Utilizing *Saccharomyces cerevisiae* to develop a system of mutualistic wastewater treatment with both chemical and biological treatments

Grant Proposal

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Executive Summary

Wastewater treatment is extremely expensive due to the copious amount of required energy and the need for tremendous volumes of different materials. Hence, the overall aim of this project is to develop a cheaper treatment method for removing wastes from textile effluents by using baker's yeast as the primary material for absorption. There will be two treatment methods within this system: A chemical treatment using chitosan harvested from the yeast for coagulating and flocculating the dye, which will be removed through a filter. After that, the yeast will biodegrade the remaining methylene blue dye within the solution. The effectiveness of this method is to be tested by purifying 1L of a 5% concentrated methylene blue dye solution. I expect this method to remove more than 99% of methylene blue dye as yeasts have shown to be an effective agent for biodegradation of this pollutant (Mazloomi et al., 2021), and chitosan, a viable coagulant (Zhao et al., 2021).

Keywords: Saccharomyces cerevisiae, chitosan, biodegradation, coagulation/flocculation

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Utilizing *Saccharomyces cerevisiae* to develop a system of mutualistic wastewater treatment utilizing both chemical and biological treatments

Effluents produced by the textile industry can be detrimental to the environment and human health (Al-Tohamy et al., 2022). Figure 1 provides a comprehensive review of the harms of textile effluents on the environment. Some of these detriments are caused by toxic dyes contained within the wastewater, which could remain within the earth and water for an extended period. These toxic chemicals could easily damage the environment by degrading the soil's quality and absorbing large amounts of oxygen, which causes plants to wither. Furthermore, when released to a body of water, dyes can inhibit sunlight from passing through to algae, which decreases the amount of oxygen produced at this location. In addition, dyes cause severe health problems in humans when ingested or inhaled, such as dermatitis, irritation, and even cancer. Hence, treatment of textile wastewater is needed to preserve the environment, ensure human safety, and maintain a clean water source for consumption and utility.

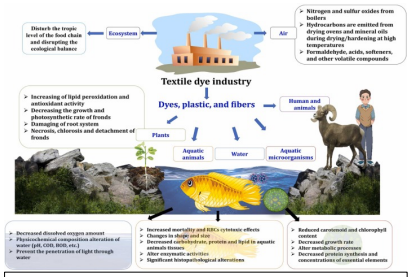

Figure 1: "Ecotoxicological impacts of dye-containing textile wastewater on the environment and living microorganisms." This figure explains the detriments of directly releasing textile effluents into the environment, emphasizing the need for wastewater treatment (Al-Tohamy et al., 2022).

Current textile wastewater treatment methods are expensive and consume a large amount of energy, which makes the technology inaccessible to low-income governments (Al-Tohamy et al., 2022). Therefore, this emphasizes the necessity of a cost-effective treatment for purifying textile effluents. The forefront of developing these treatments is using organic chemicals and microorganisms to remove dyes from the wastewater through adsorption and biodegradation, respectively (Al-Tohamy et al., 2022). Moreover, organic alternatives to current treatments are not only cheaper, but they are also much more environmentally friendly, as previous treatment methods may also leave toxic chemicals in the wastewater after treatment (Zahrim et al., 2011). The organic polymer that will be used in this research is chitosan, as it has been shown to remove 98% of methylene blue dye in a simulated wastewater solution under optimal conditions (Karthi et al., 2022). The microorganisms used for biodegradation in the experiment are fungi due to their resilience to dye, ability to produce chitin, and ability to degrade inorganic dyes in effluents (Nabilah et al., 2023).

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The process of using chitin as an adsorbent for removing waste materials from dyes has been extensively studied, and it was shown to be able to remove a significant proportion of heavy metals and methylene blue dyes from simulated wastewater (Nabilah et al., 2023). Indeed, chitin's crystalline structure allows for easy adsorption of dye particles due to its large surface area as a product of high porosity (Saravanan et al., 2022). Therefore, the dye particles adhere to the chitin's surface to create clumps that sink from the wastewater. However, chitin is mostly obtained from crustaceans, so this research examines the effectiveness of chitin produced by fungi for removing dyes from wastewater through adsorption. The importance of using fungi is that they can also be used for biodegradation of textile dyes, which allows for an efficient process combining chemical and biological treatment.

Biodegradation by fungi, although effective, can take a long time to achieve effective results due to the fungi's long growth cycle and high nutritional requirements (Jamee & Siddique, 2019). However, this limitation can be amended by combining fungi with bacteria in cell immobilization matrices (Nabilah et al., 2023). The fungi and bacteria in this research share a symbiotic relationship where the fungi produce laccase to degrade textile dyes while the bacteria produce nutrients to enhance fungal growth, which allows the fungi to produce the enzyme at a higher rate. Hence, combining fungi and bacteria produces an effective method for biodegradation, as well as improving the production of chitin to be harvested for an effective chemical treatment. Yet, the limitation of this method is that the fungi and bacteria are somewhat expensive to obtain, and the preparation process for creating the immobilized alginate beads are complex (Zhang et al., 2021). Hence, this project will utilize *Saccharomyces cerevisiae* due to the fungus' abundance in the market.

To increase the efficiency of wastewater treatment, this research aims to combine the chemical treatment, using chitin for adsorption of methylene blue dye, and the biological treatment, using Brewer's yeast for biodegradation of said dye (Ghodsi et al., 2018). This method reduces the amount of economy spent buying materials for wastewater treatment as yeasts are cost-effective, and the chitin can be continually harvested from the fungi using a two-phase extraction method (Gachhi & Hungund, 2018). The system would first begin with the chemical treatment where chitosan isolated from yeast would be mixed with a methylene blue dye solution at 2.5% concentration under optimal conditions (Karthi et al., 2022). Then, the coagulated dye will be filtered out, and a gram of yeast will be added to the solution to break down the dye. The removal rate of methylene blue dye will be measured using a spectrophotometer before and after the treatment for comparison. The expected result will be that the amount of dye removed is greater than 99%. If this result is achieved, this novel approach to wastewater treatment

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has the potential for large-scale commercial use for effectively purifying textile wastewater, replacing inorganic chemicals used for treatment that may cause harm to the environment.

Section II: Specific Aims

This proposal's objective is to draw attention to the usage of *Saccharomyces cerevisiae* for treating textile wastewater and the corresponding chitosan that could be harvested from the fungus. Due to the abundance of yeast, this engineering project contributes to the effort of developing a cheap and ecologically friendly system for recycling wastewater.

Our long-term goal is to show the effectiveness of *Saccharomyces cerevisiae* to introduce an economically efficient method for removing dyes from wastewater, where the central hypothesis of this proposal is to provide preliminary research on the role of *S. cerevisiae* within wastewater treatment as methylene blue dye is one of the most common dyes utilized in textile industries. Furthermore, obtaining chitosan, a natural coagulant for textile dyes, would increase the value of the yeast, as extracting the polymer from its cell wall is cheap, and chitosan's ability to coagulate was shown to be effective (Zhao et al., 2021). The rationale is that the yeast's effectiveness in removing methylene blue dye-and the chitosan extracted from the fungus would indicate that this approach is feasible for potentially developing a cost-effective treatment system for a wastewater facility. The work we propose here will draw attention to the usage of yeast in the treatment industry, as limited research has been done on the extraction of chitosan from yeast and the fungus' capabilities for biodegradation.

Specific Aim 1: The effectiveness of chitosan extracted from *Saccharomyces cerevisiae* for coagulating and flocculating methylene blue dye.

Specific Aim 2: The effectiveness of biodegradation of methylene blue dye by *Saccharomyces cerevisiae.*

Specific Aim 3: The effectiveness of the overall system at removing methylene blue dye after an instance.

The expected outcome of this work highlights the evidence for *Saccharomyces cerevisiae* as an eco-friendly and economically sustainable alternative for wastewater treatment. Hence, recycling wastewater would be more achievable for low-income governments, which helps with the endeavor to preserve this valuable natural resource.

Section III: Project Goals and Methodology

Relevance/Significance

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The significance of this work is that the effectiveness of yeast for biodegradation is overshadowed by bacteria's role in wastewater treatment. Hence, this research aims to shed light upon this field as yeasts have the natural ability to produce chitin that makes up their cell wall, so chitosan can be easily extracted from a population of yeast. Furthermore, yeasts are cheaper than bacteria, and fresh *Saccharomyces cerevisiae* are more accessible due to their abundance in the market. Thus, yeasts are cheaper alternatives to bacteria for creating a biological treatment of textile effluents.

Innovation

As stated before, bacteria are the focus for biodegradation of wastewater, so utilizing yeast for biological treatment will be a novel approach. Hence, this wastewater treatment method will perpetuate itself as the yeast will continually feed on the methylene blue dye and degrade it, which helps with their growth and reproduction. Thus, chitosan can be extracted from this new yeast population while still having the microorganism degrading toxic chemicals.

Methodology

Specific Aim #1:

Determine the effectiveness of the chitosan at coagulation and flocculation extracted from the cell wall of *Saccharomyces cerevisiae.* The objective is to produce chitosan from the yeast to be as effective as pure chitosan bought from a supplier and chitosan extracted from crustaceans. Our approach is to dehydrate the yeasts and put them in a solution of hot sodium hydroxide and stir it. Then, separate the yeast from the solution and wash them with distilled water (Syala et al., 2024). Next, dry the cells and put them in a hot sulfuric acid solution. Use a filter paper to extract the liquid for cooling. Then, remove the chitosan from the solution with centrifugation. Next, compare the chitosan obtained from the fungus to pre-made chitosan under the same conditions. Spectroscopy analysis will determine the effectiveness of the isolated chitosan by assessing the degree of deacetylation. Our rationale for submerging the yeast into a solution of sodium hydroxide at a high temperature is to disrupt the cell wall and degrade its components, such as proteins and glucans, to separate the chitosan. Then, the sulfuric acid solubilizes the chitosan from the cell wall, which is then separated from the solids using a filter paper. Centrifugation will separate the suspended chitosan from the solution (Araújo et al., 2020). The optimal conditions found by experimentation are to be kept the same throughout testing to provide comparable results.

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Justification and Feasibility. The method provides a way to obtain chitosan from yeast, which is instrumental for our work. One of the main reasons for using yeast, other than economic efficiency, is that it can produce chitin, which can be deacylated into chitosan. Then, the comparison is for determining the effectiveness of the extraction method for producing pure chitosan that can be used for coagulation. This data table shows the percent yield of chitosan from different fungal cells (Araújo et al., 2020). This data gives an approximation for the success of this procedure for producing chitosan than can be used, assuming that yeast cells are similar to these other fungal cells.

Table 1. Chitinous polymer production by different organisms using different cultivation modes and substrates

Product	Organism	Cultivation mode	Substrate	Production yield (%)	Reference
Chitosan	*Gongronella butleri*	Submerged	Sweet potato	12.7	25
		Solid state		9.2	
Chitosan	*Rhizopus arrhizus*	NA	Corn steep liquor and honey	3.0	26
Chitosan	*Rhizopus arrhizus*	NA	Molasses and corn steep liquor	4.9	10
Chitosan	*Cunninghamella elegans*	NA		3.3	
Chitin	*Rhizopus arrhizus*	NA		8.3	
Chitin	*Cunninghamella elegans*	NA		7.2	
Chitin	*Cunninghamella elegans*	NA	Glucose	24	27
Chitin	*Cunninghamella elegans*	Submerged	Yam bean	6.6	28
Chitin	*Aspergillus terreus*	Submerged	Glucose	34	21
Chitosan				4.8	
CGC	*Schizophyllum commune*	Submerged	Sucrose	15.2–30.2	12
CGC	*Komagataella pastoris*	Fed-batch mode	Crude glycerol	18–26	16
CGC	*Komagataella pastoris*	Fed-batch mode	Glucose	16	29
			Xylose	15	

Table 1: "Chitinous polymer production by different organisms using different cultivation modes and substrates." This table show the success of extraction methods similar to the one described (Araújo et al., 2020).

Summary of Preliminary Data. The data in Figure 2 is obtained using spectroscopy to determine the transparency of the solution after chitosan treatment. The amount of dye remaining within the solution is inversely proportional to the absorbency of the solution, as the more light rays the solution absorbs, the more apparent the methylene blue dye is within the solution. Major differences can be observed with the absorbance rate in relation to the pH. The graph shows that deviating from 7pH will reduce the effectiveness of chitosan absorption; hence, 7pH is the optimal condition for chemical treatment. In Figure 3, the light absorbance rate decreases as temperature increases, so the higher the temperature, the more effective chitosan is at coagulating. Thus, the optimal temperature would then be determined by the cost of energy to maintain the solution at that specific temperature while maintaining an effective chitosan coagulation rate. Furthermore, the preliminary data for comparing the effectiveness of yeast-harvested chitosan to other prominent sources of chitosan is needed for analysis.

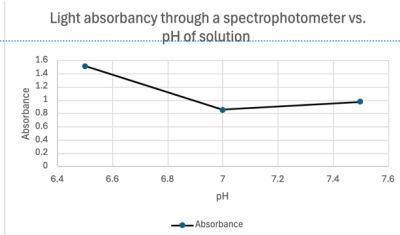

Figure 1: Graph showing the trend of pH effects on the light absorbance of the solution after treatment. As the pH deviates from 7, the solution after treatment is more opaque as shown by the increase in absorbance.

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Expected Outcomes. The overall outcome of this aim is to show that chitosan obtained from *Saccharomyces cerevisiae* is like pre-made chitosan products and those from crustaceans. This knowledge will be used to claim that research about chitosan's ability for coagulation of dye can be generalized to chitosan extracted from yeast.

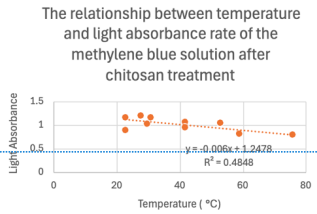

Figure 2: Graph showing the trend of temperature effects on the light absorbance of the solution after treatment. As temperature increases, light absorbance decreases.

Potential Pitfalls and Alternative Strategies. We expect the chitosan from the yeast to be of lower quality, meaning that it would have a lower degree of deacetylation, than pre-made chitosan, so the result of coagulation by the chemical may be lower than expected. However, if this is the case, then the chitosan could undergo another process of purification to enhance its quality. The purification process is to subject the chitosan to an acid bath for demineralization and deproteinization. The chitosan is also submerged in an alkaline solution for further deacetylation (Karthi et al., 2022).

Specific Aim #2:

Determine the effectiveness of biodegradation of methylene blue dye by *Saccharomyces cerevisiae*. The objective is to highlight the potential of yeast to be used as a biological treatment for textile effluents. Our approach is similar to the previous procedure set for comparison between different sources of chitosan for coagulation. We would prepare 2.5% concentrated methylene solutions, and half of them will be treated with yeast. All solutions in this experiment will be under the same conditions as the chitosan treatment. After the reaction had completed, centrifuge the samples to obtain the supernatant in preparation for spectroscopy. Compare the absorbance of the treated mixture and the control, untreated solution to determine the removal rate of methylene blue dye due to the yeast. Our rationale for this procedure fairly assesses if the yeast could be a reliable microorganism for biodegradation, so the yeast is compared to a control group that is not subjected to any treatment, so the change in methylene blue dye concentration is due to the yeast. The purpose of keeping the pH and temperature the same is to not lose any energy spent on changing these variables in an industrial setting from the previous chemical treatment method.

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Justification and Feasibility. Since yeast is the agent for this method's biological treatment, it is necessary to confirm the yeasts' effectiveness at removing one of the most common dyes used in the textile industry. This experiment will help evaluate *Saccharomyces cerevisiae's* potential as a microorganism that can benefit the treatment industry. Past articles have shown the yeast's capabilities of degrading methylene blue dyes, which provides the grounds for this project (Ghodsi et al., 2018). The table shows that yeasts have the potential to remove up to 99.16% of dyes within a simulated wastewater solution under optimal conditions, so the microorganism is capable of treating methylene blue dye. However, since the condition of this experiment differs from that of previous research, this necessitates the evaluation of the yeast's ability to degrade dyes under more stressful conditions to determine if the amount of energy saved by keeping the conditions the same is justifiable by the lower amount of degraded dye.

Table 2
Comparison of the affecting parameters on biosorption process by *S. cerevisiae* during dye removal.

Sorbent type	Modification method	Pollutant	Conditions	Removal efficiency (%) or sorption capacity (mg/g)	Ref
$ZnFe_2O_4$ nanoparticle functionalized baker's yeast, Pure yeast	Functionalized, Pre-treat	Methylene blue (MB)	Yeast dose: 2.5 g/L, dye concentration: 100 mg/L, pH: 7, time: 90 min.	S_{BET} = 108.3 mg/g (nanocomposite), S_{BET} = 79.3 (pure yeast)	Zhang and Zhang (2020)
S. cerevisiae	Pre-treat	C. I. Reactive Red 2	Yeast dose: 1.25 g/L, pH: 2, temperature: 30 °C, time: 90 min.	500 mg/g	Zhang and Wang (2013)
baker's yeast modified with Fest	Immobilization via adsorption, Pre-treat	Crystal violet	Yeast dose: 0.2 g/L, dye concentration: 100 mg/L, pH: 10, time: 150 min.	S_{BET} = 17.9 mg/g, Langmuir = 15.1 mg/g	Zafeen et al. (2016)
Poly (methacrylic acid) modified baker's yeast	Functionalized	MB, Rhodamine B (RB), Basic magenta (BM)	Yeast dose: 0.05 g/L, dye concentration: 1×10^{-3} mol/L, pH: 6.5, time: 70 min, temperature: 35 °C	MB, RB, and BM were 860.6, 267.4, and 719.4 mg/g	Yu et al. (2009)
S. cerevisiae	–	Remazol Orange RR	Yeast dose: 2 g/L, dye concentration: 200 mg/L, pH: 3, temperature: 25 °C	84.9%	Ulas and Ergun (2019)
baker's yeast modified by nano-Fe_2O_3	Functionalized, Pre-treat	Methyl violet	Yeast dose: 0.5 g/L, pH: 6, dye concentration: 300 mg/L, time: 30 min, temperature: 25 °C	60.8 mg/g	Tian et al. (2010)
S. cerevisiae immobilized on calcium alginate	Immobilization via encapsulation	Methylene green (MG)	Yeast dose: 0.25 g/L, dye concentration: 140 mg/L, pH: 5, temperature: 35 °C, time: 240 h.	17 mg/g	Godbole and Sawant (2006)
xanthate-modified baker's yeast	Functionalized	MB	Yeast dose: 0.2 g/L, dye concentration: 100 mg/L, pH: 7, temperature: 25 °C	64.5 mg/g	Song et al. (2019)
Baker's yeast modified MnO_2 composites.	Functionalized	MG	Yeast dose: 1 g/L, dye concentration: 100 mg/L, pH: 10, temperature: 25 °C, time: 120 min.	Sorption capacity: 86.7 mg/g, Langmuir = 243.9 mg/g	Santos et al. (2019)
S. cerevisiae immobilized calcium alginate (SC-A-5%), *S. cerevisiae* immobilized chitosan (SC-C-2.5%)	Immobilization via encapsulation	Orange II, Indigo Carmine	Yeast dose: 1 g/L, OII concentration: 30 mg/L, IC concentration: 50 mg/L, pH: 5	27.8% and 58.3% (alginate), 17.2% and	Rumi et al. (2021)
Yeast (*S. cerevisiae*) extract capped on the surface of Ag nanoparticles	–	MB	Yeast dose: 0.2 g/L, dye concentration: 10 mg/L, time: 6 h	80%	Roy et al. (2015)
S. cerevisiae	–	Alizarin Red S	Yeast dose: 0.4 g/L, dye concentration: 75 mg/L, pH: 3, time: 120 min	69%	Ramavandi et al. (2019)
S. cerevisiae	–	m-(4-dimethylamino) phenylazo) benzeneosulfonic acid, methyl orange	Yeast extract (0.25%, wt/vol), glucose (2%, wt/vol), and 0.2 mmol/L of the tested dye	–	Ramalho et al. (2005)
S. cerevisiae	–	Reactive Red 120	Yeast dose: 0.25 g/L, dye concentration: 16.25 mg/L, pH: 4.75, time: 52.5 min.	99.9%	Noroozia et al. (2019)
S. cerevisiae immobilized on Luffa	–	Direct Red 23	Yeast dose: 100 mg/L, pH: 2.5, time: 240 h, temperature: 30 °C	49.42% (dilute), 65.5% (powder)	Morão et al. (2017)
Chitosan Beads with Immobilized *S. cerevisiae*	–	Acid Blue 25 dye	Yeast dose: 50 g/L, dye concentration: 100 mg/L, pH: 2.5, time: 240 min.	28.2 mg/g	Mendes et al. (2021)
S. cerevisiae	–	Direct Orange 2 GL	Yeast dose: 0.5 g/L, dye concentration: 50 mg/L, pH: 4, time: 90 min	0.06 mg/g	Morales et al. (2015)
S. cerevisiae	–	MB	Yeast dose:1.32 g/L, dye concentration: 14.37 mg/L, pH: 9.35, time: 50.46 min	99.16%	Mashayeti et al. (2021)
S. cerevisiae	–	Ramazole blue (Vinyl sulfone)	Yeast dose: 0.25 g/L, dye concentration: 100 mg/L, pH: 2, time: 60 min, temperature: 25 °C	100%	Mohamed (2016)
S. cerevisiae	–	MG	Yeast dose: 1 g/L, dye concentration: 100 mg/L, pH: 6, time: 60 min, temperature: 30 °C	75.18%	Liu et al. (2019)

Table 2: "Comparison of the affecting parameters on biosorption process by *S. cerevisiae* during dye removal." This table show the success of the fungus' biodegradation of various dyes commonly used in the textile industry (Ghodsi et al., 2018).

Summary of Preliminary Data. No preliminary data have been collected on this matter, but we expect the data to show that the yeast's biodegradation of methylene blue dye was not hindered, so it is acceptable that the conditions be kept the same from the previous treatment to save electricity.

Expected Outcomes. The overall outcome of this aim is to determine the biodegradation ability of yeasts with the same conditions in the previous treatment with chitosan. We expect the yeast's removal rate of methylene blue dye to be insignificantly less than the percentage reported in past research, as *Saccharomyces cerevisiae* is resistant to environmental conditions (Al-Tohamy et al., 2022). Hence, the operating cost to run the biological treatment decreases because no effort is needed to change the pH or the temperature from one method to another. This knowledge will be used to promote yeasts as an effectual microorganism for treating wastewater over bacteria, as yeasts are more resilient.

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Potential Pitfalls and Alternative Strategies. We expect the change in the effectiveness of biodegradation by yeast to be minuscule, but if the pH and temperature greatly impact the ability of the yeast to degrade dyes, then we will adjust the pH and temperature of the solution accordingly. The optimal pH for biodegradation by *S. cerevisiae* is 9.35pH (Mazloomi et al., 2021) and about 30°C (Ghodsi et al., 2018). The pH level can be adjusted easily by using 1N NaOH solution to increase the pH and decrease the temperature of the hot plate to 30°C. However, scaling the experiment into a treatment industry will increase energy and economy consumption significantly.

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Specific Aim #3:

Determine the effectiveness of the combined system for treating a simulated wastewater solution, which is a 5% concentrated methylene blue dye (Yaseen & Scholz, 2018). Our approach is the same as the procedure mentioned for specific aim #2, with the difference that the chemical treatment will be performed before the biological treatment. Chitosan will be added to 10 of the solutions created, and the same procedure will take place, but after the reaction is done, the flocculated dye will be filtered out, and 1.5g of yeasts will be added into the solution. After the reaction is complete, spectroscopy analysis will be performed to assess the effectiveness of the system as a whole. Our rationale for a combined treatment system is to increase the removal rate of methylene blue dye from the mixture for better purification of water. The reason for the initial chemical treatment is to lessen the concentration of methylene blue dye to reduce the stress experienced by yeasts in the biological treatment. Furthermore, yeasts can be recycled after treatments as they persist after degrading the dyes, so biodegradation is cost-efficient, which is the reason why it is included.

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Justification and Feasibility. This wastewater treatment system is essentially a combination of chemical treatment using chitosan and biological treatment using *S. cerevisiae*. Hence, the justification for the specifics of this system has already been elaborated in specific aims #1 and #2. As for the overall mechanism, the inspiration for a mutualistic treatment method is that some wastewater treatment facilities are using a combination of chemical and biological treatment methods already, so this approach will be easy to implement for existing establishments (Al-Tohamy et al., 2022).

Summary of Preliminary Data. No preliminary data have been collected on this matter, and the preliminary data obtained from the two previous aims will provide a good indication for the success of this project. Hence, we expect the

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chitosan from yeast to be able to coagulate and flocculate as well as pre-made chitosan, and the yeasts to remain an effective biodegradation agents under the same conditions as the chemical treatment.

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Expected Outcomes. The overall outcome of this claim is to promote this mutualistic treatment system that is extremely easy to replicate but remains an effective and cost-efficient treatment. This knowledge will be used to emphasize the simplistic nature of the method that is still able to provide desirable results.

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Potential Pitfalls and Alternative Strategies. We expect to refine this system in accordance with the results obtained in specific aims #1 and #2. In the scenario that the results from these aims are extremely undesirable, major alterations are needed to improve the system, leading to a reevaluation of the methods to harvest chitosan from yeasts, and the yeasts' capability for degrading dyes is needed. In this case, specific aims #1 and #2 would receive revisions, as well as potentially adding a treatment method to the current system.

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Section IV: Resources/Equipment

The materials are pre-made chitosan, 5% Acetic solution, hot plates, filter papers, ice, magnetic stirrers, beakers, pipettes, *Saccharomyces cerevisiae*, 1N solution of KOH, a centrifuge, centrifuge capsules, spectrophotometer, filter paper, distilled water, and tap water. All of this equipment is readily available at the school site.

Section V: Ethical Considerations

There are no serious concerns for safety in this project. However, standard lab safety procedures still apply, such as not ingesting any chemicals present in the procedures. Methylene blue dye is carcinogenic and is toxic to humans. Furthermore, yeasts are single-celled organisms, so there are no ethical concerns in this project.

Section VI: Timeline

Specific aim 1: To be completed in early January

- Obtaining chitosan from yeasts: To be completed on December 13th
- Obtaining chitosan from crustaceans: To be completed on December 26th
- Comparison: To be completed on January 5th

Specific aim 2: To be completed in late January

- Determining the effectiveness of biodegradation by yeast: To be completed on January 20th

Specific aim 3: To be completed in mid-February

- Determining the effectiveness of the method: To be completed on February 10th

Section VII: Appendix

We are applying for a small grant for purchasing some mundane equipment in case of the materials were not all present at the school where the experimentation take place. Currently, we are lacking sufficient amount of methylene blue dye at 5% concentration, so a small grant will be able to cover that expense.

Section VIII: References

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