How does an increase in the exposure of UV A-light at 390nm from 0 mins to 35 mins affect the viability of 0.025g *Saccharomyces cerevisiae* in 200 cm³ sterilized water as measured by viable cell count in percentage using Trypan Blue on a hemocytometer?

Personal code: 

Date of Submission: 22 May 2017

Session: November 2017

Subject: Biology

Word Count: 3984
Abstract

The number of people diagnosed with cancer is increasing and I was curious to explore carcinogens and its effect on cells. With sun bathing becoming more popular in my hometown and having learned that UV radiation is present in sunlight, I wanted to explore the effect of exposure to UV light on eukaryotic cells. As a simple eukaryote, yeast cells are used to model basic actions of a human cell, forming the research question, ‘How does an increase in the exposure of UV A-light at 390nm from 0 minutes to 35 minutes affect the viability of 0.025g *Saccharomyces cerevisiae* in 200 cm$^3$ sterilized water as measured by viable cell count in percentage using Trypan Blue on a hemocytometer?’

To conduct this experiment, 0.025g *S.cerevisiae* was dissolved in 200cm$^3$ sterilized water at room temperature along with 0.020g disaccharide sugar (dextrose). 1000µL solution was transferred to 12 Petri dishes which were exposed to 3 UV-A lamps of 390nm wavelength fixed in a box. Aluminium foil was used to cover the dishes after exposing it to UV for a set amount of time in mins: 0, 5, 10, 15, 20, 25, 30 and 35. After 35 minutes, aliquots of 200µL was mixed with 300µL of Trypan Blue and then loaded onto a hemocytometer to calculate the percentage viability of the yeast cells by counting the number of viable and non-viable cells as indicated by the Trypan Blue.

The results implied that as the UV exposure time increased, the viability of *S.cerevisiae* decreased with statistically significant data. This may imply the damage caused by UV-A light on eukaryotic DNA of eukaryotes as well as the repair mechanisms such as the nucleotide excision repair a cell may undergo. These results help to predict the effect of UV in sunlight on dermatological cells.

Word Count: 298
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Introduction

Context

According to the National Cancer Institute, US, the number of people diagnosed with cancer is increasing with the number predicted to rise from 14.5 million in 2014 to 19 million in 2019 in the US (National Cancer Institute, n.d.). Research by organisations such as the Cancer Research UK have labelled UV light as a major cause of skin cancer with studies such as one published in 2011 that discovered that 86% of the malignant skin cancers in the UK was caused due to long exposures to sunlight consisting of UV radiation (Cancer Research UK, 2015). With sunbathing being popular within my culture, I wanted to explore the effect of UV on eukaryotic cells and I hope that this will help future researchers to understand more on the causes of skin cancers.

UV light can be of three types: UV-A, UV-B and UV-C. Although UV-C, having the shortest UV wavelength of 100nm to 290nm is the most harmful type of UV as it possesses the greatest amount of energy (which can be absorbed by the DNA), it is almost completely absorbed by the Earth’s Ozone layer such that it does not reach the Earth’s surface (UV Radiation, 2010). UV B on the other hand, with 290nm to 320nm wavelength is also mostly absorbed by the Ozone layer but some that reaches the Earth’s surface are significantly known to promote the development of skin cancer (UV Radiation, 2010). However, 95% of the sunlight that reaches the Earth is UV-A light (World Health Organization, 2017) with the longest UV wavelength, 320nm to 400nm such that it can penetrate through living cells more deeply than UV-B and UV-C (UV Radiation, 2010). Although less harmful due to the lesser energy possessed by UV-A than UV-B and UV-C, recent studies conducted
by organisations such as the skin cancer foundation have shown that it can initiate skin cancer. (Skincancer.org, 2013). Thus, in order to explore the effect of UV on cells as close to real life situation, UV-A light was used as the independent variable in this experiment.

According to recent studies, it is also known that 20% of the human genes in diseased cells such as cancerous cells are known to have counterparts in yeasts cells (Yourgenome.org, 2017) and as a eukaryotic cell, the yeast cells are used in this research with the assumption that yeast cells can model basic behaviour of human cells to some extent.

With these, the research question, ‘**How does an increase in the exposure of UV A-light at 390nm from 0 mins to 35 mins affect the viability of 0.025g Saccharomyces cerevisiae in 200 cm$^3$ sterilized water as measured by viable cell count in percentage using Trypan Blue on a hemocytometer?**’ was developed. This is significant as the results of this investigation may help to understand the possible effects of UV light from the sun at a DNA level as well as the extent to which UV light may not be dangerous for eukaryotic cells including human cells. This may further help researchers aiming to prevent skin cancer.

**Background Information**

**The Yeast Cell Cycle**

The yeast cells reproduce via asexual or sexual reproduction depending on environmental factors such as the availability of nutrients although asexual reproduction is the most common in yeasts (Micropop.cbs.umn.edu, 2012).

As a eukaryote, *S.cerevisiae* undergoes similar mitotic cell cycle as that of a human cell with 4 distinct stages in the cell cycle: G1 phase, S phase, G2 Phase and Mitotic phase as shown in figure 1 (Cooper, 2000).
The yeast cell commits to the cell cycle at the ‘Start’ point at the end of G\textsubscript{1} phase where the cell monitors its environment. If the yeast cell does not have the right environment, such as the availability of nutrients, the cells enter the G\textsubscript{0} phase. Once the cell commits to the cell cycle, buds start to grow at the S phase after the replication of DNA. The buds then grows throughout the cell cycle until cytokinesis after Mitosis. At cytokinesis, the yeast undergoes unequal division of the cells leading to asymmetrical budding of the yeast cell (Cooper, 2000).

If the yeast cells do not meet all the conditions necessary at the G\textsubscript{1} phase, another possibility is that the cells can undergo meiosis and reproduce through sexual reproduction. Thus, the haploid cells formed due to meiosis are smaller in size (Cooper, 2000). This knowledge may help with the analysis as to understand whether UV light may hinder yeast cells from mitosis and committing to the cell cycle.
Effect of UV light on DNA

UV radiation can cause mutations in genes as they are the most sensitive to UV radiation and absorb energy. The energy absorbed has the potential to alter the bonds in the DNA, altering their structures.

In the case of ‘Direct DNA damage’, the UV light is absorbed by the purines and pyrimidine bases in a DNA (Guengerich, 2014) and is then released (Nature.com, 2015). If the energy takes long before getting released, it results in the formation of pyrimidine dimers by two adjacent pyrimidine in the DNA covalently bonding together (Guengerich, 2014). Thus, the double helix structure is disrupted with the formation of DNA adducts (Guengerich, 2014), that leads to an inability for DNA helicase to unwind and break the double helix in the S-phase such that the cell gets arrested in the G₀ phase (Guengerich, 2014).

Repair Mechanisms

However, when a DNA is damaged, it undergoes repair mechanism such as the nucleotide exclusion repair (Sigma-Aldrich, n.d.). Through this, enzymes activate repair proteins at the site of DNA adducts which then removes the adduct and replaces it with the correct base pairs (Nature.com, 2015). At times when the cell is unable to undergo repair mechanisms, they undergo processes such as Apoptosis. (Sciencing, 2017)

Apoptosis

Apoptosis refers to the process through which eukaryotic cells such as yeast undergoes a ‘programmed cell death’ when their DNA is irreparably damaged due to lethal environmental conditions such as a long exposure of UV radiation. The cells break down into smaller membrane packets that used to be contained in the cell such that it makes space for other cells to live. (Sciencing, 2017)
Literature Review Summary

Previous studies on this topic have shown that exposure to UV light decreases the viability of UV sensitive yeast cells in particular. A study done by Chen et Al in 2011 (Chen et Al., 2011) showed that irradiation of UV on *S.cerevisiae* gave a negative trend for the population density with an increase in UV exposure time. Yet, their results were not statistically significant and failed to reject their null hypothesis. However, Russell in 2006 aimed to test the extent of damage created in DNA using UV but with different forms of protection. Her results supported her hypothesis as cells were affected by UV more without protection which was also statistically significant. Russell’s study being conducted in 2006 used YED Agar plates while Chen et Al prepared yeast by diluting yeast solution in a standard medium. Yet, Chen et Al’s methodology was mainly chosen as a reference as it focused on my research question more than Russell’s study, although it was modified using other sources during the preliminary trial as seen in the next section. In addition, all the sources used to form the protocol were within the last 10 years in order to meet the demands of improvement in technology and equipment.

Preliminary Experiment and Scope of Final Investigation:

The preliminary experiment conducted narrowed down the research question by modifying the methodology gained from the literature review. Through this trial, the most desirable concentration of the yeast solution was found to be 0.0125% (0.025g yeast in 200 cm$^3$ water) with 0.020g disaccharide sugar as it resulted in a countable number of cells in the microscope at the end of the experiment after 40 minutes (assuming that the yeast cells in the samples are counted within 5 minutes after the 35 minutes experiment). Failure in being able to count the viable cells using methylene blue as indicated by Chen et Al’s protocol (Chen et al., 2011), led to the
usage of Trypan Blue (Maria Fuentes, 2017) which gave a visible difference in the
colour of the viable and non-viable cells under the microscope. The most desirable
ratio of the Trypan Blue with the yeast solution was finalised as 3:2 although
secondary sources (Maria Fuentes, 2017) provided me with the ratio as 1:1. Different
ratios were tried out and 3:2 ratio worked best as it consumed less quantity of
Trypan Blue but also was enough to dye the non-viable cells blue. Moreover, in order
to control the amount of UV light, the protocols from MIT (The Effect of Ultra Violet
Radiation on *Saccharomyces cerevisiae*, 2017) uses aluminium foil to block the UV-
A light. This was tried in the preliminary trial and it was found to be the most suitable
protocol as it blocked the UV and also allowed the controlling of time the yeast
samples were left before the cell count. The trial also allowed the formation of a UV-
light box made of cardboard which reduced the exposure of UV radiation to people
present in the lab (See Appendix I). It was also found through this trial that having
three UV lamps of 390 nm wavelengths gave a clear difference in results between
each levels of the independent variable.

**Hypothesis**

With the literature reviewed and the background information researched, the
hypothesis is that an increase in the exposure time of UV A light will decrease the
yeast cell viability as measured by the counting of viable cells using a
hemocytometer. With an increase in UV-A radiation, the DNA double helix structure
would be disrupted to greater extents such that lesser repair mechanism takes place
that may cause apoptosis.
Investigation

Experimental Design

Independent Variable

The exposure time in minutes of UV light with 390nm wavelength from 0 minutes to 35 minutes at 5 minute intervals was the independent variable. The preliminary experiment conducted verified the suitability of this range and interval with a desirable result. Although the aim of this study is to test the effect of UV-A radiation of 390 nm on the yeast cells in order to predict the effect of UV light from sun on the development of tumours in human beings, 35 minutes is a short time span. Yet, this range was chosen as it was not possible to do longer periods of exposure time in a school environment due to the possible health risks associated with UV.

Dependent Variable

The yeast viability in percentage, calculated by counting the number of viable cells in a hemocytometer was the dependent variable. According to secondary sources such as by the Fuentes, a PhD who is well experienced using hemocytometers (Maria Fuentes, 2017) and as experienced through preliminary trials, non-viable cells appear blue when dyed with Trypan Blue whereas viable cells appear white in the dye.
To calculate the viability, the viable cells that appear white when stained with Trypan Blue and non-viable cells that appear blue when stained with Trypan Blue within 4 corner chambers in a hemocytometer (See Figure 2) under a microscope were counted and averaged by dividing by 4 and then the percentage viability of yeast cells in the aliquot in the 100 µL chamber in the hemocytometer was calculated using equation 1:

\[
Cell \text{ Viability} = \frac{\text{No. of Viable Cells}}{\text{No. of Viable Cells} + \text{No. of Non Viable Cells}} \times 100
\]

Equation 1: to calculate cell Viability (Phe-culturecollections.org.uk, n.d.)

Controlled Variables:

Temperature of the solution and the room:

Effect: Surrounding temperature differences can change the rate of metabolic reactions in the yeast as it influences enzyme activity, leading to a difference in budding rates such that apoptosis or repair mechanisms may not be consistent.

Control: The air conditioner was set at 25°C throughout the experiment and the temperature of the sterilized water was measured with a thermometer to ensure that the water is at 25°C when dry yeast was added.
Initial concentration of yeast cells:

Effect: Different initial concentrations of yeasts will give different final concentrations

Control: Different initial concentrations of yeasts will give different final concentrations

Wavelength of UV

Effect: Different wavelengths of UV light provide different exposure of radiation such that the effect of manipulating time would be affected.

Control: 3 of the same UV lamps of the same wavelength (390nm) were used throughout the experiment with all samples such that wavelength was kept constant

Ratio of Trypan Blue and Yeast solution:

Effect: Different ratio would mean different concentrations/count of cells which would vary the cell count in the hemocytometer

Control: The Trypan Blue was used in a 3:2 ratio with 300μL Trypan blue added to an Eppendorf tube to which 200μL yeast aliquot was added after irradiation

Availability of nutrients

Effect: Different availability of nutrients would mean different rates of fermentation such that the cell count would be different.

Control: 0.020g disaccharide was added to 0.025g yeast in each sample to ensure each culture had equal amounts of disaccharides.
Procedure:

As mentioned in the literature review and the preliminary experiments section, the methodology used by Chen et al. was considered and was then modified to fit the context and to obtain more reliable results. The following method was followed for the final experiment.

**Aseptic Technique:** A 500 cm$^3$ culture flask was autoclaved and 200 cm$^3$ water was sterilized for 40 minutes. The working station and all the apparatus (See appendix II) was sterilized by spraying 70% ethanol and then being wiped by a clean towel and heated using a bunsen burner as part of the aseptic techniques (Teach.genetics.utah.edu, 2015) to avoid contamination by other microorganisms. Gloves were also worn throughout the experiment and all the containers used were closed when not in use (Working with Yeast, n.d.).

Once the apparatus were sterilized, the 200 cm$^3$ sterilised water was added to the autoclaved culture flask. 0.025g *S. cerevisiae* was then weighed by firstly placing a weighing boat on a 3 decimal place top pan balance and pressing ‘tare’ to read 0.000g and then adding 0.025g yeast into the weighing boat. This was transferred to the culture flask. This step was repeated with the disaccharide (dextrose was used) to add 0.020g disaccharide to the flask. The solution was then gently swirled with the flask closed loosely with the lid until the solution turned unclear. Loosening the lid limited the entry of air-borne micro-organisms into the solution but also allowed the exchange of gases required for the respiration of the cells.

8 petri-dishes were then marked with either 0, 5, 10, 15, 20, 25, 30 or 35 indicating the UV exposure time. After swirling the flask again to suspend the cells in the solution, using a micro-pipet, 1000µl of the solution was then transferred to each of the 8 petridishes. The petri-dish marked 0 was covered with a piece of aluminium
of approximately 10cm by 10 cm and along with all the other petri-dishes with the sample, they were kept inside a UV light Box that was used as a safety precaution (See Appendix II for the set up of UV light Box). 3 UV Lamps fixed in the box were then turned on along with a stopwatch and after every 5 minutes, the petri-dishes marked with the time shown on the stopwatch (eg: 10 minutes) were covered with pieces of Aluminium foil similar to the petri-dishes marked with 0 so that all the petri-dishes remained in the UV box but was not exposed to UV light after its exposure time.

At the station, using a micropipette 300µL of the indicator, Trypan Blue was added into 8 Eppendorf tubes which also acted as a diluent so that the aliquot was diluted before observing under the microscope. Once the UV lamp was turned off after 35 minutes, the Petri-dishes were removed from the UV box and 200µL of the sample was transferred from each of the petri-dish to one of the 8 Eppendorf tubes using a micro-pipet and was shaken well.

Microscope was set up by verifying that there were no cells present on the hemocytometer. (If cells were visible before adding the sample, then spray 70% ethanol and dry the hemocytometer.) The focus of the microscope was then adjusted such that the grid lines of the hemocytometer were clearly seen (See figure 2) and the lens was magnified to x100.

100µL of the solution (since that was the minimum volume possible in the micropipet) was taken into a micropipet from the Eppendorf tube and was released gently into the gap between the coverslip and the slide in the hemocytometer until any excess aliquot reached the ‘H’ in the Hemocytometer. (See figure 3). This meant that $0.1 \times 3 \times 3 = 0.9\mu L$ was added into the hemocytometer.
The number of viable cells (white) and non-viable cells (blue) were counted using the ‘Gibco Cell Culture’ App, downloaded from the AppStore that kept the count. The cells that touched the left border and the bottom border of the square and any artifacts and moving cells were not included in the count. This procedure (from transferring of the aliquot into the hemocytometer to counting of cells) were repeated for the other 7 yeast samples in the petri-dish after cleaning the apparatus used with 70% ethanol as mentioned earlier.

The whole procedure was then repeated five times until 10 repeats were done.

**Results:**

**Qualitative Observations**

- As the yeasts were added to the sterilized water, the solution turned unclear. However, when the flask was left on the table for sometime, the yeasts settled at the bottom. This meant that the yeast cells could have been distributed unevenly within the solution.

- Through the microscope, artifacts were seen suggesting that the samples could have been slightly contaminated.

- There were cells in colonies as well as cells that were in a cluster of 2 or 3 cells.
Processed Data

The raw data obtained is presented in Appendix III and was processed (see examples in Appendix III) to obtain the following processed data.

*Table 1: A table showing the cell viability in % of 10 trials (calculated using the tables in appendix IV) and its average and standard deviation at different levels of UV light exposure at 390nm from 0 mins to 25 mins*

<table>
<thead>
<tr>
<th>Time/min</th>
<th>Cell Viability/%</th>
<th>St. Dev.</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.00</td>
<td><strong>100.0</strong></td>
<td>1.9</td>
</tr>
<tr>
<td>5.00</td>
<td>81.7</td>
<td>10.2</td>
</tr>
<tr>
<td>10.00</td>
<td>76.6</td>
<td>9.0</td>
</tr>
<tr>
<td>15.00</td>
<td>69.2</td>
<td>5.3</td>
</tr>
<tr>
<td>20.00</td>
<td><strong>80.3</strong></td>
<td>5.7</td>
</tr>
<tr>
<td>25.00</td>
<td>67.0</td>
<td>7.3</td>
</tr>
<tr>
<td>30.00</td>
<td>70.6</td>
<td>3.9</td>
</tr>
<tr>
<td>35.00</td>
<td>75.7</td>
<td>6.6</td>
</tr>
</tbody>
</table>

Note: The highlighted values are anomalies as they are far from the other values and are not included in the calculations of Average Viability. (For the calculation of these values, see Appendix IV)
Figure 4: Graph showing the relationship between the exposure time of UV light in mins (from 0 mins to 25 mins) and the cell viability in percentage measured using a hemocytometer

Trends Noted from the graph (These are explained in the Discussion):

- The longer the yeast cells are exposed to UV-A light, the lower viability of cells.
- However, it also seems that there are some non-viable yeast cells even when it has not been exposed to UV light.
- The decrease in viability slows down with greater exposure to time.
- There are anomalies in the results at 5 minutes and 30 minutes.
Statistical Test

Spearman's Correlation Rank test was used as a statistical test to check if these results were statistically significant. This test was used because the graph is not expected to be linear and is expected to be monotonic.

- H₀ (Null Hypothesis): There is no monotonic relationship between the UV light exposure time and the yeast viability.

- H₁ (Alternate Hypothesis): There is a monotonic relationship between the UV light exposure time and yeast viability.

**Table 2: Calculation of the Spearman's Correlation Rank**

<table>
<thead>
<tr>
<th>Sample number</th>
<th>UV light Exposure time (mins)</th>
<th>UV Light Exposure time Rank</th>
<th>Average Yeast Viability (%)</th>
<th>Average Yeast Viability Rank</th>
<th>Difference (d)</th>
<th>d²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.00</td>
<td>8</td>
<td>94.6</td>
<td>1</td>
<td>-7</td>
<td>49</td>
</tr>
<tr>
<td>2</td>
<td>5.00</td>
<td>7</td>
<td>75.3</td>
<td>3</td>
<td>-4</td>
<td>16</td>
</tr>
<tr>
<td>3</td>
<td>10.00</td>
<td>6</td>
<td>76.4</td>
<td>2</td>
<td>-4</td>
<td>16</td>
</tr>
<tr>
<td>4</td>
<td>15.00</td>
<td>5</td>
<td>72.7</td>
<td>4</td>
<td>-1</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>20.00</td>
<td>4</td>
<td>70.0</td>
<td>5</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>25.00</td>
<td>3</td>
<td>67.6</td>
<td>6</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>7</td>
<td>30.00</td>
<td>2</td>
<td>67.3</td>
<td>7</td>
<td>5</td>
<td>25</td>
</tr>
<tr>
<td>8</td>
<td>35.00</td>
<td>1</td>
<td>65.0</td>
<td>8</td>
<td>7</td>
<td>49</td>
</tr>
</tbody>
</table>

\[ \Sigma d^2 = 166 \]

If \( n \) is the number of samples used in this test, \( n = 8 \). Thus, \( n^3 - n = 8^3 - 8 = 504 \).
To find the Spearman's correlation rank, the following equation was used, (Spearman's Rank Correlation Coefficient-Excel guide, 2017)

\[ r = 1 - \frac{6 \sum d^2}{n^3-n} \]

\[ r = 1 - \frac{6 (166)}{504} \]

\[ = -0.976 \text{ (to 3d.p)} \]

degrees of freedom = 8 - 2 = 6

Using the table of critical values for Spearman's test from an academic website, (Media3.bournemouth.ac.uk, n.d.) my Spearman's rank value of 0.976 is greater than the critical value of 0.886 at 0.05 significance for degree of freedom of 6 such that the null hypothesis, ‘There is a monotonic relationship between the UV light exposure time and the yeast viability’ is rejected and the results of this experiment is statistically significant.

Discussion

Evaluation of Results

Figure 4 shows the effect of increasing exposure to UV-A light at 390nm on the viability of \textit{S.cerevisiae} as measured using viable cell count under a hemocytometer. The trends from the graph suggest that the longer exposure to UV-A light, the lesser viability of \textit{S.cerevisiae} cells supporting my original hypothesis of the study, ‘an increase in the exposure time of UV-A light will decrease the yeast cell viability as measured by the counting of viable cells using a hemocytometer’. As shown in figure 4, the viability of cells decreases from an average of 94.6\% at 0 seconds to 65.0\% at 35 minutes exposure to UV.
The gradient of the curve decreases as the exposure time of UV increases suggesting that some of the yeast cells may be undergoing repair mechanisms. However, the extent to which UV affects the repair mechanisms can only be confirmed the experiment having a greater range of exposure times which is therefore a limitation of the experiment.

Furthermore, the validity of this result is also limited with the high standard deviations (lowest being 1.9 and highest being 10.2) suggesting the inconsistency of the cell viability in different trials. Thus, more trials will be needed in order to identify anomalies. The anomalies in this investigation were also avoided in calculations in order to obtain reliable results.

The statistical test also provides the evidence that the results of this test are statistically significant confirming that UV-light affects the viability of the yeast cells.

However, the strengths of these results are limited as the results also suggest that there are non-viable cells even without irradiation in samples marked 0 minutes. This may suggest that there are other factors that may affect the viability and may lead to a limitation of the apparatus or the controlled variables.

**Evaluation of Procedure**

<table>
<thead>
<tr>
<th>Limitation</th>
<th>Improvements</th>
</tr>
</thead>
<tbody>
<tr>
<td>Need a greater range of the exposure time to understand the effect of long exposure of UV Light (60 mins)</td>
<td>Carry out the experiment with the range 0 seconds to 3600 secs.</td>
</tr>
<tr>
<td>Need more repeats to identify anomalies and get an accurate result</td>
<td>Carry out the experiment 20 times.</td>
</tr>
<tr>
<td>Limitation</td>
<td>Improvements</td>
</tr>
<tr>
<td>----------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Although the sterile techniques were used, the sample was still slightly</td>
<td>Wear a mask, gloves, sterile lab coat and carry out the experiment in a very</td>
</tr>
<tr>
<td>contaminated</td>
<td>sterile lab.</td>
</tr>
<tr>
<td>The yeasts may not have been at the same proportion throughout the yeast</td>
<td>Use a magnetic stirrer which will keep stirring the yeast solution</td>
</tr>
<tr>
<td>solution</td>
<td></td>
</tr>
<tr>
<td>The experiment may have been started before the cells were active</td>
<td>Use a sterilized gas tube inverted into sterilized water and look for bubbles</td>
</tr>
<tr>
<td></td>
<td>to ensure that fermentation has started before the experiment has taken place.</td>
</tr>
<tr>
<td>Some UV radiation could have penetrated through the aluminium foil</td>
<td>Use an effective UV-A light sun cream protector above the foil to ensure that</td>
</tr>
<tr>
<td></td>
<td>no UV light penetrates through the Aluminium foil that covers the petri-dish</td>
</tr>
<tr>
<td>The yeast count was not taken at the same time for the different samples.</td>
<td>Radiate lesser number of petri dish at one time so that the delay in counting</td>
</tr>
<tr>
<td>This may mean that the yeast may bud more depending on the delay in counting</td>
<td>the cells in each of the dish may be lowered.</td>
</tr>
<tr>
<td>the cells.</td>
<td>Alternatively, ask a friend to count it in another microscope so that the</td>
</tr>
<tr>
<td></td>
<td>efficiency of counting would increase</td>
</tr>
<tr>
<td>The counting of yeast cell can be subjective/easy to miscount</td>
<td>Ask a friend to recount the cells and ensure that the numbers are similar</td>
</tr>
</tbody>
</table>
Evaluation of the Sources used:

The secondary sources I have used during my research seems reliable as they were taken from published journal articles (most of them within the last 15 years), educational websites or organizations. However, as science develops rapidly, the older the secondary source, the less reliable the information is. Thus, an improvement could have been to rely on websites/books/articles published in the last 5 years.

Conclusion

In conclusion, the essay provides an answer to the research question, ‘How does an increase in the exposure of UV A-light at 390nm from 0 mins to 35 mins affect the viability of 0.025g Saccharomyces cerevisiae in 200 cm² sterilized water as measured by viable cell count in percentage using Trypan Blue on a hemocytometer?’ as the results of this investigation support the hypothesis that increase in UV-A light decreases the viability of S.cerevisiae cells despite the anomalies and the high standard deviation.

This suggests that as eukaryotic cells are exposed more to UV light, the greater irreparable damage is done to the DNAs of the eukaryotes such as yeasts as shown by the decrease in viability of yeast cells at higher UV exposure times. The large difference in the viability of cell at 0 minutes and 35 minutes may also suggest that cells can be damaged to an extent where repair mechanisms such as the nucleotide excision repair mentioned in the background knowledge cannot occur leading to apoptosis and death of cell. This may in turn be used to predict the effect of UV-A on human cells which could initiate skin cancer due to direct DNA damage. In addition, the results also conclude that small amounts of UV light could affect DNA as seen in the steep decrease in viability from 0 minutes to 5 minutes on Figure 4, although as
mentioned earlier, this may be due to other factors that were not considered. However, we should also consider that these results are only of relevant to dermatological cells and cannot be used for predictions of other cancer types or types of cells as they are not affected by exposure of UV Light from the sun.
References


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Appendices

Appendix I: Set up of a UV Box

SET UP: a UV light box with a ‘door’ attached to a string, which can be opened and closed. The box should also have 3 opening at the top such that the UV lamps will fit perfectly on the opening. This should also allow switching on and off of the lamp without having direct contact with the UV radiation as shown in figure 5 below:

*Figure 5: Set up of the UV Box*
## Appendix II : Apparatus

<table>
<thead>
<tr>
<th>Apparatus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemocytometer</td>
</tr>
<tr>
<td>Microscope with 100x lens</td>
</tr>
<tr>
<td>500 cm³ Culture Flask</td>
</tr>
<tr>
<td>200 cm³ Sterilised Water</td>
</tr>
<tr>
<td>Weighing boat</td>
</tr>
<tr>
<td>Top pan balance with 3 decimal places</td>
</tr>
<tr>
<td>1000 µL Micropipet and 3 tips</td>
</tr>
<tr>
<td>UV light box (can be made with cardboard- See appendix I)</td>
</tr>
<tr>
<td>8 Eppendorf tubes</td>
</tr>
<tr>
<td>8 Petri Dishes</td>
</tr>
<tr>
<td>2 mL Trypan Blue</td>
</tr>
<tr>
<td>1g Dextrose sugar</td>
</tr>
<tr>
<td>Cover slip of the Hemocytometer</td>
</tr>
<tr>
<td>3 UV lamps of 390nm wavelength</td>
</tr>
<tr>
<td>70% Ethanol (for sterilizing)</td>
</tr>
<tr>
<td>Bunsen burner (for sterilizing)</td>
</tr>
<tr>
<td>Clean soft towel</td>
</tr>
<tr>
<td>12 Aluminium foil of size 10cmx10cm</td>
</tr>
</tbody>
</table>
Appendix III: Raw Data Tables

<table>
<thead>
<tr>
<th>Time/min*</th>
<th>No. of cells in the 4 corner squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 1</td>
</tr>
<tr>
<td></td>
<td>Viable</td>
</tr>
<tr>
<td>0</td>
<td>433</td>
</tr>
<tr>
<td>5</td>
<td>264</td>
</tr>
<tr>
<td>10</td>
<td>294</td>
</tr>
<tr>
<td>15</td>
<td>304</td>
</tr>
<tr>
<td>20</td>
<td>347</td>
</tr>
<tr>
<td>25</td>
<td>379</td>
</tr>
<tr>
<td>30</td>
<td>324</td>
</tr>
<tr>
<td>35</td>
<td>381</td>
</tr>
</tbody>
</table>

Table 3: Raw data Table showing the viable and non-viable cell count using Trypan Blue on a hemocytometer for the first 5 trials.

<table>
<thead>
<tr>
<th>Time/min*</th>
<th>No. of cells in the 4 corner squares</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trial 6</td>
</tr>
<tr>
<td></td>
<td>Viable</td>
</tr>
<tr>
<td>0</td>
<td>225</td>
</tr>
<tr>
<td>5</td>
<td>297</td>
</tr>
<tr>
<td>10</td>
<td>223</td>
</tr>
<tr>
<td>15</td>
<td>272</td>
</tr>
<tr>
<td>20</td>
<td>130</td>
</tr>
<tr>
<td>25</td>
<td>367</td>
</tr>
<tr>
<td>30</td>
<td>283</td>
</tr>
<tr>
<td>35</td>
<td>261</td>
</tr>
</tbody>
</table>

Table 4: Raw data Table showing the viable and non-viable cell count using Trypan Blue on a hemocytometer for trial 6 to 10
Appendix IV: Example of Calculations

The Raw data in Appendix III was processed to get the percentage viability. For example, for the control that was exposed to UV for 0 minutes at trial 1:

\[ Cell \ Viability \ (\%) = \frac{\text{No. of Viable Cells}}{\text{No. of Viable Cells} + \text{No. of Non Viable Cells}} \times 100 \]

Therefore, viability = \( \frac{433}{433+0} \times 100 = 100\% \)

The percentage viability of all the data at all trials are shown in Table 1.

The percentage viability of all the trials at different UV-exposure time is then averaged by adding all the percentage viability and dividing it by the number of values added. Note: Anomalies highlighted in table 1 are avoided.

For example, for the control exposed to UV for 0 minutes,

Average Percentage viability = \( \frac{96.6+95.3+91.9+97.7+93.4+92.6+95.6+93.8}{8} = 94.6 \)

The standard deviation is also found using Microsoft Excel.