SASTA OLIPHANT SCIENCE AWARDS

SOUTH AUSTRALIAN SCIENCE TEACHERS ASSOCIATION

Prize Winner

Science Investigation

Year 11 - 12

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SOUTH AUSTRALIA

Government of South Australia

Department for Education

SASTA South Australian Science Teachers Association

Oliphant Science Awards: Scientific Investigation

Topic: Effect of Curcumin Concentration and Iron Overload on the Growth and Viability of *Saccharomyces cerevisiae.*
***Research Question*:** To what extent do curcumin concentration (0.0-2.0% w/v) and iron overload influence the growth and viability of *Saccharomyces cerevisiae,* as measured by colony surface area (mm^2) and colony-forming units (CFU/mL) following 24 hours of incubation?

Introduction

Background Information

1. *Oxidative stress and reactive oxygen species (ROS) in Saccharomyces cerevisiae*

Reactive oxygen species (ROS) are highly reactive oxygen-containing molecules, including superoxide anion radicals (O_2^-), hydrogen peroxide (H_2O_2), singlet oxygen, and hydroxyl radicals (·OH) (Sikder *et al.,* 2025). They are produced as by-products of aerobic metabolism, particularly within the mitochondrial electron transport chain (ETC), where incomplete reduction of oxygen leads to electron leakage and superoxide formation (Sikder *et al.,* 2025).

Under normal conditions, ROS levels are regulated by intracellular antioxidant systems (Tavassolifar *et al.,* 2020). When ROS production exceeds antioxidant capacity, oxidative stress occurs, resulting in damage to lipids, proteins, and DNA (Juan *et al.,* 2021). Severe oxidative stress can disrupt mitochondrial membrane potential and activate apoptosis-like pathways (Bayir & Kagan, 2008). Cells counteract ROS using enzymatic and non-enzymatic systems (Kozlov *et al.,* 2024). Superoxide dismutase (SOD) converts superoxide into H_2O_2, which catalase then breaks down into water and oxygen (Kozlov *et al.,* 2024). Glutathione acts as a non-enzymatic antioxidant by donating electrons, forming oxidised glutathione (GSSG), which is regenerated by glutathione reductase (Kozlov *et al.,* 2024).

In *Saccharomyces cerevisiae*, ROS are produced during both aerobic respiration and fermentative growth (Maslanka *et al.,* 2020). Although yeast preferentially ferments in high glucose conditions, mitochondria remain partially active, enabling continued ROS generation (Maslanka *et al.,* 2020). Additionally, NAD(P)H-dependent oxidoreductase pathways can also contribute to hydrogen peroxide production (Maslanka *et al.,* 2020).

2. *The role of iron in oxidative stress*

Iron is an essential cofactor in enzymes involved in mitochondrial electron transport, ATP synthesis, oxygen transport, and DNA replication (Liu & Chang, 2026). However, excess intracellular iron can accumulate in the labile iron pool (LIP), where it promotes ROS formation through redox cycling (Kruszewski, 2003). A key mechanism is the Fenton reaction, in which ferrous iron (Fe^{2+}) reacts with hydrogen peroxide to generate highly reactive hydroxyl radicals (Kruszewski, 2003):

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + \cdot OH + OH^-$$

Ferric iron (Fe^{3+}) can be reduced back to Fe^{2+} by cellular reductants, sustaining the cycle and amplifying ROS production (Kruszewski, 2003). Hydroxyl radicals cause severe damage to proteins, lipids, and nucleic acids, impairing mitochondrial function and reducing cell viability (Chandimali *et al.,* 2025).

To maintain iron homeostasis, *S. cerevisiae* regulates iron uptake and sequestration (Ramos-Alonso *et al.,* 2020). Under iron-replete conditions, excess iron is stored in the vacuole (Ramos-Alonso *et al.,* 2020). When this system is overwhelmed, cytosolic iron accumulates, increasing Fenton-driven oxidative stress (Ramos-Alonso *et al.,* 2020).

3. *Curcumin's antioxidant-pro-oxidant dual behaviour*

Curcumin is a polyphenol from turmeric with antioxidant, anti-inflammatory, and antimicrobial properties (Hewlings & Kalman, 2017). It scavenges ROS and enhances antioxidant enzymes such as SOD, catalase, and glutathione peroxidase (Avendano-Briseno *et al.,* 2025). Its lipophilic structure allows interaction with cellular membranes, interrupting oxidative chain reactions (Avendano-Briseno *et al.,* 2025).

However, curcumin exhibits concentration-dependent behaviour (Banerjee *et al.,* 2008). At low concentrations, it acts as an antioxidant, while at higher concentrations or in the presence of transition metals such as iron, it can act as a pro-oxidant by promoting ROS formation and oxidative stress (Banerjee *et al.,* 2008).

In yeast cells, curcumin can enter the cell and interact with intracellular components, including metal ions (Paramera *et al.,* 2011). It can also chelate iron, potentially reducing iron availability for metabolic processes (Rainey *et al.,* 2019). Therefore, curcumin may either reduce or enhance oxidative stress depending on concentration and iron conditions.

4. *Experimental Design*

This investigation examined the effect of curcumin concentration on the growth of *Saccharomyces cerevisiae* under normal and iron-induced oxidative stress conditions. Yeast naturally experiences basal ROS production even under non-stress conditions, making it a suitable model organism for studying redox-active compounds (Moradaas-Ferreira *et al.,* 1996). *Saccharomyces cerevisiae* is also easy to culture and widely used in laboratory investigations (Johnson & Echavarri-Erasun, 2011).

Iron(II) sulfate ($FeSO_4 \cdot 7H_2O$) was used to simulate iron overload and enhance ROS production via the Fenton reaction. Curcumin was investigated as a redox-active compound with potential antioxidant and pro-oxidant effects depending on concentration and iron availability. Yeast growth was assessed using CFU/mL and colony surface area as quantitative measures of viability.

Methodology

Aim: To investigate the effect of curcumin concentration (0%, 0.5%, 1.0%, 2.0% w/v) and iron overload (2 mM $FeSO_4 \cdot 7H_2O$) on the growth of *Saccharomyces cerevisiae* by comparing colony-forming units (CFU/mL) and colony surface area after 24 hours of incubation.

Hypotheses

1. *Yeast Viability (CFU/mL)*

Table 1: CFU/mL hypotheses of the experiment.

Null Hypothesis (H_0)	Neither curcumin concentration nor iron overload, nor any interaction between the two, will have a statistically significant effect on the viable population of *Saccharomyces cerevisiae*, measured as CFU/mL after 24 hours of incubation.
Alterative Hypothesis (H_1)	Low concentrations of curcumin (approximately 0.5%) are expected to maintain or slightly improve cell viability through antioxidant activity, whereas higher concentrations (1–2%) are expected to reduce viability due to concentration-dependent cytotoxic and pro-oxidant effects (Stanley *et al.,* 2025). Iron overload is expected to decrease cell viability by increasing oxidative stress within yeast cells, resulting in an interaction between curcumin concentration and iron availability (Mancardi *et al.,* 2021).

2. *Colony Surface Area (mm^2)*

Table 2: Colony surface area hypotheses of the experiment.

Null Hypothesis (H_0)	Neither curcumin concentration nor iron overload, nor any interaction between the two, will have a statistically significant effect on *Saccharomyces cerevisiae* colony surface area after 24 hours of incubation.
Alterative Hypothesis (H_1)	Increasing curcumin concentration is expected to reduce colony expansion due to the concentration-dependent inhibition of *Saccharomyces cerevisiae* growth (Stanley *et al.,* 2025). Iron overload is predicted to modify this response by increasing intracellular iron availability, partially alleviating growth inhibition at lower curcumin concentrations, whereas at higher curcumin concentrations the combined effects of iron-mediated oxidative stress and curcumin toxicity are expected to further reduce colony growth (Mancardi *et al.,* 2021).

Variables

1. Independent Variables

Table 3: Independent variables of the experiment.

Variable	How

Curcumin concentration (% w/v)	Curcumin concentration was varied to investigate its effect on *Saccharomyces cerevisiae* growth. Four concentrations were tested: 0%, 0.5%, 1.0% and 2.0% (w/v). The 0% treatment served as the experimental control to determine baseline growth in the absence of curcumin. Curcumin was diluted in 100% ethanol to increase solubility.
Iron condition	Samples were either grown under normal conditions (0 mM $FeSO_4 \cdot 7H_2O$) or iron-overload conditions produced by adding $FeSO_4 \cdot 7H_2O$ to achieve a final concentration of 2 mM. This allowed investigation of whether iron availability altered the response of yeast to curcumin.

2. *Dependent Variables*

Table 4: Dependent variables of the experiment.

Variable	How
Viable yeast concentration (CFU/mL)	After 24 hours of incubation, yeast cultures were serially diluted and plated onto YEPD agar. Colony-forming units were counted following incubation and converted to CFU/mL using the standard plate count equation. CFU/mL was used as a quantitative measure of viable yeast cells.
Colony surface area (mm^2)	Photographs of each agar plate were analysed using ImageJ software. The total colony area was determined by threshold analysis and converted from pixels to mm^2 using calibration against the known 90 mm Petri dish diameter. Colony area was used as a quantitative measure of overall yeast growth.

3. *Controlled Variables*

Table 5: Controlled variables of the experiment.

Variable	Why and how it was controlled
Incubation temperature	All liquid cultures and agar plates were incubated at 30°C, the optimum growth temperature for *Saccharomyces cerevisiae* (Lip *et al.,* 2020). Temperature strongly influences enzyme activity, metabolic rate and cell division (Peterson *et al.,* 2007). Maintaining a constant temperature prevented growth differences resulting from altered metabolic activity rather than experimental treatments.
Incubation time	All cultures were incubated for 24 hours before analysis. Controlling incubation time ensured equal opportunity for growth across all treatment groups.

Initial inoculum concentration	A 1:20 dilution of the starter culture was prepared before experimental setup so that each sample received the same initial yeast concentration, minimising variability from unequal starting populations.
Growth medium	All cultures were grown using the same batch of sterile YEPD broth and YEPD agar prepared using a standard protocol. Consistent nutrient composition ensured differences resulted from curcumin and iron treatments, not media composition.
Total sample volume	Every treatment was prepared to a final volume of 10 mL. Maintaining constant volume ensured consistent nutrient availability and treatment concentrations across all samples.
Yeast strain	All cultures originated from the same *Saccharomyces cerevisiae* live slope culture to minimise genetic and physiological variation between treatment groups (Camara *et al.,* 2024).

4. Uncontrolled Variables

Table 6: Uncontrolled variables of the experiment.

Variable	Why and how it was minimized
Natural biological variation	Individual yeast cells naturally differ in physiological state and growth rate, producing variation between biological replicates (Hardwick *et al.,* 2021). This was minimised through a sample size of 3, with all samples prepared from the same starter culture.
Curcumin degradation	Curcumin is unstable under light exposure and in aqueous solutions, resulting in gradual degradation and reduced biological activity (Appendino *et al.,* 2022). Stock solutions were prepared in amber bottles, protected from light and used shortly after preparation.

Procedure

1. Apparatus

Table 7: Apparatus used.

Material	Amount Required	Notes
Yeast (Saccharomyces cerevisiae BY4741)	24mL	1mL per sample for 24 samples
YPD Broth	340mL	240mL for 10mL per 24 samples + ~100mL for buffer
YPD Agar Plates	~30 plates	Colony count for 10 samples × 2 dilutions (experiment whether 10^{-2} or 10^{-3} work)

Distilled Water	10mL	For iron treatment dilution
Curcumin Powder	5g	For stock (10% in 50mL ethanol) to prepare 100mL stock
Ethanol (100%)	50mL	50mL dissolved with 5g curcumin for 1 mM stock.
Iron(II) Sulfate Heptahydrate	0.5g	0.278g mixed with 5mL distilled water for 2mM concentration
Sterile Test Tubes	72 tubes	72 (3×24 tubes for serial dilution)
Pipette (1-10mL)	1	For minimal contamination, I'd need around 150-200 pipette tips but since that's unrealistic, I would need to know how many tips I can use.
Micropipette (100-1000 uL)	1	
Sterile Inoculation Loop	1	For transferring yeast culture into YEPD broth
Conical Flask (100mL, 25mL)	24×25mL, 2×100mL	For initial yeast culture growth, yeast culture dilution, and yeast samples.
Beakers (150mL, 250mL)	1 of each	For curcumin stock solution preparation.
Measuring Cylinder (5mL, 10mL, 50mL)	1	For general use
Glass stirring rods	1-2	
Incubator (30 ˚C)	1	For culture growth and colony incubation
Amber Bottles	2×25mL 1×10mL	Storing curcumin stock and iron treatment
Parafilm/Tube Caps	1 roll of parafilm/30 caps	For sealing culture tubes
Sterile Glass Spreader	1	For plating
Marker	1	For labelling tubes and plates
Tube Rack	1-2	For holding serial dilution tubes securely.
Foam Stopper	25	Sealing test tubes and flask containing yeast culture
Matches	1 box	For lighting Bunsen burner
Bunsen Burner	1	Sterilizing YPD broth

2. Method

1. Preparation of Media
   1.1. YEPD broth and agar plates were prepared by laboratory staff according to a standardised protocol (Appendix A).

1.2. All media and glassware were sterilised via autoclaving to eliminate contamination.
1.3. Aseptic technique was maintained throughout all procedures to prevent microbial contamination (Figure 5).

2. Inoculation and Pre-Culture of *Saccharomyces cerevisiae*
   2.1. A sterile inoculation loop was used to transfer a visible amount of *Saccharomyces cerevisiae* from a live slope culture into 40 mL of sterile YEPD broth in a 100 mL Erlenmeyer flask.
   2.2. The culture was vortexed briefly to ensure even distribution of yeast cells.
   2.3. The flask was sealed with a sterile cotton plug to allow gas exchange while minimising contamination.
   2.4. The culture was incubated at 30°C for 24 hours to reach exponential growth phase.
3. Standardization of Yeast Concentration
   3.1. After incubation, the culture was homogenised.
   3.2. A 1:20 dilution was prepared by transferring 1 mL of culture into 19 mL of sterile YEPD broth (Figure 1).

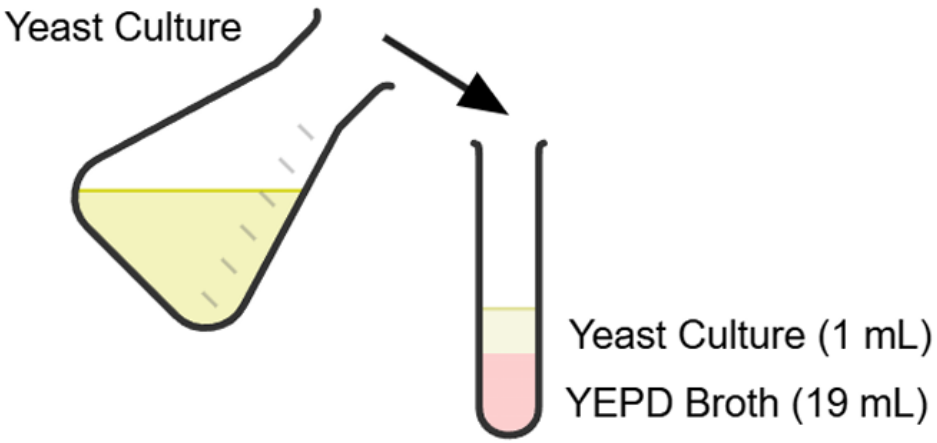


Figure 1: Diagram of 1:20 cell dilution, created on Chemix.

   3.3. The diluted culture was used as the standardised inoculum for all experimental treatments.
4. Preparation of Curcumin Stock Solution
   4.1. A 10% (w/v) curcumin stock solution was prepared by dissolving 5.0 g of curcumin powder in 50 mL of 100% ethanol in a 100mL beaker.
   4.2. The solution was heated in a water bath (100mL beaker-in- 250mL beaker setup) on a hotplate set to 60 °C to improve solubility.
   4.3. Continuous stirring was applied until the solution was fully dissolved.
   4.4. The stock solution was stored in amber bottles at room temperature and protected from light to minimise photodegradation.
5. Preparation of Iron Solution

5.1. A 2 mM $FeSO_4 \cdot 7H_2O$ working solution was prepared by dissolving 0.278 g $FeSO_4 \cdot 7H_2O$ in 5 mL sterile distilled water (Appendix B).
5.2. The solution was mixed thoroughly to ensure complete dissolution.
5.3. The solution was stored in an amber bottle wrapped in aluminium foil to prevent oxidation.

6. Experimental Design and Treatment Setup
6.1. The experiment consisted of two main conditions: samples exposed to iron-overload conditions (+$FeSO_4.7H_2O$) and samples grown under normal conditions (no $FeSO_4.7H_2O$). Each condition was tested with four curcumin concentrations (0%, 0.5%, 1%, and 2%) (Table 8).
6.2. Samples were prepared in triplicate for a total of 24 samples.

Table 8: Treatment groups for samples.

Group	Iron Condition	Curcumin Concentration
A1-A4	No $FeSO_4.7H_2O$	0%, 0.5%, 1%, 2%
B1-B4	+2mM $FeSO_4.7H_2O$	0%, 0.5%, 1%, 2%

7. Sample Preparation
7.1. Each 10mL sample was prepared in a 25 mL flask as follows (Table 9):
7.1.1. 1 mL of diluted yeast culture was added.
7.1.2. The appropriate volume of curcumin stock solution (see Table above) was added.
7.1.3. 100 µL of either sterile distilled water (for Group A) or $FeSO_4.7H_2O$ solution (for Group B) was added.
7.1.4. YEPD broth was added to reach a final volume of 10 mL.

Table 9: Volume of curcumin, yeast culture, iron/water, YPD broth to be added to each sample.

Groups		Curcumin (mL)	Yeast Culture (mL)	$FeSO_4.7H_2O$ /Water (mL)	YPD Broth (mL)
A1	B1	0.00	1.0	0.1	8.90
A2	B2	0.50	1.0	0.1	8.40
A3	B3	1.00	1.0	0.1	7.90
A4	B4	2.00	1.0	0.1	6.90

8. Incubation of Culture
8.1. All samples were incubated at 30°C for 24 hours.
8.2. Flasks were sealed with foam stoppers to allow gas exchange while preventing contamination (Figure 3).
8.3. Samples were incubated under identical environmental conditions to ensure validity of comparisons.

9. Viability Assessment (CFU/mL)
9.1. Three sterile microtubes were labelled: 10^{-1}, 10^{-2}, and 10^{-3}.
9.2. Each tube was filled with 900 µL YEPD broth.
9.3. 100 µL of yeast culture was transferred into the 10^{-1} tube and mixed thoroughly.

9.4. 100 µL from 10^{-1} was transferred into 10^{-2} and mixed.
9.5. 100 µL from 10^{-2} was transferred into 10^{-3} and mixed.
9.6. 100 µL of the 10^{-3} dilution was spread onto YEPD agar plates using a sterile spreader (Figure 2; Figure 4).

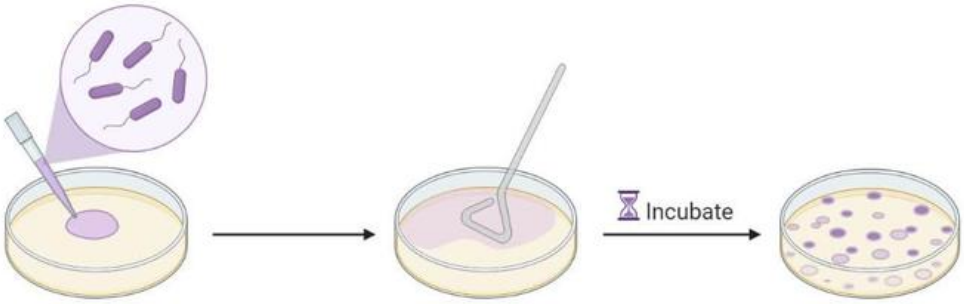


Figure 2: Diagram of spread plate method (Dahal, 2022)

9.7. Plates were incubated inverted at 30°C for 24 hours to prevent condensation interference.
9.8. Colonies were counted manually, and only plates with 30-300 colonies were used for CFU calculations.
9.9. CFU/mL was calculated using:

$$CFU/mL = \frac{colonies\ counted \times dilution\ factor}{volume\ plated\ (mL)}$$

10. Colony Area Analysis
    10.1. Plates were photographed under standardised lighting conditions.
    10.2. Images were analysed using ImageJ software.
    10.3. Threshold analysis was used to isolate colony growth from background agar.
    10.4. Total colony area (mm^2) was calculated by calibrating pixel measurements against the known 90 mm Petri dish diameter.

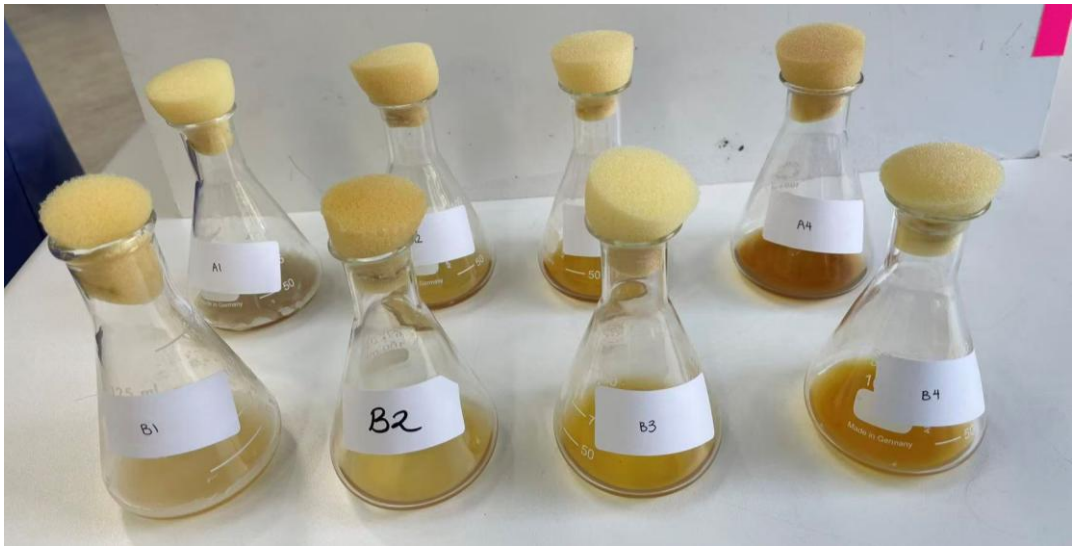


Figure 3: Flasks containing curcumin and iron/water treatment (A1-A5, B1-B5), sealed with foam stoppers.

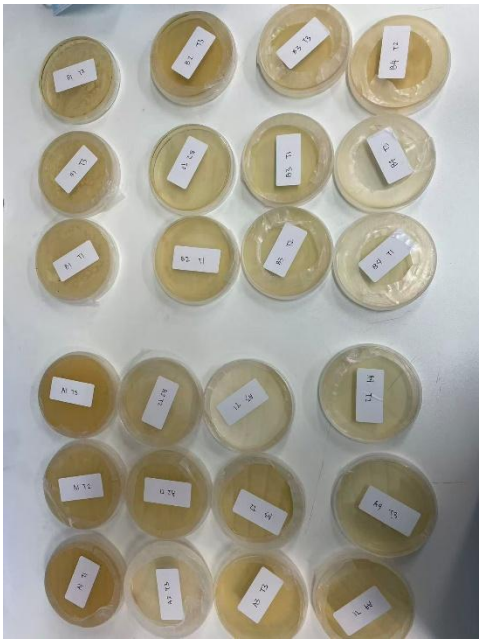


Figure 4: Plated agar plates (1:1000 dilution) for 3 replicates per treatment.

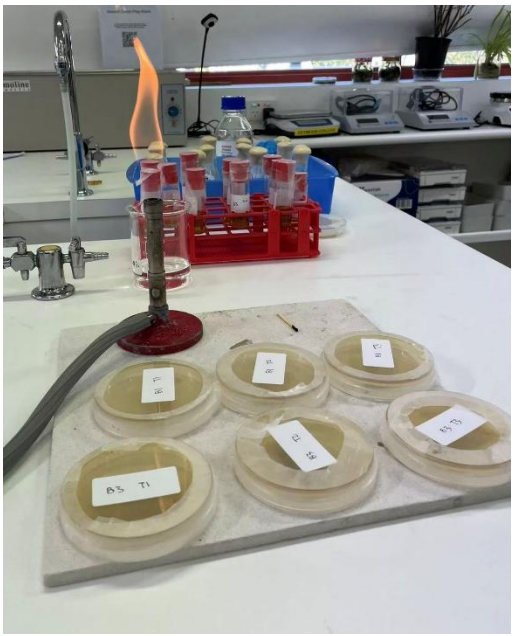


Figure 5: Experiment setup – aseptic technique with Bunsen burner flame.

Safety Concerns

Table 10: Risk assessment of the experiment.

Risk	Why	How risk was reduced
Use of Bunsen burner	Risk of burns or fire if used improperly	Long hair was tied back, loose clothing secured, burner was placed on a safety flame when not in use, never left unattended.
Presence of *Saccharomyces cerevisiae* culture	Although non-pathogenic, live cultures may cause contamination or allergic reactions in sensitive individuals (Xing *et al.,* 2022)	Gloves were worn and aseptic technique was maintained. All equipment and work surfaces were sterilised before and after use. Cultures were disposed of according to laboratory procedures.
Use of ethanol (curcumin solvent) and Iron (II) sulfate	Ethanol is highly flammable and volatile (Muslija, 2024); vapours may cause irritation. Iron (II) sulfate may cause skin/eye irritation and is harmful if ingested (Affinity Chemical, 2020)	Ethanol was kept away from open flames. Handling was done in a well-ventilated area, and containers were kept sealed when not in use. Gloves were worn when handling solutions. Direct contact was avoided.
Breakage of glassware	Can cause cuts and possible exposure to biological or chemical material	Glassware was handled carefully, inspected for cracks before use, and cleaned immediately if broken. Broken glass was disposed of in a designated sharps container.

Ethical, Environmental, and Social Concerns

Table 11: Ethical, environmental, and social concerns

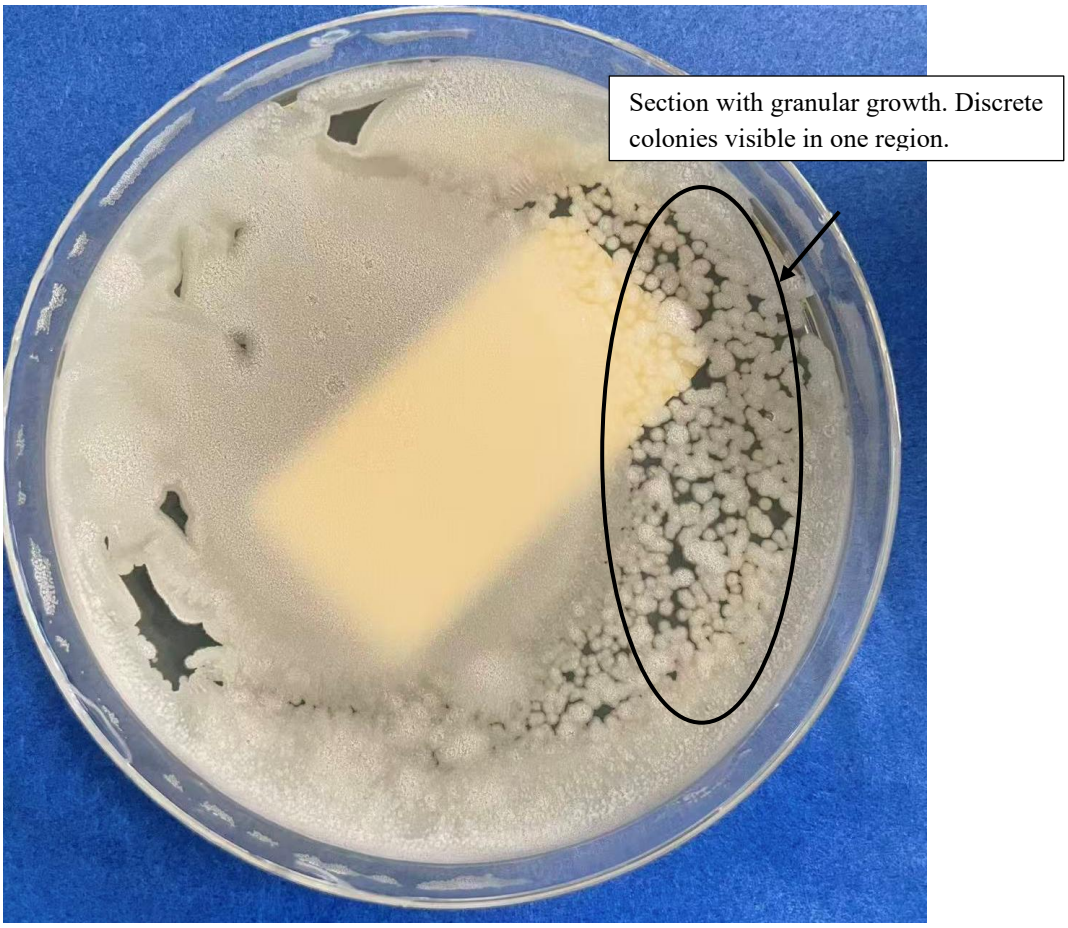
Concern	What/Why	How concern was mitigated
Ethical	Use of live microorganisms (*Saccharomyces cerevisiae*). involves working with living organisms, raising concerns about proper handling and disposal.	Cultures were handled with aseptic technique; all samples were sterilised and disposed of according to school biohazard protocols.
Environmental	Disposal of biological waste. Improper disposal of live yeast and agar plates could lead to contamination of drains or unintended growth outside of lab.	All agar plates and yeast cultures were disinfected before disposal and work areas were thoroughly sanitised.
Social	No significant social or cultural concerns are associated with this investigation.	Not applicable.

Data Collection and Processing

Qualitative Data

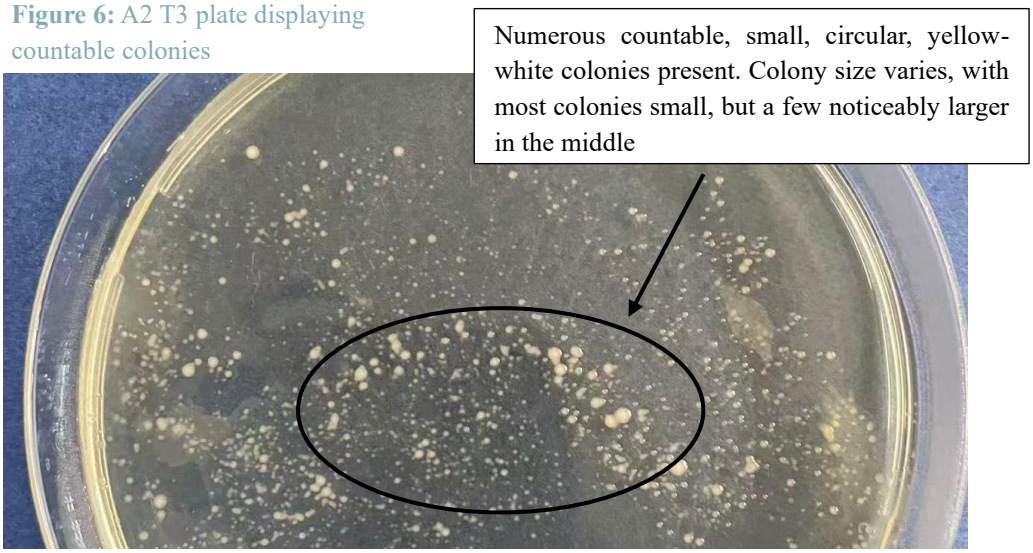
Some plates had confluent lawn growth (e.g. A1 T1):

Figure 5: A1 T1 agar plate, displaying a confluent lawn growth.



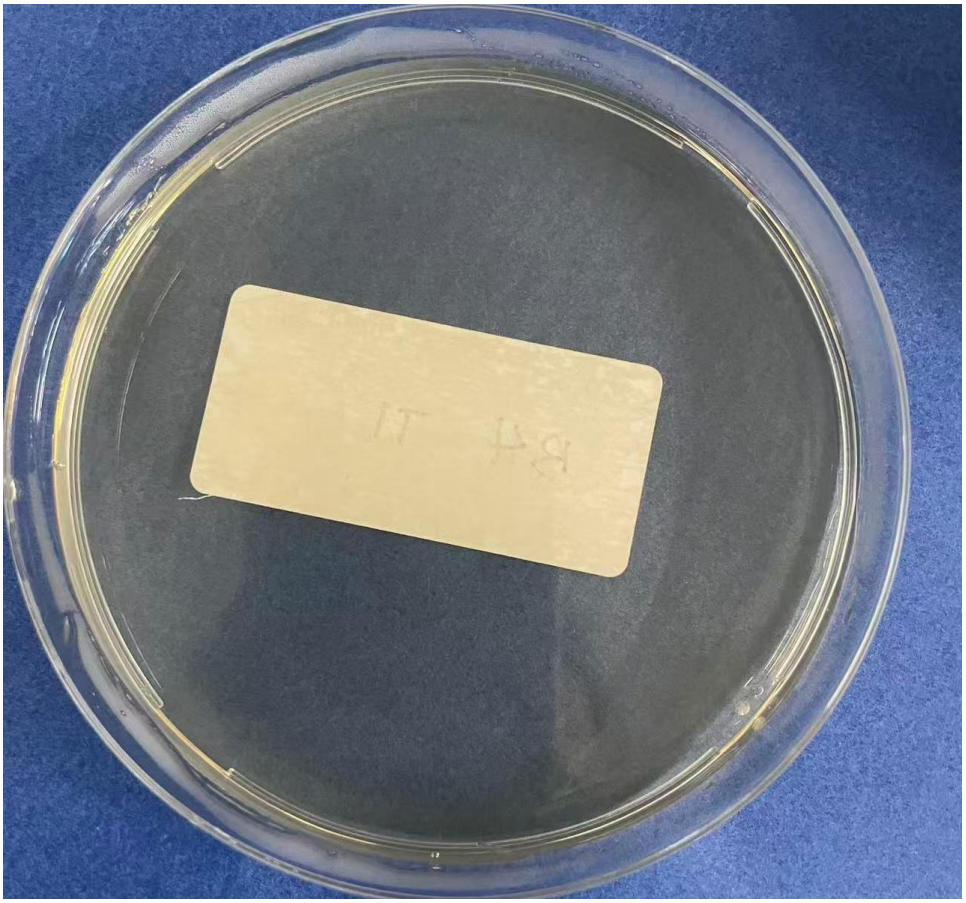
Some plates had distinct, countable colonies (A2 T3):

Figure 6: A2 T3 plate displaying countable colonies



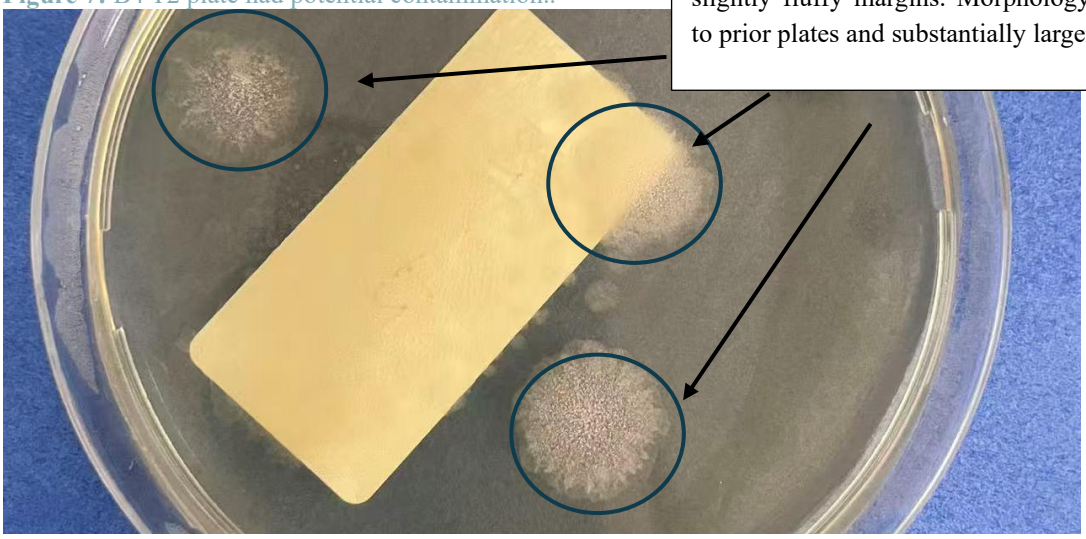
Some plates had no growth (B4 T1):

Figure 6: B4 T1 agar plate displayed no microbial growth.



Some plates had suspected contamination (B4 T2):

Figure 7: B4 T2 plate had potential contamination..



Summary

Table 12: Qualitative data from observing agar plates 24 hours after incubation.

Observation	Representative samples	Description
Confluent lawn growth	B1 T3, A1 T1, A1 T2, A1 T3	Colonies merged into a continuous pale cream lawn with only occasional individual colonies visible around the perimeter. Plates were recorded as TNTC.
Distinct colonies	B1 T2, B1 T3, B2 T3, B2 T2, B2 T1, A2 T2, A2 T3, A2 T1	Individual circular colonies were distinguishable and suitable for counting. Colonies appeared smooth, convex and cream-coloured.
Reduced growth	B3 T3, B3 T1, B3 T2, B4 T1, A3 T2, A4 T1	Very few colonies were present, with several plates containing none.
Suspected contamination	B4 T2, B2 T1, B4 T3, A4 T3, A4 T1, A2 T1	Large fluffy circular colonies differing from typical yeast morphology were observed, indicating likely contamination. These colonies were morphologically distinct from *S. cerevisiae*.

Table 13: Further observations.

Colony morphology
The colonies of *Saccharomyces cerevisiae* appeared cream to pale yellow in color and were generally circular with smooth margins. At high cell densities (A1), colonies merged to form a confluent lawn, although individual colonies remained visible in isolated regions. Plates with lower cell densities contained discrete circular colonies.
Contamination
Several plates, particularly A4 Trial 2 and B4 Trial 2, contained colonies with markedly different morphology from the surrounding yeast. These colonies were larger, white, opaque and exhibited fluffy or filamentous edges rather than the smooth circular morphology typical of *Saccharomyces cerevisiae* (Reis *et al.,* 2013). Based on these characteristics, they were excluded from CFU counting but remained included in the ImageJ colony-area measurements, meaning the surface area dataset represents total visible growth rather than confirmed yeast growth alone.

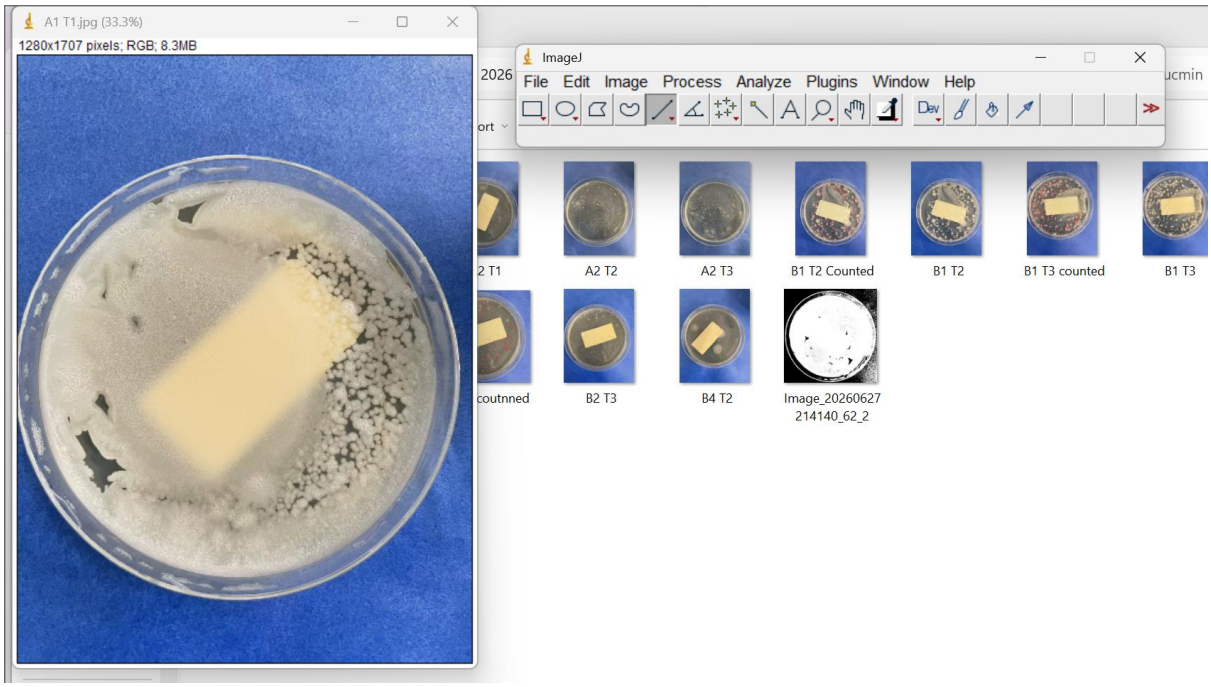
Image Analysis of Colony Coverage

1. *Raw Data*

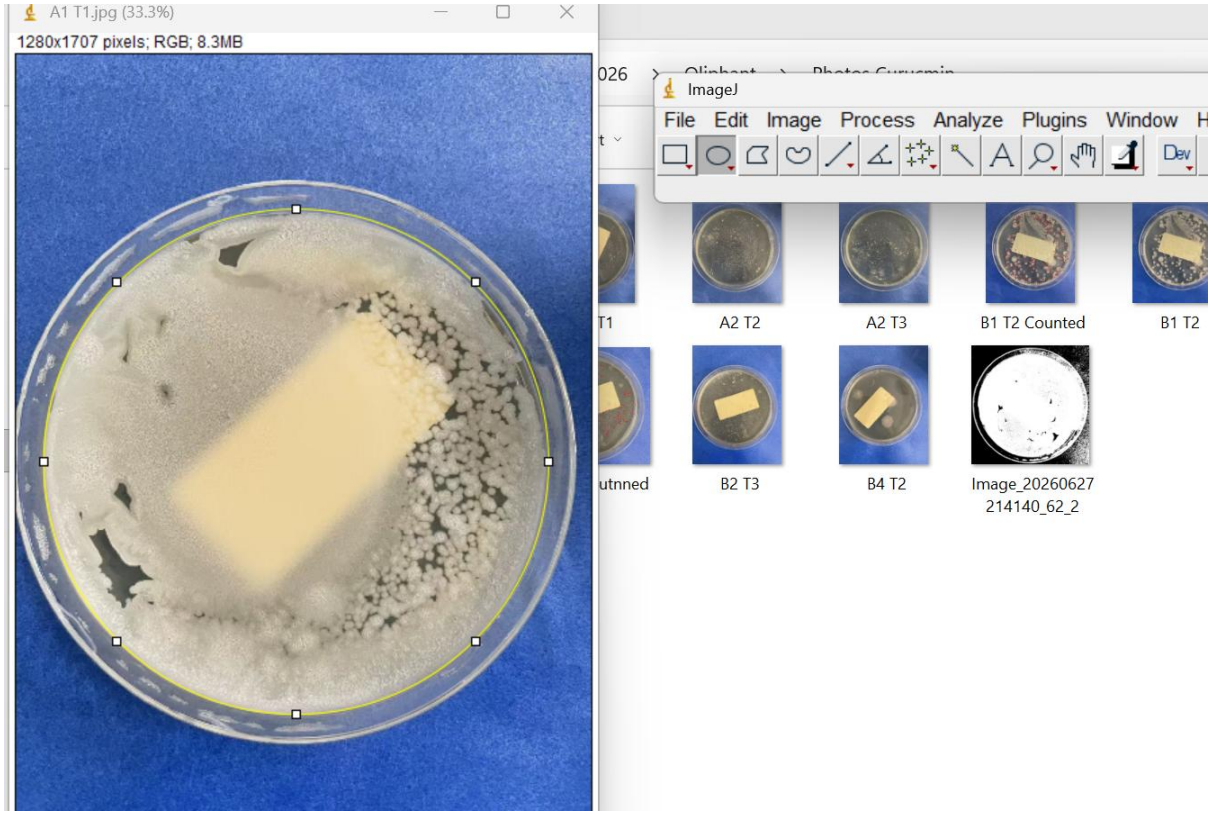
Raw photographic images of each agar plate used for ImageJ analysis are presented in Appendix C.

2. *Sample ImageJ Processing*

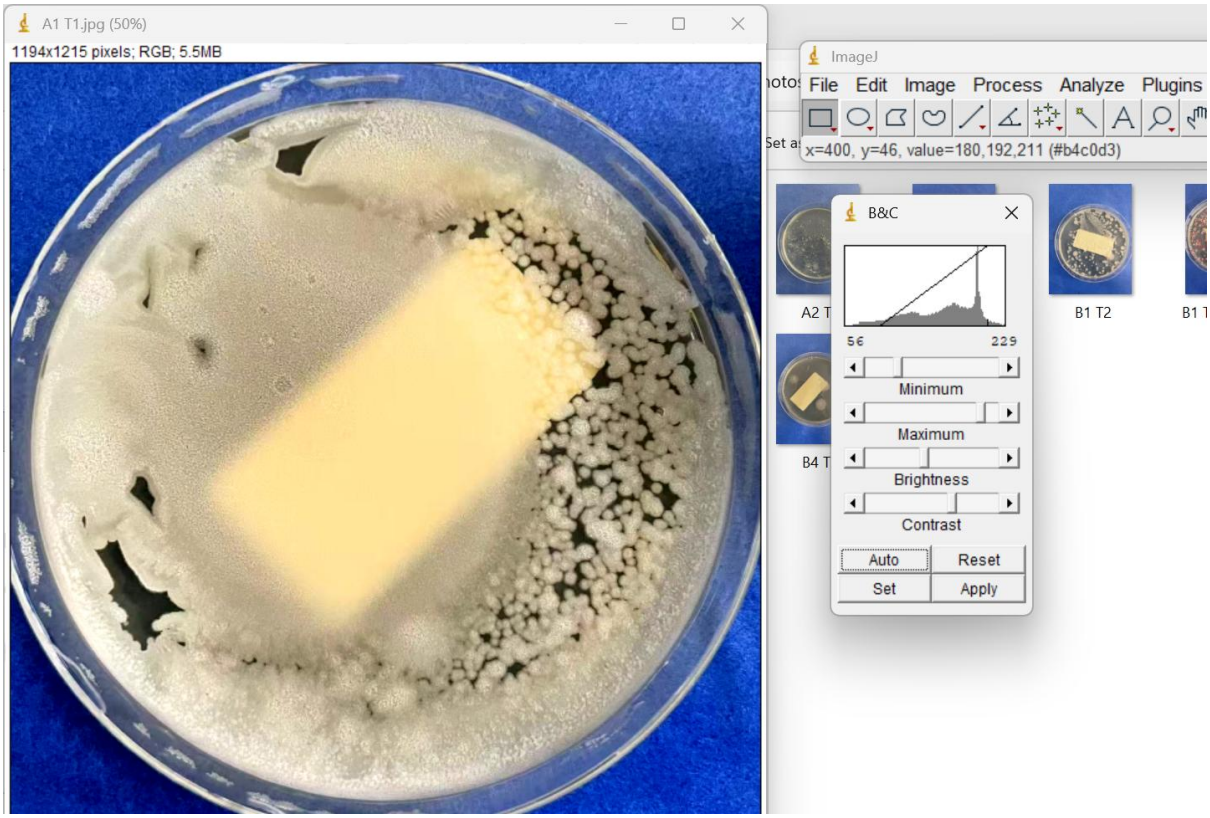
1. Import image for A1 T1



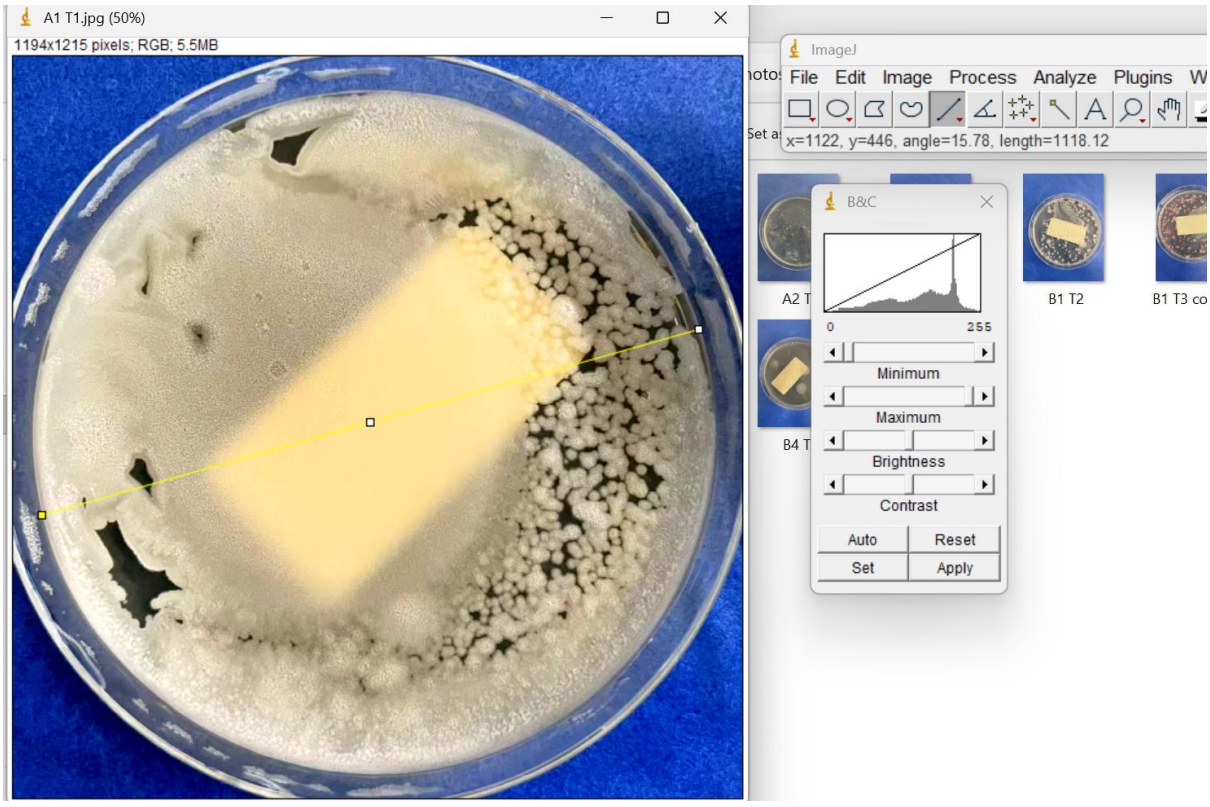
2. Crop Image to Agar Plate



3. Adjust brightness and contrast (Image > Adjust > Brightness/Contrast)

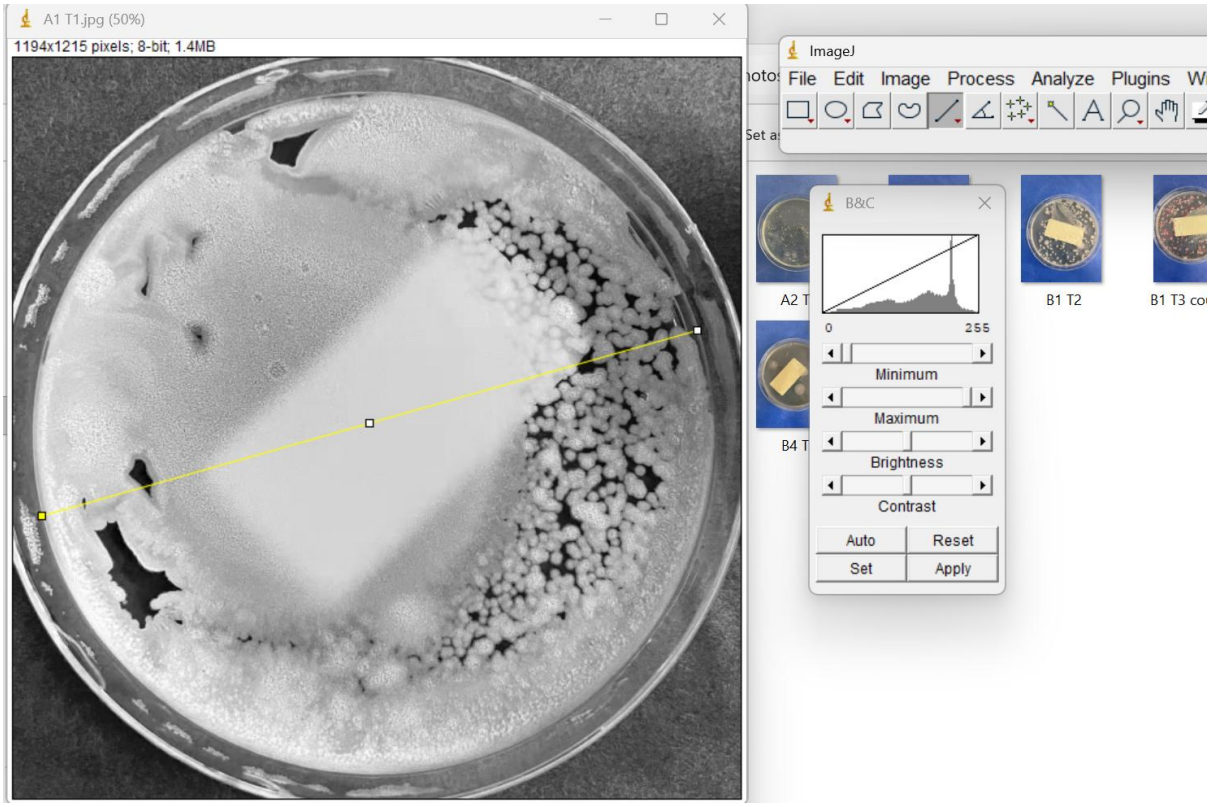


4. Calibrate Image Scale.
   Using the Straight Line Tool, a line was drawn across the widest diameter of the petri dish (known diameter = 90mm)



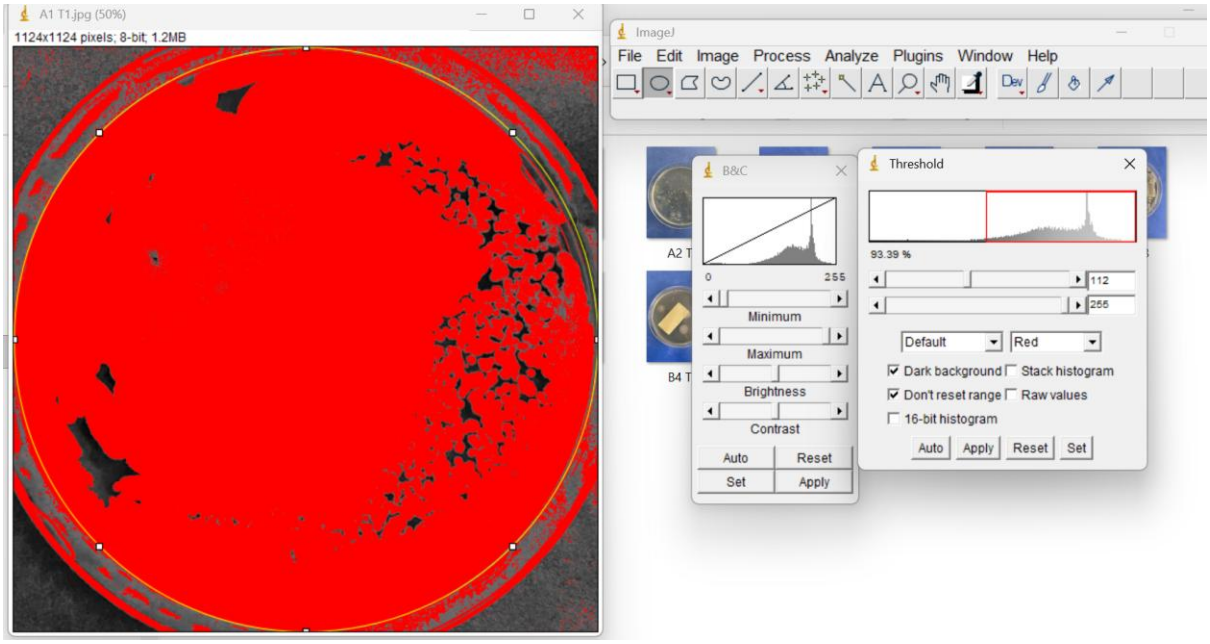
$$Dish\ width = 1118.12\ pixels$$

5. Measure Yeast Growth Area
    5.1. Convert image to greyscale (Image > Type > 8-bit)

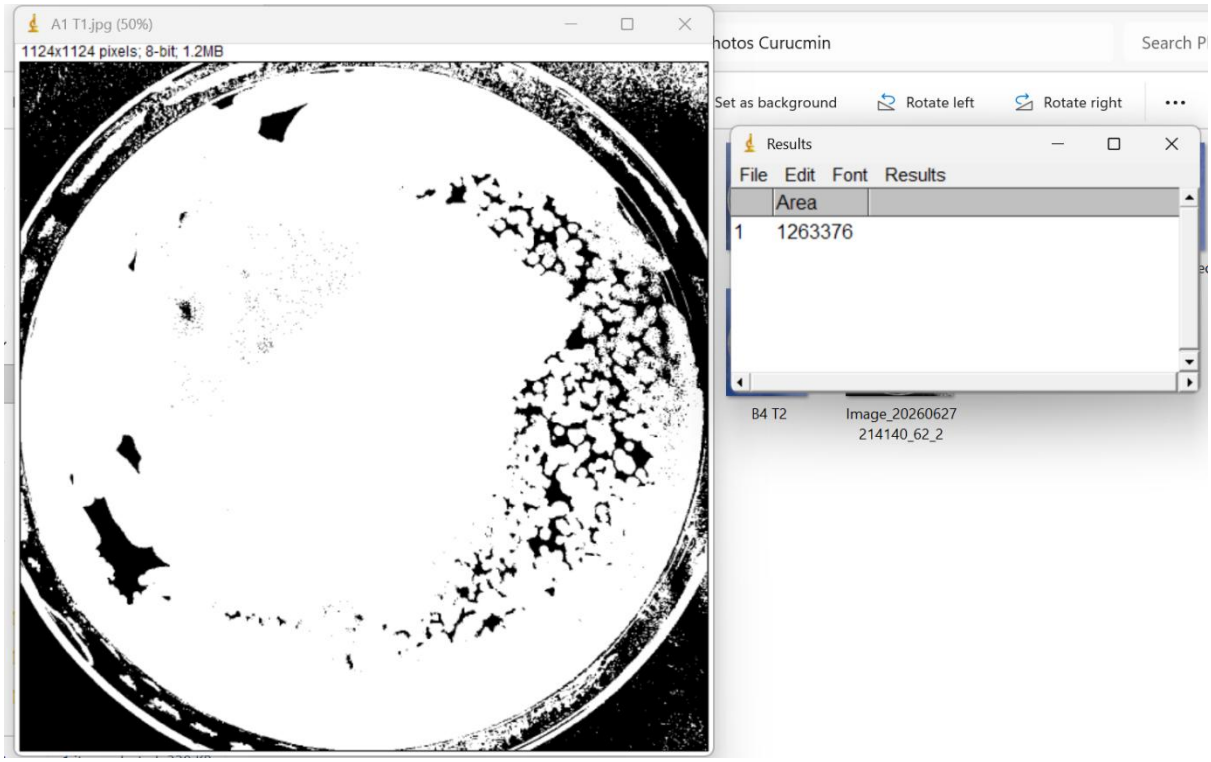


    5.2. Adjust threshold (highlighted red region) (Image > Adjust > Threshold) until yeast growth is completely highlighted

Tracing the outer edge of the agar plate allows for area calculations to disregard highlighted regions in the background/reflected on surface of agar plate.



5.3. Measure highlighted area (Analyze > Set Measurements > Area > OK)



$$Area\ of\ yeast = 1264476\ pixels^2$$

6. Determining Actual Area (mm^2)

As ImageJ reports measurements in $pixels^2$, the measured colony area was converted to mm^2 using the known 90mm diameter of the petri dish as a calibration reference.

$$Actual\ colony\ area = Yeast\ pixel\ area \times \left(\frac{90}{Dish\ Pixel\ Width}\right)^2$$

$$= 1264476 \times \left(\frac{90}{1118.12}\right)^2$$

$$= 8192.54\ mm^2$$

Where handwritten labels or markings obscured part of the agar surface (occurred consistently for all trials), the obscured region was excluded from the analysis by subtracting its area digitally using the freehand tool from the total plate area prior to calculating colony coverage. This prevented overestimation or underestimation of the measured yeast growth area.

7. Mean colony area on agar plate (mm^2) for 0.0% curcumin concentration and no iron (A1).

$$\bar{x} = \frac{\sum x}{n}$$

$$\bar{x} = \frac{(8192.54 + 8179.87 + 10251.42)}{3}$$

$$\bar{x} = 8874.61$$

8. Sample standard deviation for colony area on agar plate (s, mm^2) for 0.0% curcumin concentration and no iron (A1).

$$s = \sqrt{\frac{\sum(x_i - \bar{x})^2}{n - 1}}$$

$$= \sqrt{\frac{(8192.54 - 8874.61)^2 + (8179.87 - 8874.61)^2 + (10251.42 - 8874.61)^2}{3 - 1}}$$

$$= 1195.34\ mm^2$$

9. Coefficient of Variation (CV, %) of colony area on agar plate for 0.0% curcumin concentration and no iron (A1)

$$CV = \frac{s}{\bar{x}} \times 100$$

$$= \frac{8874.61}{1195.34} \times 100$$

$$\approx 13.47\%$$

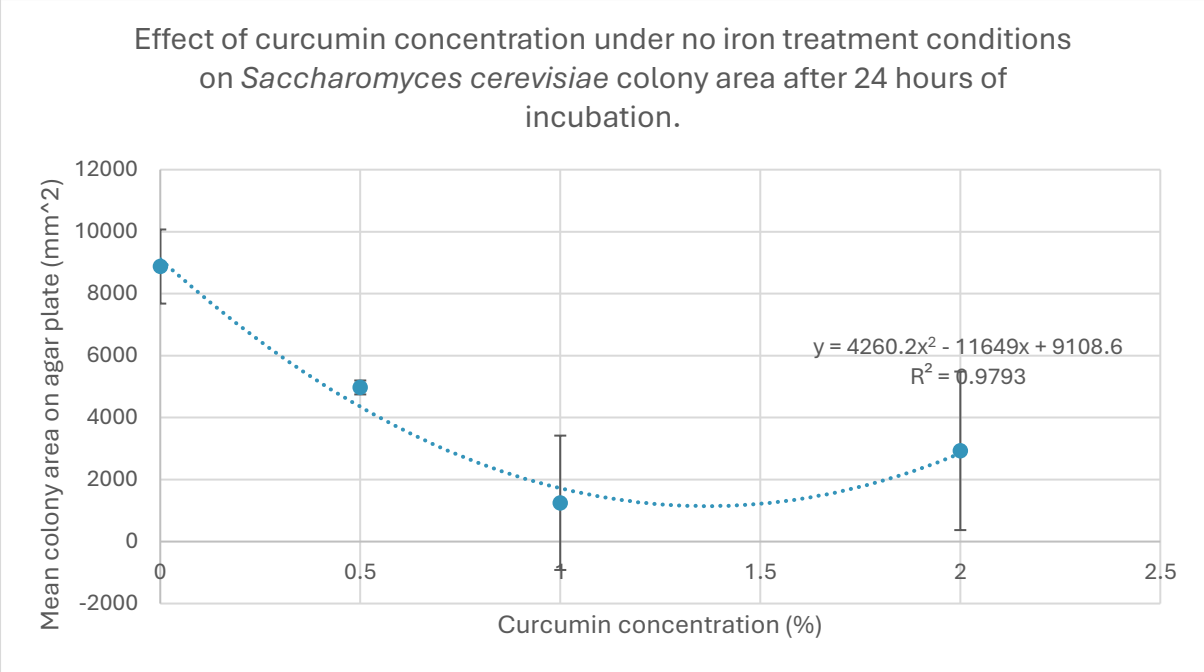
3. *Processed Data*

Table 14: Colony surface area on each agar plate (mm^2) after 24 hours of incubation.

Curcumin conc. (%)	Iron	Colony area on agar plate (mm^2)					
		Trial 1	Trial 2	Trial 3	Mean area	Standard deviation	CV (%)
0.0	Absent	8192.54	8179.87	10251.42	8874.61	1195.34	13.47
	Present	5178.69	5316.59	5027.68	5174.32	144.49	2.79
0.5	Absent	4710.82	5112.59	5095.91	4973.11	225.45	4.53
	Present	4760.19	4374.07	4870.15	4668.14	260.61	5.58
1.0	Absent	3754.20	0	0	1251.40	2167.49	173.21
	Present	0	0	0	0.00	0.00	-
2.0	Absent	4085.92	0	4699.96	2928.63	2557.88	87.34
	Present	0	3964.71	3551.13	2505.28	2181.43	87.07

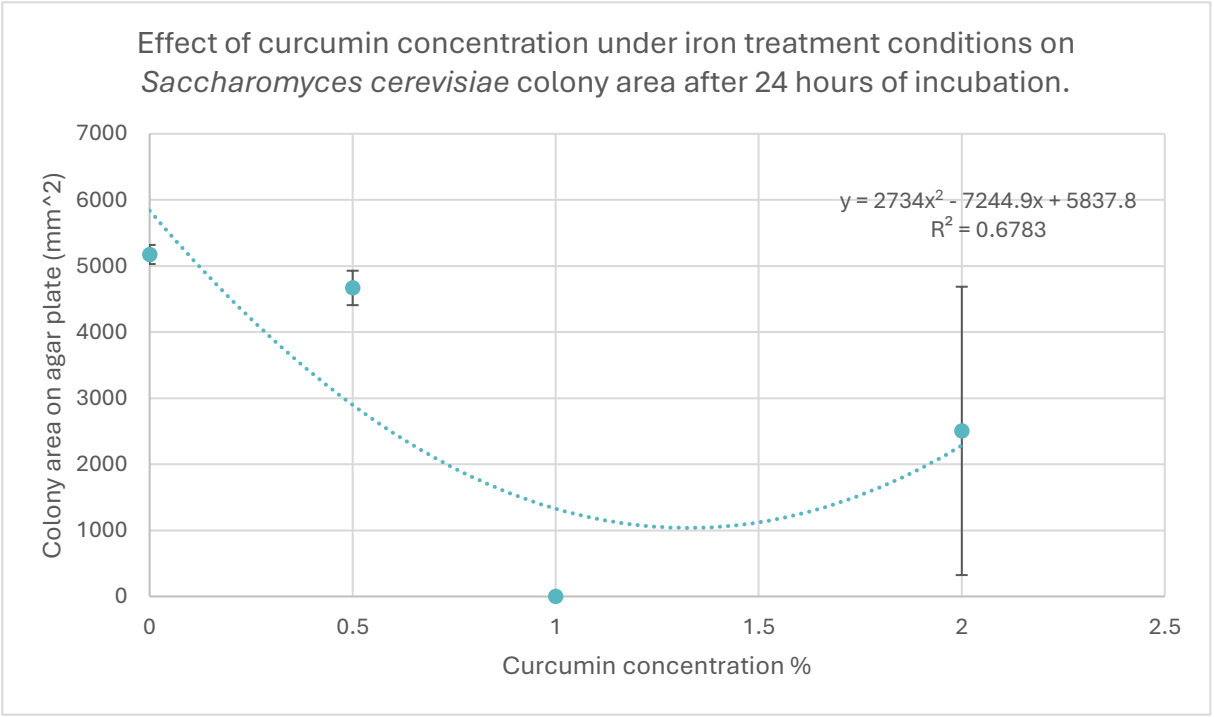
CV for 1.0% curcumin concentration, iron present is undefined due to the denominator being 0.

4. *Graphical Analysis*



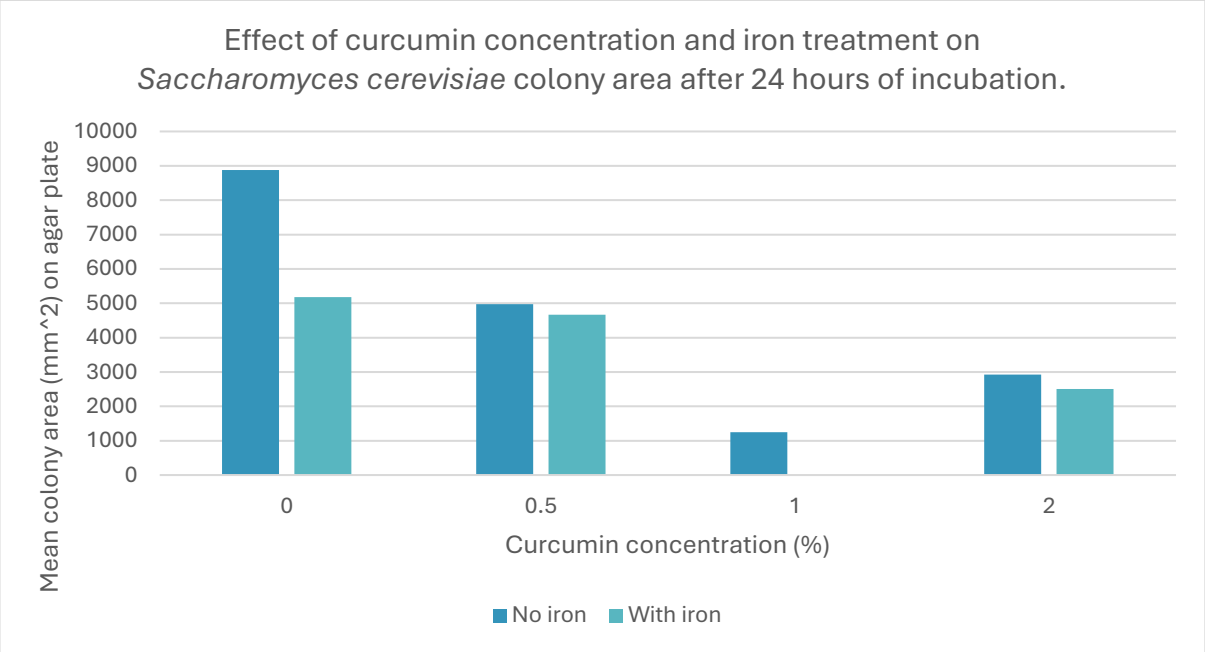
Graph 1: Scatter plot of mean *Saccharomyces cerevisiae* colony surface area (mm^2) after 24 hours of incubation without iron.

Trend: Mean colony surface area generally decreased with increasing curcumin concentration, from 8874.61mm^2 (0.0%) to 1251.40mm^2 (1.0%), with a higher mean recorded at 2.0% (2928.63mm^2). **Error Bars:** Error bars represent sample standard deviation (n=3). **Trendline:** A polynomial trendline was selected due to its close fit to the overall data trend while minimising masking of variability.



Graph 2: Scatter plot of mean *Saccharomyces cerevisiae* colony surface area (mm^2) after 24 hours of incubation with iron treatment.

Trend: Mean colony surface area generally decreased with increasing curcumin concentration, from 5174.32mm^2 (0.0%) to 0.00mm^2 (1.0%), with a higher mean recorded at 2.0% (2505.28mm^2). **Error Bars:** Error bars represent sample standard deviation (n=3). **Trendline:** A polynomial trendline was selected due to its close fit to the overall data trend while minimising masking of variability.



Graph 3: Bar graph comparing mean *Saccharomyces cerevisiae* colony surface area (mm^2) with and without iron treatment after 24 hours of incubation.

Trend: Mean *Saccharomyces cerevisiae* colony surface area was generally greater in the absence of iron than in the iron-treated groups. The difference between treatments decreased as curcumin concentration increased. No measurable colony growth was observed in the iron-treated group at 1.0% curcumin.

5. Inferential statistical analysis

1. A two-way analysis of variance (Two-Way ANOVA with replication) was conducted to determine whether curcumin concentration (0.0%, 0.5%, 1.0%, and 2.0%), iron treatment (absent or present), and the interaction between two factors had statistically significant effects on the mean colony area (mm^2) of *Saccharomyces cerevisiae* after 24 hours of incubation. The analysis was performed using data from three biological replicates per treatment group (n=3), comparing the variation attributable to each experimental factor relative to the variation within treatment groups (editage, 2023).

A significance level of $\alpha = 0.05$ was used for all statistical analyses.

Table 15: Two-way ANOVA with replication for colony surface area data.

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*
Curcumin concentration	136127900	3	45375967	20.699	0.000009
Iron	12098430	1	12098430	5.519	0.03198
Curcumin × Iron	11197140	3	3732380	1.703	0.20659
Error (Residual)	35074320	16	2192145		
Total	194497790	23			

As the p-value for curcumin concentration ($p = 0.000009$) was less than the significance level ($\alpha = 0.05$), there is sufficient evidence to conclude that curcumin concentration had a statistically significant effect on the mean colony area of *S. cerevisiae* (Minitab Blog, 2015). Therefore, the null hypothesis that curcumin concentration has no effect on colony area is rejected.

Similarly, the p-value for iron treatment (0.03198) was less than $\alpha = 0.05$, indicating that the presence or absence of iron also had a statistically significant effect on mean colony area (Minitab Blog, 2015). Therefore, the null hypothesis for iron treatment is rejected.

However, the p-value for the interaction between curcumin concentration and iron treatment (0.20659) was greater than $\alpha = 0.05$. Therefore, there is insufficient evidence to conclude that the effect of curcumin concentration depended on the presence or absence of iron (Minitab Blog, 2015). The null hypothesis for the interaction is not rejected.

As curcumin concentration showed a statistically significant effect and contained more than two treatment levels, a Tukey's Honestly Significant Difference (HSD) post-hoc test was conducted to determine which curcumin concentrations differed significantly in mean colony area. A post-hoc test was not required for iron treatment because only two treatment levels were compared (GraphPad Software, n.d.).

2. Post-Hoc Test: Tukey's HSD

$$HSD = q_\alpha(k, df_{within}) \times \sqrt{\frac{MS_{W(error)}}{n_k}}$$

2.1. Tukey's critical value (q_α) could be obtained from the studentised range (Tukey) distribution table for $k = 4$ groups, $df_{within}(Error\ (residual)) = 16$, and $\alpha = 0.05$ (Real Statistics Using Excel, 2012).

$$\therefore q_\alpha = 4.046$$

2.2.Calculating HSD

$$HSD = q_{\alpha}(k, df_{within}) \times \sqrt{\frac{MS_{Error}}{n_k}}$$

$$HSD = 4.046 \times \sqrt{\frac{2192145}{3 \times 2}}$$

$$= 2445.60mm^2$$

2.3.Calculating the mean for each curcumin concentration.

0.0%:

$$\frac{8874.61 + 5174.32}{2} = 7024.47mm^2$$

0.5%:

$$\frac{4973.11 + 4668.14}{2} = 4820.63mm^2$$

1.0%:

$$\frac{1251.40 + 0}{2} = 625.70mm^2$$

2.0%:

$$\frac{2928.63 + 2505.28}{2} = 2716.96mm^2$$

2.4.Determining the absolute mean difference (MD) between 0.0% and 0.5% curcumin concentrations from values found in Step 2.3.

$$|MD| = |mean\ 0.0\%\ colony\ area - mean\ 0.5\%\ colony\ area|$$

$$= 7024.47 - 4820.63$$

$$= 2203.84$$

Table 16: Tukey's HSD post-hoc test for colony surface area and curcumin concentration data (using mean surface area between treatments with and without iron).

Comparisons between curcumin concentrations (%)	Absolute MD	HSD Value	Is Absolute MD larger than HSD value (and therefore have statistically significant differences)?
0.0 vs 0.5	2203.84	2445.60	No
0.0 vs 1.0	6398.77	2445.60	Yes
0.0 vs 2.0	4307.51	2445.60	Yes
0.5 vs 1.0	4194.93	2445.60	Yes

0.5 vs 2.0	2103.67	2445.60	No
1.0 vs 2.0	2091.26	2445.60	No

Cell Viability (Colony Forming Units (CFU/mL)) Data

1. Raw Data

Table 17: Colony count of *Saccharomyces cerevisiae* in each petri dish after 24 hours of incubation.

			Colony count of *Saccharomyces cerevisiae* in each petri dish after 24 hours of incubation.			
			Without Iron (A)		With Iron (B)	
			1:1000	Mean	1:1000	Mean
Curcumin concentration (%)	0.0 (1)	Trial 1	TNTC	TNTC	TNTC	219.50
		Trial 2	TNTC		198	
		Trial 3	TNTC		241	
	0.5 (2)	Trial 1	228	259.67	183	188.67
		Trial 2	286		203	
		Trial 3	265		180	
	1.0 (3)	Trial 1	0	0.33	0	0
		Trial 2	1		0	
		Trial 3	0		0	
	2.0 (4)	Trial 1	0	0	0	0
		Trial 2	0		0	
		Trial 3	0		0	

Highlighted data: plates that contained potential contamination (Table 12).

2. Sample Calculations

1. CFU/mL calculation for Trial 1 of the 0.5% curcumin treatment without iron (A2 T1) (1:1000 dilution)

$$CFU/mL = \frac{Number\ of\ colonies \times Dilution\ factor}{Volume\ plated\ (mL)}$$

$$= \frac{228 \times 1000}{0.1}$$

$$= 2.28 \times 10^6\ CFU/mL$$

2. Mean CFU/mL calculation for the 0.5% curcumin treatment without iron

$$Mean\ CFU/mL = \frac{2.28 \times 10^6 + 2.86 \times 10^6 + 2.65 \times 10^6}{3}$$

$$2.60 \times 10^6\ CFU/mL$$

3. Range of CFU/mL for the 0.5% curcumin treatment without iron

$$Range = Maximum - Minimum$$

$$= 2.86 \times 10^6 - 2.28 \times 10^6$$

$$= 5.80 \times 10^5\ CFU/mL$$

3. *Processed Data*

All CFU calculations were based on the 1:1000 dilution, as this was the only dilution plated for all treatment groups. Plates recorded as TNTC (Too numerous to count) were excluded from quantitative CFU/mL calculations because accurate colony enumeration was not possible. CFU/mL values were calculated using the standard plate count equation, with 0.1mL of diluted culture plated on each agar plate.

Table 18: CFU/mL of *Saccharomyces cerevisiae* in each petri dish after 24 hours of incubation.

			CFU/mL of *Saccharomyces cerevisiae* in each petri dish after 24 hours of incubation (1:1000).	
			Without Iron (A)	With Iron (B)
Curcumin concentration (%)	0.0 (1)	Trial 1	TNTC	TNTC
		Trial 2	TNTC	1.98×10^6
		Trial 3	TNTC	2.41×10^6
	0.5 (2)	Trial 1	2.28×10^6	1.83×10^6
		Trial 2	2.86×10^6	2.03×10^6
		Trial 3	2.65×10^6	1.80×10^6
	1.0 (3)	Trial 1	0	0
		Trial 2	1.00×10^4	0
		Trial 3	0	0
	2.0 (4)	Trial 1	0	0
		Trial 2	0	0
		Trial 3	0	0

Table 19: Mean CFU/mL and range for CFU/mL data.

Curcumin conc. (%)	**Iron**	**Mean CFU/mL**	**Range (CFU/mL)**
0.0	Absent	TNTC	-
	Present	2.20×10^6	4.30×10^5
0.5	Absent	2.60×10^6	5.80×10^5
	Present	1.89×10^6	2.30×10^5
1.0	Absent	3.33×10^3	1.00×10^4
	Present	0	0
2.0	Absent	0	0
	Present	0	0

Statistical analysis (one-way or two-way ANOVA) and graphical analysis were not performed on the CFU/mL data. Several treatment groups contained plates recorded as TNTC or 0 CFU, resulting in highly censored, non-normally distributed data with insufficient measurable variation for meaningful parametric analysis. Consequently, the CFU results were used as qualitative and descriptive evidence to support interpretation of the ImageJ colony area analysis rather than as the primary quantitative dataset.

Analysis

1. *Effect of curcumin concentration on Saccharomyces cerevisiae growth*

Colony surface area generally declined as curcumin concentration increased under both iron treatment conditions (Graph 1; Graph 2; Table 14), indicating that increasing curcumin concentrations inhibited the growth of *Saccharomyces cerevisiae*.

The two-way ANOVA demonstrated that curcumin concentration had a statistically significant effect on colony surface area ($p = 0.000009$) (Table 15). Tukey's HSD post-hoc analysis further showed that mean colony area differed significantly between 0.0% and concentrations of 1.0% and above, while the comparison between 0.5% and 1.0% was also significant (Table 16). No significant difference was detected between 0.0% and 0.5% curcumin, indicating that the largest decline in growth occurred between 0.5% and 1.0%, rather than progressively across every concentration increment (Table 16).

Although growth declined under both iron conditions, the magnitude differed. In the absence of iron, mean colony area decreased from 8874.61 mm^2 at 0.0% curcumin to 4973.11 mm^2 at 0.5%, before falling sharply to 1251.40 mm^2 at 1.0% (Table 14). Under iron treatment, colony area declined from 5174.32 mm^2 to 4668.14 mm^2 between 0.0% and 0.5%, before complete inhibition of measurable yeast growth occurred at 1.0% (0 mm^2) (Table 14). These results suggest that once a threshold concentration was reached, curcumin became more inhibitory regardless of iron treatment.

The relatively high growth observed at 0.5% curcumin is consistent with curcumin's antioxidant activity. Curcumin scavenges ROS, reducing oxidative damage, while also chelating extracellular iron and lowering the availability of Fe^{2+} for the Fenton reaction (Rainey *et al.,* 2019). In addition, curcumin has been reported to accumulate within intracellular membranes, including the endoplasmic reticulum (ER), where it may reduce the intracellular LIP (Zhang *et al.,* 2018). These mechanisms may partially alleviate oxidative stress experienced by *S. cerevisiae,* allowing substantial colony growth to be maintained despite exposure to curcumin.

Growth declined dramatically at 1.0% curcumin (4668.14mm^2 & 4973.11mm^2 to 0.00mm^2 & 1251.40mm^2), consistent with the concentration-dependent transition of curcumin from an antioxidant to a pro-oxidant (Banerjee *et al.,* 2008). At elevated intracellular concentrations, curcumin promotes ROS generation and disrupts cellular redox homeostasis, increasing

oxidative stress beyond the capacity of antioxidant defence systems (Yu *et al.,* 2019). Additionally, curcumin has been reported to interfere with cytoskeletal proteins such as septins in the ER, prolonging the G1 phase of the cell cycle and delaying cytokinesis (Carver & King, 2026). Combined with ROS-mediated damage to proteins, lipids, ad DNA, these effects reduce cell proliferation and limit colony expansion.

Although mean colony area increased slightly at 2.0% curcumin (2928.63mm² without iron; 2505.28mm² with iron), Tukey's HSD indicated that this increase was not statistically significant (Table 16). Additionally, these treatments exhibited very high variability (CV = 87.07–173.21%), and qualitative observations identified contamination on several plates that contributed to the measured surface area. Therefore, the apparent increase is unlikely to represent a genuine recovery in yeast growth.

The CFU data supported these findings. Although inferential statistical analysis was not performed because several treatments produced TNTC or zero colony counts, the 0.5% treatment retained high viability (2.60×10^6 CFU/mL without iron; 1.89×10^6 CFU/mL with iron), whereas the 1.0% treatments produced negligible viable colonies (3.33×10^3 CFU/mL without iron; 0 CFU/mL with iron), and no colonies were detected at 2.0% (Table 19). The agreement between colony surface area and CFU measurements therefore strengthens the conclusion that increasing curcumin concentration progressively reduced both the growth and viability of *S. cerevisiae*. Minor differences between the two datasets occurred at higher curcumin concentrations because ImageJ quantified all visible colony coverage, whereas suspected contaminant colonies were excluded from colony counting. Consequently, colony area occasionally overestimated viable yeast growth in contaminated treatments, although the general trend was preserved.

2. *Effect of iron on yeast growth and oxidative stress*

Iron treatment significantly reduced *Saccharomyces cerevisiae* colony surface area overall (Two-way ANOVA**,** $p = 0.03198$), indicating that iron overload inhibited yeast growth (Graph 2; Table 15). The greatest reduction occurred in the absence of curcumin, where mean colony area decreased from 8874.61 mm² without iron (A1) to 5174.32 mm² with iron (B1), representing an ~41.70% reduction (Table 14). As no curcumin was present, the reduction in colony area can be attributed primarily to iron-induced ROS production and oxidative damage rather than the effects of curcumin. A smaller decrease was also observed at 0.5% curcumin (4973.11 mm² to 4668.14 mm²), indicating that iron treatment continued to reduce yeast growth even in the presence of low concentrations of curcumin.

This reduction is consistent with iron-induced oxidative stress (Lin *et al.,* 2011). Although iron is essential for mitochondrial respiration and numerous metabolic enzymes, excess intracellular Fe^{2+} increases the labile iron pool and promotes the Fenton reaction (Lin *et al.,* 2011). In this reaction, ferrous iron catalyses the decomposition of hydrogen peroxide to produce highly reactive hydroxyl radicals (·OH), which rapidly oxidise proteins, lipids and DNA (Freinbichler *et al.,* 2011). Unlike other reactive oxygen species, hydroxyl radicals cannot be detoxified

enzymatically and therefore cause extensive cellular damage (Rezayian *et al.,* 2019). The resulting oxidative stress impairs mitochondrial function, disrupts ATP production and ultimately reduces cell growth and viability (Rezayian *et al.,* 2019).

Although iron significantly reduced colony area overall, its effect became less apparent at higher curcumin concentrations (Graph 3). This is likely because yeast growth was already severely inhibited by curcumin at 1.0% and 2.0%, leaving little remaining growth for iron to further reduce.

3. Interaction between curcumin concentration and iron treatment

The two-way ANOVA indicated no statistically significant interaction between curcumin concentration and iron treatment ($p = 0.20659$), suggesting that the effect of curcumin on *Saccharomyces cerevisiae* colony surface area did not significantly depend on the presence of iron (Table 15). Although iron reduced growth overall and curcumin became increasingly inhibitory with increasing concentration, these effects appeared to act largely independently.

One possible explanation is that curcumin acts as an iron chelator, reducing the availability of Fe^{2+} required for the Fenton reaction (Rainey *et al.,* 2019). By lowering the intracellular LIP, curcumin may partially limit iron-mediated hydroxyl radical production, reducing the extent to which iron overload further increases oxidative stress (Rainey *et al.,* 2019). This may explain why the difference between iron-treated and untreated cultures became progressively smaller as curcumin concentration increased.

Additionally, at 1.0% and 2.0% curcumin, yeast growth was already severely inhibited by curcumin alone, producing a floor effect whereby colony growth approached zero regardless of iron treatment (Haqiqatkhah, 2025). Consequently, any additional inhibitory effect of iron was difficult to detect. Interpretation of the interaction was also limited by the high variability observed at higher curcumin concentrations, contamination on several plates, and the relatively small sample size (n=3).

Evaluation

Precision/Reliability

Coefficient of variation (CV) values for colony surface area ranged from 2.79% to 173.21%, indicating highly variable precision between treatments (Table 14). Precision was greatest at 0.0% curcumin with iron (CV = 2.79%) and lowest at 1.0% curcumin without iron (CV = 173.21%), where the standard deviation exceeded the mean, indicating substantial variability between replicates (Table 14). Similarly, CFU/mL measurements showed the greatest range at 0.5% curcumin without iron (5.80×10^5 CFU/mL) and no variation at 2.0% curcumin, where no viable colonies were detected (Table 19). Overall, precision decreased at higher curcumin concentrations, likely due to reduced colony numbers and contamination.

The coefficient of determination indicated a stronger relationship between curcumin concentration and colony area in the absence of iron ($R^2 = 0.9793$) than in the presence of iron ($R^2 = 0.6783$), suggesting greater unexplained variation under iron treatment (Graph 1; Graph 2). This is reflected by overlapping error bars at 1.0% and 2.0% curcumin (Graph 1) and the large standard deviation for 2.0% curcumin with iron (2181.43 mm^2) (Graph 2), reducing confidence in differences between these treatments. Furthermore, the small sample size (n = 3) limited the ability to reduce random error through averaging, decreasing the overall reliability of the results.

Table 20: Random errors of the experiment.

Error Source	Evidence	Effect on Results	Improvements
Inconsistent inoculum distribution during plating	Yeast suspension was manually mixed and pipetted without continuous agitation prior to plating	Uneven cell distribution across plates likely contributed to variability in colony area and CFU counts, particularly where replicate plates showed large differences at the same treatment concentration (e.g. >0.5% curcumin)	Use vortex mixing before each aliquot and use a shaking incubator/orbital shaker during culture preparation to maintain homogenous cell suspension
Contamination of agar plates	Several high-curcumin plates showed atypical large, fluffy, non-opaque colonies inconsistent with yeast morphology	Contaminant colonies likely inflated ImageJ-measured colony surface area and increased variability, particularly at 1.0% and 2.0% curcumin, reducing reliability of growth measurements	Improve aseptic technique, use sterile laminar flow conditions.
Subjective colony identification and measurement thresholding	CFU counts excluded colonies thought to be non-yeast based on morphology; ImageJ thresholding required manual adjustment for each image	Introduces inconsistency between datasets and potential undercounting (CFU) or overestimation (ImageJ), particularly in mixed or unclear colony morphology conditions (A3, B3, A4, B4 treatments which experienced most contamination).	Use automated colony counting software or predefined threshold parameters; validate colony identity using microscopy or staining

Accuracy

A comparison of results could be made against a study by Minear *et al.,* which investigated the effect of curcumin on *Saccharomyces cerevisiae* growth (Minear *et al.,* 2011). Their study demonstrated that curcumin inhibits yeast growth primarily through iron chelation, reducing the intracellular LIP and inducing an iron starvation response (Minear *et al.,* 2011). This aligns closely with this investigation, where both colony surface area and CFU data showed a consistent concentration-dependent decrease in growth, with near-complete inhibition at higher curcumin concentrations (≥1.0%). Minear *et al.* also reported that iron supplementation can rescue curcumin-induced growth inhibition, whereas in this investigation iron treatment instead reduced colony area overall (Minear *et al.,* 2011). This difference may be due to experimental conditions, as Minear *et al.* used liquid culture and controlled iron availability, whereas this study used agar plates where iron availability and uptake by cells may differ, altering its overall effect on growth (Minear *et al.,* 2011). Despite these differences, the overall trend of a suppression in growth due to curcumin levels is consistent between studies. Minor differences in magnitude are likely due to variation in exposure time, growth medium, and measurement method. Overall, the agreement between datasets supports moderate–high accuracy of the present results.

Table 21: Systematic errors of the experiment.

Error Source	Evidence	Effect on Results	Improvements
Curcumin solubility limitations	Visible precipitation of curcumin in aqueous medium despite ethanol being used as a solvent	Actual curcumin concentration likely varied between treatments, meaning the intended concentration gradient may not have been achieved. This reduces accuracy of dose–response interpretation	Use DMSO as a solvent to dissolve curcumin fully.
Ethanol as a solvent confounder	Ethanol was required to dissolve curcumin and may not have been held constant across all treatments	Ethanol may independently inhibit *Saccharomyces cerevisiae* growth, potentially exaggerating the apparent inhibitory effect attributed to curcumin/iron (Zhang *et al.,* 2015).	Standardize ethanol concentration across all treatments (including controls) so solvent effects are controlled and isolated from curcumin effects

Conclusion

Curcumin concentration and iron treatment both influenced the growth of *Saccharomyces cerevisiae* over the 24-hour incubation period. Overall, the results partially supported the alternative hypotheses. Increasing curcumin concentration significantly reduced both colony surface area and cell viability, with the greatest decline occurring between 0.5% and 1.0% curcumin, aligning with a concentration-dependent transition from antioxidant to pro-oxidant activity. Iron treatment also significantly reduced colony growth overall, indicating that excess iron increased oxidative stress. However, contrary to the hypotheses, no statistically significant interaction between curcumin concentration and iron treatment was detected.

The prediction that low curcumin concentrations would maintain yeast growth through antioxidant activity was supported, while higher concentrations ($\geq 1.0\%$) produced substantial growth inhibition. Although iron was expected to partially alleviate curcumin-induced iron-starvation at lower concentrations, it instead reduced colony growth, suggesting that within this investigation, iron-mediated oxidative stress outweighed any beneficial effect of increased iron availability.

Validity was moderate. Controlled variables, including incubation time, growth medium, inoculum source and incubation temperature, improved comparability between treatments. However, contamination on several plates and curcumin solubility limitations may have influenced the accuracy of growth measurements.

Reliability was moderate. Statistical analysis demonstrated significant effects of both curcumin concentration and iron treatment, and the agreement between colony surface area and CFU measurements strengthened confidence in the observed trends. However, high variability at 1.0% and 2.0% curcumin concentrations, together with the small sample size (n=3), reduced precision and limited observation of treatment interactions.

Overall, increasing curcumin concentration progressively inhibited the growth and viability of *Saccharomyces cerevisiae*, while iron overload further reduced growth through oxidative stress. Future investigations using larger sample sizes, improved curcumin solubility, and more objective methods of assessing yeast viability, such as OD_{600} spectrophotometry or fluorescent ROS probes, would improve accuracy and provide deeper insight into the interaction between curcumin and iron.

Word Count: 2200 (excluding titles, headings, captions, figures, tables, logbook)

Reference List

Affinity Chemical (2020) *Ferrous Sulfate Environmental and Toxicological Information*, *Affinity Chemical*. Available at: https://www.affinitychemical.com/ferrous-sulfate-environmental-and-toxicological-information/ (Accessed: June 28, 2026).

Appendino, G. *et al.* (2022) "Shedding light on curcumin stability," *Fitoterapia*, 156(105084), p. 105084. Available at: https://doi.org/10.1016/j.fitote.2021.105084.

Avendaño-Briseño, K.A. *et al.* (2025) "Antioxidant effect of curcumin and its impact on mitochondria: Evidence from biological models," *Journal of Xenobiotics*, 15(5), p. 139. Available at: https://doi.org/10.3390/jox15050139.

Banerjee, A. *et al.* (2008) "Concentration dependent antioxidant/pro-oxidant activity of curcumin studies from AAPH induced hemolysis of RBCs," *Chemico-Biological Interactions*, 174(2), pp. 134–139. Available at: https://doi.org/10.1016/j.cbi.2008.05.009.

Banerjee, A. *et al.* (2008) "Concentration dependent antioxidant/pro-oxidant activity of curcumin studies from AAPH induced hemolysis of RBCs," *Chemico-Biological Interactions*, 174(2), pp. 134–139. Available at: https://doi.org/10.1016/j.cbi.2008.05.009.

Bayir, H. and Kagan, V.E. (2008) "Bench-to-bedside review: Mitochondrial injury, oxidative stress and apoptosis--there is nothing more practical than a good theory," *Critical Care (London, England)*, 12(1), p. 206. Available at: https://doi.org/10.1186/cc6779.

Cámara, E. *et al.* (2024) "Saccharomyces cerevisiae strains performing similarly during fermentation of lignocellulosic hydrolysates show pronounced differences in transcriptional stress responses," *Applied and Environmental Microbiology*, 90(5), p. e0233023. Available at: https://doi.org/10.1128/aem.02330-23.

Carver, M. and King, N. (2026) "Septins regulate cytokinesis and multicellular development in the closest living relatives of animals," *bioRxivorg*. Available at: https://doi.org/10.64898/2026.04.05.716596.

Chandimali, N. *et al.* (2025) "Free radicals and their impact on health and antioxidant defenses: a review," *Cell Death Discovery*, 11(1), p. 19. Available at: https://doi.org/10.1038/s41420-024-02278-8.

Dahal, P. (2022) Spread Plate Method- Definition, Principle, Procedure, Uses, Microbe Notes. Available at: https://microbenotes.com/spread-plate-technique/ (Accessed: June 29, 2025).

editage (2023) *What Is ANOVA (Analysis of Variance): Definition, Types, Uses & Assumptions*, *Educational Articles For Researchers, Students And Authors - Editage Blog*. Available at: https://www.editage.com/blog/anova-types-uses-assumptions-a-quick-guide-for-biomedical-researchers/ (Accessed: June 28, 2026).

Freinbichler, W. *et al.* (2011) "Highly reactive oxygen species: detection, formation, and possible functions," *Cellular and Molecular Life Sciences*, 68(12), pp. 2067–2079. Available at: https://doi.org/10.1007/s00018-011-0682-x.

GraphPad Software, LLC (no date) *GraphPad Prism 11 Statistics Guide - When it makes sense to not correct for multiple comparisons*, *Graphpad.com*. Available at: https://www.graphpad.com/guides/prism/latest/statistics/stat_when_to_not_correct_for_2.htm (Accessed: June 28, 2026).

Haqiqatkhah, M.M. (2025) "Floor and ceiling effects," *MATILDA Preprints* [Preprint], (2025-05–23). Available at: https://matilda.fss.uu.nl/articles/floor-ceiling-effects.html.

Hardwick, J.M. *et al.* (2021) "Editorial: Yeast differentiation: From cell-to-cell heterogeneity to replicative aging and regulated cell death," *Frontiers in Cell and Developmental Biology*, 9, p. 823447. Available at: https://doi.org/10.3389/fcell.2021.823447.

Hewlings, S.J. and Kalman, D.S. (2017) "Curcumin: A review of its effects on human health," *Foods (Basel, Switzerland)*, 6(10), p. E92. Available at: https://doi.org/10.3390/foods6100092.

Johnson, E.A. and Echavarri-Erasun, C. (2011) "Yeast Biotechnology," in *The Yeasts*. Elsevier, pp. 21–44.

Juan, C.A. *et al.* (2021) "The chemistry of reactive oxygen species (ROS) revisited: Outlining their role in biological macromolecules (DNA, lipids and proteins) and induced pathologies," *International Journal of Molecular Sciences*, 22(9), p. 4642. Available at: https://doi.org/10.3390/ijms22094642.

Kozlov, A.V., Javadov, S. and Sommer, N. (2024) "Cellular ROS and antioxidants: Physiological and pathological role," *Antioxidants (Basel, Switzerland)*, 13(5), p. 602. Available at: https://doi.org/10.3390/antiox13050602.

Kruszewski, M. (2003) "Labile iron pool: the main determinant of cellular response to oxidative stress," *Mutation Research*, 531(1–2), pp. 81–92. Available at: https://doi.org/10.1016/j.mrfmmm.2003.08.004.

Lin, H. *et al.* (2011) "Genetic and biochemical analysis of high iron toxicity in yeast: iron toxicity is due to the accumulation of cytosolic iron and occurs under both aerobic and anaerobic conditions: Iron toxicity is due to the accumulation of cytosolic iron and occurs under both aerobic and anaerobic conditions," *The Journal of Biological Chemistry*, 286(5), pp. 3851–3862. Available at: https://doi.org/10.1074/jbc.M110.190959.

Lip, K.Y.F. *et al.* (2020) "Selection and subsequent physiological characterization of industrial Saccharomyces cerevisiae strains during continuous growth at sub- and- supra optimal temperatures," *Biotechnology Reports (Amsterdam, Netherlands)*, 26(e00462), p. e00462. Available at: https://doi.org/10.1016/j.btre.2020.e00462.

Liu, Y. and Chang, Y.-Z. (2026) "The regulation of mitochondrial ferritin on mitochondrial redox balance is essential to cell fate decision," *Redox Biology*, 92(104124), p. 104124. Available at: https://doi.org/10.1016/j.redox.2026.104124.

Mancardi, D. *et al.* (2021) “Iron overload, oxidative stress, and ferroptosis in the failing heart and liver,” *Antioxidants (Basel, Switzerland)*, 10(12), p. 1864. Available at: https://doi.org/10.3390/antiox10121864.

Maslanka, R., Zadrag-Tecza, R. and Kwolek-Mirek, M. (2020) “Linkage between carbon metabolism, redox status and cellular physiology in the yeast Saccharomyces cerevisiae devoid of SOD1 or SOD2 gene,” *Genes*, 11(7), p. E780. Available at: https://doi.org/10.3390/genes11070780.

Minear, S. *et al.* (2011) “Curcumin inhibits growth of Saccharomyces cerevisiae through iron chelation,” *Eukaryotic Cell*, 10(11), pp. 1574–1581. Available at: https://doi.org/10.1128/EC.05163-11.

Minitab Blog (2015) *What Can You Say When Your P-Value is Greater Than 0.05?*, *Minitab.com*. Available at: https://blog.minitab.com/en/blog/understanding-statistics/what-can-you-say-when-your-p-value-is-greater-than-005 (Accessed: June 28, 2026).

Moradas-Ferreira, P. *et al.* (1996) “The molecular defences against reactive oxygen species in yeast,” *Molecular Microbiology*, 19(4), pp. 651–658. Available at: https://doi.org/10.1046/j.1365-2958.1996.403940.x.

Muslija, N. (2024) *Ethanol*, *oxytec*. Available at: https://www.oxytec.com/en/pollutants/ethanol-3/ (Accessed: June 28, 2026).

Paramera, E.I., Konteles, S.J. and Karathanos, V.T. (2011) “Microencapsulation of curcumin in cells of Saccharomyces cerevisiae,” *Food Chemistry*, 125(3), pp. 892–902. Available at: https://doi.org/10.1016/j.foodchem.2010.09.063.

Peterson, M.E. *et al.* (2007) “The dependence of enzyme activity on temperature: determination and validation of parameters,” *The Biochemical Journal*, 402(2), pp. 331–337. Available at: https://doi.org/10.1042/BJ20061143.

Rainey, N.E. *et al.* (2019) “Iron chelation by curcumin suppresses both curcumin-induced autophagy and cell death together with iron overload neoplastic transformation,” *Cell Death Discovery*, 5(1), p. 150. Available at: https://doi.org/10.1038/s41420-019-0234-y.

Ramos-Alonso, L. *et al.* (2020) “Iron regulatory mechanisms in Saccharomyces cerevisiae,” *Frontiers in Microbiology*, 11, p. 582830. Available at: https://doi.org/10.3389/fmicb.2020.582830.

Reis, V.R. *et al.* (2013) “Characteristics of Saccharomyces cerevisiae yeasts exhibiting rough colonies and pseudohyphal morphology with respect to alcoholic fermentation,” *Brazilian Journal of Microbiology*, 44(4), pp. 1121–1131. Available at: https://doi.org/10.1590/S1517-83822014005000020.

Rezayian, M., Niknam, V. and Ebrahimzadeh, H. (2019) “Oxidative damage and antioxidative system in algae,” *Toxicology Reports*, 6, pp. 1309–1313. Available at: https://doi.org/10.1016/j.toxrep.2019.10.001.

Sikder, M.M. *et al.* (2025) "Reactive oxygen species: Role in pathophysiology, and mechanism of endogenous and dietary antioxidants during oxidative stress," *Chonnam Medical Journal*, 61(1), pp. 32–45. Available at: https://doi.org/10.4068/cmj.2025.61.1.32.

Stanley, M. *et al.* (2025) "Evaluation of the impact of curcumin concentrations on the viability of human periodontal ligament cells: An in vitro study," *Journal of Indian Society of Periodontology*, 29(2), pp. 147–152. Available at: https://doi.org/10.4103/jisp.jisp_461_24.

Tavassolifar, M.J. *et al.* (2020) "The influence of reactive oxygen species in the immune system and pathogenesis of multiple sclerosis," *Autoimmune Diseases*, 2020, p. 5793817. Available at: https://doi.org/10.1155/2020/5793817.

Xing, H. *et al.* (2022) "Recent advances in the allergic cross-reactivity between fungi and foods," *Journal of Immunology Research*, 2022, p. 7583400. Available at: https://doi.org/10.1155/2022/7583400.

Yu, T. *et al.* (2019) "Curcumin induces concentration-dependent alterations in mitochondrial function through ROS in C2C12 mouse myoblasts: YU et al," *Journal of Cellular Physiology*, 234(5), pp. 6371–6381. Available at: https://doi.org/10.1002/jcp.27370.

Zhang, L. *et al.* (2018) "Curcumin induces endoplasmic reticulum stress-associated apoptosis in human papillary thyroid carcinoma BCPAP cells via disruption of intracellular calcium homeostasis," *Medicine*, 97(24), p. e11095. Available at: https://doi.org/10.1097/MD.0000000000011095.

Zhang, Q. *et al.* (2015) "Substrate and product inhibition on yeast performance in ethanol fermentation," *Energy & Fuels: An American Chemical Society Journal*, 29(2), pp. 1019–1027. Available at: https://doi.org/10.1021/ef502349v.

Appendices

Appendix A

PDF for YEPD preparing instructions: YEPD(Yeast extratePeptone Dextrox) MEDIUM protocol.pdf

Appendix B

$FeSO_4$ 2mM calculation

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

Where C1=stock concentration, V1=0.1mL added, C2=2mM (desired final concentration), V2=10mL (final sample volume)

$$C_1 \cdot (0.1) = 2(10)$$

$$0.1C_1 = 20$$

$$C_1 = 200\ mM$$

So stock solution must be 200mM $FeSO_4.7H_2O$

Need 5mL:

$$m = C \times V \times M_r$$

M=mass in grams, C=concentration in mol/L, V=volume in L, Mr=molar mass

$FeSO_4.7H_2O$ molar mass=278.01g/mol

200mM=0.200mol/L

5mL=0.005L

$$m = 0.200 \times 0.005 \times 278.01$$

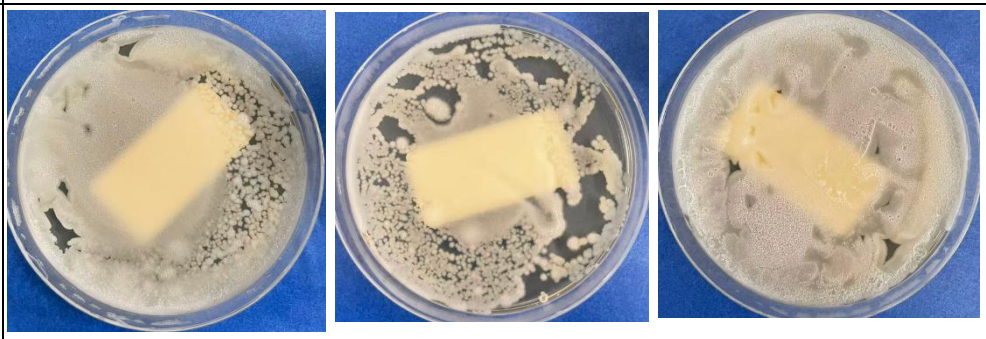
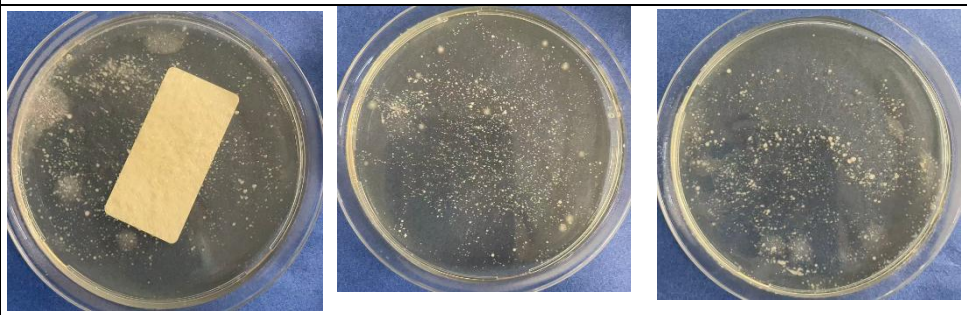
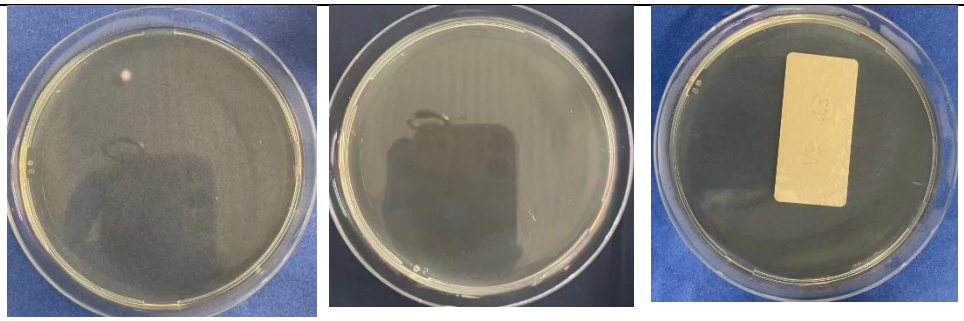
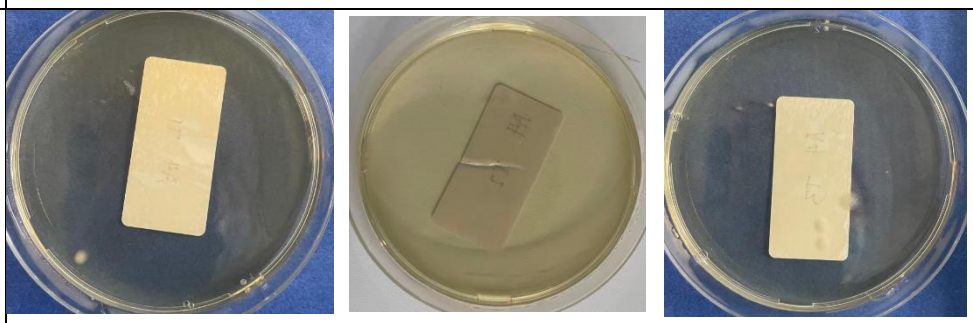
$$m = 0.27801$$

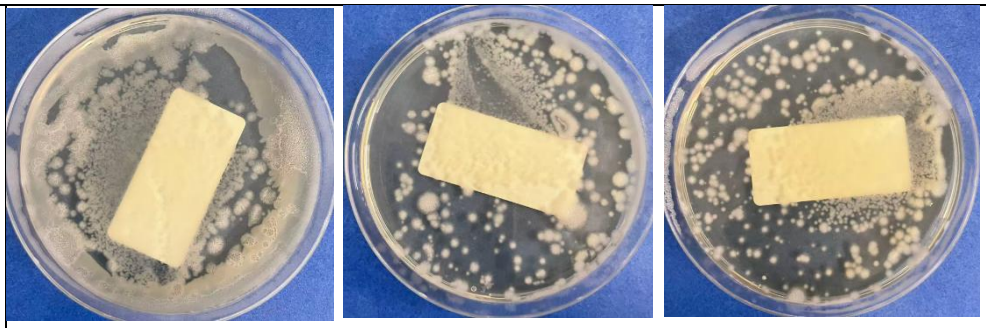
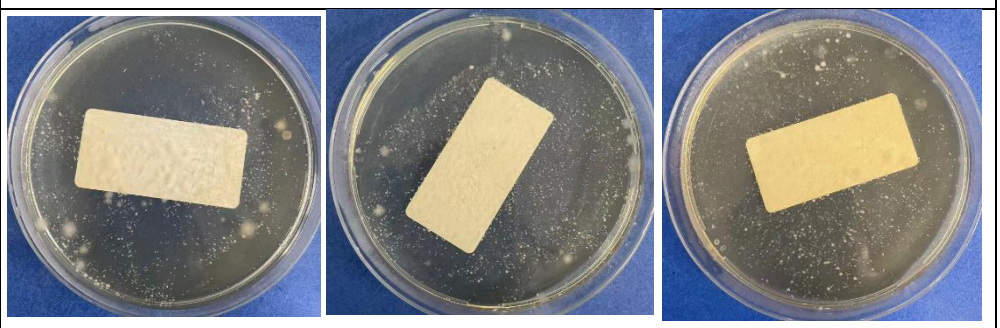
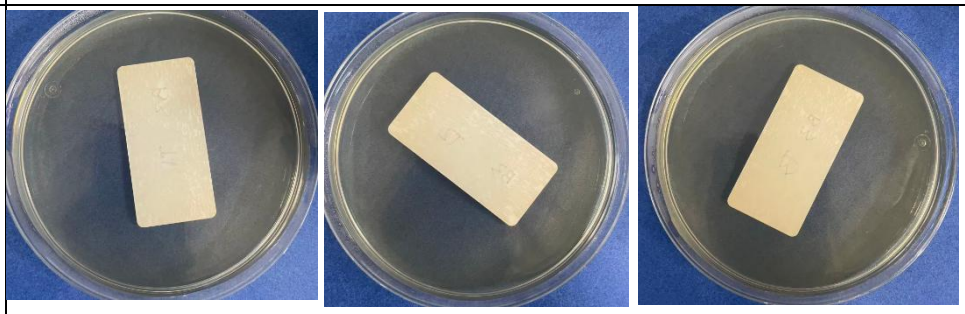
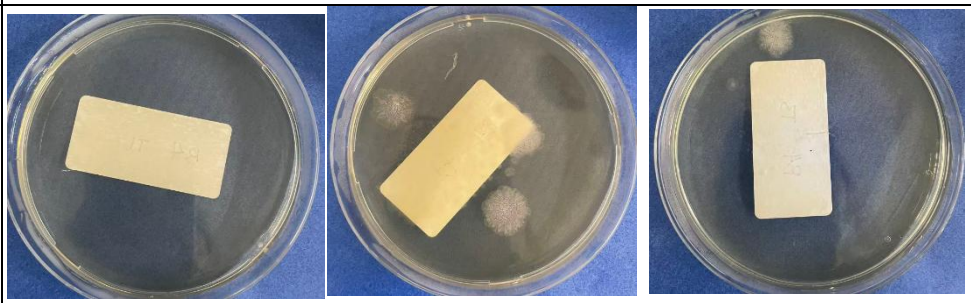
Therefore, 0.278g $FeSO_4.7H_2O$ is needed to be dissolved in 5mL of distilled water – forms stock solution of 200mM.

Pipette 0.1mL out of 200mM for final concentration of 2mM $FeSO_4$.

Appendix C

Raw data for CFU/mL (agar plate photos)

Treatment Group	Results
A1 Trial 1 Trial 2 Trial 3	
A2 Trial 1 Trial 2 Trial 3	
A3 Trial 1 Trial 2 Trial 3	
A4 Trial 1 Trial 2 Trial 3	

B1 Trial 1 Trial 2 Trial 3	
B2 Trial 1 Trial 2 Trial 3	
B3 Trial 1 Trial 2 Trial 3	
B4 Trial 1 Trial 2 Trial 3	

Acknowledgements

- Ms Panchal and Mr Braini (Lab Technicians) prepared YPD agar, YPD broth and laboratory apparatus, supervised experimental procedures, and provided guidance on aseptic technique
- Ms Salvi assisted with the preparation and execution of serial dilutions.
- Dr O'Halloran provided advice on *Saccharomyces cerevisiae* physiology and behaviour, plating methodology, colony-forming unit (CFU) calculations, and appropriate incubation conditions.
- Vivian Jiang helped take photos.

AI (ChatGPT) was used to assist with checking spelling and ensure correct grammar and punctuation.

Logbook

5/01/2026

I started brainstorming possible inquiry ideas. Last year, I completed an inquiry investigating the antioxidant and pro-oxidant characteristics of curcumin and explored how curcumin concentration and hydrogen peroxide (simulating oxidative stress) affected yeast growth. I knew that I wanted to continue with this topic, particularly as I thoroughly enjoyed researching for it. I was also slightly unhappy with the data I collected from last year, as I ran out of time to conduct serial dilutions and calculate CFU/mL.

Through last year's research, I realized 'iron chelation' kept popping up frequently, so I decided that I wanted to investigate something related to iron and curcumin.

I stumbled upon an article by Minear *et al.* (2011): https://pubmed.ncbi.nlm.nih.gov/21908599/ that I used in my analysis last time, and I realized I could make iron concentration another IV (along with curcumin).

Therefore, I did some research and came up with a really rough draft:

Effect of curcumin concentration on intracellular free iron levels and oxidative stress in yeast under iron overload conditions.

IV:

- Iron concentration
- Curcumin concentration

DV (common dyes used):

- Intracellular free iron levels – measured with iron-sensitive fluorescent dye – Phen Green SK
    - https://pmc.ncbi.nlm.nih.gov/articles/PMC4739508/
- Reactive oxygen species (ROS) levels – measured with ROS-sensitive fluorescent dye – DCFDA
    - https://pubmed.ncbi.nlm.nih.gov/40418478/
- Yeast viability or growth – measured by either optical density (OD600) or colony forming units (CFUs)
    - I didn't get the chance to do this last yr so I wanted to finish it this yr

Why iron overload

- Iron overload creates free iron inside cells that produces ROS through the Fenton reaction, causing oxidative stress – lets you specifically test how curcumin chelates free iron and reduces oxidative stress

Why measure both iron and ROS

- Iron measurement shows if curcumin reduces free intracellular iron
- ROS measurement shows biological effect of free iron on oxidative stress

Why viability

- To see if changes in iron and ROS actually affect cell survival/growth

Equipment needed

- Fluorescent dyes (Phen Green SK and DCFA)
- Fluorescence microscope or microplate reader
- Spectrophotometer for OD600 and agar plates/incubator for CFUs
- Image analysis software for microscope

[29/01/2026-6/02/2026]

To explore the topic more, I decided to make a basic deconstruct and brainstorm.

Links:

- https://pmc.ncbi.nlm.nih.gov/articles/PMC5664031/
- https://www.sciencedirect.com/science/article/pii/S0002916522027241
- https://pmc.ncbi.nlm.nih.gov/articles/PMC6757914/
- https://journals.asm.org/doi/10.1128/aem.02202-12
- https://www.sciencedirect.com/topics/earth-and-planetary-sciences/fenton-reaction
- https://pubmed.ncbi.nlm.nih.gov/8597169/
- https://www.sciencedirect.com/topics/chemistry/fenton-reaction

Effect of **curcumin concentration** on **intracellular free iron levels** and **oxidative stress** in yeast under **iron overload conditions**.

Curcumin concentration

- Primary bioactive substance in turmeric
- Targets multiple signaling molecules and has been shown to benefit inflammatory conditions, metabolic syndrome and pain – due to ant-inflammatory & antioxidant effects
- Poorly absorbed when orally ingested alone – common methods of improving absorption are to pair curcumin with piperine or lipids

Oxidative stress

- Has role in many conditions like cancer, Alzheimer's disease and heart disease
- Imbalance of free radicals and antioxidants in body = leads to cell damage
- Too many free radicals and not enough antioxidants – causes excess free radicals start to harm body's cells and tissues
- Free radicals can harm if in excess while antioxidants can help protect body from damage
- Free radicals and antioxidants are 2 different types of molecules that play role in body function – requires both
- When body converts food into energy, by-product = free radicals – they support work of immune system but only need low → moderate levels
- Free radicals are unstable molecules – missing an electron, need a certain number of electrons to be stable
- Free radicals search for electrons to take from other molecules – puts healthy molecules at risk – can snatch electrons from other molecules, damaging them and making them unstable
- Antioxidants can donate one of their electrons to a free radical – makes it complete so it doesn't steal
- Thus require enough antioxidants to satisfy free radicals otherwise they go scavenging = oxidative stress
- ROS damages plasma membranes and organelle membranes, altering membrane fluidity

Intracellular free iron levels

- Iron is essential in mammalian cells
- An excess of iron is often toxic (like all multivalent cations?) so there must be a balance between iron accumulation, its concentration within cellular compartments and its release to maintain cell viability
- Disruption of iron balance has association with damage to the CNS

Association to oxidative stress:

- Free iron within the cytosol is known as the labile iron pool (LIP) which is available for use in cellular processes
- Iron participates in the metabolism of reactive oxygen species (ROS) – thus elevation of free iron may be damaging
- Excess iron is expected to give rise to ROS – causes cellular damage through oxidative stress, mitochondrial damage and ferroptosis
- To prevent iron-induced damage, cells store excess iron in ferritin, a protein complex that sequesters iron within its core and exports excess iron through ferroportin
- Ferrous iron (Fe^{2+}) has potential for catalytic reaction with hydrogen peroxide to generate hydroxyl radial – most reactive and dangerous form of ROS encountered within cells
- Fenton reaction = chemical process where ferrous iron catalyzes the decomposition of hydrogen peroxide to generate highly reactive hydroxyl radicals and ferric iron

Iron overload conditions

- When excess iron accumulates in cells – primarily in the liver, heart and endocrine glands – due to genetic mutations or secondary causes e.g. frequent blood transfusions or chronic hemolysis
- Excess iron exceeds the storage capacity of ferritin, resulting in production of toxic reaction oxygen species (ROS) which causes cellular and tissue damage
- Excess iron – specifically non-transferrin-bound iron (NTBI) enters cells and triggers Fenton reaction, creating ROS
- Iron overload disease = any disease where body absorbs and retains too much iron (iron overload) e.g. hemochromatosis

11/02/2026

More brainstorming ad topic refining:

Independent variables:

- Curcumin concentration (0+4 levels)
- Iron condition – absent, present
    - Iron is redox catalyst for ROS production – fenten reaction; ROS is a result of redox imbalance; curcumin is redox modulator

DVs:

- CFU
- ROS
    - Iron absent: does curcumin itself change oxidative stress levels?
    - Iron present: does curcumin reduce oxidative stress under iron-induced conditions?
    - Comparison: how much extra oxidative stress is caused by iron exposure?
    - How do changes in ROS correlate to CFU?
    - However, is indirect ad is affected by many factors, not just iron

I decided that 3 DVs was too many, and I can probably infer free iron levels from ROS measurements.

When iron is absent, curcumin can still alter yeast metabolism – but becomes a baseline control – is curcumin itself harmful or beneficial without stress

Essentially, iron causes oxidation, curcumin tries to regain balance, ROS shows balance, CFU shows biological damage

Reframing:

- Experiment about iron-induced redox imbalance model in yeast

- DOSE DEPENDENT

https://pmc.ncbi.nlm.nih.gov/articles/PMC3914732/

what to use to simulate iron overload?

Ferrous Sulfate – $FeSO_4$ - was to used as it is ioninc, dissociates into Fe^{2+} and SO_4^{2-} in water – Fe2+ directly participates in Fenten reaction

https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2026.1789479/full

[taken and adapted from last year's method]

1. **Preparation of Media**
   1.1 YEPD broth and agar plates were prepared by laboratory staff following a standard protocol (see instructions in Appendix).
   1.2 All materials were autoclaved to ensure sterility, and aseptic techniques were followed throughout the procedure
2. **Inoculation of Yeast Culture**
   2.1. A sterile inoculation loop was used to collect a visible quantity of *Saccharomyces cerevisiae* from a live slope tube.
   2.2. The yeast was transferred into 40 mL of sterile YEPD broth in a 250 mL Erlenmeyer flask and briefly vortexed to disperse the cells evenly.
   2.3. A cotton ball was used to cover the neck to allow for gas exchange whilst filtering microorganisms in the air.
   2.4. The culture was incubated at room temperature for three days.
   2.5. During the first 6-8 hours, the flask was gently swirled every 1-2 hours to improve oxygen availability.
   2.6. After incubation, a 1:20 dilution was prepared by mixing 1 mL of the turbid yeast culture with 19 mL of sterile broth to standardize cell concentration.
3. **Curcumin Preparation**
   3.1. A 10% (w/v) curcumin stock solution was prepared by dissolving 10 g of curcumin powder in 100 mL of 50% ethanol.
   3.2. The solution was vortexed thoroughly to ensure full dissolution.
   3.3. The solution was stored in amber bottles at room temperature, protected from light.
   3.4. Working concentrations of curcumin (0%, 0.25%, 0.5%, 1% and 2%) were prepared by adding the respective volumes of stock solution per 10 mL sample, as shown below:

Final Curcumin Concentration	Volume of 10% Stock (per 10 mL sample)
0.25%	0.25mL
0.5%	0.5mL
1%	1mL
2%	2mL

4. **Oxidative Stress Induction**
   4.1. A working solution of ferrous sulfate was prepared by []
   4.2. The solution was stored []
5. **Experimental Setup**

1.1. The experiment consisted of two main conditions: samples exposed to iron-overload conditions (+$FeSO_4$) and samples grown under normal conditions (no $FeSO_4$). Each condition was tested with five curcumin concentrations (0%, 0.25%, 0.5%, 1%, and 2%).

1.2. Samples were prepared in triplicate for a total of 30 samples.

Group	Iron Condition	Curcumin Concentration
A1-A5	No $FeSO_4$	0%, 0.25%, 0.5%, 1%, 2%
B1-B5	+____$FeSO_4$	0%, 0.25%, 0.5%, 1%, 2%

6. Sample Preparation

6.2 Each 10 mL sample was prepared as follows:

6.2.1 1 mL of diluted yeast culture was added.

6.2.2 The appropriate volume of curcumin stock solution (see Table above) was added.

6.2.3 100 µL of either sterile distilled water (for Group A) or __$FeSO_4$ solution (for Group B) was added.

6.2.4 YEPD broth was added to reach a final volume of 10 mL.

7. Incubation of Culture

7.2 All samples were incubated at 30 ˚C for 24 hours.

7.3 Lids were loosened or replaced with breathable seals to allow gas exchange.

8. ROS Assessment – DCFDA Staining and measurement – ROS assay

8.1. following incubation, 1mL of each culture was aseptically transferred into a sterile 1.5mL microcentrifuge tube

8.2. samples were centrifuged at 3000-5000 × g for 5 mins to pellet yeast cells

8.2. cells were washed 1 or 2 times with phosphate-buffered saline (PBS) – washing: cell suspension spun in centrifuge, old liquid removed, fresh PBS added to resuspend cells

8.2. A fresh working solution of DCFDA was prepared by diluting stock solution (10microM) in PBS to a final working concentration of 5 microM – diluted by DMSO

8.3. washed cell pellets were resuspended in 1.0mL of DCFDA working solution

8.4. incubate cells with the diluted DCFDA solution for 45 mins at 37 degrees in dark

8.5. wash cells 1 or 2 times with 1 times PBS, then resuspended in 1mL PBS for measurement

8.6. Perform live cell microscopy with fluorescence microscopy or plate reader

9. Viability Assessment – Colony Counting

<u>Serial Dilutions</u>

1. Label three sterile microtubes: Tube 1 (10^{-1}), Tube 2 (10^{-2}), Tube 3 (10^{-3}).
2. Add 900 µL of sterile water to each tube.
3. Take 100 µL from each yeast sample and add to Tube 1. Mix by pipetting.
4. Transfer 100 µL from Tube 1 to Tube 2. Mix well.
5. Transfer 100 µL from Tube 2 to Tube 3. Mix well.

<u>Plating and Incubation</u>

- Plate 100 µL of the most appropriate dilution (aiming for 30-300 colonies) onto a YEPD agar plate.
- Spread evenly using a sterile glass spreader.
- Incubate plates at 30˚C for 24-28 hours, inverted to avoid condensation.

CFU Calculation

- Count distinct colonies and calculate CFU/mL using the formula:

$$\frac{CFU}{mL} = \frac{Colonies\ Counted\ \times Dilution\ Factor}{0.1}$$

(0.1 mL = volume plated)

analysis: visually score cells for brightness and compare between control and samples or use image analysis methods to compare signal between digital photographs of cells

how to cellular assay:

file:///C:/Users/BWu.67041/Downloads/DCFDA-H2DCFDA-Cellular-ROS-assay-protocol-book-v2a-ab113851%20(website)_2.pdf

buffer, dye for intracellular ROS level

https://www.sciencedirect.com/science/article/pii/S2213231717301775

washing cells

https://www.researchgate.net/post/How-to-wash-cell-pellets-with-1XPBS-without-disrupting-cell

specific:

https://www.sciencedirect.com/science/article/pii/S2213231717301775

1/05/2026

Draft 2:

10. Preparation of Media

1.3 YEPD broth and agar plates were prepared by laboratory staff following a standard protocol (see instructions in Appendix).

1.4 All materials were autoclaved to ensure sterility, and aseptic techniques were followed throughout the procedure

11. Inoculation of Yeast Culture

2.7. A sterile inoculation loop was used to collect a visible quantity of *Saccharomyces cerevisiae* from a live slope tube.

2.8. The yeast was transferred into 40 mL of sterile YEPD broth in a 250 mL Erlenmeyer flask and briefly vortexed to disperse the cells evenly.

2.9. A cotton ball was used to cover the neck to allow for gas exchange whilst filtering microorganisms in the air.

2.10. The culture was incubated at room temperature for three days.

2.11. During the first 6-8 hours, the flask was gently swirled every 1-2 hours to improve oxygen availability.

2.12. After incubation, a 1:20 dilution was prepared by mixing 1 mL of the turbid yeast culture with 19 mL of sterile broth to standardize cell concentration.

12. Curcumin Preparation

3.1. A 10% (w/v) curcumin stock solution was prepared by dissolving 10 g of curcumin powder in 100 mL of 50% ethanol.

3.2. The solution was vortexed thoroughly to ensure full dissolution.

3.3. The solution was stored in amber bottles at room temperature, protected from light.

3.4. Working concentrations of curcumin (0%, 0.5%, 1% and 2%) were prepared by adding the respective volumes of stock solution per 10 mL sample, as shown below:

Final Curcumin Concentration	Volume of 10% Stock (per 10 mL sample)
0.5%	0.5mL
1%	1mL
2%	2mL

13. Iron Overload Conditions

4.1. A working solution of iron(II) sulfate heptahydrate was prepared by mixing 0.278g of $FeSO_4.7H_2O$ with 5mL of sterile distilled water (Appendix__) (This dilution allowed the addition of 100microL of solution to each sample to achieve the final concentration of 2mM $FeSO_4$)

4.2. The solution was stored in an amber bottle wrapped in foil.

14. Experimental Setup

1.3. The experiment consisted of two main conditions: samples exposed to iron-overload conditions (+$FeSO_4.7H_2O$) and samples grown under normal conditions (no $FeSO_4.7H_2O$). Each condition was tested with five curcumin concentrations (0%, 0.5%, 1%, and 2%).

1.4. Samples were prepared in triplicate for a total of 30 samples.

Group	Iron Condition	Curcumin Concentration
A1-A4	No $FeSO_4.7H_2O$	0%, 0.5%, 1%, 2%
B1-B4	+2mM $FeSO_4.7H_2O$	0%, 0.5%, 1%, 2%

15. Sample Preparation

15.2 Each 10 mL sample was prepared as follows:

15.2.1 1 mL of diluted yeast culture was added.

15.2.2 The appropriate volume of curcumin stock solution (see Table above) was added.

15.2.3 100 µL of either sterile distilled water (for Group A) or $FeSO_4.7H_2O$ solution (for Group B) was added.

15.2.4 YEPD broth was added to reach a final volume of 10 mL.

Groups		Curcumin (mL)	Yeast Culture (mL)	$FeSO_4.7H_2O$ /Water (mL)	YPD Broth (mL)
A1	B1	0.00	1.0	0.1	8.90
A2	B2	0.50	1.0	0.1	8.40
A3	B3	1.00	1.0	0.1	7.90
A4	B4	2.00	1.0	0.1	6.90

16. Incubation of Culture

16.2 All samples were incubated at 30˚C for 24 hours.

16.3 Lids were loosened or replaced with breathable seals to allow gas exchange.

17. ROS Assessment – DCFDA Staining and measurement – ROS assay

8.1. following incubation, 1mL of each culture was aseptically transferred into a sterile 1.5mL microcentrifuge tube

8.2. samples were centrifuged at 3000-5000 × g for 5 mins to pellet yeast cells

8.2. cells were washed 1 or 2 times with phosphate-buffered saline (PBS) – washing: cell suspension spun in centrifuge, old liquid removed, fresh PBS added to resuspend cells

8.2. A fresh working solution of DCFDA was prepared by diluting stock solution (10microM) in PBS to a final working concentration of 5 microM – diluted by DMSO

8.3. washed cell pellets were resuspended in 1.0mL of DCFDA working solution

8.4. incubate cells with the diluted DCFDA solution for 45 mins at 37 degrees in dark

8.5. wash cells 1 or 2 times with 1 times PBS, then resuspended in 1mL PBS for measurement

8.6. Perform live cell microscopy with fluorescence microscopy or plate reader

18. Viability Assessment – Colony Counting

Serial Dilutions

6. Label three sterile microtubes: Tube 1 (10^{-1}), Tube 2 (10^{-2}), Tube 3 (10^{-3}).
7. Add 900 µL of sterile water to each tube.
8. Take 100 µL from each yeast sample and add to Tube 1. Mix by pipetting.
9. Transfer 100 µL from Tube 1 to Tube 2. Mix well.
10. Transfer 100 µL from Tube 2 to Tube 3. Mix well.

Plating and Incubation

- Plate 100 µL of the most appropriate dilution (aiming for 30-300 colonies) onto a YEPD agar plate.
- Spread evenly using a sterile glass spreader.

- Incubate plates at 30˚C for 24-28 hours, inverted to avoid condensation.

CFU Calculation

- Count distinct colonies and calculate CFU/mL using the formula:

$$\frac{CFU}{mL} = \frac{Colonies\ Counted\ \times Dilution\ Factor}{0.1}$$

(0.2 mL = volume plated)

https://www.sciencedirect.com/science/article/pii/S0944501324001058

*$FeSO_4$ 2mM calculation

$$C_1 \cdot V_1 = C_2 \cdot V_2$$

Where C1=stock concentration, V1=0.1mL added, C2=2mM (desired final concentration), V2=10mL (final sample volume)

$$C_1 \cdot (0.1) = 2(10)$$

$$0.1C_1 = 20$$

$$C_1 = 200\ mM$$

So stock solution must be 200mM $FeSO_4.7H_2O$

Need 5mL:

$$m = C \times V \times M_r$$

M=mass in grams, C=concentration in mol/L, V=volume in L, Mr=molar mass

$FeSO_4.7H_2O$ molar mass=278.01g/mol

200mM=0.200mol/L

5mL=0.005L

$$m = 0.200 \times 0.005 \times 278.01$$

$$m = 0.27801$$

Therefore, I need 0.278g $FeSO_4.7H_2O$ dissolved in 5mL of distilled water – forms stock solution of 200mM

Pipette 0.1mL out of 200mM for final concentration of 2mM $FeSO_4$

5/05/2026 (Term 2 Wk 2)

I had talked to the lab techs and realized that fluorometric assays would be unfeasible as our school didn't have a centrifuge, fluorescence microscopy or plate reader. I considered whether I wanted to email local universities to ask if I could borrow their facilities to conduct my fluorometric assays. However, I realized that it was too short notice and it would be unlikely for them to respond before Week 7 (when I planned to do my experiment). Therefore, I had to make adjustments to the method again:

19. Preparation of Media

1.1. YEPD broth and agar plates were prepared by laboratory staff following a standard protocol (see instructions in Appendix).

1.2. All materials were autoclaved to ensure sterility, and aseptic techniques were followed throughout the procedure

20. Inoculation of Yeast Culture

2.13. A sterile inoculation loop was used to collect a visible quantity of *Saccharomyces cerevisiae* from a live slope tube.

2.14. The yeast was transferred into 40 mL of sterile YEPD broth in a 250 mL Erlenmeyer flask and briefly vortexed to disperse the cells evenly.

2.15. A cotton ball was used to cover the neck to allow for gas exchange whilst filtering microorganisms in the air.

2.16. The culture was incubated at 30 degrees for 24 hours.

2.17. After incubation, a 1:20 dilution was prepared by mixing 1 mL of the turbid yeast culture with 19 mL of sterile broth to standardize cell concentration.

21. Curcumin Preparation

3.1. A 10% (w/v) curcumin stock solution was prepared by dissolving 5 g of curcumin powder in 50 mL of 100% ethanol in a 100mL beaker.

3.2. The 100mL beaker was placed on a hotplate within a 250mL beaker half-filled with water to act as a water bath. The hotplate was turned to __ degrees.

3.3. The solution was stirred thoroughly to ensure full dissolution.

3.4. The solution was stored in amber bottles at room temperature, protected from light.

3.5. Working concentrations of curcumin (0%, 0.5%, 1% and 2%) were prepared by adding the respective volumes of stock solution per 10 mL sample, as shown below:

Final Curcumin Concentration	Volume of 10% Stock (per 10 mL sample)
0.5%	0.5mL
1%	1mL
2%	2mL

22. Iron Overload Conditions

4.1. A working solution of iron(II) sulfate heptahydrate was prepared by mixing 0.278g of $FeSO_4.7H_2O$ with 5mL of sterile distilled water (Appendix__) (This dilution allowed the addition of 100microL of solution to each sample to achieve the final concentration of 2mM $FeSO_4$)

4.2. The solution was stored in an amber bottle wrapped in foil.

23. Experimental Setup

1.5. The experiment consisted of two main conditions: samples exposed to iron-overload conditions (+$FeSO_4.7H_2O$) and samples grown under normal conditions (no $FeSO_4.7H_2O$). Each condition was tested with five curcumin concentrations (0%, 0.5%, 1%, and 2%).

1.6. Samples were prepared in triplicate for a total of 24 samples.

Group	Iron Condition	Curcumin Concentration
A1-A4	No $FeSO_4.7H_2O$	0%, 0.5%, 1%, 2%
B1-B4	+2mM $FeSO_4.7H_2O$	0%, 0.5%, 1%, 2%

24. Sample Preparation

24.2 Each 10 mL sample was prepared in a 25mL flask as follows:

24.2.1 1 mL of diluted yeast culture was added.

24.2.2 The appropriate volume of curcumin stock solution (see Table above) was added.

24.2.3 100 µL of either sterile distilled water (for Group A) or $FeSO_4.7H_2O$ solution (for Group B) was added.

24.2.4 YEPD broth was added to reach a final volume of 10 mL.

Groups		Curcumin (mL)	Yeast Culture (mL)	$FeSO_4.7H_2O$ /Water (mL)	YPD Broth (mL)
A1	B1	0.00	1.0	0.1	8.90
A2	B2	0.50	1.0	0.1	8.40
A3	B3	1.00	1.0	0.1	7.90
A4	B4	2.00	1.0	0.1	6.90

25. Incubation of Culture

25.2 All samples were incubated at 30˚ C for 24 hours.

25.3 Foam stoppers for gas excchange

26. Viability Assessment – Colony Counting

Serial Dilutions

11. Label three sterile microtubes: Tube 1 (10^{-1}), Tube 2 (10^{-2}), Tube 3 (10^{-3}).
12. Add 900 µL of sterile water to each tube.
13. Take 100 µL from each yeast sample and add to Tube 1. Mix by pipetting.
14. Transfer 100 µL from Tube 1 to Tube 2. Mix well.
15. Transfer 100 µL from Tube 2 to Tube 3. Mix well.

Plating and Incubation

- Plate 100 µL of the third dilution onto a YEPD agar plate (trial runs shows that 10^{-3} was most suitable although some plates had lawn and others had no growth).
- Spread evenly using a sterile glass spreader.
- Incubate plates at 30 ˚C for 24 hours, inverted to avoid condensation.

CFU Calculation

- Count distinct colonies and calculate CFU/mL using the formula:

$$\frac{CFU}{mL} = \frac{Colonies\ Counted\ \times Dilution\ Factor}{0.1}$$

(0.3 mL = volume plated)

27. Colony area calculation

9.1. Images of agar plates were uploaded unto ImageJ and annalysed to determine total area of growth.

Apparatus

Material	Amount Required	Notes
Yeast (Saccharomyces cerevisiae BY4741)	24mL	1mL per sample for 24 samples
YPD Broth	340mL	240mL for 10mL per 24 samples + ~100mL for buffer
YPD Agar Plates	~30 plates	Colony count for 10 samples × 2 dilutions (experiment whether 10^{-2} or 10^{-3} work)
Distilled Water	10mL	For iron treatment dilution
Curcumin Powder	5g	For stock (10% in 50mL ethanol) to prepare 100mL stock
Ethanol (100%)	50mL	50mL dissolved with 5g curcumin for 1 mM stock.
Iron(II) Sulfate Heptahydrate	0.5g	0.278g mixed with 5mL distilled water for 2mM concentration
Sterile Test Tubes	72 tubes	72 (3×24 tubes for serial dilution)
Pipette (1-10mL)	1	For minimal contamination, I'd need around 150-200 pipette tips but since that's unrealistic, I would need to know how many tips I can use.
Micropipette (100-1000 uL)	1	
Sterile Inoculation Loop	1	For transferring yeast culture into YEPD broth
Conical Flask (100mL, 25mL)	24×25mL, 2×100mL	For initial yeast culture growth, yeast culture dilution, and yeast samples.
Beakers (150mL, 250mL)	1 of each	For curcumin stock solution preparation.
Measuring Cylinder (5mL, 10mL, 50mL)	1	For general use
Glass stirring rods	1-2	
Incubator (30 ˚C)	1	For culture growth and colony incubation
Amber Bottles	2×25mL 1×10mL	Storing curcumin stock and iron treatment
Parafilm/Tube Caps	1 roll of parafilm/30 caps	For sealing culture tubes

Sterile Glass Spreader	1	For plating
Marker	1	For labelling tubes and plates
Tube Rack	1-2	For holding serial dilution tubes securely.
Foam Stopper	25	Sealing test tubes and flask containing yeast culture
Matches	1 box	For lighting Bunsen burner
Bunsen Burner	1	Sterilizing YPD broth

After reflecting on last year's method, I came up with a few adjustments:

Instead of 50% ethanol to dissolve curcumin, I decided to heat 100% ethanol up in a water bath and dissolve curcumin that way. This was because my curcumin did not dissolve fully last time, and majority of it was left as a solid at the bottom of the flask. As I didn't want that to happen again, I decided to try 100% ethanol. I considered whether 100% ethanol would negatively affect the yeast, but eventually determined that the concentrations I would use would be too minimal to damage yeast.

As I couldn't conduct fluorometric assays, I decided I had to conduct colony counting.

Instead of incubating the initial yeast culture over 3 days, I decided to decrease the timeframe to 24 hours:

https://pmc.ncbi.nlm.nih.gov/articles/PMC8391128/

3/06/2026 (Term 2 Wk 7)

I started my experiment today. I inoculated the yeast in YPD broth and left it in the incubator at 30 degrees for overnight. There were a lot of things I had forgotten about aseptic technique, which I had to relearn – such as keeping a Bunsen burner on, flaming the neck of the flask I was inoculating the yeast in, and wiping the bench down with ethanol before beginning.

I prepared my curcumin stock solution – heating the ethanol I was dissolving the curcumin in definitely helped dissolve the curcumin. In fact, most of the curcumin had dissolved.

I also made the iron stock solution – I had to double all the quantities I was using to make measuring them out easier.

4/06/2026 (Term 2 Wk 7)

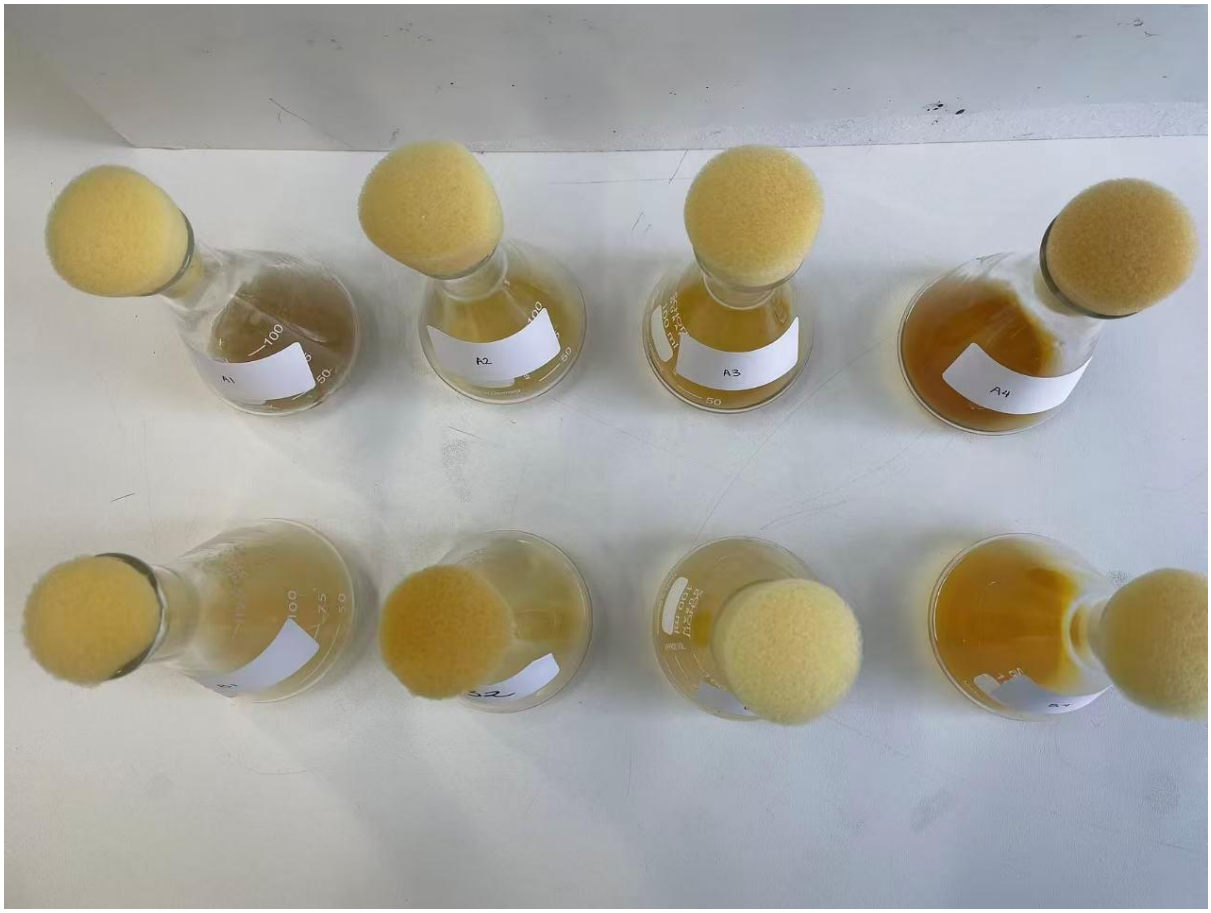
There was growth! The culture looked slightly cloudy which I assumed was yeast growth.

I prepared all my samples according to the table:

Groups		Curcumin (mL)	Yeast Culture (mL)	$FeSO_4.7H_2O$ /Water (mL)	YPD Broth (mL)
A1	B1	0.00	1.0	0.1	8.90
A2	B2	0.50	1.0	0.1	8.40

A3	B3	1.00	1.0	0.1	7.90
A4	B4	2.00	1.0	0.1	6.90

The micropipettes were fun to use. I placed all flasks in the incubator at 30 degrees. The solutions were of different colours (which I took as a good sign) from different curcumin amounts:



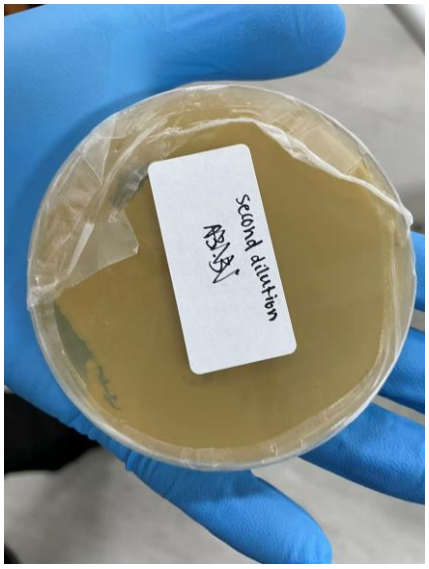
5/06/2026 (Term 2 Wk 7)

As it will the weekend tomorrow, I took all flasks out to place in the fridge to slow yeast growth. I only wanted them to grow for 24 hours on the agar plates so I decided to plate them on Monday instead.

8-9/06/2026

I was running out of time, so I first made a trial with 3 different dilutions to see which serial dilution I would do.

Results for the control (A1 T1):



While the third dilution still had some lawn, it was the best out of all of them, so I decided to dilute all to 1:1000.

10-11/06/2026

I got my final results. Unfortunately, a lot A1 and B1 trials had a lawn, while the A3, B3, A4, and B4 did not have any growth. I didn't have the time or agar plates left to redo my dilutions. Therefore, I decided to add a second dependent variable – area of colony. This was because my CFU data would definitely be extremely messy, particularly for the lawns, so this way, I could at least get quantitative data out of the lawned plates.

I found a software called ImageJ that could help me calculate area.

I was really disappointed afterwards as I had hoped to gain clean CFU data.

13-21/06/2026

Write-up

25-28/06/2026

I did some final edits and finished.

OSA RISK ASSESSMENT FORM

for all entries in (✓) ☐ Models & Inventions and ☐ Scientific Inquiry

This must be included with your report, log book or entry. One form per entry.

STUDENT(S) NAME: Benita Wu ID:

SCHOOL: Seymour College

Activity: Give a brief outline of what you are planning to do.

Investigating the effect of curcumin concentration on yeast cells with/without added iron. 0%, 0.5%, 1%, and 2% curcumin concentrations will be investigated, along with treatment groups with and without iron, before being plated on agar plates. Colony Forming Units will be calculated

Are there possible risks? Consider the following:

- Chemical risks: Are you using chemicals? If so, check with your teacher that any chemicals to be used are on the approved list for schools. Check the safety requirements for their use, such as eye protection and eyewash facilities, availability of running water, use of gloves, a well-ventilated area or fume cupboard.
- Thermal risks: Are you heating things? Could you be burnt?
- Biological risks: Are you working with micro-organisms such as mould and bacteria?
- Sharps risks: Are you cutting things, and is there a risk of injury from sharp objects?
- Electrical risks: Are you using mains (240 volt) electricity? How will you make sure that this is safe? Could you use a battery instead?
- Radiation risks: Does your entry use potentially harmful radiation such as UV or lasers?
- Other hazards.

Also, if you are using other people as subjects in an investigation you must get them to sign a note consenting to be part of your experiment.

Risks	How I will control/manage the risk
Cross contamination/exposure to Saccharomyces cerevisiae Use of bunsen burner - fire hazard Use of iron hydroxide and 100% ethanol	Aseptic technique used throughout; equipment sterilized, and workspace will be sanitized before and after usage. Cell cultures disposed of according to school biohazard policies. Bunsen burner will be left in safety flame and never left unattended. Gloves, goggles, and necessary lab protection will be worn

(Attach another sheet if needed.)

Risk Assessment indicates that this activity can be safely carried out

RISK ASSESSMENT COMPLETED BY (student name(s)): Benita Wu

SIGNATURE(S):

☑ By ticking this box, I/we state that my/our project adheres to the listed criteria for this Category.

TEACHER'S NAME: Swati Salvi

SIGNATURE: DATE: 1/06/2026