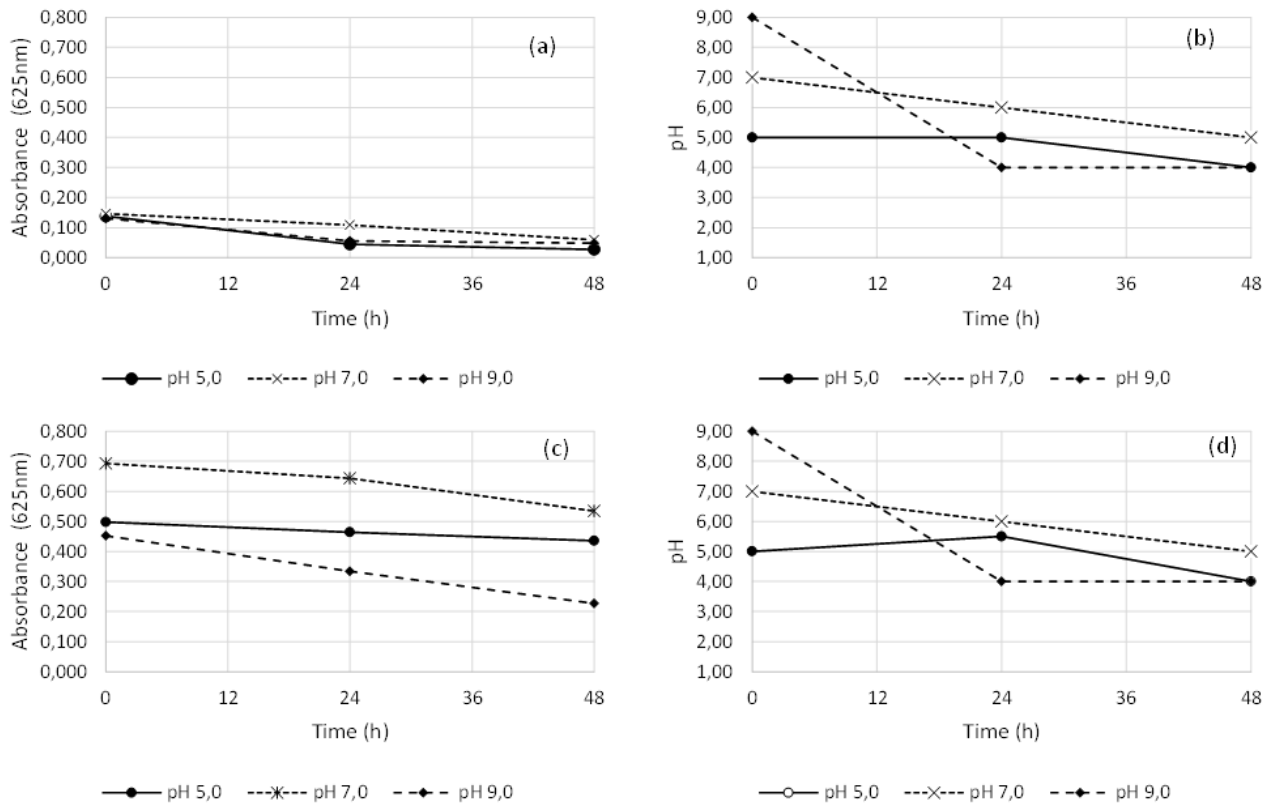


Figure 2. (a) and (b) variation of absorbance at 625 nm and pH of solutions at 5 ppm; (c) and (d) variation of absorbance at 625 nm and pH of solutions at 20 ppm.

The differences observed in the absorbances at time zero for the same dye concentration are due to the pH interference, which is corrected in the concentration calculation using a standard curve for each pH value studied.

The pH values after 48h of incubation were between 6.0 and 5.0, that is, stabilizing below the zero-load point of the adsorbent.

Percentage of dye removal after 48 h incubation with viable kefir grains or bioadk

Experiments performed with BioAdK with initial dye concentration of 5 ppm and pH of 9.0 and 5.0, showed %RC

results statically equal to those obtained with the incubation of viable kefir grains, the highest value obtained was $94.0 \pm 1.2\%$. It was observed (Figure 3) that the two tests performed with kefir grains containing active microorganisms did not differ significantly (at 95% confidence), indicating that the evaluated process was able to act on the decolouration in the two conditions of initial dye concentration.

On the other hand, BioAdK presented %RC ranging from 15% (pH 5.0, 20 ppm of dye) to 86% (pH 5.0, 5 ppm of dye), indicating that both the initial dye concentration and the pH of the process interfere in the adsorption of the dye to the insoluble extracellular polymer of kefir.

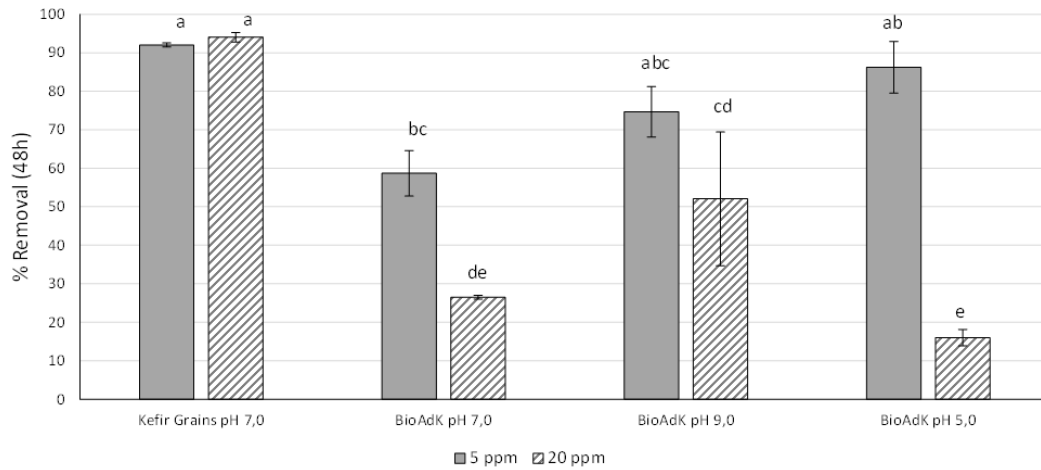


Figure 3. Percentage of blue dye removal from aqueous solutions for experiments performed with kefir grains containing active microorganisms (active kefir grains pH 7.0) and with BioAdK (pH 7.0, 9.0, and 5.0). The statistical comparison is represented by the letters on the bars, with the same letters indicating that there is no significant difference between the experiments, using the Tukey test at 95% confidence.

The decolouration of the dye solutions obtained in the two processes studied can be seen in Figures 4 and 5, in which there is a sample of the aliquots analysed after centrifugation. In the experiments in which kefir grains containing active microorganisms were used, it was observed that the colour of the dye solution changed dramatically in relation to the control (without the presence of kefir grains), presenting a more greenish colour. After centrifuging the aliquots, it could be noted that there was the formation of a bluish-green precipitate. These two facts are indications that the dye may have undergone changes (degradation) due to microbial action and was still adsorbed or coagulated in the polysaccharides soluble substances released by kefir grains in the incubation medium. This observation was the great motivator for conducting the adsorption tests on the insoluble matrix of kefir grains.

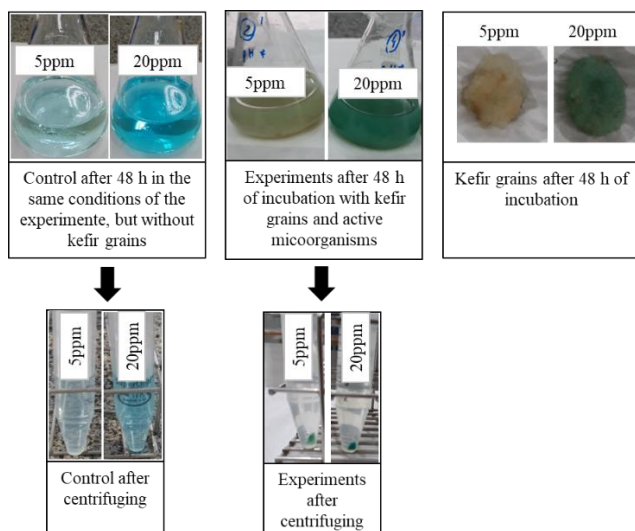


Figure 4. Experiment images of kefir grains with active microorganisms after 48 h of incubation.

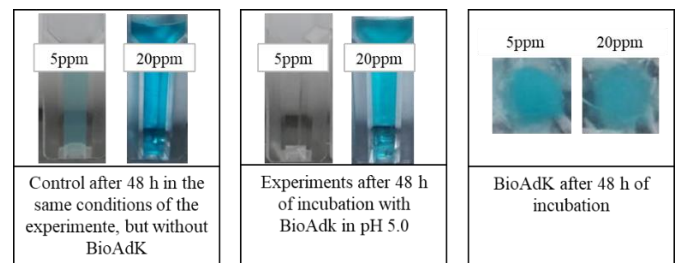


Figure 5. Experiment images of BioAdk after 48h of incubation at pH 9.0

It is possible to notice that the kefir grains and the BioAdK were coloured after the decolouration processes of the textile dye solutions. Processes for desorption of the dye from the surface of the grains and the BioADK can improve the proposed use of these materials for the treatment of textile effluents, allowing their reuse.

As reported by Elbana et al. (2010), lactic acid bacteria may require anaerobic processes to degrade azo-type textile dye structures. The same may be necessary for anthraquinone dyes. Thus, evaluating the incubation of kefir grains with viable microorganisms under anaerobiosis or alternating anaerobic and aerobic conditions may be relevant to increase the efficiency of the decolouration process of solutions with reactive blue dye.

Amount of dye adsorbed after 48 h incubation with bioadk

The amount of dye adsorbed at equilibrium (q_e) was determined for the adsorption process. In this way, BioAdK was incubated for up to 118 h in the dye solution at different concentrations, and pH evaluated. No significant difference was observed, at 95% confidence, between the %RC at 48 h and 118 h, except in the process carried out at pH 9.0 and 20 ppm of dye, in which the final dye concentration reached $12.9 \pm 3, 9$ ppm (72% concentration reduction), corroborating the %RC result for this pH value. The values of q_e (mg of dye per g of BioAdK) are shown in Figure 6.

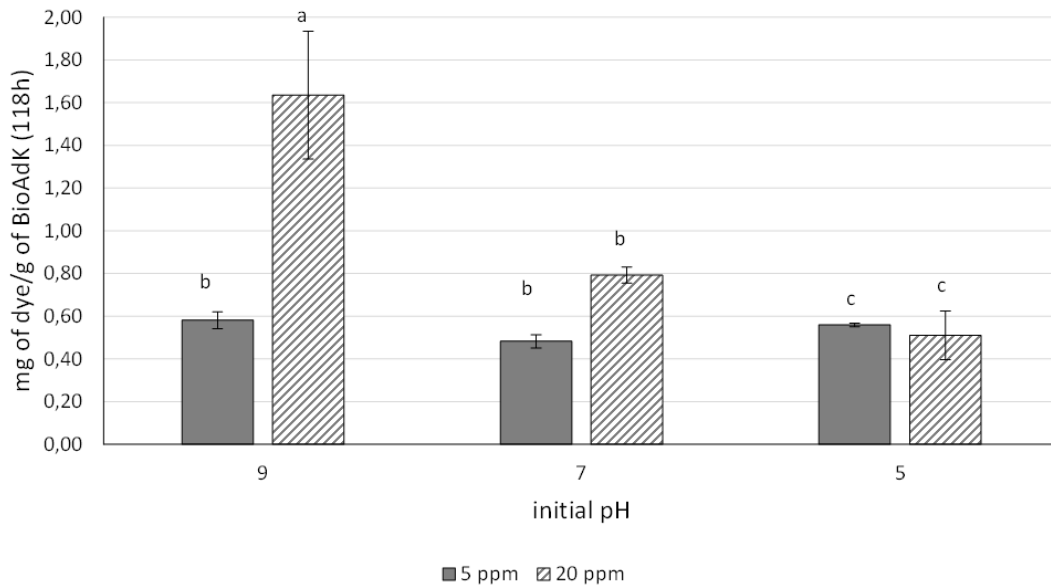


Figure 6. Amount of dye adsorbed at equilibrium (q_e) in mg of dye per gram of BioAdK. The statistical comparison is represented by the letters on the bars, with the same letters indicating that there is no significant difference between the tests, using the Tukey test at 95% confidence.

The highest q_e value (1.64 ± 0.30 mg of dye/g BioAdK) was obtained when BioAdK was applied at an initial pH of 9.0 and dye concentration of 20 ppm, indicating that, for this process of decolourizing aqueous solution of reactive blue dye, pH 9.0 was more effective. This data agrees with what was found by Ribas et al. (2019), who found that the ability to remove the reactive blue dye by adsorption on wheat husk was greater at a pH close to 9.0, followed by a pH close to 7.0, and finally a pH of 5.0. They also found that a pH below 3.0 led to a significant increase in dye adsorption. However, the adsorption capacity of wheat husk was four times higher than that observed here with BioAdK.

Thus, considering that the adsorption capacity of BioAdK was relatively low, the decolouration process of reactive blue textile dye solutions using kefir grains involving live microorganisms proved to be more effective, allowing greater decolouration even at high concentrations of the dye in solution.

Conclusions

Water kefir grains, containing viable microorganisms or their insoluble polysaccharide matrix (BioAdk) free of microorganisms proved to be materials capable of decolourizing anthraquinone C. I. Reactive Blue 4 HFG textile dye (Dianativo®). Colour reduction of up to 92% of the solutions were observed. Besides, kefir grains are easy to obtain and require few resources for their multiplication. Hence, dye decolouration process can be improved to be more expressive, while toxicity tests of the treated solutions can complement the benefit of this application.

Acknowledgments

The authors would like to thank the Federal Institute of Science and Technology of São Paulo – Avaré Campus, and the Brazilian National Council for Scientific and Technological Development (CNPq), for their support and

financial incentive to the project. The authors would also like to thank the Bioprocesses laboratory (Faculty of Animal Science and Food Engineering - FZEA / USP) as well as the Danny Color Corantes company for donating the materials that made the development of this study possible.

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