Design Brief

Yeast biosensor for simple detection of lactose-metabolizing pathogens through a galactose-inducible promoter*

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Diseases caused by contaminated water, such as typhoid fever and cholera, indicate that there may be harmful bacteria in the drinking water. It is important to be able to identify unsafe drinking water to determine the presence of harmful pathogens. A functional, self-renewing biosensor can be made accessible and cost-effective, which is necessary to detect unsafe water in resource-constrained settings. We propose utilizing yeast to build a biosensor for these purposes. The yeast Saccharomyces cerevisiae, commonly known as baker's yeast, is a good candidate for a biosensor due to its resistance to various environmental conditions, including temperature and nutrient concentrations. S. cerevisiae was transformed with the pESC-URA plasmid to express the green fluorescent protein (GFP) in the presence of galactose, a byproduct of the catabolism of lactose. Lactose, a disaccharide composed of galactose and glucose, can be metabolized by bacteria that have the lac operon, which produces the enzyme β-galactosidase to cleave lactose into its component monosaccharides. In the presence of lactose-metabolizing pathogens, such as Escherichia coli, GFP transcription is induced and detected through colorimetric analysis under ultraviolet light. However, S. cerevisiae does not produce β-galactosidase and thus does not induce GFP transcription. Our biosensor consists of the transformed yeast in a lactose solution. The sensitivity of the biosensor was tested by varying the concentration of E. coli initially in the solution. Understanding the capability of producing a biosensor is essential to ensure safe drinking water and effective water filtration methods. This critical first step is also needed to better understand the prevalence of waterborne diseases.

Keywords: Yeast biosensor, *Saccharomyces cerevisiae*, *Escherichia coli*, self-renewing biosensor, waterborne illness

A multitude of areas around the globe suffer from a lack of clean water sources. While many efforts have been initiated to resolve this issue, some are costly, which can harm communities, especially undeveloped ones. Moreover, some solutions are highly unsustainable, such as large wastewater treatment plants, due to the high energy consumption and release of greenhouse gasses into the atmosphere (Gagné, 2023). Therefore, a more efficient, sustainable, and attainable solution for small-scale wastewater treatment must be found, and water treatment begins with the successful detection of harmful agents.

There are several dangers associated with

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drinking unclean water. Most prominently, pollutants include heavy metals, plant nutrients, and micropollutants (Gagné, 2023). However, pathogenic contaminants are perhaps the most problematic because these diseases can be highly transmissible, causing large outbreaks in populations. Evidently, clean water is not available to the entirety of the public, which is highly dangerous; for example, drinking water contaminated with pathogens such as *Salmonella* and *Vibrio* or *Helicobacter pylori* can cause severe gastrointestinal disease or even gastric cancer (Magana-Arachchi & Wanigatunge, 2020). Moreover, in 2016, over 829,000 deaths were attributed to diarrheal disease, which is closely linked to waterborne pathogens such as *Salmonella* and *Vibrio* (Magana-Arachchi & Wanigatunge, 2020). Having a quicker and overall, more efficient process through the use of a biosensor allows communities to stay safe. The main purpose of this experiment is to provide a method to test for safe water.

The use of yeast as the host organism for a biosensor is beneficial in several ways. First, yeast spp. (particularly *Saccharomyces cerevisiae*) are quite resilient and are able to withstand relatively harsh conditions, including variable temperatures, pH, and contaminants such as alcohol. This is necessary so that they do not simply die when making contact with a sample solution and so that they can be easily and safely stored. Simplicity of storage is also important since yeast can be stored easily in an "active-dry" state. Finally, since yeasts are eukaryotic, they are able to implement more complicated biological pathways that may allow more sophisticated detection techniques in the future (Adeniran et al., 2015). For instance, eukaryotic cells such as yeast are much more able to properly fold proteins, unlike prokaryotes (Figure 1).

Yeast-based pathogen biosensors have already been implemented in some studies, such as the Patrol Yeast, which targets toxic substances in food (Dong et al., 2023). However, the benefit of our method is its simplicity. This would be useful in underdeveloped areas that may not have access to the most sophisticated equipment but do have some basic lab equipment. Also, once created, the genetically modified yeast can reproduce and become self-renewing so that it does not have to be constantly replaced. Creating this practical and applicable biosensor is important to increase accessibility to clean water and reduce the risk of waterborne disease in populations that do not have guaranteed clean water supplies.

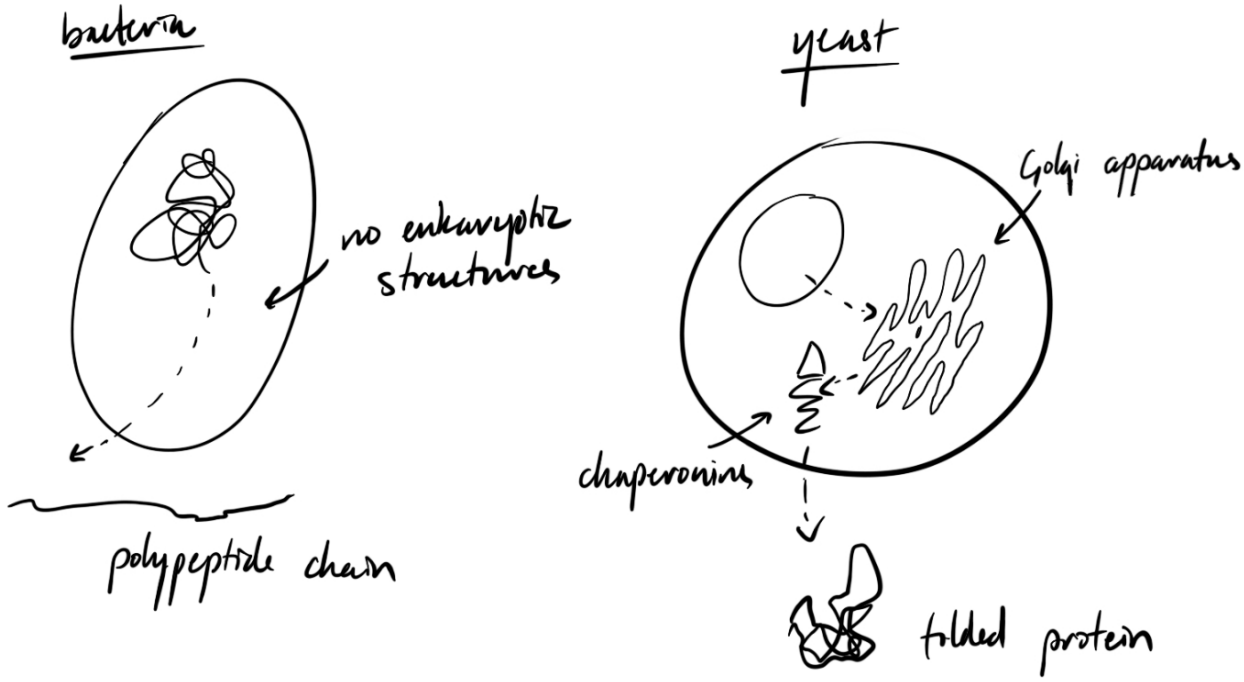


Figure 1. Protein synthesis in bacteria vs. yeast.

Materials and methods

Preparation of yeast

To create the yeast biosensor, a yeast chassis with a selectable marker was needed so that only transformed colonies would appear. When plated on complete minimal media without uracil, *S. cerevisiae* with the URA3 marker that does not have a plasmid is not able to grow. Yeast colonies were isolated on YED and CM without uracil agar plates (TekNova, Hollister, CA, USA), and disposable inoculating loops (Carolina Biological Supply, Burlington, NC, USA) were used to streak the plates.

The pESC-URA plasmid was chosen as a base since it has both uracil and a galactose promoter (GAL1). Then, for easy detection and colorimetric analysis, the fluorescent marker eGFP was chosen as the protein produced in the presence of galactose. Due to limited resources, we did not insert the gene ourselves and ordered 4 µg of lyophilized plasmid synthesized by GenScript Biotech (Piscataway, NJ, USA). The plasmid was resuspended in sterilized water by centrifuging at 6,000 x g for 1 min and adding 20 µL of water (GenScript).

Yeast transformation reagents (Zymo Research, Irvine, CA, USA) were used to transform *S. cerevisiae* with the pESC-URA-eGFP. The attached Frozen-EZ Yeast Transformation II protocol included creating a pellet, washing, and resuspending the yeast cells; adding 0.5 µg plasmid DNA in 50 µL of competent cells; incubating for 45 minutes; plating the transformed cells on YED agar (TekNova); and incubating for 2–4 days (Zymo Research, 2021). Both the transformed and untransformed yeast were also plated on CM-URA agar to check that the transformation was successful.

Following the incubation, the transformed yeast was ready to be used as a biosensor.

Escherichia coli culture

The HB101 strain of *E. coli* (Bio-Rad, Hercules, CA, USA) was used since it is nonpathogenic and safe to work with in a BSL-1 laboratory. To resuspend the lyophilized cells, 0.3 mL of LB broth was added and mixed. The broth was then incubated at room temperature for 1 day.

Then, a serial dilution was performed to observe the effect of concentration on the detection ability of the biosensor (Figure 2). A total of 10 µL of the previously prepared LB broth was mixed with 90 µL of water in a microcentrifuge tube labeled 1 (Carolina Biological Supply). Then, 20 µL of the dilution was plated onto an LB plate (TekNova), and another 10 µL was used for the next dilution. This was repeated for a total of seven plates. Next, 80 µL of the original LB broth was placed in a tube labeled 0, and 20 µL was plated; 20 µg was removed from the last sample and discarded. Then, the whole procedure was repeated for two sets of serial dilutions. The 16 plates were incubated at 30°C for 1 day.

The microcentrifuge tubes were kept and immediately used for detection. It is important that they are not incubated so that the bacterial concentration in the samples is the same as the amount observed from the serial dilutions.

Detection of E. coli

Three colonies of the prepared yeast were inoculated into 150 µg of distilled water. Six colonies of the yeast were inoculated into another 150 µg of water. Then, 0.5 g of lactose was added to each sample. Next, 20 µL of the three-colony biosensor solution was added to each of the first set of eight microcentrifuge tubes; 20 µL of the six-colony biosensor solution was added to each of the second set of eight microcentrifuge tubes. The tubes were left at room temperature.

Using a black light and the CarolinaRGBColorimeter app (Carolina Biological Supply), the presence of GFP was analyzed in each of the 16 microcentrifuge tubes every hour for 5 hours.

Although *S. cerevisiae* and *E. coli* strain HB101 are both nonpathogenic and safe to use in BSL-1 laboratories, all plates and equipment were autoclaved or safely discarded following the experiment.

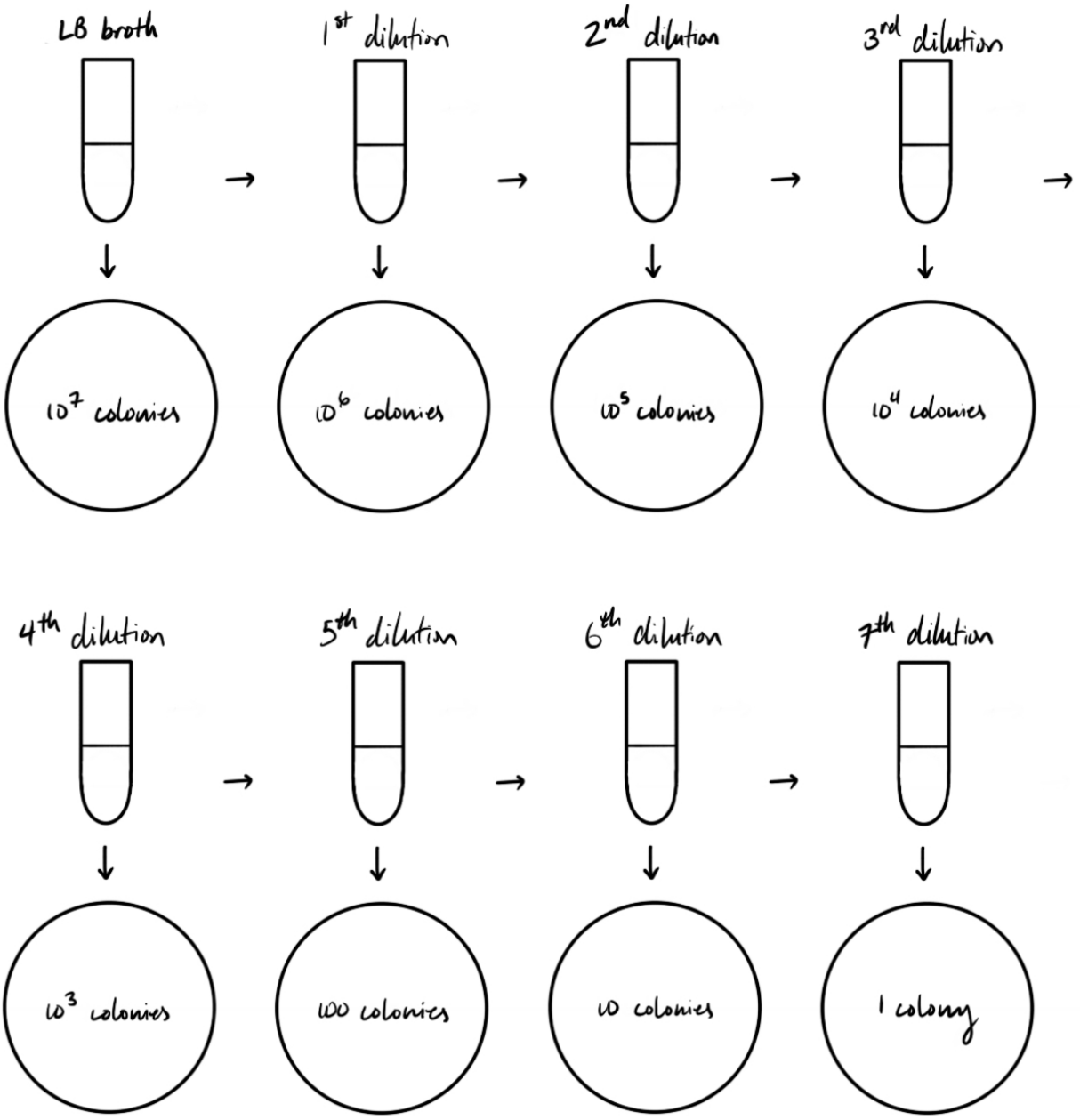


Figure 2. Serial dilution (with hypothetical data). Each subsequent dilution results in a 10x less concentrated solution (tenfold dilution).

Results

Colorimetric Analysis

For each recorded time, two photos were taken, as shown in Figure 3. The photos were converted to black and white images, and the colors of the liquids in the tubes were analyzed with the RGB values (all red/green/blue values are the same since the images are in grayscale). The higher of the values from the two photos was recorded in Table 1.

Time

In order to track the effect of time on the visibility of the biosensor, the brightness was recorded and analyzed at several different times, as shown above in Figure 3. These data

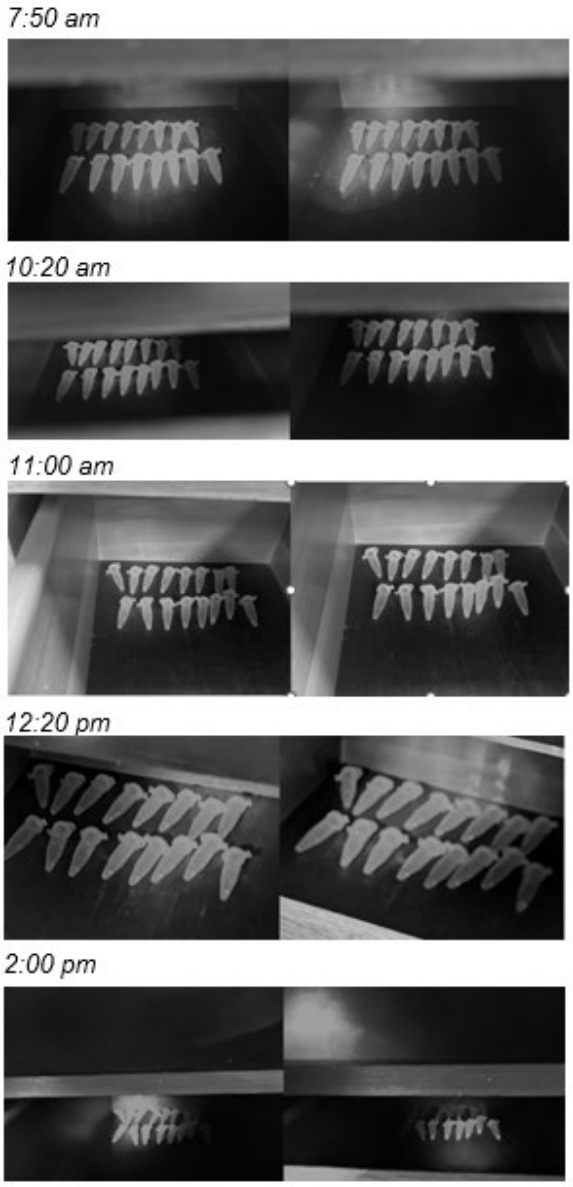


Figure 3. Biosensor solution with contaminated water samples under black light over time.

are represented graphically below in Figures 4–6.

E. coli concentration

Growth was found on all plates, but only the sample 7 plates had a suitable number of colonies to count. Since a tenfold serial dilution was performed, the concentration of the other samples was calculated by multiplying the concentration by 10 for each previous step of the dilution.

Yeast concentration

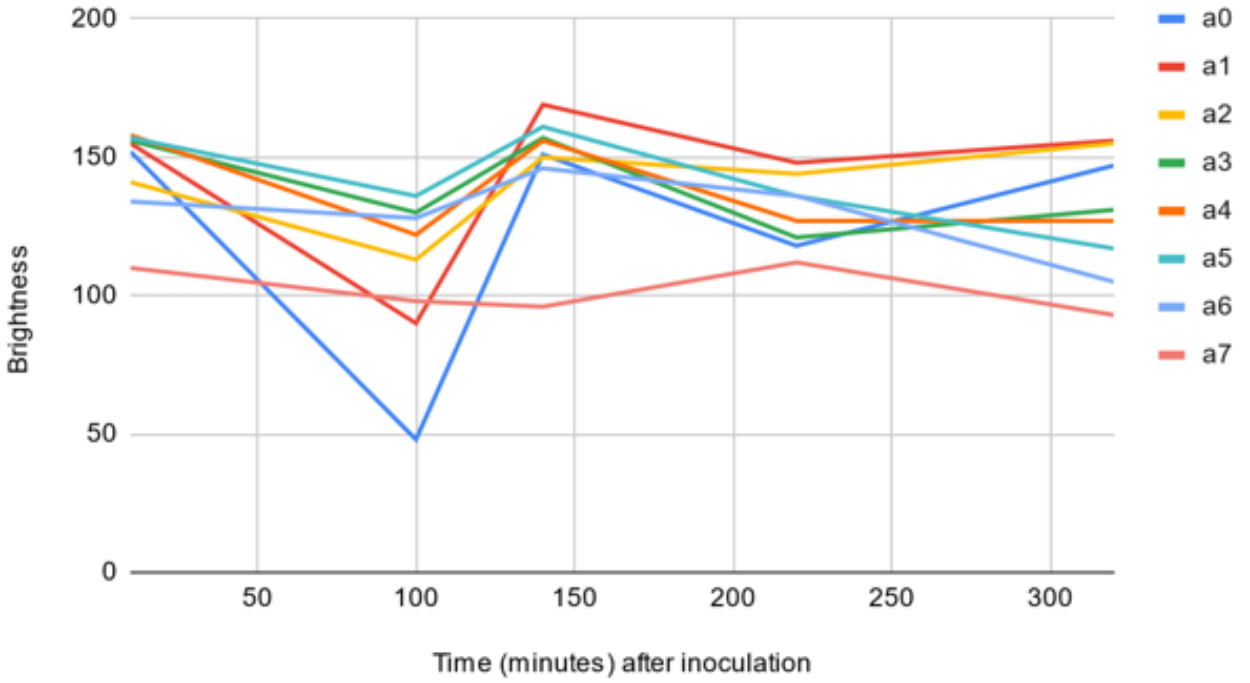
The samples in set A were inoculated with fewer yeast colonies than those in set B, and the brightnesses of these two sets were recorded and analyzed separately. As depicted in Figures 6 and 8, the samples in set A were consistently less bright than the samples in set B.

Discussions

The colorimetric analysis techniques used in this report were highly inconsistent and yielded results that are likely inaccurate. To begin, the camera used to take the images must be consistent. Cell phone cameras typically adjust brightness automatically, which results in inconsistent representations of brightness (e.g., dimmer samples will appear brighter in a photo after adjustment). Also, different cameras capture the same image slightly differently, so the resulting images could give a different brightness. Furthermore, the intensity of the black light must be consistent. Shining a small handheld black light in different places results in different intensities depending on where the light is pointed, which also changes the brightness of the samples. This was an issue with this data set; as Figure 8 shows, especially in set A, the highest and lowest concentrations are lower than the rest of the trend, which is likely a result from the dimmer edges of the black light illuminating the samples on either end. A standardized setup—using one camera that does not auto adjust, a larger black light that shines equally on all samples and is fixed in a certain position, and a darker room to minimize other visible light—could fix many of these issues

Table 1. Colorimetric analysis of analytes at different times

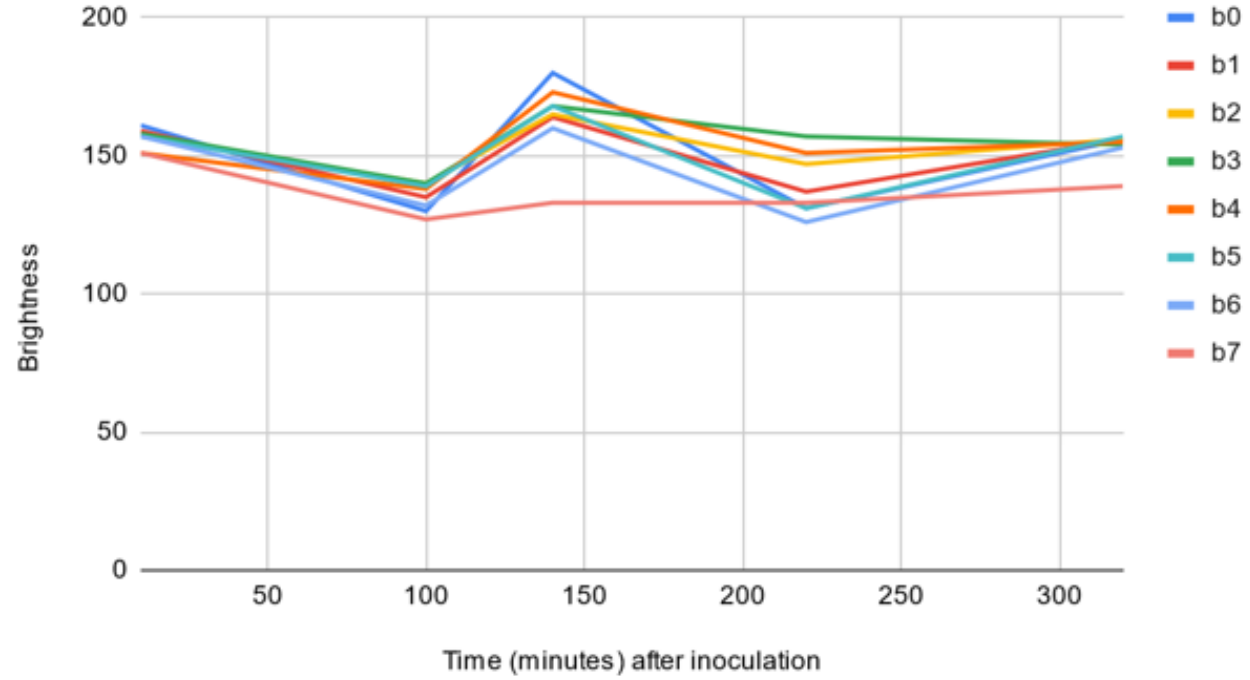
	a0	a1	a2	a3	a4	a5	a6	a7	b0	b1	b2	b3	b4	b5	b6	b7
7:50	152	155	141	156	158	157	134	110	161	159	157	158	151	157	157	151
10:20	48	90	113	130	122	136	128	98	130	135	140	140	138	139	132	127
11:00	151	169	150	157	156	161	146	96	180	164	165	168	173	168	160	133
12:20	118	148	144	121	127	136	136	112	131	137	147	157	151	131	126	133
2:00	147	156	155	131	127	117	105	93	156	156	156	154	155	157	153	139



Figures 4. Graphs of brightness over time for each sample in set A.

and produce more reliable data.

Because of the unreliable data, the exact effect of time on brightness is inconclusive. Qualitatively, the brightness of the samples seemed to increase over time, but the data do not reflect this. In Figures 4–6, the general trend should be upward since as the bacteria metabolize lactose, more galactose is produced, and eGFP production is induced, increasing eGFP concentration and therefore brightness. However, the graphs show that the sample brightnesses fluctuated over time. Interestingly, each sample's brightness followed mostly the same pattern,



Figures 5. Graphs of brightness over time for each sample in set B.

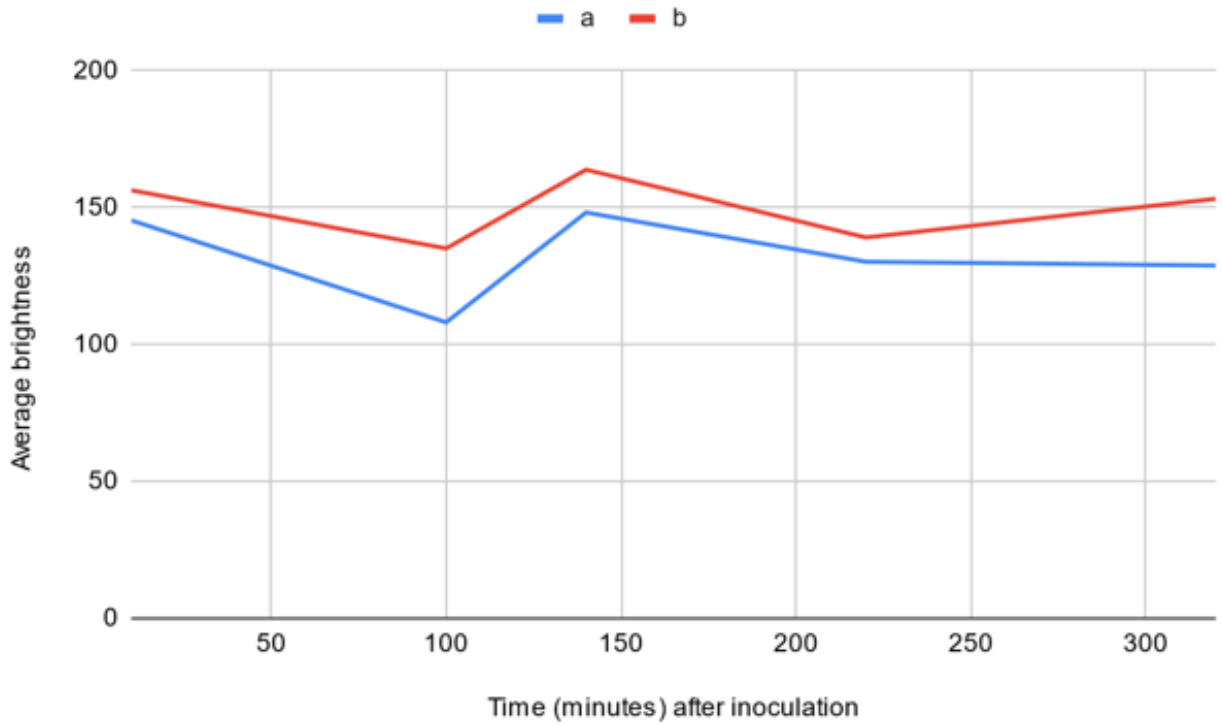


Figure 6. Graph of average brightness for sets A and B over time.

strengthening the implication that the different imaging setups caused unwanted variations in the measured brightness.

The effect of bacterial concentration on brightness is similarly inconclusive, although there is a slight upward trend. Additionally, the concentrations of *E. coli* tested are far too high to be applicable to drinking water since there can be no cells detected in 100 mL of water (WHO Housing and Health Guidelines), which is equivalent to less than 0.01 CFU/mL. The lowest concentration tested was over one hundred thousand times the acceptable concentration. The efficacy of the biosensor at typically acceptable levels of bacteria must be tested for it to be practically useful. Thus, the biosensor must be tested with much lower concentrations, which can be accomplished by either diluting the solution with a greater ratio of water or by beginning with a less concentrated stock.

The concentration of the biosensor did seem to affect the brightness of the samples. Set B, which had more yeast colonies added, was consistently brighter than set A (Figure 6, Figure 8). However, due to the small sample size and inconsistent imaging techniques, the exact relationship between yeast concentration and sample brightness cannot be determined from this data set.

The effectiveness of this system is limited by the rate of galactose transfer out of the *E. coli* by lactose permease (lacY) following catabolism by lactase. The exact rate can be described through Michaelis–Menten mechanics but depends on a variety of factors, including intracellular and extracellular concentration (which can vary greatly), membrane composition, and the ability of the specific strain to transport galactose (Rothfield, 1971). If the majority of the galactose stays in the *E. coli* and is immediately metabolized without ever inducing expression in the yeast, it will be impossible to detect even though there is *E. coli* present. Thus, for this system to be most effective, the galactose cannot be digested by the pathogen before it reaches the yeast. The

Table 2. Bacterial concentration in samples.

Vial number	Colony count	Concentration (CFU/mL)
0	No distinct colonies/too many to count	1.1e10
1	-	1.1e9
2	-	1.1e8
3	-	1.1e7
4	-	1.1e6
5	-	1.1e5
6	-	1.1e4
7	25	1.1e3

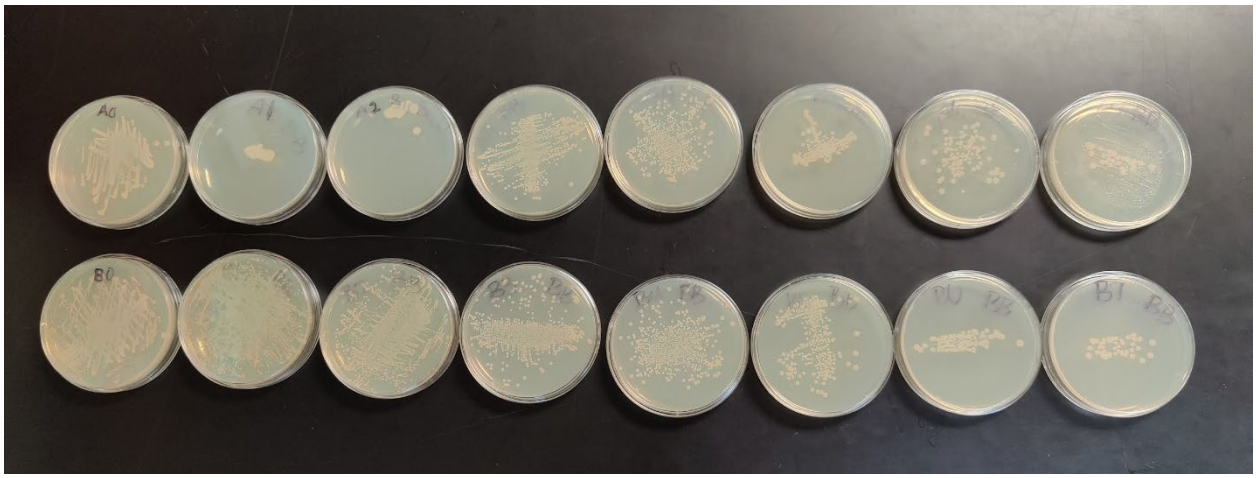


Figure 7. LB plates with E. coli growth from serial dilution.

effect of this can be minimized by increasing the concentration of yeast, which could be a viable solution due to the ease of replicating the biosensor. Another possible solution is to use a modified form of lactose that can be cleaved by wild-type *E. coli* and, after catabolism, produces a product that cannot be further digested but can still induce expression in the GAL1,10 promoter.

Moreover, many important waterborne pathogens do not have the lac operon and so cannot be detected with this system (Magana-Arachchi & Wanigatunge, 2020). Although *E. coli* can be an important indicator of harmful fecal contamination (Odonkor & Mahami, 2020), it is not the only source of water contamination. Thus, other more complex processes are likely necessary to implement a useful biosensor with this approach.

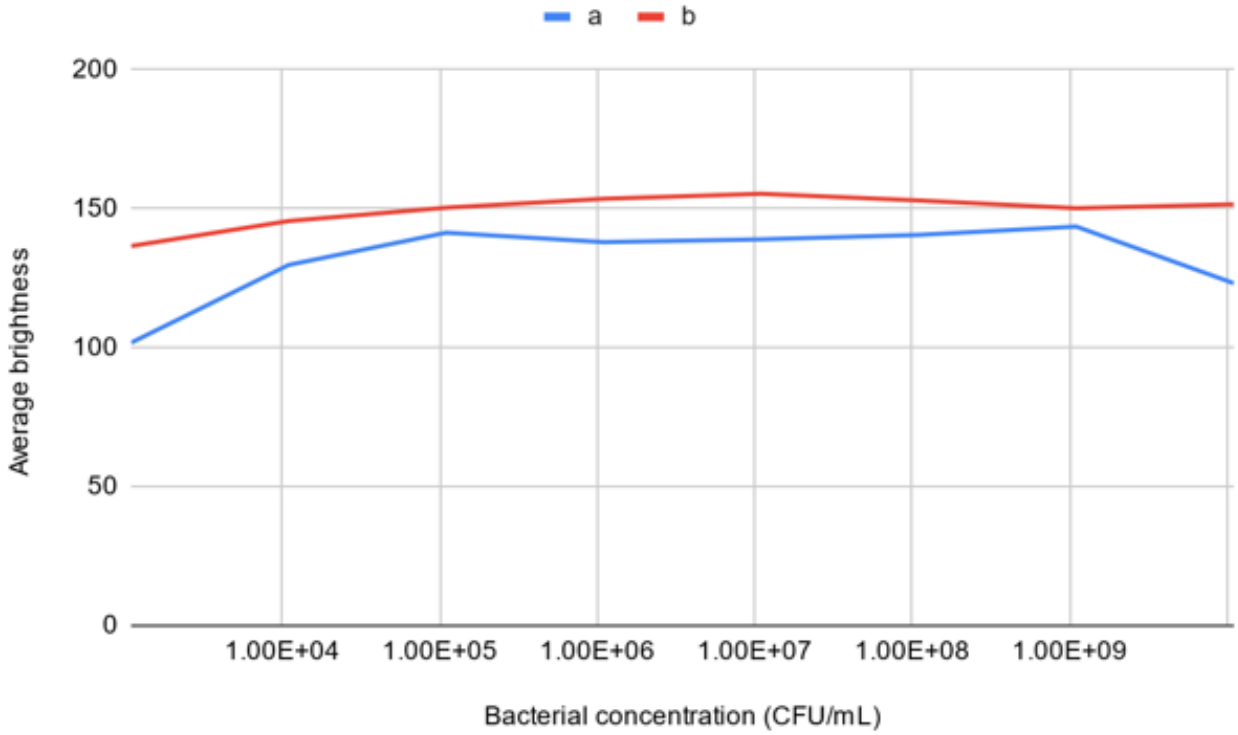


Figure 8. Semi-log graph of average brightness vs. bacterial concentration.

Next steps

The most pressing concern is to establish that the biosensor works for the lower concentrations in which it could be practically useful. After ensuring the functionality of this yeast as a biosensor, research should be performed to determine the most effective manner of long-term storage. For example, yeast could be stored lyophilized and mixed with lactose powder or in a pre-made liquid solution. The safety of each possibility must be evaluated so as to minimize contamination. Also, the viability of the cells following long-term storage must be evaluated. Furthermore, differing environmental factors such as temperature should be tested and considered so that the practical application of the biosensor in real conditions can be tested. Finally, an experiment testing for the optimal concentration of yeast in the solution is needed to optimize and standardize the conditions for successful detection.

In addition, the mechanisms for the detection of other non-lactose metabolizing pathogens must be considered since many waterborne pathogens can cause illness but do not have a lac operon (Magana-Arachchi & Wanigatunge, 2020). This system may still be useful for these other pathogens, but more research is needed to determine the exact processes.

Author contributions

All authors contributed to performing research and generating research ideas. A.P., J.T., E.R., and J.W. discussed sponsorships with companies such as TekNova and Zymo Research and obtained the required materials for the experiment. R.R., A.N, J.T., J.W., and A.V. detailed the specifics of the procedure. J.J., A.V., A.K, J.T., and S.S. were responsible for describing the implications of the experiment. All members participated in experimentation, and all members drafted and proofread the manuscript.

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