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Wagner Artifon

Bioflocculant production from industry residues and its application in textile wastewater treatment

Florianópolis
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Thesis submitted to the Postgraduate Program in Chemical Engineering at Federal University of Santa Catarina for obtaining the Doctoral Degree in Chemical Engineering.
Advisor: Prof. Dr. Débora de Oliveira
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The present work at the doctoral level was evaluated and approved on April 28, 2023, by an examining board composed of the following members:

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I dedicate this work to those who have been alongside me.

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The world might not understand if you do not choose everybody else's road. The worst would be trying to fit in. It is a relentless and arduous feeling to take the pathway less traveled by, but you have everything you need to be the person you once admired.

RESUMO

A indústria têxtil é um setor que produz efluentes tóxicos e recalcitrantes cujo descarte inadequado acarreta em impacto ambiental e potencial efeito deletério à saúde humana. Nesse sentido, os sistemas de floculação caracterizam-se como uma opção econômica de tratamento e uma rota que tem atraído atenção para esta área é o efeito floculante de componentes biológicos. Conhecidos como biofloculantes, essas moléculas extracelulares são, principalmente, polissacarídeos e proteínas produzidas por microrganismos que apresentam eficiência na precipitação de partículas suspensas ou substâncias diluídas em soluções. Em contrapartida, alguns resíduos industriais, como o lêvedo de cerveja e o lodo ativado, apresentam concentração rica em componentes biológicos. Neste contexto, este trabalho visou a utilização destes resíduos na produção de biofloculantes assim como sua aplicação no tratamento de efluentes têxteis. Primeiramente, produziu-se um biofloculante a partir do lêvedo de cerveja, sendo este aplicado em um efluente têxtil real contendo o corante rodamina e em uma solução sintética contendo o corante flavina. Este biofloculante foi produzido via hidrólise alcalina, sendo os parâmetros de temperatura, concentração de NaOH e massa de levedura otimizados via planejamento fatorial completo 2^3. A composição do agente floculante é de 46% de proteínas e 29% de carboidratos e a sua eficiência na precipitação dos corantes em solução foi avaliada em termos do pH, temperatura, agitação e dosagem de floculante. Remoções de cor acima de 80 e 90% foram obtidas para os sistemas com rodamina e flavina, respectivamente. Experimentos adicionais de demanda bioquímica de oxigênio, demanda química de oxigênio, carbono orgânico total, metais leves e biodegradabilidade foram realizados para caracterizar o efluente real tratado e não tratado. Na sequência, uma metodologia semelhante foi aplicada na produção de um biofloculante de lodo ativado, o qual foi empregado no tratamento de água contendo azo corantes reativos e dispersos. A hidrólise alcalina empregada resultou em um sobrenadante com composição química de 27% de proteínas, 12% de carboidratos e 12% de lipídeos. Os ensaios de floculação mostraram considerável influência do pH e, juntamente com as análises de potencial zeta, fez-se possível a inferência do mecanismo de floculação dominante. Resultados de remoção de cor acima de 90% foram obtidos para ambos os sistemas e um teste de biodegradabilidade evidenciou a baixa presença de material celular remanescente. Testes comparativos com o floculante comercial policloreto de alumínio evidenciam a eficiência do biofloculante. Por fim, foi possível verificar o potencial dos biofloculantes no tratamento de efluentes têxteis assim como a possibilidade de valorização de resíduos industriais como fonte de componentes biológicos.

Palavras-chave: efluentes da indústria têxtil; biofloculantes; corantes; lêvedo de cerveja; lodo ativado.

RESUMO EXPANDIDO

Introdução

A indústria têxtil apresenta grande relevância no Brasil assim como no Estado de Santa Catarina. Este setor, no entanto, é caracterizado como um dos que mais gera impactos ambientais, tanto pelo seu elevado consumo de água em seus processos internos como pela alta carga de corantes e compostos químicos que são liberados nos recursos hídricos sem o devido tratamento. Os corantes são definidos como as substâncias agentes da coloração dos artigos têxteis, possuindo estruturas químicas distintas e compostas por grupos que lhes confere cor e grupos responsáveis pela sua solubilidade em água e pelas ligações com as fibras têxteis. Assim, devido às suas estruturas, muitos corantes são compostos recalcitrantes, que possuem uma forte resistência à degradação, e podem apresentar propriedades tóxicas, cancerígenas e até mutagênicas, prejudicando a saúde animal e humana quando em níveis acima dos aceitáveis na hidrosfera. Dessa forma, a busca por tratamentos eficientes e economicamente viáveis é o foco de pesquisas. Uma alternativa muito empregada no tratamento destes efluentes é o uso de agentes floculantes, os quais promovem a agregação e precipitação de substâncias dissolvidas e suspensas em água. No entanto, os floculantes mais utilizados, inorgânicos e sintéticos, estão relacionados com pesquisas que atestam preocupações em relação aos seus usos na saúde humana e ambiental. Pressuposto isso, um caminho alternativo consiste no uso de biofloculantes, componentes biológicos oriundos de microrganismos que apresentam efeito floculante em efluentes. Esses biofloculantes apresentam em sua composição majoritária derivados de carboidratos e proteínas que são substâncias produzidas e liberadas por microrganismos ou obtidos através da lise celular. Pesquisas recentes têm apresentado resultados preliminares de produção de biofloculantes a partir de compostos biológicos variados. Este fato abre uma janela de pesquisa que visa a conversão de resíduos industriais, com elevada carga orgânica e baixo valor agregado, em agentes floculantes. Um exemplo de material é o lêvedo de cerveja, um resíduo da indústria cervejeira com rica composição química que muitas vezes é descartado devido a sua produção em excesso nos fermentadores. Após o bagaço de malte, a levedura in natura é o segundo maior resíduo do setor cervejeiro, sendo estimado que a cada mil litros de cerveja sejam produzidos dois kg de lêvedo (massa seca). Outro exemplo de resíduo industrial é o lodo ativado de estações de tratamento de efluente industrial e doméstico. Por ser o resultado do crescimento microbiano em lagoas de estabilização ou reatores biológicos, sua geração é inevitável. Considerando a indústria têxtil como exemplo, é estimado que para cada milhão de metros cúbicos de efluente sejam produzidos aproximadamente 25 m^3 de lodo ativado. Portanto, este material apresenta potencial risco ambiental, demandando manejo e destinação adequados.

Objetivos

O principal objetivo deste trabalho foi desenvolver e caracterizar compostos biofloculantes produzidos a partir de resíduos industriais, assim como avaliar as suas atividades floculantes no tratamento de efluentes contendo corantes comerciais. Para isso, os seguintes objetivos específicos foram determinados: (i) definir o lêvedo de cerveja e o lodo ativado como matérias-primas a serem utilizadas, separadamente, na produção de componentes floculantes; (ii) otimizar a extração de biofloculantes dos diferentes resíduos a partir de hidrólise alcalina; (iii) caracterizar os agentes floculantes produzidos em termos de composição química e grupos funcionais superficiais; (iv) quantificar a atividade floculante dos biofloculantes na precipitação de clarificação de efluentes contendo corantes comerciais utilizados na indústria têxtil.

Metodologia

Os dois biofloculantes foram produzidos de forma individual. Para o biofloculante de lêvedo de cerveja, o resíduo foi coletado dos fermentadores de uma indústria local, sendo o material lavado, adensado à concentração de 30% e armazenado para ser utilizado nos experimentos. Um planejamento experimental 2^3 fatorial completo foi aplicado para a otimização da hidrólise alcalina do lêvedo, sendo as variáveis de estudo a concentração de lêvedo, a molaridade de NaOH e a temperatura de hidrólise. O material resultante da etapa de hidrólise foi centrifugado e o líquido sobrenadante foi definido como o biofloculante cru. A resposta do planejamento experimental foi a remoção da cor obtida em um sistema de floculação definido como 20 mL de efluente real contendo o corante rodamina e 1 mL de biofloculante, sendo o pH ajustado a 3. Após 30 min de reação, a solução foi centrifugada a 5000 rpm (2240 G) por 5 min para precipitação do material floculado e o líquido sobrenadante foi utilizado para determinação da fração de corante não precipitado. Após a etapa de otimização, o biofloculante obtido foi caracterizado em termos de grupos funcionais e composição química via análise centesimal. A atividade floculante foi avaliada perante o efluente real contendo o corante rodamina e uma solução com o corante flavina (150 mg/L). Para isso, o sistema de floculação foi definido como anteriormente, sendo o pH do meio floculante variado na faixa entre 2 e 9 acompanhado por análise de potencial zeta. Parâmetros adicionais de floculação, como dosagem de biofloculante, temperatura e agitação, também foram considerados no estudo. Finalmente, por se tratar de um efluente proveniente da indústria têxtil, fez-se necessária a caracterização em termos de DBO, DQO, TOC, metais leves e biodegradabilidade. Para o biofloculante obtido a partir do lodo ativado, o resíduo foi coletado em uma lagoa de tratamento de efluente de uma indústria têxtil local, sendo seco e moído em moinho de bolas. O sistema para a otimização da hidrólise alcalina foi definido em termos da massa de lodo adicionada (0,5 e 4,0 g) em 50 mL de solução NaOH (0,5 M), sendo o processo conduzido na temperatura de 100 °C por 10 min. Após a hidrólise, a solução resultante foi centrifugada e o líquido sobrenadante definido como o biofloculante. A atividade floculante dos compostos extraídos foi determinada pela remoção da cor em solução contendo o corante vermelho reativo 194 (150 mg/L). Neste sistema, 200 µL de sobrenadante foram adicionados a 20 mL de solução com corante, sendo o pH ajustado a 3 e deixado reagir por 30 min. Após este tempo, a solução passou por centrifugação e a fase líquida foi utilizada na determinação da remoção da cor. Uma cinética foi conduzida para otimizar o tempo de hidrólise. O biofloculante de lodo obtido nas condições ótimas também foi caracterizado em termos de grupos funcionais e composição química. O efeito da faixa de pH de 3 a 8 no sistema de floculação, assim como o potencial zeta, foi avaliado perante soluções contendo o corante vermelho reativo 194 e o corante vermelho disperso 343 (150 mg/L). Um teste de biodegradabilidade foi aplicado para verificar a possível presença de debris celular no efluente tratado. Por fim, um estudo comparativo entre o biofloculante produzido e o floculante comercial (PAC) foi realizado levando em consideração suas dosagens em ambos os sistemas com os corantes reativo e disperso.

Resultados e Discussão

Considerando o biofloculante extraído do lêvedo de cerveja, os parâmetros do processo de extração temperatura, massa de levedura e concentração de NaOH foram otimizados em 100 °C, 3 g e 2 M, respectivamente, de acordo com o sistema de hidrólise. O tempo de hidrólise foi definido em 5 min. De acordo com a análise centesimal, o conteúdo aquoso e volátil corresponde a 95,51%, a fração de carboidratos 29% e a de proteínas 46%. Os ensaios de floculação mostraram que o sistema é extremamente dependente do pH com os melhores índices de remoção de cor, 85% para efluente com rodamina e 95% para solução com flavina,

no pH 2. Os resultados de potencial zeta confirmam a neutralização das cargas elétricas superficiais das partículas em pH reduzido, possibilitando assim a desestabilização das moléculas de corante e formação de flocos. Testes de floculação mostraram baixo efeito da agitação na faixa entre 50 e 150 rpm, boa estabilidade térmica do biofloculante sob diferentes temperaturas (20-50°C), saturação do sistema com flavina para dosagens de biofloculante acima de 0,8 mL por 20 mL de efluente e crescimento gradual na remoção de cor no sistema com rodamina. Testes de caracterização do efluente real tratado, operados em sistema de floculação com pH 3 e dosagem de 1 mL de biofloculante para 20 mL de efluente, indicaram remoção de 72% da cor com aumento considerável nos índices de COT, DBO e DQO, resultados estes atrelados à permanência de debris celular oriundos do processo de hidrólise. Devido a isso, fez-se necessário o teste adicional de biodegradabilidade, o qual indicou a maior biodegradabilidade do efluente tratado, sugerindo a fácil assimilação microbiológica dos componentes orgânicos remanescentes da levedura. Para o biofloculante produzido a partir do lodo ativado seco, o sistema de hidrólise foi definido como 3 g de lodo em 50 mL de solução de NaOH (0,5 M) mantido na temperatura de 100 °C durante 10 min. A caracterização via análise centesimal do agente floculante resultou em frações de proteínas, carboidratos e lipídeos de 27, 12 e 12% em base seca, respectivamente. Os experimentos de floculação mostraram o aumento da efetividade do biofloculante de acordo com a acidificação do meio reacional, sendo a melhor condições obtida em pH 5 com precipitação de 91% do corante reativo e 100% do corante disperso. Os resultados de potencial zeta indicam a neutralização das cargas do material presente no meio, com valores oscilando próximo ao ponto de zero carga, de acordo com a redução do pH. Para o teste de biodegradabilidade, as concentrações de carbono orgânico total do efluente tratado se apresentaram reduzidas, indicando a baixa permanência de debris celular no meio. O estudo comparativo entre o floculante comercial e o biofloculante produzido mostrou que para a solução contendo o corante reativo uma adição de ~120 mg/L de PAC se equivale a adição de ~350 mg/L de biofloculante em base seca. Já para o corante disperso, remoções da cor acima de 90% foram obtidas com adições de ~60 mg/L de PAC ou ~270 mg/L de biofloculante em base seca.

Conclusão

Os efluentes contendo corantes têxteis apresentam risco potencial se liberados no meio ambiente sem o devido tratamento. Neste contexto, os biofloculantes são uma alternativa na clarificação destes efluentes e este trabalho evidencia que resíduos industriais são uma interessante fonte de compostos biológicos que podem ser convertidos em agentes floculantes. Ambos os biofloculantes obtidos via hidrólise alcalina apresentaram potencial na remoção de cor das soluções contendo corantes comerciais, com destaque para o biofloculante oriundo do lodo ativado, o qual teve maior eficiência com menor dosagem. A composição química dos dois agentes floculantes produzidos foi majoritariamente proteínas e carboidratos, sendo os grupos funcionais superficiais característicos por induzir a formação de flocos. O pH do meio floculante se mostrou crítico na desestabilização das moléculas possibilitando a formação de coágulos, efeito este comprovado pelas análises paralelas de potencial zeta do meio. Finalmente, a produção de biofloculantes a partir dos resíduos abordada neste trabalho, além de ser uma alternativa ao uso de floculantes comerciais, reduz os custos com gerenciamento e direciona estes materiais a uma aplicação ambientalmente correta.

Palavras-chave: efluentes da indústria têxtil; biofloculantes; corantes; lêvedo de cerveja; lodo ativado.

ABSTRACT

The textile industry is a segment that generates toxic and recalcitrant effluents, which inappropriate disposal implies an environmental impact and might affect human health. In this sense, flocculating systems are conventionally considered a cost-effective treatment of dye-containing wastewaters, and one methodology that has attracted attention to this research field is the flocculating effect of biological components. Known as bioflocculants, these extracellular molecules are, mainly, carbohydrates and proteins produced by microorganisms that present efficacy on precipitating suspended particles or diluted substances. On the other hand, some industry residues, such as spent brewer's yeast and excess-activated sludge, present considerable biological content and require management. In this context, this work aims at the use of these residues in bioflocculants production as well as their application in dye-containing wastewaters. Firstly, it is presented a bioflocculant produced from spent brewer's yeast and its application in real textile wastewater containing rhodamine dye and in a synthetic solution with flavine dye. The bioflocculant was produced by alkaline hydrolysis and the parameters of temperature, NaOH concentration, and yeast mass were optimized by a full 2^3 factorial design. The flocculant agent composition consisted of 46% proteins and 29% carbohydrates and its performance on dye precipitation was evaluated in terms of flocculation system pH, temperature, agitation, and flocculant dosage. Color removal results near 80 and 90% were obtained for rhodamine and flavine systems, respectively. Further experiments considering biochemical oxygen demand, chemical oxygen demand, total organic carbon, light metals content, and biodegradability were performed to infer the characteristics of the real textile wastewater containing rhodamine before and after treatment. Additionally, a similar approach was applied to produce a bioflocculant from excess-activated sludge, which was employed on clarifying water containing reactive and disperse azo dyes. The alkaline hydrolysis employed on the substrate resulted in a supernatant with a chemical composition of 27% protein, 12% carbohydrate, and 12% lipid. During the flocculation assays, the pH presented relevant influence and associated with zeta potential analysis, it was possible to define the governing flocculation mechanism. Color removal outcomes above 90% were attained under slightly acidic pH for both dyes and a biodegradability test showed low cellular debris content after flocculation. Furthermore, a comparative study with the commercial flocculant poly aluminum chloride (PAC) points out the performance of the produced bioflocculant. Finally, we can verify the feasibility of bioflocculants in clarifying textile wastewaters and the potential of industrial residues as a source of valuable biological components.

Keywords: textile industry wastewaters; bioflocculants; dyes; spent brewer's yeast; excess-activated sludge.

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LIST OF ABBREVIATIONS

ANOVA – Analysis of variance

BOD – Biochemical oxygen demand

COD – Chemical oxygen demand

COT – Carbono orgânico total

DBO – Demanda bioquímica de oxigênio

DQO – Demanda química de oxigênio

EPS – Extracellular polymeric substances

FA – Flocculation activity

FTIR – Fourier transformed infrared spectroscopy

GNP – Gross national product

IC – Inorganic carbon

LI – Lipid

OD – Optical density

PAC – Poly aluminum chloride

PS – Polysaccharide

PR – Protein

TOC – Total organic carbon

TSS – Total suspended solids

TC – Total carbon

UA – Uronic acid

SUMMARY

CONCEPTUAL DIAGRAM OF THE WORK

Valorize the spent brewer's yeast and the excess-activated sludge into a flocculating agent and evaluate its effect on the treatment of dye-containing wastewaters.

Why?

- The disposal of textile industry wastewaters consists in an environmental concern and requires treatment in accordance.
- The reuse of wastewaters within the industry aims at abating water demand.
- The spent brewer's yeast and excess-activated sludge are two rich sources of biological compounds and urge valorization.

Who has already studied?

- The valorization of spent brewer's yeast is in the beginning and some works report its usage as a flocculant agent precursor.
- To the best of our knowledge, there is no report that correlates the production of bioflocculant with the hydrolysis of spent brewer's yeast and excess-activated sludge for dye precipitation.

Hypothesis

- The inner components of spent brewer's yeast and excess-activated sludge present a flocculation effect.
- The produced bioflocculant shows efficacy in the precipitation of commercial dyes from the textile industry.
- The treated wastewater presents proper physical and chemical parameters to be discharged into aquatic resources.

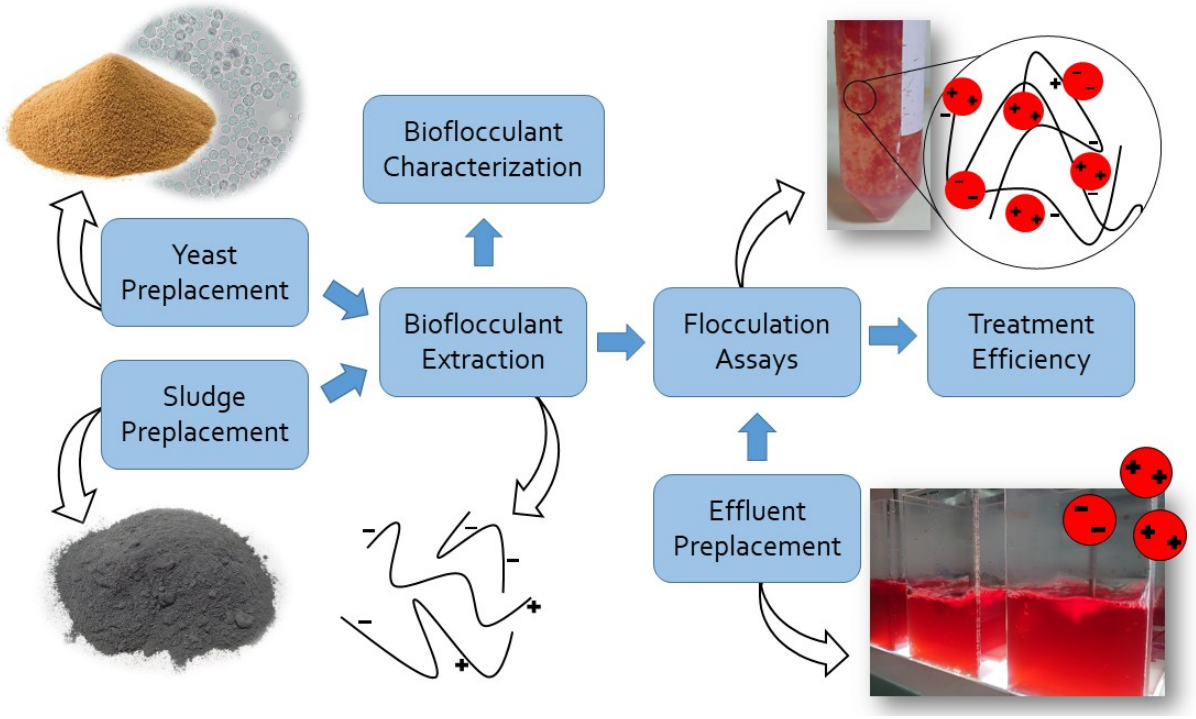
Methodology

- Bioflocculant extraction from spent brewer's yeast and excess-activated sludge.
- Characterization of the bioflocculant.
- Flocculation assays.
- Characterization of treated and untreated wastewaters.

Answers

- Optimization of the bioflocculant extraction.
- Definition of flocculation system parameters.
- Characterization of the bioflocculant and the treated effluent.
- Elucidation of bioflocculation mechanisms.

A schematic representation of the entire project design is presented below. It comprehends the preplacement of the industry residues (spent brewer's yeast and the excess-activated sludge) and the textile wastewaters. Then, each residue follows separately to the bioflocculant extraction by alkaline hydrolysis. The bioflocculant is characterized in terms of functional groups and chemical composition; subsequently, it follows to flocculation assays with the effluents. Finally, the efficacy of the flocculation treatment is determined with respect to the physical and chemical parameters of the treated and untreated wastewaters.



1 INTRODUCTION

The textile industry is responsible for the production of fibers, yarn, and all raw materials related to clothing manufacturing. Brazil is a worldwide front-runner in textile products such as summer clothing, jeanswear, and homewear (ABIT, 2022). The textile industry represented 5% of Brazilian GNP (Gross National Product) in 2020 and it is the second largest employer segment in the country, which makes Brazil the biggest textile chain in the Western world. On the national scenario, the State of Santa Catarina, more specifically the mesoregion of Vale do Itajaí, stands up as a major producer of textile articles. In 2019, the textile sector accounted for almost 20% of industrial enterprises in Santa Catarina, with more than 7,000 clothing-related companies (ROCHA, 2019).

This massive industrial segment has a relevant role in the Brazilian developing economy, but it also brings some environmental concerns. The huge water requirements for dyeing and washing processes within the industry and the aquatic systems contamination due to inappropriate dyeing components disposal are the primarily associated issues. Toxicity and carcinogenic are common characteristics of some textile dyes and their derivatives, which along with their recalcitrant properties, may induce deleterious effects on the ecosystem and human health (CRINI 2006).

In wastewater treatment plants flocculation is a well-known technique with the ability to promote the precipitation of compounds in the water. Recent studies have reported that biomolecules produced and released during microbial growth, can induce floc formation and settle down suspended and dissolved substances in wastewaters. The also called bioflocculants are mainly composed of polysaccharides and proteins and are normally produced during fermentation (ARTIFON *et al*, 2021).

In this context, the motivation of this study lies in the demand for techniques that can efficiently treat textile industry effluents. In this work, a different approach from the literature was employed to obtain two new bioflocculants. These biomolecules were extracted by alkaline hydrolysis from two industrial residues, the spent brewer's yeast and the excess-activated sludge, aiming at valorizing these compounds and evaluating their performance on dye-containing wastewaters treatment.

1.1 OBJECTIVES

1.1.1 General objective

To develop and characterize bioflocculants compounds from industrial residues and evaluate their flocculation activity in the treatment of dye-containing wastewaters from the textile industry.

1.1.2 Specific objectives

- Valorize the spent yeast residue of the brewing industry.
- Properly assign a usage for excess-activated sludge from the textile wastewater treatment plant.
- Optimize the bioflocculant extraction from industrial residues.
- Characterize the produced bioflocculants in terms of functional groups and chemical composition.
- Evaluate the flocculant activity of the extracted compounds from spent brewer's yeast on real and synthetic dye-containing wastewaters.
- Assess the efficacy of bioflocculant from excess-activated sludge on treating azo dye-containing wastewaters.

1.2 THESIS OUTLINE

For better understanding, this thesis is structured in five chapters as follows.

Chapter 1 is this introductory one, which provides the scope, work motivation and objectives. Chapter 2 comprises the theoretical foundation, an already published review, related to the textile industry wastewaters, dyestuffs, and bioflocculants.

Chapters 3 and 4 present a study related to bioflocculant production. The former is a published article, which optimizes flocculant agent from spent brewer's yeast and evaluates its efficacy on real and synthetic textile wastewaters. The second is a submitted manuscript, which comprises the usage of excess-activated sludge as a bioflocculant source as well as employs this compound on azo dye-containing wastewaters.

Finally, Chapter 5 summarizes the final remarks and highlights suggestions for future work.

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2 DYESTUFFS FROM TEXTILE INDUSTRY WASTEWATERS: TRENDS AND GAPS IN THE USE OF BIOFLOCCULANTS

This chapter is based on the review article: "ARTIFON, W. *et al*. Dyestuffs from textile industry wastewaters: Trends and gaps in the use of bioflocculants. **Process Biochemistry**, v. 111, p. 181–190, 2021."

ABSTRACT

Regarding environmental impact, the textile industry is one of the most relevant sectors. Worldwide, it is estimated that 700,000 tons of dyes are generated annually, and 12% of them are inappropriately disposed into aquatic resources. In this sense, flocculating systems can be a cost-effective treatment of dye-containing wastewater. One route that has attracted attention to this research field is the flocculating effect of biological components. Known as bioflocculants, these extracellular molecules, mainly polysaccharides and proteins, are produced by microorganisms and present relevant effects on precipitating suspended particles or diluted substances from solutions. However, the flocculating phenomena related to bioflocculants and dyes are not fully comprehended. The complex structure of extracellular substances and the variance in dye molecules make their interactions hard to predict. This review aims to critically discuss the current state of the art and future trends related to bioflocculants and dye-containing wastewaters from the textile industry. It includes biological systems of bioflocculant-producing strains, chemical properties of bioflocculants and dyes, and physical parameters employed in flocculation processes. This review would lead to a better understanding of challenges and corresponding strategies to open up new ways of bioflocculation in wastewater treatment.

Keywords: textile industry; bioflocculant; dyes; wastewater treatment.

2.1 INTRODUCTION

The increasing challenges related to water availability have restricted environmental legislation, including industrial wastewater disposal. Thus, it is essential to improve and develop cost-effective wastewater treatments and encourage the reuse of water (SONAI *et al.*, 2016). In this sense, the textile industry is one of the most water consumers and polluters. In addition, there are perspectives for textile industry expansion in developing countries (SUBRAMANI; THINAKARAN, 2017) and dye-containing wastewaters from the textile industry are composed of recalcitrant molecules (CHEN, L. *et al.*, 2017). Hence, the inappropriate disposal of textile wastewaters into aquatic bodies interferes with the entire aquatic community since there are toxicological effects that hamper light penetration. Consequently, the photosynthetic aquatic community is primarily affected (ELISANGELA *et al.*, 2009).

It is worth noting that inorganic and synthetic flocculating agents are widely applied to dye-containing wastewaters. However, they are related to environmental and human health concerns, for instance, acrylamide has neurotoxic and carcinogenic properties (RUDÉN, 2004), aluminum salts may induce Alzheimer's disease (CAMPBELL, 2002), among others. In this sense, bioflocculation is a promising alternative. Flocculating agents can be classified into three groups: (I) inorganic, such as aluminum sulfate and poly-aluminum chlorides; (II) synthetic, such as polyacrylamide and polyethyleneimine; and (III) natural or bioflocculants, such as chitosan, alginate, and extracellular polymeric substances (EPS) (SALEHIZADEH; SHOJAOSADATI, 2001; SHIH *et al.*, 2001). On the other hand, bioflocculants produced by microorganisms are considered an eco-friendly alternative (GAO *et al.*, 2009). The dye-bioflocculant interactions are induced by microbial flocculants' chemical structure, which makes the suspended material settle down by adsorption, bridging, and charge neutralization (LI *et al.*, 2009; ZHAO; LIU; ZHOU, 2013).

Several review papers have reported the bio-aggregation dynamics, bioflocculant producers, and bioflocculation modeling. The information is segregated themselves, that is, they do not provide a correlation among them. In this sense, this review aims to explore the current state of the art of bioflocculation systems for dye-containing wastewaters, including the available data for chemical interactions, bioflocculant strains, and flocculation system parameters in order to deeply discuss this promising research and technological field.

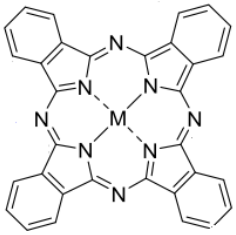
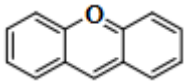
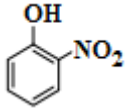
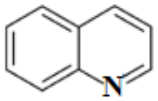
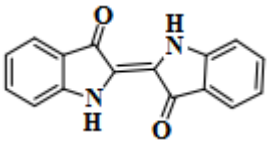
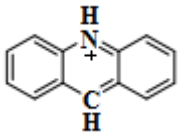
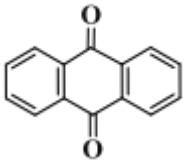
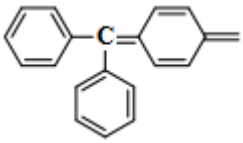
2.2 DYE-CONTAINING WASTEWATERS FROM THE TEXTILE INDUSTRY

Dyes are compounds responsible for giving color to a material (NOREEN *et al.*, 2020). The chemical structure of dyes is composed of two main parts, (I) the chromophore group, which confers the color to the textile fibers, and (II) the auxochrome that supplements the chromophore and can even enhance solubility and binding to fibers (GUPTA; SUHAS, 2009). Dyes have a wide range of chemical structures (BELPAIRE *et al.*, 2015). Nevertheless, the chemical characteristic that corresponds to each color is unique (RAMAN; KANMANI, 2016). In this sense, Table 2.1 presents the classification of textile dyes according to their chemical classes and the color index system (LE COZ, 2005). Most dyes present chemical functional groups such as carboxylic, amine, azo groups, sometimes conjugated with aromatic structures (TEMESGEN; GABBIYE; SAHU, 2018). The increase of aromatic nucleus in the assemblage of the dye matrix augments double bonds and molecule complexity (BENKHAYA; M' RABET; EL HARFI, 2020). Due to these complex structures, synthetic dyes usually present very low degradability-recalcitrance (FRITZKE *et al.*, 2020).

Benkhaya et al. (2018) classified the textile dyes based on their application and fiber attraction, as acid, azoic, basic, direct, disperse, reactive, sulfur, and others. The Color Index list comprises> 8,000 synthetic dyes and ≈ 40,000 trade names that indicate the class, color, and order of dyes (YUAN *et al.*, 2020). According to Subramani and Thinakaran (2017), ≈ 700,000 tons of dyes are produced annually, in which the textile industry consumes ≈ 466 tons of dyes/year, that is, two-thirds out of total. Furthermore, ≈ 12.5% out of total (87.500 tones) are disposal inadequately into the environment (ASGHAR *et al.*, 2015; SUBRAMANI; THINAKARAN, 2017).

The textile industry is a massive water-consuming sector (HENDAOUI *et al.*, 2018; SUBRAMANI; THINAKARAN, 2017). Furthermore, the wastewater generated in this industrial sector is considered a relevant source of pollution due to characteristics such as oxidative substances, persistent color, low biodegradability, and alkalinity (O'NEILL *et al.*, 1999). These liquids are a complex mixture of substances such as acids (e.g., polycyclic acids), alkalis (e.g., NaOH), auxiliaries (e.g., enzymes), surfactants (e.g., alcohol sulfates), heavy metals (e.g., copper), dyes and pigments based on organic chlorine (SARAYU; SANDHYA, 2012). In addition, the liquid discharged is a descendent runoff from a series of chemical baths, which produces high volumes (HARANE; ADIVAREKAR, 2017).

Table 2.1: Chemical structure of textile dye groups.

Group of Dye	Chemical Structure	Reference
Azo	**-N=N-**	(SHANKARLING; DESHMUKH; JOGLEKAR, 2017)
Phthalocyanines		(URBANI *et al.*, 2019)
Xanthene		(RAUF; HISAINDEE, 2013)
Nitro		(RAUF; HISAINDEE, 2013)
Quinoline		(WAINWRIGHT; KRISTIANSEN, [*s. d.*])
Indigo		(ARENAS *et al.*, 2017)
Acridine		(RAUF; HISAINDEE, 2013)
Azine	**=N-N=**	(BELYAEV *et al.*, 2021)
Anthraquinone		(LI *et al.*, 2017)
Triarylmethane		(RAUF; HISAINDEE, 2013)

Source: Artifon *et al*. (2021)

The textile dyes show high molecular stability to chemical oxidation and light degradation (recalcitrant molecules). In addition, they may present biomagnification, toxicity,

and even carcinogenic properties which affect humans health (BELTRÁN-HEREDIA; SÁNCHEZ-MARTÍN; DELGADO-REGALADO, 2009; CRINI, 2006; SECULA; CREŽESCU; PETRESCU, 2011). Hence, textile wastewaters are a concern that should be carefully treated to mitigate their environmental impact.

In the textile industry, an ideal wastewater treatment plant should include coagulation/flocculation operations to induce the precipitation of major suspended and soluble substances and reduce the demand for biological treatment downstream. It is important to highlight that the textile dyeing sludge formation is inevitable in this process (WANG, X. *et al.*, 2019), and it will be formed in two distinct steps. The first one is generated in the decanters as a result of flocculant agent-dye complexation and its precipitation, and the second one is formed during the biological treatment, and it is rich in biomass content due to aerobic microbial activity. According to Huang et al. (2011), ≈ 25 m^3 of sludge are generated per million tons of textile wastewater. In 2016, the China Environment Statistical Yearbook revealed that > 4.5 million tons of textile dyeing sludge were produced (80% moisture content) (MAN *et al.*, 2018). The textile sludge is composed of toxic organic matter and heavy metals, among others (XIE *et al.*, 2018).

Some procedures have been considered to minimize the deleterious environmental effects of textile dyeing sludge, such as incineration (HUANG *et al.*, 2011), oxidation (MAN *et al.*, 2018), gasification (WANG, M. *et al.*, 2019), pyrolysis/carbonization (SOHAIMI *et al.*, 2017), use of landfills, dewatering (WEI *et al.*, 2018). Although the textile dyeing sludge treatment from conventional wastewater systems is reported in the literature, the activated sludge from bioflocculation of dyes is a non-covered area. After bio-aggregation, the bioflocculant and dye complex deserves attention and appropriate disposal.

2.3 BIOFLOCCULANTS

Bioflocculants are specific microbial extracellular molecules. Their biosynthesis is related to the cellular attachment on a surface that assists biofilm formation, so, the components are environmentally resistant with high adsorption properties (NOUHA *et al.*, 2018). The produced bioflocculants can be used in a wide range of wastewater treatments (LI *et al.*, 2020; OKAIYETO *et al.*, 2016; SHAHADAT *et al.*, 2017), such as color removal (HUANG *et al.*, 2014), metal removal (CHEN *et al.*, 2016), sludge dewatering (MORE *et al.*, 2012), microalgae harvesting (DONG *et al.*, 2019), and among others.

2.3.1 Chemical composition

The chemical composition of extracellular polymeric substances varies according to microbial strain and growth conditions, among others. Nevertheless, the composition of bioflocculants is roughly constant (mainly carbohydrates and minority protein, nucleic, lipids, and non-secreted fraction represented by humic substances) (CHOUCHANE *et al.*, 2018; J. *et al.*, 2019).

The polysaccharides produced by microorganisms are classified as homo and heteropolysaccharides (ELKADY *et al.*, 2017; MONSAN *et al.*, 2001). Homopolysaccharides, such as cellulose, dextran, and curdlan, are typically neutrally charged with different branching degrees and can be classified according to their most present linkage: α-D-glucans, β-D-glucans, and fructans (CZACZYK; MYSZKA, 2007). Heteropolysaccharides, such as alginate, xanthan, and gellan gum, present distinct physical properties according to their monosaccharides and branching (MORE *et al.*, 2014). The uronic acid content and their derivates, such as acetate ester, pyruvate ketals, succinates, phosphates, and sulfates, define the molecular charge of bioflocculant (MORE *et al.*, 2014). The bioflocculant polysaccharide content and properties depend on several factors related to microbial growth (NOUHA *et al.*, 2018). The extracellular products released during the microbial consortium growth also present a wide range of compositions (KARTHIGA DEVI; NATARAJAN, 2015). Different carbon and nutrient sources (LI *et al.*, 2013) and extraction methods (NGUYEN *et al.*, 2016) also interfere with flocculant efficiency.

Proteins and enzymes are relevant components of flocculation systems. Non-enzymatic proteins such as lectins, responsible for producing carbohydrates matrix network (FLEMMING; WINGENDER, 2010), and glycoproteins, accountable for form-giving and shape-maintaining function of the bacteria (UPRETI; KUMAR; SHANKAR, 2003), are, usually, present in the bioflocculant matrix. Liu et al. (2010) reported a consistent amount of proteins when strains were fed with different carbon sources. Nevertheless, the EPS presented differences in protein content when varying nitrogen sources and availability (HOA; NAIR; VISVANATHAN, 2003).

The bioflocculant matrix also includes minor components such as nucleic acid and humic substances. The former is an intracellular substance released after cell lysis and found in large amounts in wastewaters after microbial growth (FLEMMING; WINGENDER, 2010). Whereas the humic substances, generated by the hydrolysis of organic substances, are associated with several substances such as amino acids, pectin, lignin, and carbohydrates.

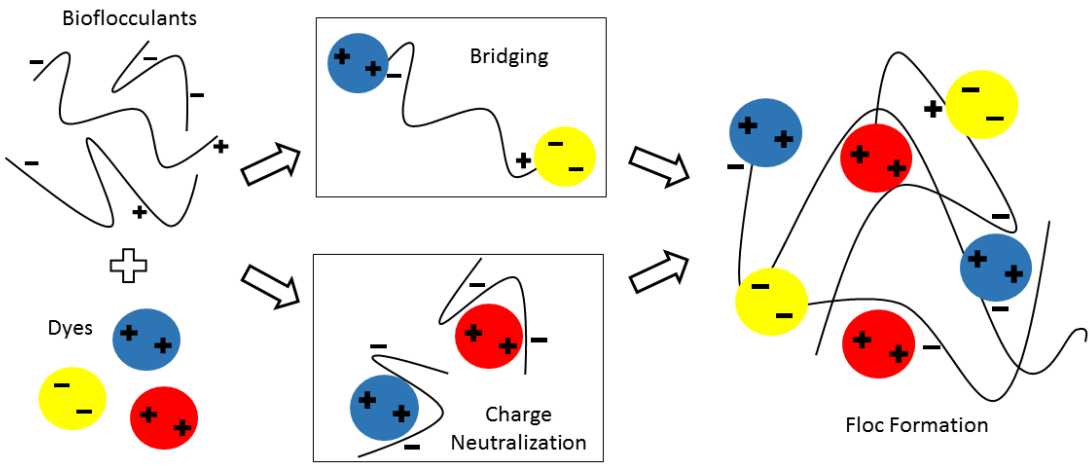
Distinct groups represent the humic substances such as the fulvic and humic acids that correspond to the soluble fragments and the insoluble fraction named humin (PEÑA-MÉNDEZ; HAVEL; PATOČKA, 2005).

Several functional groups are inherent to bioflocculant configuration. Hydroxyl and aliphatic structures, typical from carbohydrates, and carboxyl and amide groups, classic from proteins, integrate the main functional groups responsible for floc formation. This chemical composition of bioflocculants and their molecular functional groups is strictly related to microbial strain, carbon source, nitrogen source, cultivation parameters, and extraction techniques. These properties are essential to determine their effects on flocculation aptitude and application suitability (NOUHA *et al.*, 2018).

2.3.2 Flocculation mechanisms

The mechanisms related to the chemical-flocculating agents are well-known. However, the bioflocculating agents have not been fully explored. The bioflocculating agents act, very likely, by bridging and charge neutralization mechanisms (HE *et al.*, 2009; SALEHIZADEH; SHOJAOSADATI, 2001). Figure 2.1 illustrates the contribution of both phenomena on the formation of a floc between dyes and bioflocculants.

Figure 2.1: Flocculation mechanisms scheme of charged dyes promoted by bioflocculants.



Source: Artifon *et al.* (2021)

The presence of biopolymers in the flocculation systems creates threads or fibers. The bridging phenomenon occurs when the bioflocculant acts as a neutralizing and stabilizing agent to the charged particles suspended in the medium by forming bridges in between (SALEHIZADEH; SHOJAOSADATI, 2001). This process could initiate due to chemical

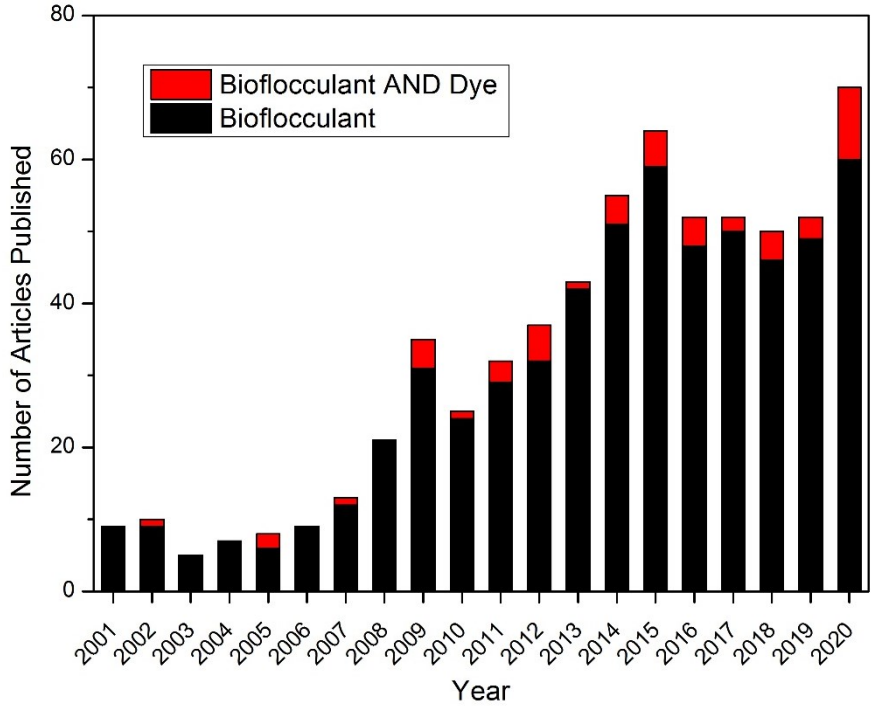
reaction, van der Waals force, static or simple hydrogen bonds. Even neutral bioflocculant could link particles by extending itself into the solution from a distance greater than the interparticle repulsion edge. Bioflocculants with high molecular weight may promote larger flocs by binding particles during flocculation reaction, which induces an increase in floc density, size, and resistance to shear (WU; YE, 2007). The functional groups on the surface of the bioflocculant as well as their configuration induced by pH are key factors for the bridging effect of attractive adsorption sites (ZHANG *et al.*, 2010).

The presence of negatively charged particles provides electrostatic repulsion forces in a solution, which are stronger than the van der Waals forces and prevent settling and floc establishment. In the charge neutralization process, the surface charge density of the particles is reduced by the adsorption sites of the bioflocculant (SALEHIZADEH; SHOJAOSADATI, 2001). The repulsive electrostatic interactions among particles give place to attractive forces, which destabilize particles and start the flocculation. It has been found that low molecular biomolecules are quite effective for charge neutralization (YANG *et al.*, 2016). Several studies reported the enhancement of bioflocculation efficacy by adding metallic cations such as K^{+}, Mg^{2+}, Ca^{2+}. These ions can assist the neutralization of negative charges (functional groups) and contribute to bridge formation (BUTHELEZI; OLANIRAN; PILLAY, 2012; CHOUCHANE *et al.*, 2018). The flocculation process starts with the formation of small flocs; the charge of the particles decreases negativity augmenting attraction forces and making the flocs larger by the time contact with the suspension (LEVY; MAGDASSI; BAR-OR, 1992).

2.4 BIOFLOCCULANT-BASED TREATMENT FOR DYE-CONTAINING TEXTILE WASTEWATERS

The flocculating effect of microbial substances was reported for the first time in the 18^{th} century (PASTEUR, 1876). Since then, bioflocculants have been applied in several fields for coagulation systems (LI *et al.*, 2020). In order to identify the most recent trend on bioflocculant-based treatment for dye-containing textile wastewaters, research on the Scopus database was carried out considering the terms (I) "Bioflocculant" and (II) "Bioflocculant" AND "Dye" that are presented in the title, abstract, and keywords of published papers and patents. The analysis of data revealed an increasing trend in the last 20 years. Figure 2.2 shows publications related to the terms I (entire bar) and II (red part) from 2001 until 2020. During this period, 649 articles were found for "Bioflocculant" and 50 for "Bioflocculant" AND "Dye", which represent an average mean of ≈ 8%.

Figure 2.2: Trends in bioflocculant and dye researching field publications in the last 20 years.



Source: Artifon *et al*. (2021)

Furthermore, the number of patents during the same period was 57 and 12 for I and II, respectively. Regarding the countries, China was the first one with 359 published articles, followed by India with 63. Additionally, the National Natural Science Foundation in China is reported to be the most prominent funding sponsor.

Bioflocculants from several microorganism strains have been used on textile wastewaters. It presents coagulation effects on a variety of dyes. In this sense, Tables 2.2 and 2.3 summarize essential data for bioflocculant-producing strains and specific experimental conditions applied on the removal of commercial dyes from textile wastewater, respectively.

Table 2.2: Bioflocculant producer strains and their chemical composition.

(continue)

Microorganism Origin	Strain	Bioflocculant Chemical Composition	Reference
Laboratory strain	*Aspergillus parasiticus*	PS 76%; PR 22%	(DENG; YU; TING, 2005)
Laboratory strain	*Staphylococcus* sp. and *Pseudomonas*	-	(ZHI-QIANG *et al.*, 2007)
Soil	*Klebsiella mobilis*	PS 100%	(WANG *et al.*, 2007)
Conglutination mud	*Rothia* sp.	PS 100%	(GAO *et al.*, 2009)
Cassava-processing wastewater sludge	-	PS 42%; PR 27%	(LIU *et al.*, 2009)
Conglutination mud	ZHT3-9 and ZHT4-13	-	(WEI *et al.*, 2011)
Activated sludge	*Bacillus subtilis* *Exiguobacterium acetylicum* *Klebsiella terrigena* *Staphylococcus aureus* *Pseudomonas pseudoalcaligenes* *Pseudomonas plecoglossicida*	-	(BUTHELEZI; OLANIRAN; PILLAY, 2012)
Soil	*Paenibacillus elgii*	PS 100%	(LI *et al.*, 2013)
Laboratory strain	*Acinetobacter baumannii*	PS 14%; PR 3%	(LI *et al.*, 2015)
Laboratory strain	*Rhodococcus erythropolis*	PS 91%; PR 8%; 1% DNA	(PENG *et al.*, 2014)
Laboratory strain	*Bacillus licheniformis* (Bl) *Bacillus firmus* (Bf)	PS 167 ug/mL; PR 48 ug/mL PS 90 ug/mL; PR 30 ug/mL	(KARTHIGA DEVI; NATARAJAN, 2015)
Activated sludge	*Haloplanus vescus*	PS 79%; PR 21%	(ZHONG *et al.*, 2016)
Egyptian Ecosystem	*Bacillus velezensis* (Bv) *Bacillus mojavensis* (Bm) *Pseudomonas* (Ps)	detailed amino acids and sugar composition	(ELKADY *et al.*, 2017)
Seawater	*Alteromonas* sp.	PS 70%; PR 22%	(CHEN, Z. *et al.*, 2017)

Table 2.2: Bioflocculant producer strains and their chemical composition.

(end)

Microorganism Origin	**Strain**	**Bioflocculant Chemical Composition**	**Reference**
Hydrocarbon contaminated sediments	*Kocuria rosea*	PS 72%; PR 2.8%; UA 16%	(CHOUCHANE *et al.*, 2018)
Papermill sludge	*Pseudomonas boreopolis*	-	(GUO *et al.*, 2018)
Laboratory strain	Periphytic biofilms	PS 53%; PR 20%	(SUN *et al.*, 2018)
Soil	*Bacillus* sp.	PS 97%; PR 1.4%	(BISHT; LAL, 2019)
Laboratory strain	*Serratia* sp.	PS 67%; PR 0.54%; LI 10.5%	(KUMAR *et al.*, 2019)
Ruditapes philippinarum conglutination mud	*Vibrio* and *Bacillus*	PS ~100%	(MU *et al.*, 2019)
Ramie degumming wastewater	*Alcaligenes faecalis*	PS 75%; PR 21%	(CHEN *et al.*, 2020)
Water treatment plant	*Pseudomonas monteilii* (Pm) *Paenibacillus xylanilyticus* (Px) *Bacillus pumilus* (Bp) *Pseudomonas putida* (Pp)	PR 84%; PS 3.6%; LI 0.8% PR 85.5%; PS 8.2%; LI 1.6% PR 83.5%; PS 4.3%; LI 1.25% PR 82%; PS 7.4%; LI 1.5%	(SAHA *et al.*, 2020)

PS (Polysaccharide); PR (Protein); UA (Uronic Acid); LI (Lipid)

Source: Artifon *et al*. (2021)

Table 2.3: Dye removal efficacy of bioflocculants and experimental conditions.

(continue)

Dye/Charge nature	pH	Temperature (°C)	Bioflocculant dosage	Cation added	Dye removal	Reference
Direct blue 1/Anionic	6	Ambient	50 mL broth/L	-	58%	(DENG; YU; TING, 2005)
Reactive orange 16/Anionic	6				68%	
Reactive blue 4/Anionic	6				92%	
Basic blue B/Cationic	6				2%	
Acid yellow 25/Anionic	6				93%	
Acid orange 8/Anionic	6				62%	
Acid Blue 45/Anionic	6				43%	
Indigo/Neutral	9	Ambient	17 mL broth/L	Ca^{2+}	86%	(ZHI-QIANG *et al.*, 2007)
Disperse yellow/Non-ionic	7	Ambient	4 mL broth/L	Ca^{2+}	85%	(WANG *et al.*, 2007)
Disperse violet/Non-ionic					91%	
Reactive light-yellow/Anionic					60%	
Reactive turquoise blue/Anionic					65%	
Methylene blue/Cationic	Natural	Ambient	200 mg/L	-	86%	(GAO *et al.*, 2009)
Crystal violet/Cationic					98%	
Malaquite green/Cationic					99%	

Table 2.3: Dye removal efficacy of bioflocculants and experimental conditions.

(continue)

Dye/Charge nature	pH	Temperature (°C)	Bioflocculant dosage	Cation added	Dye removal	Reference
Methylene blue/Cationic Fast blue/Anionic	7	Ambient	5 mg/L	Ca^{2+}	83% 78%	(LIU *et al.*, 2009)
Methylene blue/Cationic Crystal violet/Cationic Malaquite green/Cationic Ink Blue/Anionic	3-11 3-11 3-11 9-11	10-50 10-50 10-50 10-20	200 mg/L 200 mg/L 600 mg/L 400 mg/L	Ca^{2+}	96% 96% 95% 82%	(WEI *et al.*, 2011)
Whale dye/Anionic Mediblue dye/Anionic Fawn/Anionic Mixed dyes	7 7 10 10	35-45	10 mg/L	Ca^{2+}, Mn^{2+}, Mg^{2+}, CTAB	97% 81% 95% 82%	(BUTHELEZI; OLANIRAN; PILLAY, 2012)
Methylene blue/Cationic Red X-GRL/Cationic Anionic and Neutral	Natural	Ambient	15 mg/L	-	65% 72% <50%	(LI *et al.*, 2013)
Congo red/Anionic	7	Ambient	0.009 mg/gDS	Ca^{2+}	79%	(LI *et al.*, 2015)
Reactive brilliant red/Anionic Direct sky blue/Anionic Dispersive yellow/Non-ionic	7	Ambient	30-65 mg/L	Cu^{2+}	23% 87% 74%	(PENG *et al.*, 2014)

Table 2.3: Dye removal efficacy of bioflocculants and experimental conditions.

(continue)

Dye/Charge nature	pH	Temperature (°C)	Bioflocculant dosage	Cation added	Dye removal	Reference
Orange G/Anionic Methylene blue/Cationic Crystal violet/Cationic Malachite green/Cationic	Natural	Ambient	800 mg/L	-	Bl; Bf* 67%; 58% 89%; 90% 90%; 84% 83%; 72%	(KARTHIGA DEVI; NATARAJAN, 2015)
Acid brilliant scarlet GR/Anionic	2	Ambient	150 mg/L	Ca^{2+}	95%	(ZHONG *et al.*, 2016)
Basic yellow/Cationic	7	Ambient	-	-	Bv; Bm; Ps* 91%; 89%; 88%	(ELKADY *et al.*, 2017)
Congo Red/Anionic Direct Black/Anionic Methylene Blue/Cationic	11 11 11	Ambient	100 mg/L 200 mg/L 180 mg/L	-	98% 98% 72%	(CHEN, Z. *et al.*, 2017)
Reactive blue/Anionic Acid yellow/Anionic Basic red/Cationic Basic blue/Cationic	Natural	Ambient	15 mg/L	Ca^{2+}	76% 73% 23% 11%	(CHOUCHANE *et al.*, 2018)
CBBR-250/Anionic	Natural	Ambient	300 mg/L	Ca^{2+}	89%	(GUO *et al.*, 2018)
Aniline blue/Anionic	-	Ambient	1.2 mg/L	Ca^{2+}	56%	(SUN *et al.*, 2018)

Table 2.3: Dye removal efficacy of bioflocculants and experimental conditions.

(end)

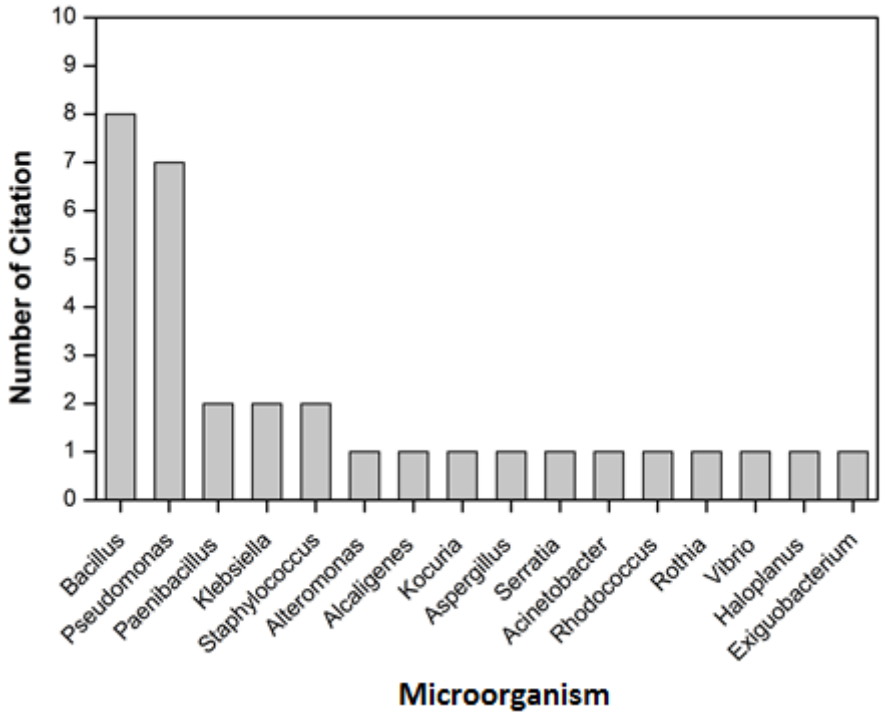
Dye/Charge nature	pH	Temperature (°C)	Bioflocculant dosage	Cation added	Dye removal	Reference
Indigo/Non-ionic	7.8	Ambient	2% (w/v)	-	83%	(BISHT; LAL, 2019)
Trypan blue/Anionic	7	Ambient	-	Ca^{2+}	40%	(KUMAR *et al.*, 2019)
Acridine orange/Cationic					80%	
Methyl orange/Anionic					25%	
Bromothymol blue/Anionic					75%	
Aniline blue/Anionic					60%	
Crystal violet/Cationic					95%	
Methylene blue/Cationic	7.5	Ambient	26000 mg/L	-	99%	(MU *et al.*, 2019)
Crystal violet/Cationic			10000 mg/L		89%	
Malaquite green/Cationic			14000 mg/L		99%	
Dispersive Blue/Non-ionic	8	Ambient	1000 mg/L	-	86%	(CHEN *et al.*, 2020)
Congo red/Anionic Rhodamine-B/Cationic	6-8	25-40	10-14 mg/L	Na^{+} k^{+} Ca^{2+} Mn^{2+} Mg^{2+} Al^{3+} Fe^{3+} CTAB	Pm; Px; Bp; Pp* 79%; 96%; 90%; 69% 54%; 98%; 77%; 88%	(SAHA *et al.*, 2020)

*Refers to the producer strain (see Table 2.2).
Source: Artifon *et al*. (2021)

2.4.1 Bioflocculant-producing strains

New strategies for color removal by applying bioflocculants produced by low-cost and widely available microorganisms have been studied (LEE; CHANG, 2018; REBAH; MNIF; SIDDEEG, 2018). The properties of produced bioflocculants depend dramatically on the parameters employed on the microorganism cultivation. Figure 2.3 correlates the bioflocculant-producing strains to the number of times that they were cited as bioflocculant-producing for dye removal. *Bacillus* sp. and *Pseudomonas* sp. are the genus most reported on these and many other applications (LEE; CHANG, 2018).

Figure 2.3: Bioflocculant strain producers applied for dye removal.



Source: Artifon *et al*. (2021)

Isolated *Bacillus* sp. from soil was employed to produce an efficient bioflocculant to remove indigo dye from wastewater (BISHT; LAL, 2019). The reduction in dye color, chemical oxygen demand (COD), total suspended solids (TSS), and chloride ions were 83, 92, 74, and 82%. Regarding the composition of bioflocculating agents from the same microbial genus, Karthiga Devi and Natarajan (2015) cultivated *Bacillus licheniformis* and *Bacillus firmus*. They compared them according to their capacity for producing bioflocculant agents. *B. licheniformis* produced 80% more bioflocculant, which resulted in a better dye removal efficacy, and 77% of it corresponds to polysaccharides. whereas *B. firmus* produced fewer flocculant agents 75% of

them were polysaccharides. It is also possible to highlight that dye removal with bioflocculant from the same genus strains, at the same experimental conditions, is alike. Elkady et al. (2017) also compared two *Bacillus* strains and obtained a very close color removal rate for basic yellow dye.

Similarly, *Pseudomonas montelli* and *Pseudomonas putida* were compared among four different microorganisms by Saha et al. (2020), and significant differences between polysaccharide content, specific growth rate, and bioflocculant yield were found. The color removal results also presented variances. *P. montelli* presented a higher dye removal for the anionic dye congo red, and *P. putida* was more efficient in removing the cationic dye rhodamine-B. These results are attributed to the differences in chemical composition and functional groups inherent to each strain.

The bioflocculant-producing strains seem to be a cost-effective strategy for textile wastewater treatment, particularly *Bacillus* sp. and *Pseudomonas* sp.. Wastewaters in general present several microorganisms that are responsible for the precipitation of substances. The screening of new bioflocculant-producing strains, their specific components, and the optimization of their growth conditions represent a promising research area.

2.4.2 Bioflocculant chemical composition and flocculation mechanism

Polysaccharides and proteins present long molecular chains with many active radicals known as the main components of bioflocculants. However, these biomolecules comprehend a wide range of components that, along with their intermediates from extraction and purification steps, demand in-depth research to detail their chemical composition entirely. The interaction between bioflocculants and dyes, which covers physical properties and functional groups exposed on both components, is still a non-fully comprehended phenomenon.

Considering the chemical composition of bioflocculant presented in Table 2.2, it is possible to conclude that polysaccharides generally constitute the most fraction of bioflocculants. The characterization of the bioflocculant produced by *Paenibacillus elgii* indicated glucose, glucuronic acid, xylose, mannose, and homogeneous carbohydrates (LI *et al.*, 2013). On the other hand, EPS extracted from periphytic biofilms presented most heteropolysaccharides (SUN *et al.*, 2018), which are cited to increase flocculation yield (ZHONG *et al.*, 2016). The detailed fractions of amino acids and reducing sugar compounds

that constitute the bioflocculant agents are reported in the literature. Their contribution to the stabilization and precipitation of dye molecules is not deeply reported/investigated.

The hydroxyl groups, classical for carbohydrates, are responsible for inducing the bridging effect on dyes (BISHT; LAL, 2019). It is indicated that the floc formation between dye and bioflocculant occurs by hydrophilic and hydrophobic interactions; amine and hydroxyl groups interact with negative charge surfaces and may be responsible for hydrogen-bridging. Macromolecules as proteins and carbohydrates with long molecular chains and many active radicles give the bioflocculant the ability to neutralize negative charges and absorb colloids. It leads to the formation of heavy three-dimensional flocs that later suffer settlement (ZHI-QIANG *et al.*, 2007).

Elkady et al. (2017) screened three bioflocculant-producing strains to compare color removal on synthetic and real wastewater with basic yellow, a cationic dye. The much lower flocculating activity was reported for real wastewater when compared to synthetic dye solution due to the higher hydrogen ions concentration in the real wastewater and its competition with cationic ions of dye. Low dye concentrations exhibited rapid sorption, and equilibrium was attained quickly, indicating a monolayer coverage formation by the dye molecules onto the surface of the bioflocculant. The kinetic study carried out presented a correlation with the Langmuir model and predicted a single-stage equilibrium operation in the batch biosorption process. The negative value of free energy confirmed the affinity between bioflocculant and dye, and it indicated the dye sorption is a spontaneous reaction.

The presence of uronic acid content on bioflocculant provides hydroxyl groups to the molecular chain and is directly related to adsorption and flocculating phenomena (DE PHILIPPIS; COLICA; MICHELETTI, 2011). Chen et al. (2020) cultivated the strain *Alcaligenes faecalis* in ramie degumming wastewater medium for bioflocculant releasing. The liberation of uronic acid from cells and a high level of proteins favored the number of active sites for selective adsorption due to the amino and carboxyl groups. They also presented a dye removal efficacy of 86% dispersive blue solution. The authors reported that under acidic pH, the carboxylate and amine groups might accept hydrogen ions. It results in an electrostatic repulsion force between bioflocculant and dye, conducting to lower flocculation activity. Another study reported that *Alteromonas* sp., isolated from seawater, was used to produce a bioflocculant for treating congo red, methylene blue, and direct blue wastewaters; the results were 98, 98, and 72%, respectively (CHEN, Z. *et al.*, 2017). The dye removal results suggest

that the bioflocculant showed a better affinity with anionic dyes, probably due to the availability and strength of positive charges in the solution.

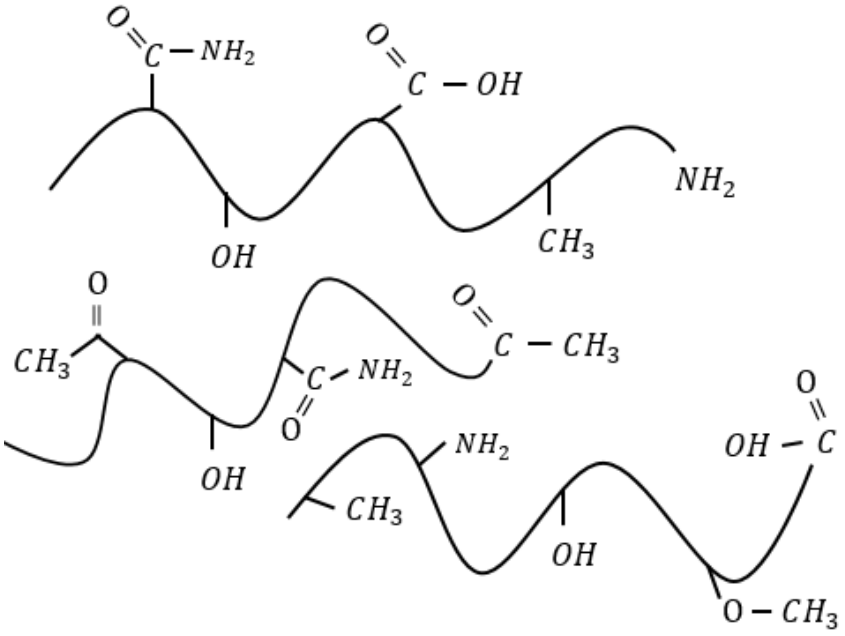
The cultivation of the strain *Kocuria rosea* was optimized to produce extracellular polymeric compounds for flocculation (CHOUCHANE *et al.*, 2018). The flocculant was employed on reactive blue, acid yellow, basic red, and basic blue, and the color removal reached 76, 73, 23, and 11%, respectively. The results indicated a higher affinity between bioflocculant and anionic dyes, mainly with reactive blue, attributed to the anthraquinone structure that contains hydroxyl groups responsible for binding the dye to EPS molecules. The size of dye molecules and its inherent number of sulfonic groups act as a barrier inhibiting other molecules' adsorption and may be another reason for this attraction (DENG; YU; TING, 2005).

The chemical functional groups present on the surface of bioflocculant play an essential role in bridging and adsorption processes (CHEN, Z. *et al.*, 2017). Further analysis conducted by several authors revealed the most common chemical functional groups schematically represented in Figure 2.4. The most groups reported are hydroxyl (BISHT; LAL, 2019; GAO *et al.*, 2009; PENG *et al.*, 2014), carboxyl (CHOUCHANE *et al.*, 2018; ELKADY *et al.*, 2017; SUN *et al.*, 2018), methoxyl (ELKADY *et al.*, 2017; KARTHIGA DEVI; NATARAJAN, 2015; SAHA *et al.*, 2020), and amine groups (DENG; YU; TING, 2005; ELKADY *et al.*, 2017; ZHONG *et al.*, 2016) all related to flocculation activity (CHOUCHANE *et al.*, 2018). Other chemical groups are acetyl and amide (CHOUCHANE *et al.*, 2018), methyl (KUMAR *et al.*, 2019), and carbonyl (SUN *et al.*, 2018).

The produced compounds reported by Karthiga Devi and Natarajan (2015) presented hydroxyl, carboxyl, and methoxyl functional groups; the authors described that the second could stretch out by electrostatic repulsion and it may outcome in more effective sites for adsorption. On the opposite side, the EPS compounds produced by both strains presented strong affinity with methylene blue, crystal violet, and malachite green (cationic dyes), nevertheless lower attraction for orange G (anionic dye). Buthelezi et al. (2012) cited that acid dyes are the most difficult to remove due to their inert chemical structure. Li et al. (2013) also reported a bioflocculant obtained from *Paenibacillus elgii* that presented better color removal ability for cationic dyes and indicated two mechanisms for adsorption. The first is related to the electrostatic attraction of two oppositely charged ions due to the large number of adsorption sites the long molecules of EPS offers (VERMA; DASH; BHUNIA, 2012). The second is based on the high amount of mannose present in the EPS, which may promote van der Waals interactions and hydrogen bonding (BLACKBURN, 2004). Table 2.3 presents more outcomes

from studies that corroborate this higher affinity between bioflocculants and cationic dyes (KUMAR *et al.*, 2019; LIU *et al.*, 2009; WEI *et al.*, 2011). The possible explanation may lie in the relation between composition and functional groups of both dye and flocculant agents (BUTHELEZI; OLANIRAN; PILLAY, 2012; GAO *et al.*, 2009).

Figure 2.4: Schematic representation of bioflocculants and their most reported chemical functional groups.



Source: Artifon *et al*. (2021)

Similarly, *Klebsiella mobilis* was cultivated in diluted wastewater for bioflocculant extraction. The bioflocculant produced was more active on removing dispersive dyes, yellow 85%, and violet 91%, than reactive ones, light-yellow 60%, and turquoise blue 65%. The higher molecular volumes and hydrophobicity inherent to reactive dyes are pointed out to justify the outcomes (WANG *et al.*, 2007).

Several studies describe the color removal yields due to the interaction between chemical groups present in both dye and flocculant agent structures. However, the identification of chemical groups from dyes and extracellular substances is often superficial, which makes the comparison among data not conclusive. Further evaluation of dye-bioflocculant pairs should be conducted to infer their chemical interactions and make it possible to correlate groups of dyes with bioflocculant components.

2.4.3 Effect of temperature on bioflocculant

Regarding the textile industry that discharges dyeing-bath at high temperatures, wastewater treatment may be conducted at high temperatures. Thus, thermally stable bioflocculants are desirable during the flocculation step. Although most bioflocculant wastewater treatments studies are carried out at room temperature, a few reports correlated the bioflocculation with temperature variation on dye solutions (SAHA *et al.*, 2020; WEI *et al.*, 2011). Wei et al. (2011) applied the bioflocculant obtained from two isolated strains from *R. philippinarum* conglutination mud on Methylene blue, Crystal violet, Malaquite green, and Ink blue dyes. The temperature range (10-50 °C) interfered critically with the dye removal of ink blue in solution. Nevertheless, no significant color removal-temperature effect was observed for Methylene blue, Crystal violet, and Malaquite green. The removal of ink blue dye decreased according to an increase in temperature. It indicates that proteins are the main responsible for the flocculation of this dye due to its low thermal stability at high temperatures.

Similarly, Saha et al. (2020) evaluated the temperature range 25-45 °C and the highest bioflocculation activity on dyes occurred at 40 °C. This increase in color removal directly correlated to higher temperature indicates an endothermic interaction between agents. Buthelezi et al. (2012) found the best temperature for removal of whale, mediblue, and fawn dyes around 40 °C. The analysis of these results indicates that the optimal operation condition is near room temperatures, independently of bioflocculant sources. It is worth noting that it is desirable to operate at mild temperatures due to lower environmental impact and energy demand.

2.4.4 Effect of pH on bioflocculant

The pH of a solution is a relevant factor due to interference in the adsorptive process by dissociation of functional groups on active sites (ELKADY *et al.*, 2017). For instance, the increase in pH decreases the electrostatic attraction, resulting in negative properties for bioflocculant molecules due to the complete deprotonation of functional groups. It may generate electrostatic repulsion and inhibit the approximation between flocculant and dye (DENG; YU; TING, 2005). However, the color removal by bioflocculants is often evaluated at not regulated pHs - usually neutral or slightly alkaline (Table 2.3) (BUTHELEZI; OLANIRAN; PILLAY, 2012). Elkady et al. (2017) described that the flocculant efficacy increased linearly from pH 1 up to 7, and tended to deplete as it becomes more alkaline. It is inferred that at pH 7 bioflocculants molecules became negatively charged due to the ionization of hydroxyl and

carboxyl groups. It enhances the sorption of the positive dye cations by electrostatic forces of attraction (XUE; LI, 2008).

Saha et al. (2020) studied the pH range 6-10 and the best pH for congo red and rhodamine removal was 8 and 7, respectively. The surface charge, structural features, and bridging ability are all related to the solution pH (PAN; SHI; ZHANG, 2009). The positively charged sites at the surface of the bioflocculant are present at lower pH, and it probably favors the anionic dye removal due to its negative charge. The repulsion between Rhodamine-B monomeric cationic form and positively charged bioflocculant surface may interfere in dye removal (KHAN; DAHIYA; ALI, 2012). On the other hand, Zhong et al. (2016) evaluated the pH range 2-10. They reported the color removal of wastewater containing acid brilliant scarlet GR increased with the decreasing pH to nearly 2, achieving a maximum removal of 82 and 95% on COD and color removal. The authors suggested that dye can present variant speciation forms at different pH levels. This parameter can change the redox potential of dye and the bioflocculant, making the dye removal intensely dependent on pH.

The pH can significantly affect the surfaces of bioflocculants and dyes, that is, on the bioflocculation systems. The optimal dye removal at neutral or slightly basic pH is aligned to the eco-friendly profile of bioflocculants.

2.4.5 Effect of bioflocculant concentration

There is a wide range of bioflocculant dosage often used (Table 2.2) in terms of concentration (mg/L) or volume of growth broth (mL broth/L) from the cultivation medium. Karthiga Devi and Natarajan (2015) reported that dye removal activity decreased due to excess flocculant agent dosage. Very likely, the saturation of polysaccharides on the surface of particles hamper the interaction with dye. On the other hand, Mu et al. (2019) described the removal (>99%) of methylene blue and malachite green dyes when a high concentration of bioflocculants (more than 10 g/L) was applied.

The synergistic effects between bioflocculants and commercial flocculants are already reported. In this sense, Huang et al. (2014) and Huang et al. (2015) obtained an increment of 7% on removal of dispersive yellow dye by applying bioflocculant simultaneously with polyaluminum chloride and aluminum sulfate. The adsorption and bridging effect due to bioflocculant can positively result in flocs size aluminum-based coagulant systems. The authors highlighted that the removal mechanism is not dominated by charge neutralization since there

are effects of bioflocculant increments on zeta potential. The sweep of colloids inside coagulant precipitates and adsorption may control the flocculation process. Floc breakage factors were also evaluated and suffered augment by insertion of bioflocculant, which indicates that the dispersive yellow flocs are more robust against shear stress.

Bioflocculant dosage is intimately related to the color removal of dye-containing wastewaters, that is, the biopolymer efficiency in promoting complexation with dyes. In this sense, production and purification steps must be optimized to generate high-efficient bioflocculant compounds.

2.4.6 Effect of cations on bioflocculant

Low concentrations of cations as K^{+}, Mn^{2+}, Mg^{2+}, Ca^{2+}, Fe^{3+} were applied with bioflocculant for dye removal-synergistic effects (BISHT; LAL, 2019; LI *et al.*, 2013; LIU *et al.*, 2009; SAHA *et al.*, 2020). Buthelezi et al. (2012) reported that flocculation results were strongly impacted by pH, temperature, bioflocculant concentration, and divalent cations (Mn^{2+}, Mg^{2+}, Ca^{2+}), which resulted in the enhancement of dye removal. The presence of metal cation was essential, for it caused sweeping and bridging of the dye (CHOUCHANE *et al.*, 2018). Metal cations are conductive and induce an electrostatic repulsion between dyes and flocculants due to neutralization and stabilization of negative charges of their functional groups (BUTHELEZI; OLANIRAN; PILLAY, 2012). Similar results related to cation addition on enhancing dye precipitation are reported (GUO *et al.*, 2018; LI *et al.*, 2015; SUN *et al.*, 2018) and the most efficient and commonly used one is the divalent Ca^{2+}.

The simultaneous application of cations with bioflocculants for dye removal-treatment systems is an interesting alternative since it reduces the bioflocculant requirements and leads to more economically feasible processes.

2.5 PROSPECTION ON BIOFLOCCULANT-BASED TREATMENT FOR DYE-CONTAINING TEXTILE WASTEWATERS

Biopolymers have been identified as an emerging solution to promote the flocculation of substances in several wastewater systems. However, some researching areas should be further explored to glean insights into the bioflocculation field. Most studies related to bioflocculant and dyes report laboratory-scale experiments with synthetic dye solutions. The

lack of pilot-scale implementation with real textile wastewater limits the evaluation of this promising technique.

Moreover, the usage of laboratory culture media as carbon and nitrogen sources, necessary for microorganism growth and bioflocculant releasing, is a drawback of the process due to the associated high cost. Industrial and agriculture residues or wastewaters may be considered as alternative culture media. It reduces the demand for treatment and serves as low-cost carbon and nutrient source for microbial growth. It is also important to highlight the wide quantity of biomass, such as algae or brewing and food industry subproducts, that may present potential as flocculant agents by themselves after conversion. Bioflocculant production might valorize these subproducts, that is, a promising research area.

Furthermore, the flocculation mechanisms associated with bioflocculant-dye systems should be carefully evaluated. The wide range of dye molecule structures and the not identified sub-fraction components of bioflocculants may generate controversial data and mask relevant results. In this sense, the detailed composition and identification of both bioflocculants and dyes are essential to reveal the unique influence of each component and, then elucidates the bio-aggregation mechanisms. Moreover, different groups of dyes may behave unpredictably in the presence of bioflocculants with different chemical compositions. Therefore, any treatment system should be aligned to each class of dyes applied by the textile industry.

In addition, the inappropriate disposal of textile dyeing sludge biomass with toxic and recalcitrant properties, very likely, leads to deleterious effects on the environment. The development of textile dyeing sludge purification methods, valorization techniques, as well as their chemical identification are remarkable researching areas. Thus, new treatment systems and traditional approaches should be investigated.

2.6 CONCLUSION

The elucidation of the bioflocculant process is a key factor for color removal and detoxifying dye-containing wastewaters. In this sense, this review comprises researching studies on the effects of biological, chemical, and physical parameters related to bioflocculants and dyes available in the literature. The flocculation and settlement of dyes are attributed to biomolecules, such as polysaccharides and proteins, which induce bio-aggregation by bridging and charge neutralization. The most prominent bioflocculant-producing strains for color removal applications were *Bacillus* sp. and *Pseudomonas* sp. Bioflocculant dosage,

temperature, and pH parameters considerably influence flocculation efficacy. Moreover, the best color removal results for both anionic and cationic dyes were attained mostly around neutral pH and at room temperature. In some studies, low cation ions addition improved dye precipitation for stabilization of functional group negative charges. Bioflocculants are cost-effective, eco-friendly, and have great potential to be employed to remove textile industrial dyes from wastewaters.

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3 PRODUCTION OF BIOFLOCCULANTS FROM SPENT BREWER'S YEAST AND ITS APPLICATION IN THE TREATMENT OF EFFLUENTS WITH TEXTILE DYES

This chapter is based on the research article: "ARTIFON, W. *et al*. Production of bioflocculants from spent brewer's yeast and its application in the treatment of effluents with textile dyes. **Journal of Water Process Engineering**, v. 49, p. 102997, 2022."

ABSTRACT

Advances in chemical synthesis have increased the complexity and degradation resistance of dyes used in textile processes. This fact interferes drastically with conventional biological wastewater treatment and demands further methods to avoid environmental damage. In this sense, this work presents a bioflocculant produced by the alkaline hydrolysis of spent brewer's yeast, a residue from the brewing industry, as well as the evaluation of its performance in the precipitation of recalcitrant dyes. The optimum hydrolysis system was accessed by a full 2^3 factorial design in terms of NaOH concentration, yeast mass, and temperature, followed by a kinetic study. The solid fraction composition of the produced flocculant agent is 46% of proteins and 29% of polysaccharides. The performance of the bioflocculant was evaluated in two different systems, real textile wastewater containing rhodamine and a synthetic solution with flavine. The effects of the flocculation variables pH, temperature, agitation, and flocculant agent dosage were taken into consideration. Color removal outcomes above 80 and 90% were attained for rhodamine and flavine systems, respectively. Further analysis of real textile wastewater for treated and untreated samples in terms of BOD, COD, TOC, and light metals was performed. A biodegradability test showed the employed treatment could increase microbial assimilation of the sample and reduce its persistent compounds. This work shows, for the first time, the feasibility of the bioflocculant from spent brewer's yeast and features its potential applicability to recalcitrant compounds precipitation in textile wastewaters.

Keywords: waste valorization; flocculant agent; rhodamine dye; flavine dye.

3.1 INTRODUCTION

The textile industry is known as one of the most water-consuming sectors (SUBRAMANI; THINAKARAN, 2017). It is estimated that a quantity near 100 L of water is required for dyeing and washing single jeans pants (HENDAOUI *et al.*, 2018). This high-water demand is summed with the presence of a diverse range of compounds that are involved in the industry's inner processes. Persistent color, toxicity, and low degradability are relevant characteristics of dye-containing wastewaters that can further constrain the efficacy of conventional biological treatment approaches (SARAYU; SANDHYA, 2012). The inappropriate disposal of these wastewaters may interfere with aquatic ecosystems, by reducing photosynthesis capacity and turning water unsuitable for the human supply (ELISANGELA *et al.*, 2009).

Several techniques have been employed for textile wastewater clarification and decontamination. Advanced oxidative process (SHAJEELAMMAL *et al.*, 2022), adsorption (LYU *et al.*, 2022), ultrafiltration (OYARCE *et al.*, 2021), and electrocoagulation (HENDAOUI *et al.*, 2018) are some examples of recently employed methodologies that present high efficiency on dye degradation/separation but are still associated with high-cost treatment systems for industrial wastewaters. Flocculation and coagulation are cost-effective treatment systems that present efficiency in the industrial field. However, some inorganic and synthetic flocculating agents have been related to environmental and human health concerns (CAMPBELL, 2002; RUDÉN, 2004). Bioflocculants are an eco-friendly alternative once it is constituted of natural components, mainly carbohydrates and proteins, from microorganisms. According to Artifon et al. (2021), studies that report the application of bioflocculants on dye-containing wastewaters treatment have increased in recent years. Sun et al. (2018) produced polymeric substances by hydrolyzing periphytic biofilms and obtained 56% of color removal in aniline blue solution. Color removal outcomes higher than 80% are reported by Saha et al. (2020) when applying extracellular substances from several microorganisms cultivation medium on congo red dye system. However, the relatively low extracellular polymeric substances content released by pure culture strains imposes constraints on the application of these substances on a wide scale (SUN *et al.*, 2018). On the other hand, the extraction of biopolymer components from microbial aggregates, such as industrial residues, implies high bioflocculant yield and low-cost requirements (ARTIFON *et al.*, 2021).

The spent brewer's yeast is a residue of the brewing industry that has attracted interest due to its rich chemical composition (MARSON *et al.*, 2019; VÉLEZ-ERAZO *et al.*, 2020). In the fermentation process, the growth of biomass yields an average ratio of 2.0 kg of yeast (dry weight) per ton of beer produced (GARCÍA *et al.*, 2020). After brewing spent grain, the spent brewer's yeast is considered the second-largest byproduct in this industry (AMORIM; PINHEIRO; PINTADO, 2019). These facts highlight the huge amount of wet biomass generated and the demand for proper management of this residue. Traditionally, its applications have comprehended mainly animal and human nutrition (FERREIRA *et al.*, 2010), but different applications focused on the valorization of spent brewer's yeast into other components of interest are becoming significant (PULIGUNDLA; MOK; PARK, 2020). Biopolymer (GARCÍA *et al.*, 2020) and added-value molecules recovery (MARSON *et al.*, 2019), bioactive peptides source (OLIVEIRA *et al.*, 2022), and flocculant for algae harvesting font (PROCHAZKOVA; KASTANEK; BRANYIK, 2015), are some recent applications related to this brewing residue.

Before taking advantage of the inner chemical components of yeasts in a determined processing medium its wall membrane must be lysed. The disruption eases the access to intracellular compounds, increases their solubility, and makes them available for the next processing steps (MARSON *et al*., 2019; VÉLEZ-ERAZO *et al*., 2020). Different approaches are normally employed in general cell lysis. It includes the mechanical category with milling and homogenization techniques, and non-mechanical, which comprises physical, chemical, enzymatic, and electrical procedures (PULIGUNDLA; MOK; PARK, 2020). In this sense, this work milestone is to feature a bioflocculant produced by the spent brewer's yeast from the brewing industry and, for the first time, evaluate its flocculating potential in the dye-containing textile industry wastewaters treatment.

3.2 MATERIALS AND METHODS

3.2.1 Source of materials

The spent brewer's yeast was donated by a local brewing industry after the fermentation process. The collected biomass was washed twice with distilled water, concentrated to 30% (wt/wt) at 60 °C, and kept at 4 °C awaiting experiments.

The produced bioflocculant was tested in two different textile wastewaters systems containing dispersive dyes from TMX (Brazil). The first was a real effluent from a washing

bath obtained from a local textile industry containing rhodamine trillon FRBT ($OD_{520\ nm}$) and the second was a synthetic solution containing flavine trillon F10GT (150 mg/L, $OD_{420\ nm}$). Both dyes persist after conventional biological degradation and demand further treatment before being discharged into aquatic resources.

3.2.2 Bioflocculant extraction and flocculation activity

The bioflocculant extraction was performed in an alkaline medium (SUN *et al.,* 2018). The hydrolysis of the yeast was optimized in terms of NaOH concentration, yeast concentration, and temperature by a full 2^3 factorial design with three center points and two axial points. The variable levels applied are presented in Table 3.1. Tests were conducted in a thermostatic bath for 1 h in falcon tubes containing 10 mL of distilled water, the yeast mass, and 2 mL of NaOH solution. Run at the temperature of 100 °C was carried on in a heating mantle with a condenser. All results were analyzed using the software Statistica 8.0 (Statsoft Inc., Tulsa, OK, USA), considering a significance level of 95%.

Table 3.1: Levels of variables in full 2^3 factorial design.

Levels	-1.68	-1	0	1	1.68
NaOH (M)	0.32	1.00	2.00	3.00	3.68
Yeast mass (g)	1.32	2.00	3.00	4.00	4.68
Temperature (°C)	30.0	44.2	65.0	85.8	100.0

Source: Artifon *et al*. (2022)

The centrifuged hydrolysate containing the bioflocculant produced by the optimized process was purified with pre-cooled (-20 °C) absolute ethanol at a fourth of the supernatant volume. The resulting solution was maintained at 4 °C overnight for stabilization and subsequently followed by centrifugation to obtain the precipitated bioflocculant compound (SUN *et al*., 2018), which was used for functional group determination. The extraction step was conducted twice over the same raw material to increase yield. The relation between the precipitated bioflocculant dried at 60 °C and the dried yeast mass determined the extraction yield.

After the extraction step, the supernatant from hydrolyzed solution was obtained by centrifugation at 5000 rpm (2240 G) for 5 min and 1 mL was used in flocculation assays with 40 mL of rhodamine dye-containing wastewater. The pH of each sample was adjusted to 3, vigorously mixed, and allowed to flocculate for 30 min before centrifugation at 5000 rpm (2240

G) for 5 min. The centrifugation step was necessary to settle down the still-suspended flocs formed and standardize optical measurements. A centrifuged sample of the wastewater at pH 3 with no addition of bioflocculant was used as a control. The flocculation activity (FA) was measured by optical density (OD) with a spectrometer (Femto, model Cirrus 80) and calculated according to Eq. 3.1.

$$FA = (b - a)/b \times 100\% \quad \text{(Eq. 3.1)}$$

where a and b are the OD of the sample and control, respectively.

After extraction optimization, a kinetic study was conducted to evaluate the effect of hydrolysis time on bioflocculant released from yeast. The experiments ranged from 0 to 1 h and the FA was measured as described above.

3.2.3 Bioflocculant characterization

The functional groups presented in purified bioflocculant were examined by Fourier transformed infrared spectroscopy (FTIR – Agilent Cary 660 Spectrometer). The compounds of crude bioflocculant were determined by a centesimal composition analysis. Proteins and sodium content were determined following the Association of Official Analytical Chemists (AOAC, 2016). Lipids, moisture and volatile and mineral residue were quantified according to the methodology described by Adolfo Lutz Institute (2018). All experiments were conducted in triplicate and the carbohydrate content was calculated as the difference between 100 and the sum of the percentage of ash, lipid, moisture, and protein.

3.2.4 Flocculation assays

The effect of pH of the bioflocculation system on color removal of effluent containing rhodamine and synthetic solution with flavine was evaluated using the previously described flocculation procedure. That is, 1 mL of the supernatant of the optimized extraction process was added to 40 mL of dye solution (ratio 1:40). The final pH was adjusted in the range between 2 and 9 with NaOH or HCl (1 M), the system was allowed to react for 30 min, and later centrifuged at 5000 rpm (2240 G) for 5 min. Zeta potential analysis (MALVERN Zetasizer Nanosizer) was performed on flocculation system for the same pH range and the same

bioflocculant dosage. Bioflocculant in distilled water was used as a blank for zeta potential. Experiments were conducted in duplicate.

The effect of agitation on flocculation was carried out in a jar test system composed of a squared-sized vessel (12x12 cm) and a straight-blade impeller (7.5x2.5 cm). 500 mL of wastewater and the bioflocculant were vigorously mixed at 200 rpm for 10 s and set at an agitation that ranged between 50 and 150 rpm for 30 min. The effects of temperature (20-50 °C) and bioflocculant dosage (0-1.2 mL) were separately evaluated in a flocculation system containing 40 mL of wastewater. All flocculation assays were conducted twice, at pH 3, and followed by centrifugation and color removal quantification.

The treated and untreated rhodamine dye-containing wastewater was evaluated in terms of COD (Merk Test Kits), BOD (SABESP, 1997), light metals (Na^+, K^+, Ca^{2+}, Mg^{2+}) (Atomic Absorption Spectrophotometer AA-6300, Shimadzu), and TOC (Total Organic Carbon Analyzer TOC-V_{CPH}, Shimadzu). A biodegradability comparison was accessed by an adapted version of OECD 301 A "DOC Dye-away test". The test was performed with fresh activated sludge using 400 mL of diluted treated and untreated solutions as medium under dark conditions, constant agitation, and 22 °C. Samples were taken in duplicate, for 28 days, and evaluated by a TOC analyzer.

3.3 RESULTS AND DISCUSSION

3.3.1 Bioflocculant extraction

Table 3.2 presents the experimental results of color removal from a full 2^3 factorial design. These outcomes were statistically analyzed to evaluate the effects of each variable on the flocculation activity of the resulting bioflocculant. A quadratic model (Eq. 3.2) was proposed to describe experimental data and all linear and quadratic terms were statistically significant ($p < 0.5$). The proposed model was validated by analysis of variance (ANOVA) as presented in Table 3.3.

$$Color\ Removal\ (100x)(\%) = -81.482 + 66.363(1) - 13.474(1)^2 - 1.868(2) - 1.375(2)^2 + 2.190(3) - 0.008(3)^2 + 7.463(1)(2) - 0.275(1)(3) - 0.039(2)(3) \quad \text{(Eq. 3.2)}$$

The counterplots in Figure 3.1 show the predicted results from modeling. In Figure 3.1 a) it is possible to evaluate the behavior of variable (2). The considerable color removal obtained

at the lowest addition of yeast mass (Run 11) points out the ability of the produced bioflocculant on rhodamine precipitation. Further yeast mass increments improved its availability in the medium for hydrolysis, which enhanced the bioflocculant liberation and lead to better flocculation results (Run 12). In addition, the inferior axial point considered for NaOH concentration presented the lowest color removal response (Run 9). Outcomes quickly became better as the values attain the central point (2 M) and presented low disparity for further increments. A similar NaOH concentration was employed by Sun et al. (2018).

Table 3.2: Matrix of experimental design (real values) and color removal results.

Run	NaOH (M) (1)	Yeast (g) (2)	Temperature (°C) (3)	Color Removal (%)
1	3	4	86	92.93
2	3	4	44	87.82
3	3	2	86	85.37
4	3	2	44	69.28
5	1	4	86	72.97
6	1	4	44	37.09
7	1	2	86	87.60
8	1	2	44	56.06
9	0.32	3	65	3.10
10	3.68	3	65	92.33
11	2	1.32	65	70.53
12	2	4.68	65	93.20
13	2	3	30	60.96
14	2	3	100	90.92
15	2	3	65	89.56
16	2	3	65	90.38
17	2	3	65	89.72

Source: Artifon *et al.* (2022)

In Figure 3.1 b) it is worth noting the positive effect on flocculation activity as temperature augments and reaches the range near the axial point of 100 °C. At the same time, the variation in yeast concentration in the hydrolysis medium shows minimal effect under different temperatures. Both outcomes indicate that temperature plays an important role in the

hydrolysis system by minimizing yeast requirements, increasing efficiency in releasing and solubilizing bioflocculant in the supernatant fraction.

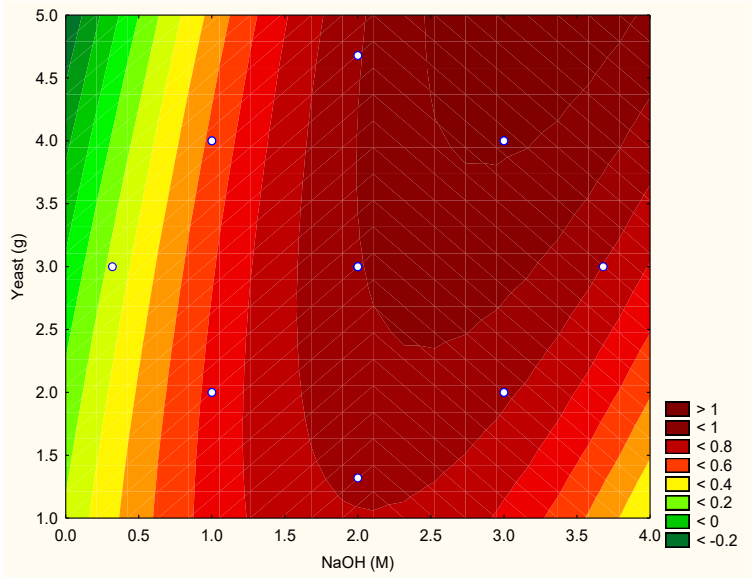
Table 3.3: ANOVA Table for color removal results.

Source of Variation	Sum of squares	Degree of Freedom	Mean Square	$F_{calculated}$
Regression	0.82609	9	0.09179	5.099
Residual	0.12599	7	0.01800	
Total	0.95200	16		

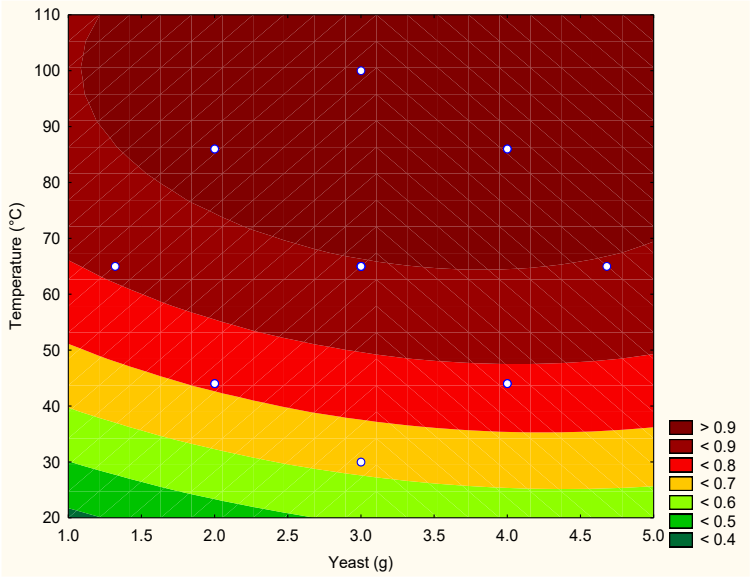
Regression Coefficient: R=0.95; $F_{0.95;\,9;\,7} = 3.67$
Source: Artifon *et al*. (2022)

Figure 3.1: Contour plots for color removal. a) Yeast versus NaOH. b) Temperature versus Yeast.

a)



b)



Source: Artifon *et al*. (2022)

The variable values of 2 M for NaOH concentration, 3 g of yeast mass, and 100 °C as temperature were chosen as optimal conditions based on the factorial design results. Focus on minimizing the brewery residue demand was taken into account once higher yeast mass addition will improve bioflocculant release but reduce its production yield. The kinetic study on the hydrolysis process conducted under the optimized values showed that the supernatant produced reached its maximum flocculation activity of 95% after 5 min of hydrolysis and a constant decay to 88% was observed till the end of the period. The reduction in flocculation activity can be attributed to the exposition of bioflocculant to the operating temperature, which may promote excessive hydrolysis and degrade extracellular polymeric substances. To minimize the remaining spent brewer's yeast precipitated after hydrolysis and centrifugation, the cellular debris were further hydrolyzed for a second and third time, under the same methodology, to produce a supernatant with 47% and 18% of its initial flocculant activity, respectively.

3.3.2 Bioflocculant characterization

The centesimal analysis of the supernatant from alkaline extraction presented in Table 3.4 showed that the total water and volatile content correspond to 95.51%. Moreover, the two main chemical components of the soluble bioflocculant are proteins and polysaccharides, which correspond to 46 and 29% of the solid fraction, respectively. The composition of the bioflocculant is in agreement with the typical composition encountered in Baker's yeast (FELDMANN, 2005).

Table 3.4: Centesimal analysis of the supernatant from alkaline extraction.

Component	Proportion (g/100g)
Protein	2.08 ± 0.02
Polysaccharides	1.3
Lipid	<0.5
Mineral residue	1.10 ± 0.09
Sodium	0.46 ± 0.02
Moisture and volatile	95.51 ± 0.03

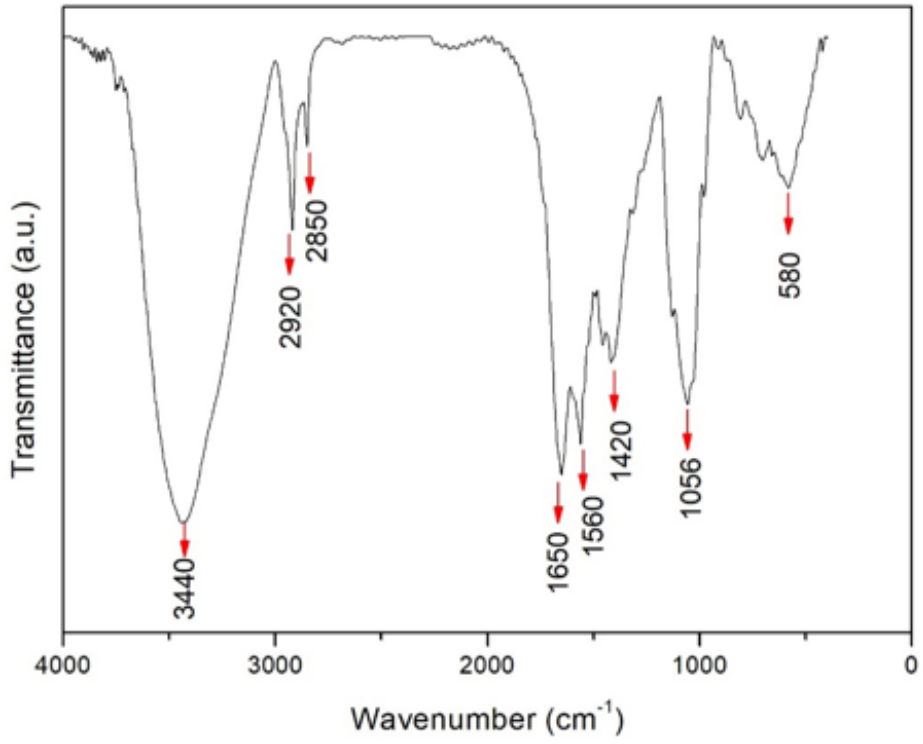
Source: Artifon *et al*. (2022)

The active radicles on the long molecular chains of polysaccharides and proteins are the components responsible for neutralizing negative charges and absorbing colloids, which induces floc formation and precipitation (SHENG; YU; LI, 2010; ZHI-QIANG *et al.*, 2007).

Differently from common microbial extracellular polymeric substances, the bioflocculant from alkaline yeast cell lysis presents a higher proteins/polysaccharides ratio. Sun et al. (2018) determined the contribution of protein content to the flocculation efficacy and indicated that it plays a significant role in the system.

The FTIR spectra analysis depicted in Figure 3.2 infers the chemical nature of the purified bioflocculant. The spectrum presented a broad and strong band at 3440 cm^{-1} that indicates stretching vibration of the hydroxyl group (O-H), which is directly related to adsorption and flocculating phenomena (DE PHILIPPIS; COLICA; MICHELETTI, 2011). Short bands at 2920 and 2850 cm^{-1} correspond to aliphatic C-H bending (BUTHELEZI; OLANIRAN; PILLAY, 2012; GONG *et al.*, 2008) and are classic for carbohydrates (SAHA *et al.*, 2020). The strong peaks at 1650 and 1560 cm^{-1} indicate C=O from amides and amino-sugars (SUN *et al.*, 2018), these characteristic protein groups confirm the high content of this component. The prominent peak around 1420 cm^{-1} is characteristic of C=O asymmetric stretching of carboxylate ions in bioflocculant molecules (SAHA *et al.*, 2020). The intense adsorption peak at 1056 cm^{-1} is a typical characteristic of sugar derivatives (GAO *et al.*, 2009; GONG *et al.*, 2008).

Figure 3.2: FTIR spectrum depicting functional groups.



Source: Artifon *et al*. (2022)

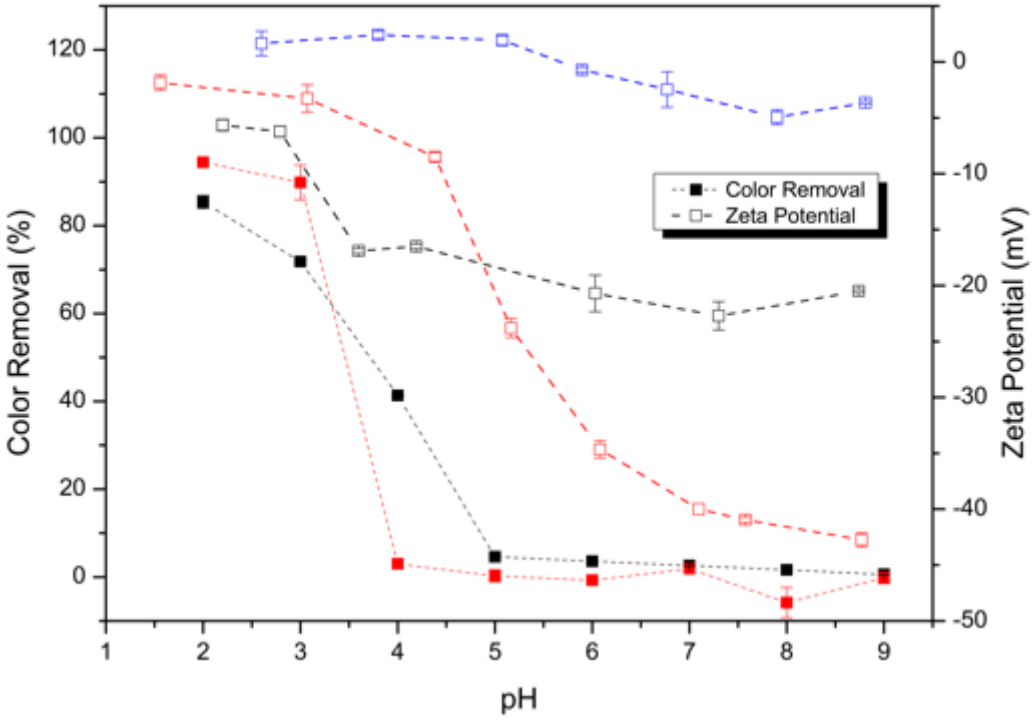
3.3.3 Flocculation assays

The pH of a flocculating system is a critical factor due to its interference in the dissociation of functional groups on active sites of bioflocculants and dyes (ELKADY *et al.*, 2017). The structural features, surface charge, and bridging ability are all related to the solution pH (PAN; SHI; ZHANG, 2009). The color removal results from pH variation presented in Figure 3.3 depict the high flocculation rate as the system becomes more acid, reaching 85 and 95% of color removal at pH 2 for rhodamine and flavine dye systems, respectively. As the pH becomes less acid, the color removal results decrease abruptly and attain less than 5% above pH 5 for both systems. This increase in pH intensifies the deprotonation of bioflocculant molecules and confers negative properties to their surface. The resulting electrostatic repulsion inhibits the approximation between bioflocculant and dye molecules and promotes decay in flocculation (DENG; YU; TING, 2005; ZHONG *et al.*, 2016).

The correlation between zeta potential by medium pH and dye precipitation by bioflocculant activity is essential to infer the predominant flocculation mechanism and is not always cited in the literature (ARTIFON *et al.*, 2021). For instance, an insignificant dye removal promoted by pH reduction itself points out that the bioflocculant in the medium acts as a stabilizing agent by forming bridges between dye molecules. On the other hand, when zeta potential reaches values proximal to zero the system loses stability due to the neutralization of surface charges and favors floc formation (PEFFERKORN, 2006). From Figure 3.3, the abrupt decrease in color removal in the range between pH 2 and 5 for both dyes and their stable behavior at higher pH values are directly correlated to zeta potential outcomes. The decrease is even higher in the flavine dye system in pH between 3 and 4. This behavior can be attributed to a sudden protonation of hydroxyl, carboxyl, and amino groups associated with the xanthene structure of rhodamine and the nitrogen heterocyclic ring structure of flavine dye (ARTIFON *et al.*, 2021). Since the flavine solution is a one-component system, this dispersive dye molecule tends to suffer less interference in the medium when compared to rhodamine molecules within washing bath wastewater, making it rapidly change its redox potential with pH variation. Once zeta potential is a critical factor that interferes with particle steadiness, the neutralization of charges induces floc formation among particles and makes them settle. It indicates that dye removal is intensely dependent on pH and that charge neutralization plays a central role in dye precipitation (HUANG *et al.*, 2014). The zeta potential blank assay for bioflocculant showed a variance from -4.9 to 2.4 mV in the pH range between 8.8 and 2.6 and it crossed the isoelectric

point at pH 5.6. It indicates that the bioflocculant molecules themselves present a net electrical charge near zero and suffer ionization of hydroxyl and carboxyl groups as the pH becomes alkaline (XUE; LI, 2008).

Figure 3.3: Effect of pH in the zeta potential of rhodamine (black) and flavine (red) flocculation systems, and in the bioflocculant in distilled water system (blue).

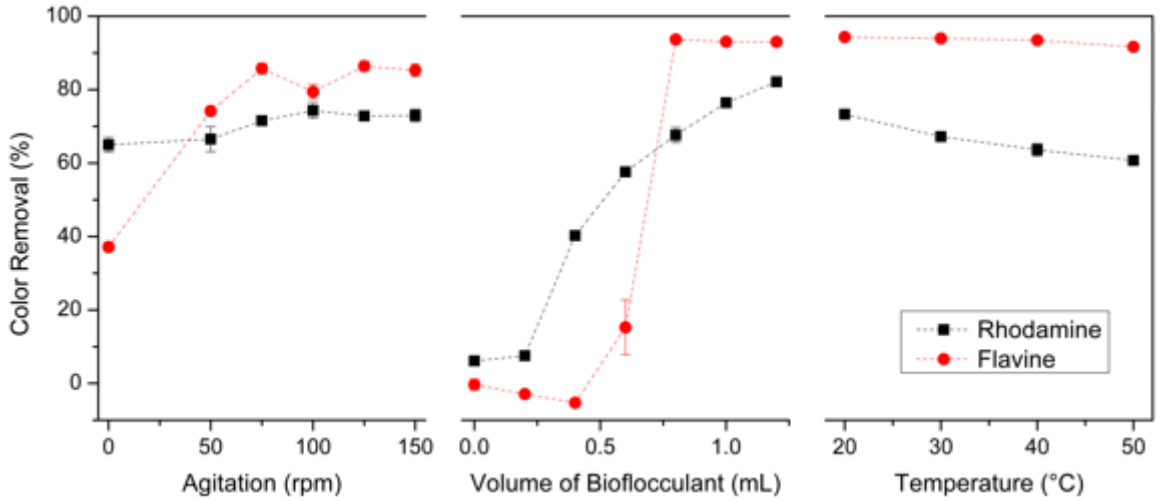


Source: Artifon *et al*. (2022)

The effect of agitation, temperature, and bioflocculant dosage parameters were evaluated separately and are presented in Figure 3.4. Flocculant agent increments influenced considerably the dye removal results. Firstly, considering rhodamine dye, the addition of 0.4 mL of bioflocculant resulted in >40% on color removal. Further increments improved outcomes at a slower rate; it indicates the system is reaching saturation by an excess of flocculant agent (KARTHIGA DEVI; NATARAJAN, 2015). By adding 1.2 mL of bioflocculant the color removal result obtained was >82%. Considering flavine dye, the addition of 0.4 mL of bioflocculant increased turbidity in the medium, further increments up to 0.8 mL rapidly resulted in a color removal >93% and reached saturation. The overdose of biomolecules may promote an incomplete dispersion in the medium and inhibit further flocculation activity (SAHA *et al.*, 2020). A parallel study seeking for an associative effect between the produced bioflocculant and the commercial flocculant $Al_2(SO_4)_3$ resulted in no significant result. The agitation range studied did not show to interfere significantly with the outcomes for rhodamine dye. A light increment of 8% on color removal was observed between

50 and 100 rpm. On the other hand, the increase in agitation considerably augmented the removal of flavine dye from 0 to 75 rpm range by favoring floc formation.

Figure 3.4: Effect of agitation (pH 3, bioflocculant dosage 1:40, ambient temperature), bioflocculant dosage (pH 3, no agitation, ambient temperature), and temperature (pH 3, bioflocculant dosage 1:40, no agitation) in the flocculation system containing rhodamine.



Source: Artifon *et al*. (2022)

The textile industry wastewater is a descendent runoff of a series of chemical and hot baths, so the temperature is a parameter to be considered during the wastewater treatment step. The color removal results decreased by 13% and 3% as the temperature of the flocculation system increased from 20 to 50 °C for rhodamine and flavine, respectively. It indicates that the temperature interferes negatively in the bioflocculant activity, mainly due to the low thermal stability of proteins at high temperatures due to denaturalization (WEI *et al.*, 2011) and the increase in thermal movement of particles in the medium (LIU *et al.*, 2009).

3.3.4 Characterization of rhodamine dye-containing wastewater

Due to the possible presence of textile additives and salts in real wastewaters, the solution containing rhodamine dye deserves special attention. Thus, a series of additional tests were conducted to infer its properties and the effect of bioflocculant addition as presented in Table 3.5. Considering the optimized condition with 72% color removal, the concentration of sodium ions in the wastewater of 257 mg/L indicates remaining salt incidence from the dyeing process, its concentration increased to 354 mg/L due to the presence of sodium hydroxide in the bioflocculant supernatant. K^{+}, Ca^{+2}, and Mg^{+2} light metals ions, in this order, comprise the

main components of yeast mineral content (BERTOLO *et al.*, 2019). It explains their concentration increase in the treated sample medium, especially potassium ions. Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD) and Total Carbon (TC) parameters presented a considerable increase after treatment (3x), which is attributed to the yeast debris from hydrolysis. It is possible to highlight that the augment of TC is attributed only to Total Organic Carbon (TOC) increase, once Inorganic Carbon (IC) concentration decreased after treatment.

Table 3.5: Physical-chemical characterization of rhodamine wastewater before and after treatment.

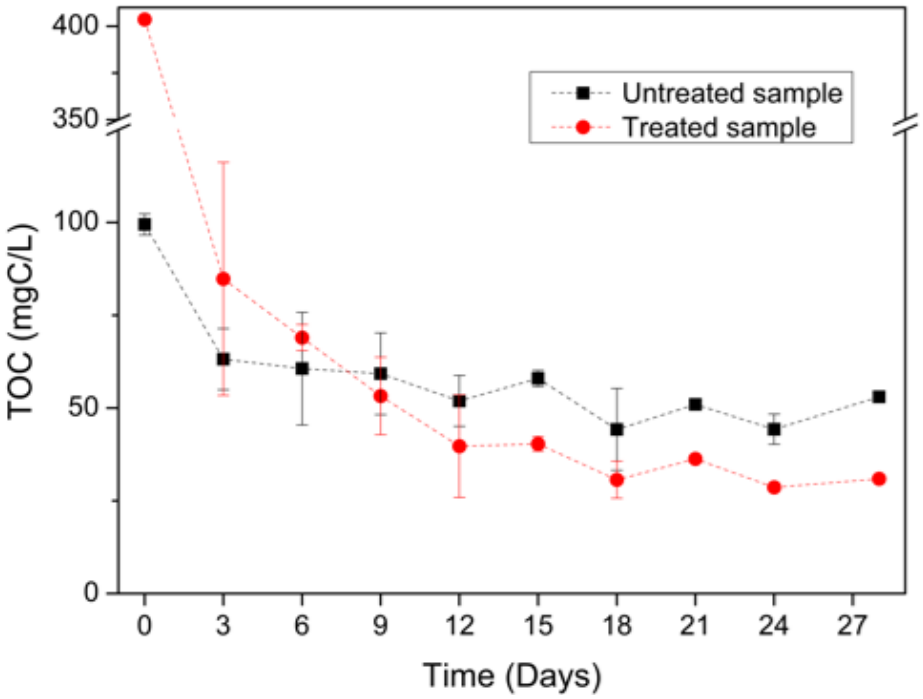
Parameter	Untreated wastewater	Treated wastewater
Color	Dark red	Light red
Odor	Non-objectionable	Non-objectionable
Absorbance (λ_{max} 520 nm)	1.917	0.537
pH	6.85	3.00
COD (mg O_2/L)	430±21	1262±29
BOD (mg O_2/L)	213±28	714±8
TC (mg C/L)	139±6	408.0±0.6
IC (mg C/L)	40±3	4.4±0.2
TOC (mg C/L)	99±3	403±0
Na^+ (mg/L)	257.0±0.8	354.4±0.6
Mg^{+2} (mg/L)	1.183±0.006	1.31±0.05
Ca^{+2} (mg/L)	2.86±0.04	3.09±0.07
K^+ (mg/L)	9.34±0.01	23.05±0.01

Source: Artifon *et al*. (2022)

When it comes to the effect of bioflocculant activity in dye-containing systems, the literature normally expresses the outcomes in terms of color removal only, and the presence of flocculant agent remaining fragments is not mentioned (ARTIFON *et al.*, 2021). In this work, a biodegradability test was performed for the high increase in TOC concentration and oxygen demand in the treated sample. The data in Figure 3.5 shows the high biodegradability of the treated sample in comparison to the untreated solution in terms of TOC through 28 days. It is clear to see the abrupt decrease in TOC values right on the third day of biodegradation kinetic for both samples, after day 12 the outcomes reach steadiness. By the end of the test, the treated and untreated samples presented a TOC concentration of 30.9±0.6 mgC/L and 53±1 mgC/L,

which represent a biodegradability index of 92 and 46%, respectively, when considering the initial concentration. It points out that yeast debris can be easily assimilated by microorganisms in conventional wastewater treatment. In comparison to untreated, the treated solution presented higher biodegradability and lower presence of dyestuff recalcitrant components.

Figure 3.5: Total organic carbon (TOC) outcomes from biodegradability test for treated and untreated samples of rhodamine dye-containing wastewater.



Source: Artifon *et al*. (2022)

3.4 CONCLUSIONS

The spent brewer's yeast is a valuable resource of biological substances that deserves valorization. In this study, the alkaline hydrolysis of the yeast cell showed to be a promising pathway to release inner polymeric substances that can be applied in several fields. The bioflocculant produced possesses efficiency on flocculation activity without cation further addition and, at acid pH, precipitates two potential recalcitrant dyes, rhodamine and flavine. Although the presence of the bioflocculant increased the total organic carbon concentration in the medium, the biodegradability test showed this excess can be easily mineralized by microbial activity, which resulted in a final solution with both lower organic and recalcitrant components. This study not only proves the potential of the bioflocculant from yeast hydrolysis in the treatment of recalcitrant dye-containing wastewaters but also features the new applicability of this brewery industry byproduct.

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4 EXCESS-ACTIVATED SLUDGE AS A SOURCE OF BIOFLOCCULANT FOR DYE-CONTAINING WASTEWATERS TREATMENT

This chapter is based on a manuscript submitted to *Water Research* in March 31, 2023.

ABSTRACT

Textile dyes are a major environmental concern related to the textile industry field due to their chemical complexity. The azo group, which corresponds to the most generated dyes, presents this molecular bond that hampers biodegradability, constrains conventional wastewater treatment, and demands further treatment approaches. In this work, it is presented a bioflocculant produced from textile industry excess activated sludge capable of precipitating Reactive Red 194 and Disperse Red 343 azo dyes. The alkaline hydrolysis employed on dried activated sludge to assess bioflocculant was optimized and the produced substance was characterized in terms of chemical composition, resulting in 27% protein, 12% carbohydrate, and 12% lipid contents. The chemical structure of dyes, the pH, and the respective potential zeta of the flocculation medium were taken into consideration during assays to conclude the flocculation mechanism. Flocculation assays resulted in color removal outcomes > 90% for both dyes at slightly acidic pH. A test comparing initial and final biodegradability confirmed the low cellular debris content after treatment as well as their microbial assimilation. The activity of the bioflocculant was evaluated with commercial naphthalene-based dispersant and the results were compared to color removal promoted by poly aluminum chloride (PAC). This work shows the feasibility of the low-cost activated sludge as a bioflocculant source and its potential in clarifying wastewater with azo dyes.

Keywords: waste valorization; flocculant agent; azo dyes; reactive red 194; disperse red 343.

4.1 INTRODUCTION

The textile industry activity corresponds to one of the major water-consuming segments (SUBRAMANI; THINAKARAN, 2017). Furthermore, dyes and chemicals related to dyeing and washing processes within the industry increase the concerns associated with textile wastewater. The azo dyes comprehend a group with a characteristic chemical structure (-N=N-), which confers the dye chemical stability against photolysis and prevents the microbial attack, that is, lowering biodegradability and maintaining color persistency (HENDAOUI *et al.*, 2018). These conditions constrain the biological conventional wastewater treatment efficiency, which demands prior management to avoid environmental impact and deleterious human consequences (SARAYU; SANDHYA, 2012).

Flocculation/coagulation operations are standard techniques used to treat textile wastewaters that present cost-effectiveness in comparison to advanced oxidative processes, ultrafiltration, adsorption, and others. However, some inorganic and synthetic flocculating agents have been associated with cancer risk (RUDÉN, 2004) and Alzheimer's disease (CAMPBELL, 2002), human health concerns that should be avoided. In this context, bioflocculants appear as an eco-friendly substitute to treat and clarify wastewater in general (SHAHADAT *et al.*, 2017). These compounds are mainly proteins and polysaccharides from microorganisms that present the ability to form flocs and precipitate dissolved and suspended substances in an aqueous medium (NOUHA *et al.*, 2018). The application of extracellular polymeric substances in textile wastewater treatment has increased in recent years, but the relatively low biological content released by culture strains cultivation creates barriers to the wide-scale application (ARTIFON *et al.*, 2021). So, the utilization of residues as a source of polysaccharides and proteins as flocculating agents is an unexplored field with great potential. Artifon et al. (2022) produced polymeric substances by hydrolyzing spent brewer's yeast and obtained color removal higher than 80% over synthetic and real textile wastewaters containing disperse dyes. Color removal results above 55% were reported by Sun et al. (2018) when using a bioflocculant produced by the hydrolysis of periphytic biofilms in an aniline blue solution.

During conventional wastewater treatment, the formation of activated sludge is inevitable and needs to be handled by the plant. It results in biomass production during microbiological growth, tends to exceed the plant capacity, and requires eventual removal (JUNG; XING; MATSUMOTO, 2001). In the textile industry, it is estimated that every million tons of textile wastewater produce nearly 25 m^3 of activated sludge (WANG, X. *et al.*, 2019). So, this

treatment plant residue deserves attention and appropriate disposal. The disposal of this sludge in industrial waste deposits is considered a major environmental liability, presenting risks of environmental contamination and high repair, environmental and social costs in case of weather accidents. The techniques often used for sludge handling include incineration (HUANG *et al.*, 2011), gasification (WANG, M. *et al.*, 2019), pyrolysis (SOHAIMI *et al.*, 2017), disposal in landfills, and dewatering (WEI *et al.*, 2018). In this context, this study aims at featuring a bioflocculant produced by the alkaline hydrolysis of excess activated sludge from a textile industry wastewater treatment plant, and, additionally, assess its potential on clarifying reactive and disperse azo dye-containing textile wastewaters.

4.2 MATERIALS AND METHODS

4.2.1 Source of materials

The activated sludge was obtained from the wastewater treatment system of a local textile industry. The material was dried under sunlight and grounded in a ball mill. The flocculation assays were performed separately with two synthetic solutions (150 mg/L) containing the Disperse Red 343 ($OD_{584\,nm}$, CAS: 99031-78-6, MW: 424.5 g/mol) and Reactive Red 194 ($OD_{539\,nm}$, CAS: 23354-52-1, MW: 896.2 g/mol) dyes represented in Figure 4.1 a) and b), respectively. The commercial dispersant CORACLEAN IP, a viscous naphthalene-based dispersant, was kindly donated by CORATEX Company, Brazil. The poly aluminum chloride 18% (PAC, alumina content 17.21%) was obtained from PROJESAN Company, Brazil.

4.2.2 Bioflocculant extraction

The extraction of the bioflocculant substances from activated sludge was obtained by alkaline hydrolysis of the crude material conducted at 100 °C in a heating mantle with a condenser (ARTIFON *et al*., 2022). Firstly, the amount of activated sludge mass added to 50 mL of NaOH solution (0.5 M) was varied from 0.5 to 4.0 g (30 min each). Later, a kinetic study of the hydrolysis with the optimized sludge mass was performed during 30 min. The flocculation activity of the extracted compounds was determined with the supernatant of the hydrolyzed solution obtained by centrifugation at 5000 rpm (2240 G) for 5 min. 200 μL of supernatant was added to 20 mL of the reactive dye-containing solution (150 mg/L), the pH was set at 3, vigorously agitated, and allowed to react for 30 min before centrifugation at 5000

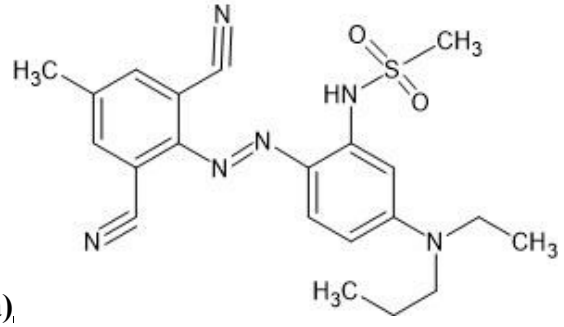
rpm (2240 G) for 5 min. The centrifugation was necessary to standardize optical measurements by settling down the suspended particles. A centrifuged sample of dye solution at pH 3 containing no bioflocculant was used as a control. The flocculation activity (FA) was accessed by optical density (OD) with a spectrometer (Femto, model Cirrus 80) and calculated according to Eq. 4.1.

$$FA = (b - a)/b \times 100\% \quad \text{(Eq. 4.1)}$$

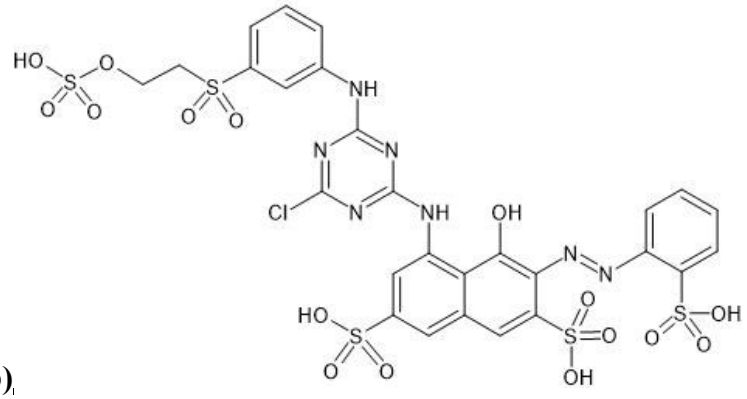
where a and b are the OD of the sample and control, respectively.

The supernatant with the bioflocculant produced under optimal conditions was purified for characterization with pre-cooled (-20 °C) absolute ethanol at a fourth of the supernatant volume. The resultant solution was kept at 4 °C overnight for stabilization and followed by centrifugation to obtain the precipitated flocculant agent (SUN *et al*., 2018), which was used for functional group determination.

Figure 4.1: Chemical structure of the dyes used in this study. a) Disperse Red 343; b) Reactive Red 194.



a)



b)

Source: Author (2023)

4.2.3 Bioflocculant characterization

Centesimal composition analysis was performed to determine the compounds of the dried milled activated sludge and the crude bioflocculant. Lipids, moisture, volatile, and mineral residue were quantified according to the methodology described by Adolfo Lutz Institute (2018). Proteins and sodium content were determined following the Association of Official Analytical Chemists (AOAC, 2016). The procedures were conducted in triplicate and the carbohydrate content was defined as the difference between 100 and the sum of the percentage of ash, lipid, moisture, and protein. The functional groups present on the surface of dried activated sludge and on the purified bioflocculant were examined by Fourier transformed infrared spectroscopy (FTIR – Agilent Cary 660 Spectrometer).

4.2.4 Flocculation assays

The effect of the pH of the bioflocculation system on color removal of effluent-containing reactive and disperse dyes in the study was evaluated. For the former, the procedure consisted of the addition of 200 μL of the supernatant of the extraction process into 20 mL of dye solution, the final pH was adjusted in the range between 3 and 8 with NaOH or HCl (1 M), the system was allowed to react for 30 min and later centrifuged at 5000 rpm (2240 G) for 5 min. For the second, the same procedure was followed, except for the centrifugation step, which was reduced to 1000 rpm (90 G) for 10 min due to the low solubility, a characteristic of disperse dyes. Zeta potential analysis (MALVERN Zetasizer Nanosizer) was performed for the same experimental conditions. Bioflocculant in distilled water was used as a blank for zeta potential and experiments were conducted twice.

A biodegradability test was conducted to estimate the presence of activated sludge debris on the treated sample as well as to compare its biodegradability to the original recalcitrant dye solution. This effect was accessed by an adapted version of OECD 301 A “DOC Dye-away test”. The test was performed with fresh activated sludge and 400 mL of treated and untreated dye solutions as a medium under dark conditions, constant agitation, and 22±1 °C. Samples were taken throughout 28 days, in duplicate, and evaluated by a TOC analyzer (Total Organic Carbon Analyzer TOC-V_{CPH}, Shimadzu). The biodegradability index was calculated as defined by OECD and represented in Eq. 4.2.

$$D_t(\%) = \left(1 - \frac{C_t - C_{b(t)}}{C_0 - C_{b(0)}}\right) * 100 \quad \text{(Eq. 4.2)}$$

where D_t is the degradation; C_0 and C_t are the mean TOC concentration in the inoculated culture medium containing test substance at initial and at time t, respectively; $C_{b(0)}$ and $C_{b(t)}$ are the mean TOC concentration in the blank inoculated mineral medium at initial and at time t, respectively. Only the dye solution containing reactive dye was used for the biodegradability test once the disperse one is withheld during the filtration step before TOC analysis.

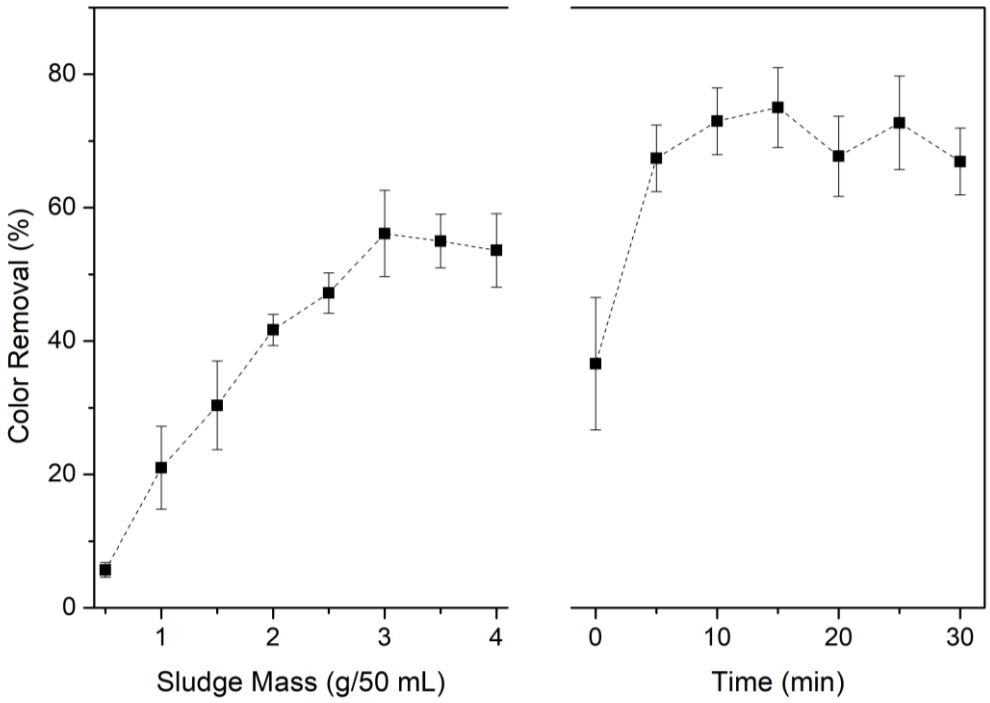
The effect of bioflocculant dosage in the range of 0 to 250 µL (0 to 250 mg) was evaluated in a flocculation system containing 20 mL of synthetic solution. The inhibitory effect of a naphthalene-based dispersant was accessed by adding this chemical to the flocculation experiments, the dispersant concentration was defined as 0.5 and 1.0 g/L for reactive and disperse dyes, respectively. To compare flocculation activity under these conditions, a similar set of experiments was conducted using PAC as a flocculant instead of the bioflocculant. All flocculation assays were conducted twice, at optimal pH, and followed by centrifugation and color removal quantification.

4.3 RESULTS AND DISCUSSION

4.3.1 Bioflocculant extraction

The extraction step was evaluated in terms of sludge load and hydrolysis kinetic. Figure 4.2 presents the results for color removal, which indicates the flocculation activity of the hydrolyzed supernatant. Flocculation activity increased proportionally from the sludge load starting point to 3 g of sludge/mL of solution, and no further increment was attained with sludge addition. The increase in sludge mass increased its accessibility in the hydrolysis medium, boosting the bioflocculant release until a certain point. The kinetic study on the alkaline hydrolysis of sludge presented that the supernatant generated attained its maximum flocculation activity of 73-75% at 10-15 min and 67% at 30 min. This light reduction in color removal may be attributed to the excessive degradation of the bioflocculant, mainly protein content, due to the exposure time to the operating temperature (ARTIFON *et al.*, 2022). Considering these results, the extraction medium was defined as 3 g of sludge in 50 mL of NaOH (0.5 M) aqueous solution, and the hydrolysis conditions were set at 100 °C for 10 min.

Figure 4.2: Flocculation activity for extraction step: sludge load (30 min each) and hydrolysis kinetic (3 g of sludge mass).



Source: Author (2023)

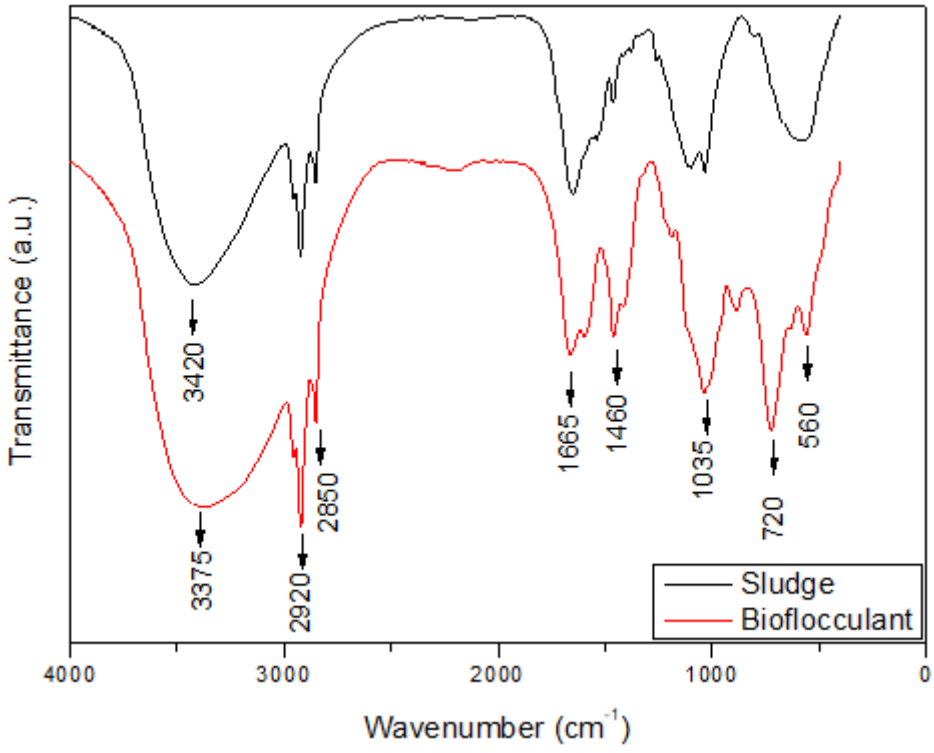
4.3.2 Bioflocculant characterization

Table 4.1 represents the centesimal analysis of both dried activated sludge and the supernatant from alkaline extraction used as bioflocculant. The dried and milled activated sludge presented a moisture and volatile content of 11.68%. When considering only solid fraction components, the protein, carbohydrate, and lipid content correspond to 35, 8.6, and 15%, respectively. A high protein content is characteristic of raw sludge (SUN *et al.*, 2016), which reverberates in the hydrolysate. For the hydrolysis supernatant used as bioflocculant, the moisture and volatile content correspond to 94.49% of the bioflocculant mass. Proteins, carbohydrates, and lipids represent 27, 12, and 12%, respectively, of the solid fraction.

The functional groups present on the surface of the dried and milled activated sludge and the purified bioflocculant were evaluated by FTIR spectra analysis and are depicted in Figure 4.3. Firstly, dried activated sludge presented a broad and strong band at 3420 cm^{-1}, a characteristic stretching vibration of the hydroxyl group (O-H) (GONG *et al.*, 2005). Bands at 2920 and 2850 cm^{-1} are related to C-H bending and known for carbohydrates presence (GONG *et al.*, 2008). The prominent peak at 1665 cm^{-1} indicates C=O from amino acids and amino-sugars (SUN *et al.*, 2018), which are classic for groups derived from proteins. The peak around

1075 cm^{-1} implies the presence of C-O groups (GONG *et al.*, 2008). The band between 1000 and 1200 cm^{-1} is characteristic of sugar derivatives (GAO *et al.*, 2009; GONG *et al.*, 2008). The same peaks are present on the bioflocculant sample spectra and, additionally, peaks at 1460 and 720 cm^{-1} were found, which can be associated with C=O asymmetric stretching of carboxylate ions in bioflocculant surface (ARTIFON *et al.*, 2022; SAHA *et al.*, 2020) and C-H rocking in long chain alkanes, respectively. The FTIR analysis confirms the chemical nature of the bioflocculant and its source by showing the heteropolysaccharides and protein derivatives presence.

Figure 4.3: FTIR spectrum depicting functional groups present on the surface of dried sludge and purified bioflocculant.



Source: Author (2023)

Table 4.1: Centesimal analysis of the activated sludge and the bioflocculant.

Component	Activated Sludge (g/100g)	Bioflocculant (g/100g)
Protein	31.41 ± 0.02	1.52 ± 0.09
Polysaccharides	7.62	0.64
Lipid	13.63 ± 0.14	0.65 ± 0.10
Mineral residue	29.06 ± 0.04	2.70 ± 0.06
Sodium	0.813 ± 0.001	0.698 ± 0.003
Fiber	6.6 ± 0.2	<0.5
Moisture and volatile	11.68 ± 0.07	94.49 ± 0.03

Source: Author (2023)

4.3.3 pH effect on flocculation assays

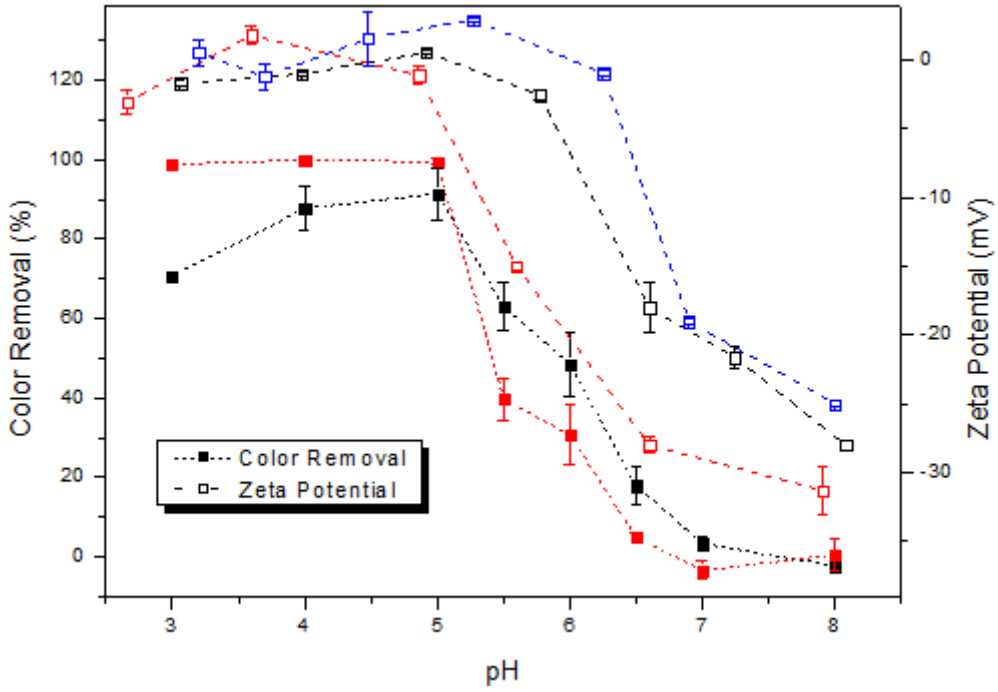
The pH of a medium interferes critically with the dissociation of functional groups present on active sites of flocculant agents (ELKADY *et al.*, 2017). So, it is possible to infer that pH promotes variations in structural features, bridging aptitude, and surface charge of bioflocculants (PAN; SHI; ZHANG, 2009). Figure 4.4 depicts the color removal where high flocculation rates for both dyes in the study are achieved as the pH became acidic. The flocculation activity reaches 91 and 100% for reactive and disperse dye, respectively, at pH 5, and decreases gradually to values near zero as pH reaches 7 for both systems. It is known that higher pH confers negative properties to biomolecules due to deprotonation, which increases repulsion among particles, hampers the bioflocculant-dye complex formation, and lowers the flocculation rate (DENG; YU; TING, 2005; ZHONG *et al.*, 2016). pH below 5 brings some resolubilization for reactive dye but not for disperse one, which can be attributed to the high solubility nature of the former under the high protonation degree of bioflocculant.

The zeta potential for the blank sample presented a net electrical charge near zero from pH 3 to 6.4, decreasing rapidly to -19 mV above pH 6.9. It points out the bioflocculant molecules suffer ionization of hydroxyl and carboxyl groups in alkaline conditions, giving negative properties to their structures (XUE; LI, 2008). Discussion on the effect of pH on zeta potential relating bioflocculant-dye complex systems is scarcely found in the literature (ARTIFON *et al.*, 2021). From Figure 4.4 it is possible to infer that low flocculation activity at pH above 7 is related to the high negative charges from bioflocculant stated by zeta potential measurements. As the negative charges on biomolecules are rapidly neutralized by medium

acidification in the range of 7 to 5 the color removal increases correspondently. This effect of charge stabilization on flocculation activity indicates that dye-bioflocculant complex formation is strongly dependent on pH.

For the system in the study, the Reactive Red 194 is an anionic dye that contains three sulfonic groups and one sulfate-ethyl-sulfone group, resulting in pka values below zero. These characteristics make the molecule maintain its negative charge even at very acidic conditions. It implies that the charge neutralization of the reactive dye is not the driving force that promotes its flocculation, but the charge neutralization of bioflocculant molecules which bond to dye by bridging effect, forms the starting flocs, and brings the suspension into precipitation.

Figure 4.4: Effect of pH in color removal and the zeta potential of reactive (black) and disperse (red) dyes flocculation systems, and in the bioflocculant in distilled water (blue).



Source: Author (2023)

To summarize simply, dye flocculation is not a consequence of reduced solubility, but a consequence of the bond formed between dye and flocculant. The sulfonic acid groups (RSO_2OH) present in the reactive dye provide stability in water, improving the solubility of the dye, due to its surfactant properties of reducing surface tension. The greater the number of sulfonic groups in the dye molecule, the greater its solubility. In addition, sulfonic acids are excellent proton (H^+) donors. Since the amines (present in the bioflocculant) have unpaired pairs of electrons, they are protonated in the acidic aqueous medium, receiving the proton H^+, due to its basic character. Therefore, the protonation of the $–NH_2$ terminal groups of the

bioflocculant occurs by the transfer of the proton from the $–SO_2OH$ terminal group of the dye or the acidified medium. When the bioflocculant is in an acidic aqueous bath, the $–S_2OO^–$ ions are neutralized by the H^+ proton and the bioflocculant becomes cationic, ideal for binding the anion of the acid dye to the NH_3^+ cation of the bioflocculant.

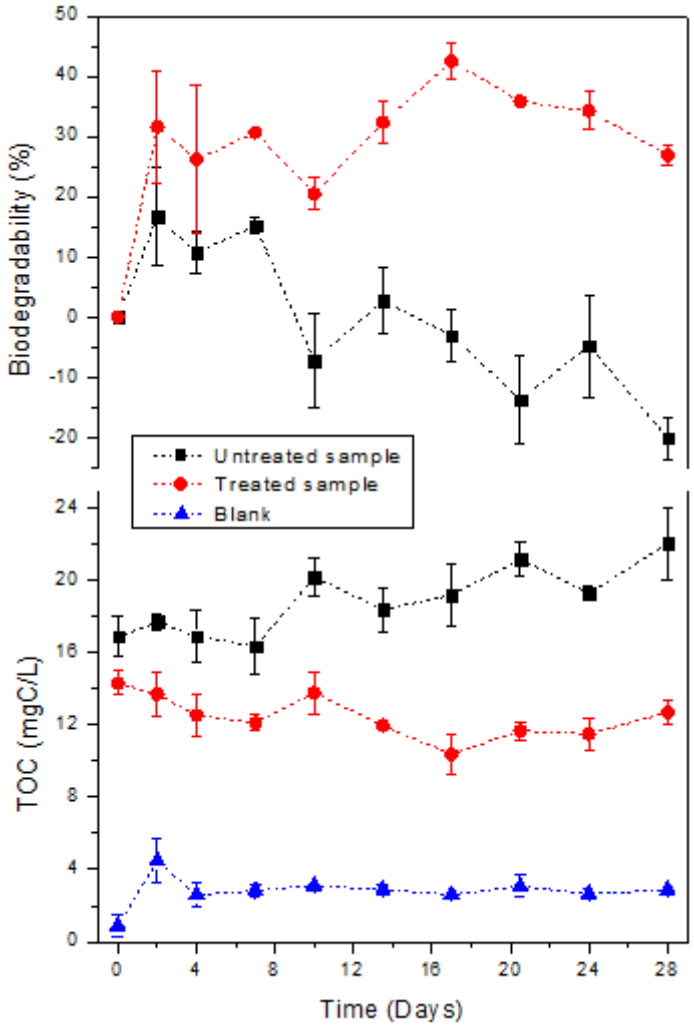
4.3.4 Biodegradability test

Due to the bioflocculant nature, some biological components may endure in the medium after flocculation treatment. This is a relevant fact for the bioflocculant removes color, but its remaining content may increase dissolved organic carbon after treatment (ARTIFON *et al.*, 2022). Soluble chemical oxygen demand substances are present after sludge hydrolysis and tend to increase concomitantly with the temperature of the reaction as a result of protein denaturation (SUN *et al.*, 2016). So, in this investigation, the biodegradability test was conducted to evaluate the biodegradation persistence of reactive dye and possible cellular debris present in the treated sample. Figure 4.5 presents the crude TOC outcomes throughout 28 days as well as the respective quantified biodegradability calculated according to OECD 301 A.

Firstly, the initial TOC concentration in the blank sample increased rapidly from 0.89 to 4.48 mgC/L in the first two days, indicating the releasing of dissolved organic compounds by the microbial activity and, after this period, the concentration reached equilibrium. The outcomes from untreated samples showed stabilization near 17 mgC/L for initially collected samples and a light increase to 20 mgC/L, which can also be attributed to the non-degradation of the reactive dye associated with the liberation of organic compounds by bacteria. On the other hand, the results of the treated samples presented a starting TOC concentration of 14.30 mgC/L, mostly represented by the cellular debris from bioflocculant once the color removal after flocculation was 92%. After day 13 the medium reached equilibrium, meaning no further degradation. These results can be better understood when expressing the calculated biodegradability at each point defined by Eq. 2. The treated sample presented a biodegradability result >30% against 16% from the untreated sample right in the second kinetic day. This index value increased for the treated sample to >42% on day 17, ending at 27% on day 28. On the opposite, the untreated sample attained a biodegradability index of 16% on the second day but decreased to negative values of -20%, accordingly to TOC concentration results. This difference in the biodegradability test is a relevant achievement, not only to justify a prior treatment of

this reactive dye-containing wastewater but to prove the assimilation of the cellular debris from alkaline hydrolysis by the microbial activity in a conventional biological treatment plant.

Figure 4.5: Total Organic Carbon (TOC) outcomes and respective biodegradability results for blank, treated, and untreated samples of reactive dye-containing solution.



Source: Author (2023)

4.3.5 PAC and bioflocculant assays

The naphthalene-based dispersant is a common chemical used within the textile industry for both reactive and disperse dyeing. For the former, it is used during the washing step after dyeing to remove deposited reactive dyes from cotton fibers, and, for the second, it is used during a polyester dyeing bath to improve the dispersion of the dye in the medium. However, this dispersant addition constrains coagulation during the wastewater treatment step. So, in this study, the interference of the dispersant was assessed against PAC and the produced

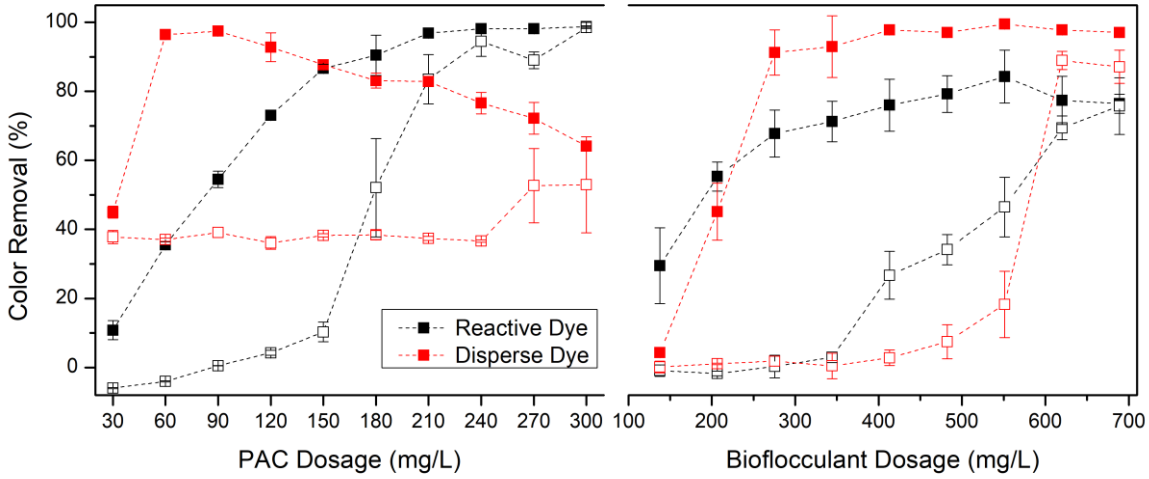
bioflocculant. Figure 4.6 presents the color removal attained by varying both flocculants dosage in reactive and disperse dyes medium. Also, results considering the presence and absence of dispersant in the concentration of 1.0 g/L for the reactive dye system and 0.5 g/L for the disperse dye system. All tests were conducted in duplicate at the optimal pH of 5.

Considering the moisture and volatile content from Table 4.1, it is possible to determine the solid content of the crude bioflocculant (5.51%) as well as its dry mass added to the 20 mL dye-solution system. The bioflocculant dosage added to the dye medium ranged between 137.75 and 688.75 mg/L (50 and 250 µL of crude bioflocculant), the color removal results for reactive dye started at 30 at the initial dosage, increased to 84% with 551 mg/L (200 µL) of bioflocculant, and slightly decreased with further additions. This result at the final stage is referred to as the saturation of bioflocculant in the medium, which promotes an incomplete dispersion and inhibits its activity (SAHA *et al.*, 2020). When dispersant was added to the medium, significant outcomes started at 413 mg/L (150 µL) of bioflocculant dosage, increasing until 76% at the final point. Disperse dye presented similar behavior. Color removal at the starting point was 4%, which increased quickly to 91% with 275 mg/L (100 µL) of bioflocculant and stabilized near 97% with further additions. When in presence of dispersant, the flocculation activity in the medium was 18% for 551 mg/L (200 µL) bioflocculant addition and increased abruptly to 89% with the next addition.

To compare the produced bioflocculant activity, experiments with the commercial flocculant agent PAC were considered into account in the concentration range between 30 and 300 mg/L in the dye medium. The color removal for the reactive dye system was 11% at the starting point and increased accordingly with PAC addition to 97% at 210 mg/L. The dispersant presence in the system inhibited PAC activity at initial points, slowing up color removal outcome to 10% at 150 mg/L. After that, further PAC addition ruptured the dispersant effect and attained 94% of flocculation activity at 240 mg/L. For disperse dye, a PAC concentration of 30 mg/L presented 45% dye precipitation, but at 60 mg/L the color removal increased drastically to 96%, decreasing continuously as the PAC content increased. This is a common effect related to the excess of flocculation polymers in a medium, they reestablish particle charges by wrapping their surfaces and hamper floc formation. PAC additions to the medium with naphthalene-based dispersant presence showed a low variation in the color removal outcomes, which remained at 37% baseline, only slightly increasing at final PAC dosages. These results show the retardant effect of dispersant reduces by more than 50% of PAC efficiency at 60 mg/L, which tends to be overcome with further PAC additions.

From Figure 4.6 it is also possible to compare PAC and bioflocculant performance on color removal. For reactive dye, an equivalent color removal >70% is attained at 120 mg PAC/L or 345 mg/L of bioflocculant (dry mass). For disperse dye, 90% of the component is precipitated with 60 mg PAC/L addition or 275 mg/L of bioflocculant (dry mass). These are relevant outcomes for a bioflocculant produced from a biomass residue that is considered an issue within the textile industry. This bioflocculant produced from excess activated sludge could valorize this material and minimize commercial flocculant agent demand.

Figure 4.6: Color removal outcomes considering PAC and bioflocculant (dry mass) variation for reactive and disperse dyes. Tests in the absence (■) and presence (□) of dispersant.



Source: Author (2023)

4.4 CONCLUSIONS

The excess activated sludge is an issue for wastewater plants due to the management it demands and possible environmental concerns it may cause under inappropriate disposal. This paper features the possibility of employing this biomass residue with low-cost handling into a valuable component to be used within the industry to treat wastewater and minimize commercial flocculant requirements. The bioflocculant proposed inhere presents efficiency on precipitating recalcitrant azo dyes at slightly acidic conditions and its potential could be further explored in several fields. Moreover, the bioflocculant is capable to overcome the dispersant effect and is further comparable to PAC precipitation efficacy on dye-containing wastewater systems. The use of bioflocculants has the advantage of the circular economy effect, the reduction of chemical additives in the process and being an eco-friendly route.

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5 FINAL REMARKS

5.1 CONCLUSIONS

The rich content of dye in textile industrial effluent is a concerning subject due to its effects on aquatic life and surrounding environmental systems. The effects on human health are also an unease once dyes residues have the potential to contaminate superficial and underground water systems that are essential for water suppling. Furthermore, water recovery within the industry is an essential subject that needs studies and development for environmental and economic purposes.

Industrial residues might require proper management and expensive procedures for correct disposal. The experimental work covered by this thesis presents the potential of bioflocculants produced from spent brewer's yeast and excess-activated sludge on precipitating textile dyes. The alkaline hydrolysis showed great effects by substantially improving the solubilization of flocculating compounds and making them available in the supernatant fraction of the hydrolysate. Both bioflocculants chemical composition were mainly proteins and carbohydrates, and the functional groups presented on their surfaces are known for inducing flocculation. Flocculation assays pointed out the intense effect of pH on the flocculation systems, and zeta potential analysis indicated the influence of adsorption and charge neutralization phenomenon as flocculation mechanism.

Considering the bioflocculants' efficiency, it is possible to affirm that the one from activated sludge presented a higher effect than the one from yeast on floc-formation and precipitation of dyes due to the reduced dosage requirements. Even considering the biomass weight used during yeast hydrolysis is lower due to its 70% of moisture, the bioflocculant releasing during activated sludge hydrolysis surpasses the yeast approach on bioflocculant solubilization and, consequently, flocculation activity. It could be attributed to the alkaline hydrolysis easiness on rupturing bacteria cell and release their inner components than rupturing yeast cell due to its complexity. Furthermore, the slightly acidic pH demanded for bioflocculant from activated sludge is an advantage. A pH reduction to 3, as required for the bioflocculant from yeast, increases considerably chemical demand and challenges treated water discharge. Additionally, the activated sludge presents higher availability and lower aggregated value due to its inherent impurities, which makes it a greater source of biological components.

5.2 SUGGESTIONS FOR FUTURE WORKS

Some challenges require further investigation on the bioflocculant research field.

- Polymeric substances from bacteria, extracellular or cellular rupture resulting compounds, are a complex mixture of biological material which identification to molecular level requires high-cost analysis but could better explain the bioflocculant-dye floc formation.
- The chemical structure of the dye is essential to start a flocculation assay. Many studies on literature do not bring it into account, which could lead to a superficial discussion. To promote a better understanding of the flocculation mechanism I would suggest for future works to know the dye chemical structure before starting.
- Others industry residues could be tested to have their potential as flocculant agents after proper hydrolysis evaluated. Some examples are blood, feathers, bristles, and algae from food industry and lignin from paper mills.
- Finally, the performance of both bioflocculants herein produced could be evaluated on the clarification or decontamination of wastewaters containing heavy metals, organic compounds, or even on algae harvesting.