Research Question

How does pH of a water sample affect its biological oxygen demand over a course of 5 days, as found using the Winkler method?

Introduction

The knowledge area of **applications of redox reactions in environmental sciences** has always piqued my interest, encouraging me to undertake my investigation on something related to concept of redox reactions. During a classroom lecture, while studying Winkler's method and Biological Oxygen Demand (BOD), I was intrigued to know **water quality and level of pollution in the pond near my home**. This motivated me in choosing to study the BOD for a water sample from the pond (*Figure 1*) as well as investigate how, if at all, the pH of the water sample would affect its BOD value. I have also studied in class that **most of fish species cannot survive if dissolved oxygen concentration is below 5.00ppm** (Talbot et al. 283–321), hence lack of aquatic life in the pond as seen in *Figures 2 and 3*, has made me hypothesise that **dissolved oxygen concentration for water sample from this pond at day 1 must be less than 5.00ppm**.



Figure 1 – Pond nearby my home Background Information



Figure 2 – Water in pond is clear



Figure 3 - No sign of aquatic life like fishes

Understanding Biological Oxygen Demand (BOD)

Biological oxygen demand relates to amount of oxygen needed to oxidise any organic material present in water. A water sample is collected from water bodies to find its BOD, measured in parts per million (ppm), at a particular temperature over a measured time period, typically 5 days (Bylikin et al. 209–226).

BOD is found by subtracting the final dissolved oxygen concentration, measured at day 5 from the initial dissolved oxygen concentration recorded at day 1 (Kognity). Generally, **a higher BOD value relates to higher pollution level in the water sample**. This is because when BOD is high, amount of organic matter like sewage or wastewater, present in water sample, would also be high since organic matter would require dissolved oxygen to break down, thereby increasing demand of oxygen, hence a higher BOD value (The Editors of Encyclopaedia Britannica). Untreated wastewater typically is between a pH range of 6.0 to 8.0 (Trygar), hence suggesting that **higher pH water would have higher BOD**.

The dissolved oxygen in water bodies is used by various types of bacteria and green algae, aerobically, to oxidise the organic matter and break it down such as by oxidising carbon to CO_2 and hydrogen to H_2O . This process results in bacteria and algae multiplying, leading to an **algal bloom or eutrophication** (Bylikin et al. 209–226). A high BOD value indicates a decline in water quality to nurture aquatic life. This occurs since dissolved oxygen is principally utilised by algae and bacteria in oxidising organic matter and aquatic life has lower levels of dissolved oxygen to rely on for survival, thus endangering their lives. Normally, majority of fish species require a minimum of 5.00ppm of dissolved oxygen to survive in water bodies (Talbot et al. 283–321).

Understanding the Winkler Method

Dissolved oxygen concentration of a water sample is determined using the Winkler method, by utilising redox reactions in titration. The water sample's chemical environment is fixed by adding certain chemical substances. Firstly, Manganese (IV) ions are oxidised from Manganese (II) ions in presence of dissolved oxygen under alkaline conditions, observed by formation of brown precipitate. Next, under acidic conditions, Potassium Iodide is added to be oxidised to form Iodine. Finally, the mixture is titrated with Sodium Thiosulfate, using starch as an indicator for end-point, to determine amount of Iodine, thereby concentration of dissolved oxygen according to the molar ratios shown: (Kognity)

1 mol of $O_2 \rightarrow 2$ mol of $I_2 \rightarrow 4$ mol of $S_2O_3^{2-}$

The various redox reactions can be highlighted as below: (Kognity)

Oxidation of Mn (II) ions to Mn (IV) ions

$$2Mn^{2+}(aq) + 4OH^{-}(aq) + O_2(aq) \rightarrow 2MnO_2(s) + 2H_2O(l)$$

Oxidation of I^- ions to I_2

$$MnO_2(s) + 2I^-(aq) + 4H^+(aq) \rightarrow Mn^{2+}(aq) + I_2(aq) + 2H_2O(l)$$

Reduction of I₂ to I⁻ through titration

$$2S_2O_3^{2^-}(aq) + I_2(aq) \rightarrow S_4O_6^{2^-}(aq) + 2I^-(aq)$$

Aim of Investigation

This investigation focuses on studying whether **pH of water bodies affects its quality** in terms of concentration of dissolved oxygen, thereby **the biological oxygen demand** over a set period, in this case 5 days.

This research will be done by utilising a water sample with its original pH, a typical low pH for water bodies affected by acid rain and, a typical high pH. The pH will be altered by adding acids and alkalis of a particular pH to the water sample.

Hypothesis

The more alkaline a water sample is, the higher is its pH, perhaps from presence of untreated sewage or wastewater (Trygar), hence the higher is its BOD, resulting from greater effects of eutrophication in presence of organic matter in wastewater or untreated sewage (Jones and Jones 303–304).

Variables

<u>Independent Variable</u>

The independent variable in this study is the **pH of water sample** whose BOD is being measured, by calculating the concentration of dissolved oxygen at day 1 and day 5. Three variations of pH are utilised in this investigation:

- 1. The sample of water with its **natural pH**.
- 2. Altering the pH of water sample to an acidic pH of 3.0 4.0, achieved by adding Hydrochloric acid.
- 3. Altering the pH of water sample to an **alkaline pH of 9.0 10.0**, achieved by adding Sodium Hydroxide.

<u>Dependent Variable</u>

The concentration of dissolved oxygen as found on day 1 and day 5, thereby **BOD of the water sample at different pH values**, is the dependent variable for this study. The concentration of dissolved oxygen would be determined by the average titre/volume of $Na_2S_2O_3$ used in redox titration.

Controlled Variables

Controlled Variables	How is it used in the study?	How is it being controlled?
Water sample	To calculate its BOD	Using the same water sample for
		all three pH variations
Concentration of Na ₂ S ₂ O ₃	Redox titration	Using 0.0393 mol/dm ³ for all
		three pH variations
Concentration and number of	To provide acidic conditions for	Using 6 drops of 80.0%
drops of H ₂ SO ₄	Iodide ions to be oxidised	concentrated H ₂ SO ₄ for all three
		pH variations
Concentration and number of	To provide alkaline conditions	Using 6 drops of 1%
drops of MnSO ₄	for Manganese ions to be	concentrated MnSO ₄ for all three
	oxidised	pH variations
Concentration and number of	To provide iodide ions to be	Using 6 drops of 1%
drops of KI	oxidised	concentrated KI for all three pH
		variations
Room Temperature over 5-day	Providing a constant environment	Storing water samples in a cool
period	during incubation of water	dark cupboard area away from
	samples	sunlight

Chemicals and Apparatus

Chemicals Required	Purpose of requirement	Potential Dangers/Risks	Precautions Taken
0.0393 mol/dm ³ solution	Redox titration	Skin and eye irritation	Hair was tied back, and
of Na ₂ S ₂ O ₃		(Harper College)	safety goggles, lab coat
Solution of MnSO ₄	To provide alkaline	Skin and eye irritation	and gloves were worn
	conditions for Manganese	and respiratory irritation	throughout the
	ions to be oxidised	if inhaled (Numinor)	experimental procedure.
80% concentrated solution	Providing acidic	Severe skin burns and	This helped minimise
of H ₂ SO ₄	environment to oxidise	respiratory irritations	exposure to chemicals.
	Iodide ions.	(The Martin Companies)	Laboratory room was kept
Solution of KI	To provide iodide ions to be	Skin, eye and respiratory	ventilated efficiently to
	oxidised	irritations (Pestell	reduce chances of direct
		Minerals & Ingredients)	inhalation of chemicals.
Starch indicator	To determine end-point in	No dangers (G-	
	redox titration	Biosciences)	
NaOH and HCl	To alter pH of water	Corrosive to skin and	
	samples where pH is	eyes, irritation from	
	independent variable	inhalation (Merck)	
		(Sciencelab.com)	

Apparatus Required	Purpose of Requirement
Six 250cm ³ Volumetric flasks	To prepare and store water samples with all
	chemical reagents
2 Dropping Pipettes	To add drops of reagents to volumetric flask
$50 \text{cm}^3 \text{Burette} (\pm 0.05 \text{cm}^3)$	To contain solution of $Na_2S_2O_3$ for redox titration
Three 250cm ³ Conical Flasks (± 10.0 cm ³)	To contain water samples for redox titration
pH meter (± 0.02 pH)	To measure pH variations of water samples

Methodology of Conducting Investigation

- 1. 3 bottles of same water sample were collected from a nearby pond shown in *Figure 1*.
- 2. Bottles were left in open air overnight for oxygen saturation to ensure initial dissolved oxygen concentration would be known (Kognity).
- 3. The lids were replaced, and water samples were stored in air-tight bottles to prevent any air bubbles from being formed.
- 4. The following day, marked as day 1, water samples were carefully poured into 6 separate 250cm³ volumetric flasks A, A₁, B, B₁, C, C₁, ensuring no formation of air bubbles. pH in Flask A and A₁ was kept unaltered, pH in Flask B and B₁ was lowered by adding 6 drops of HCl and pH in Flask C and C₁ was raised by adding 6 drops of NaOH. The resulting pH for each flask was recorded using pH meter.
- 5. Next, after few hours, redox titration was performed using the Winkler method, on Flasks A, B, and C to record concentration of dissolved oxygen in each Flask:

- Using a dropping pipette, 6 drops of 1% concentrated MnSO₄ solution was added to Flask A, which was inverted and shaken in multiple attempts and left to settle for 10 minutes until brown precipitate of MnO₂ sedimented at the bottom.
- 6 drops of concentrated 80.0% concentrated H₂SO₄ was carefully added to Flask A using a dropping pipette. Then, 6 drops of 1% concentrated KI was added and left until settled precipitate solubilised to give a brownish solution.
- iii. This brownish water sample was then transferred to a 250cm^3 conical flask. A 50cm^3 burette was filled with solution of 0.0393 mol/dm³ of Na₂S₂O₃. Titration was performed by rotating stopcock to run Na₂S₂O₃ solution slowly using left hand and simultaneously swirling the conical flask using right hand, until a paleyellow colour was noticed.



Figure 4 – Experimental Setup for Titration



Figure 5 – pH Meter reading for Flasks A and A_1

- iv. Lastly, 6 drops of starch indicator were added to conical flask, giving its contents a blue colour, and titration was continued until the water sample in conical flask turned colourless, indicating end-point.
- v. The volume of $Na_2S_2O_3$ used in titration was noted and titration was repeated four more times. The five titration readings were used to get an average titre for Flask A.
- 6. Steps i-v were repeated for Flasks B and C.
- 7. Flasks A₁, B₁, and C₁, were incubated in a dark cupboard in laboratory for 5 days, at a constant room temperature.
- 8. At Day 5, Winkler method was used to record dissolved oxygen concentration of incubated water samples, thus steps i-v were repeated for each water sample contained in Flasks A₁, B₁, and C₁.
- 9. Each time reactions were completed, contents of apparatus were carefully cleansed and disposed off in basin of school laboratory, hence abiding by environmental considerations of disposal.

Table 1 – pH of Water Samples						
FlaskChemical AddedpH (±0.02pH)						
A and A ₁	None	7.10				
B and B ₁	Hydrochloric Acid	4.10				
C and C_1	Sodium Hydroxide	9.90				

Raw Data Collection

Day 1

Table 2 - Titration results for Flask A – pH 7.10						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	

Initial Na ₂ S ₂ O ₃ volume/cm ³	50.0	50.0	50.0	50.0	50.0
$(\pm 0.05 \text{ cm}^3)$					
Final Na ₂ S ₂ O ₃ volume/cm ³	46.8	47.1	46.8	46.9	46.8
$(\pm 0.05 \text{ cm}^3)$					
$Na_2S_2O_3$ volume used/cm ³	3.20	2.90	3.20	3.10	3.20
$(\pm 0.10 \text{cm}^3)$					
Colour change observed	Blue to				
	colourless	colourless	colourless	colourless	colourless
Average Titre/cm ³ (± 0.10 cm ³)			3.12		
(<u>+</u> 3.21%)					

Table 3 - Titration results for Flask B – pH 4.10					
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial Na ₂ S ₂ O ₃ volume/cm ³	50.0	50.0	50.0	50.0	50.0
$(\pm 0.05 \text{ cm}^3)$					
Final Na ₂ S ₂ O ₃ volume/cm ³	48.8	49.0	48.9	49.0	49.0
$(\pm 0.05 \text{ cm}^3)$					
$Na_2S_2O_3$ volume used/cm ³	1.20	1.00	1.10	1.00	1.00
$(\pm 0.10 \text{cm}^3)$					
Colour change observed	Blue to				
	colourless	colourless	colourless	colourless	colourless
Average Titre/cm ³ (± 0.10 cm ³)			1.06		
(<u>±</u> 9.43%)					

Table 4 - Titration results for Flask C – pH 9.90					
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial Na ₂ S ₂ O ₃ volume/cm ³	50.0	50.0	50.0	50.0	50.0
$(\pm 0.05 \text{ cm}^3)$					
Final Na ₂ S ₂ O ₃ volume/cm ³	43.5	43.9	44.0	43.9	43.8
$(\pm 0.05 \text{ cm}^3)$					
Na ₂ S ₂ O ₃ volume used/cm ³	6.50	6.10	6.00	6.10	6.20
$(\pm 0.10 \text{cm}^3)$					
Colour change observed	Blue to				
	colourless	colourless	colourless	colourless	colourless
Average Titre/cm ³ (± 0.10 cm ³)			6.18		
(±1.62%)					

Day 5

Table 5 - Titration results for Flask A1 – pH 7.10						
Trial 1Trial 2Trial 3Trial 4Trial 5						
Initial Na ₂ S ₂ O ₃ volume/cm ³ (± 0.05 cm ³)	50.0	50.0	50.0	50.0	50.0	
Final Na ₂ S ₂ O ₃ volume/cm ³	48.0	48.0	47.9	48.0	47.8	

$(\pm 0.05 \text{cm}^3)$					
$Na_2S_2O_3$ volume used/cm ³	2.00	2.00	2.10	2.00	2.20
$(\pm 0.10 \text{cm}^3)$					
Colour change observed	Blue to				
	colourless	colourless	colourless	colourless	colourless
Average Titre/cm ³ (± 0.10 cm ³)			2.06		
(±4.85%)					

Table 6 - Titration results for Flask B1 – pH 4.10						
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5	
Initial Na ₂ S ₂ O ₃ volume/cm ³	50.0	50.0	50.0	50.0	50.0	
$(\pm 0.05 \text{ cm}^3)$						
Final Na ₂ S ₂ O ₃ volume/cm ³	49.2	49.3	49.2	49.2	49.2	
$(\pm 0.05 \text{ cm}^3)$						
$Na_2S_2O_3$ volume used/cm ³	0.80	0.70	0.80	0.80	0.80	
$(\pm 0.10 \text{cm}^3)$						
Colour change observed	Blue to					
	colourless	colourless	colourless	colourless	colourless	
Average Titre/cm ³ (± 0.10 cm ³)			0.78			
(±12.8%)						

Table 7 - Titration results for Flask C1 – pH 9.90					
	Trial 1	Trial 2	Trial 3	Trial 4	Trial 5
Initial Na ₂ S ₂ O ₃ volume/cm ³	50.0	50.0	50.0	50.0	50.0
$(\pm 0.05 \text{ cm}^3)$					
Final Na ₂ S ₂ O ₃ volume/cm ³	45.9	46.0	45.8	46.0	46.0
$(\pm 0.05 \text{ cm}^3)$					
Na ₂ S ₂ O ₃ volume used/cm ³	4.10	4.00	4.20	4.00	4.00
$(\pm 0.10 \text{ cm}^3)$					
Colour change observed	Blue to				
	colourless	colourless	colourless	colourless	colourless
Average Titre/cm ³ (± 0.10 cm ³)			4.06		
(±2.46%)					

<u>Analysis</u>

Analysing and Processing Raw Data

Preparing a solution of $Na_2S_2O_3$

Mass of $Na_2S_2O_3$ used = 3.1025g

Molar mass of $Na_2S_2O_3 = 158g/mol$

Number of moles $=\frac{mass}{molar mass} = \frac{3.1025}{158} = 0.01963$ moles of Na₂S₂O₃ used in preparing solution

Volume of water used = $500 \text{cm}^3 = 0.5 \text{dm}^3$

Uncertainty Calculations

% Uncertainty = $\frac{absolute uncertainty}{measured value} \times 100$ Mass of Na₂S₂O₃ used = $\frac{0.01}{3.1025} \times 100 = \pm 0.322\%$ Volume of water used = $\frac{0.15}{500} \times 100 = \pm 0.030\%$ Concentration of Na₂S₂O₃ = $\frac{number \ of \ moles}{volume \ of \ solution}$ = $\frac{0.01963}{0.5}$ = 0.0393mol/dm³

Concentration of $Na_2S_2O_3 = 0.322 + 0.030 = \pm 0.352\%$

$\therefore \text{ Concentration of Na}_2S_2O_3 = 0.0393 \text{mol/dm}^3 (\pm 0.352\%)$

Dissolved Oxygen Concentration for Flask A

Day 1 – Titration

Concentration of $Na_2S_2O_3 = 0.0393$ mol/dm³

Average Titre/Volume of $Na_2S_2O_3 = 3.12cm^3$

Number of moles = Concentration × Volume = $0.0393 \times \frac{3.12}{1000}$ = 1.23×10^{-4} moles of Na₂S₂O₃ are used in titration

4 mol of
$$S_2O_3^{2-} \rightarrow 1$$
 mol of O_2

 $\therefore \text{ Number of moles of } O_2 \text{ in water sample} = \frac{number of moles of thiosulfate ion}{4} = \frac{1.23 \times 10^{-4}}{4} = 3.07 \times 10^{-5}$

Mass of O₂ in water sample = Number of moles × Molar Mass = $3.07 \times 10^{-5} \times 32 = 9.81 \times 10^{-4}$ g = 0.981mg

Volume of water sample = $250 \text{cm}^3 = 0.25 \text{dm}^3$

Concentration of dissolved $O_2 = \frac{mass \ of \ Oxygen}{volume \ of \ water} = \frac{0.981}{0.25} = 3.92 \text{mg/dm}^3 = 3.92 \text{ppm}$

:: Concentration of dissolved O₂ in Flask A at day 1 = 3.92 ppm ($\pm 7.56\%$)

Dissolved Oxygen Concentration for Flask A₁

Day 5 – Titration

Concentration of $Na_2S_2O_3 = 0.0393 \text{mol/dm}^3$

Average Titre/Volume of $Na_2S_2O_3 = 2.06$ cm³

Number of moles = Concentration × Volume = $0.0393 \times \frac{2.06}{1000} = 8.10$ × 10⁻⁵ moles of Na₂S₂O₃ are used in titration

4 mol of
$$S_2O_3^2 \rightarrow 1$$
 mol of O_2

 $\therefore \text{ Number of moles of } O_2 \text{ in water sample} = \frac{number of moles of thiosulfate ion}{4} = \frac{8.10 \times 10^{-5}}{4} = 2.02 \times 10^{-5}$

Mass of O₂ in water sample = Number of moles × Molar Mass = $2.02 \times 10^{-5} \times 32 = 6.48 \times 10^{-4}$ g = 0.648mg

Volume of water sample = $250 \text{cm}^3 = 0.25 \text{dm}^3$

Concentration of dissolved $O_2 = \frac{mass \ of \ Oxygen}{volume \ of \ water} = \frac{0.648}{0.25} = 2.59 \text{mg/dm}^3$ = 2.59ppm

: Concentration of dissolved O₂ in Flask A₁ at day 5 = 2.59 ppm ($\pm 9.20\%$)

Uncertainty Calculations

Day 1 – Titration

Concentration of $Na_2S_2O_3 = \pm 0.352\%$

Average Titre/Volume of $Na_2S_2O_3 = \pm 3.21\%$

Number of moles of $Na_2S_2O_3$ used in titration = $0.352 + 3.21 = \pm 3.56\%$

Number of moles of O_2 in water sample = $\pm 3.56\%$

Mass of O_2 in water sample = $\pm 3.56\%$

Volume of water sample $=\frac{10}{250} \times 100$ = +4.00%

Concentration of dissolved $O_2 = 4.00$ + 3.56 = $\pm 7.56\%$

Uncertainty Calculations

Day 5 – Titration

Concentration of $Na_2S_2O_3 = \pm 0.352\%$

Average Titre/Volume of $Na_2S_2O_3 =$

<u>+</u> 4.85%

Number of moles of Na₂S₂O₃ used in titration = $0.352 + 4.85 = \pm 5.20\%$

Number of moles of O_2 in water sample $= \pm 5.20\%$

Mass of O_2 in water sample = $\pm 5.20\%$

Volume of water sample $=\frac{10}{250} \times 100$ = $\pm 4.00\%$

Concentration of dissolved $O_2 = 4.00$ + 5.20 = $\pm 9.20\%$

BOD for Flask A – pH 7.10

Uncertainty Calculations

 $BOD = Day 1 \text{ dissolved } O_2 \text{ conc. in Flask A}$ $- Day 5 \text{ dissolved } O_2 \text{ conc. in Flask A}_1$

 \therefore BOD = 3.92 - 2.59 = 1.33ppm

Day 1 dissolved O₂ conc. in Flask A = $\frac{7.56}{100} \times 3.92$ = ± 0.296 ppm Day 5 dissolved O₂ conc. in Flask A₁ = $\frac{9.20}{100} \times 2.59 = \pm 0.238$ ppm BOD = $0.296 + 0.238 = \pm 0.534$ ppm

\therefore BOD for water sample in Flask A at pH 7.10 = 1.33ppm (± 0.534 ppm)

Similar calculations were carried out to determine BOD values for Flasks B and B₁, C and C₁.

Table 8 – Processed Results				
Flask	pH (±0.02pH)	Day 1 Dissolved O ₂	Day 5 Dissolved O ₂	BOD/ppm
		concentration/ppm	concentration/ppm	
А	7.10	3.92 (±0.296ppm)		1.33 (±0.534ppm)
A1	7.10		2.59 (±0.238ppm)	
В	4.10	1.33 (±0.184ppm)		0.349 (±0.353ppm)
B ₁	4.10		0.981 (±0.169ppm)	
С	9.90	7.77 (±0.464ppm)		2.65 (±0.813ppm)
C ₁	9.90		5.12 (±0.349ppm)	

Analysing Results

Dissolved oxygen concentration value at day 1 for neutral water sample in Flask A is 3.92ppm (± 0.296 ppm). Value of dissolved oxygen concentration being **lower than 5.00ppm**, despite adding positive uncertainty of 0.296ppm, **explains absence of aquatic life** in this pond as I had observed, showcased in *Figure 3*.

Figure 6 below portrays graphical relationship obtained between the independent variable – pH of water sample and dependent variable – BOD of water sample measured in parts per million, summarising my processed results shown in *Table 8*.



A positive linear relationship is observed with pH of water sample being directly related to BOD, hence increasing the pH raises BOD value. The data points are roughly consistent with best-fit line, as confirmed by the \mathbf{R}^2 value of 98.9% or 0.9891. This consistency continues even when considering measurement uncertainties. At higher pH values, the uncertainties for BOD are higher as reflected through larger vertical error bars, also depicted in *Table 8*. Uncertainty of pH meter was consistent at (±0.02pH) but is not visible as horizontal error bars being a minute value. At pH 4.10, BOD value is calculated as 0.349ppm with uncertainty (±0.353ppm), however, since negative uncertainty is larger than BOD value itself, i.e., 0.353>0.349, graph illustrates a possible negative value for BOD at pH 4.10. Nevertheless, BOD values cannot be negative, hence only positive uncertainty of +0.353ppm must be considered for pH 4.10.

Conclusion and Evaluation

Alkaline water with higher pH has greater BOD value because of **presence of substances like sewage or wastewater** (Trygar), making water alkaline. Water bacteria break down organic matter present in sewage or wastewater, for which dissolved oxygen is utilised, hence **depleting oxygen levels** and **raising BOD**. Conversely, since microbial growth is normally favoured in **pH range of 6.5 to 8.5**, majority of bacteria and other microorganisms would **barely survive in low pH water**, hence decreasing BOD since rate of decomposition of organic matter would be lowered (Environmental Business Specialists). This supports positive direct relationship between pH and BOD of water sample, as illustrated in *Figure 6*.

Moreover, for typical domestic wastewater, as alkalinity in terms of CaCO₃ concentration rises from 50ppm to 200ppm, **BOD**₅ **increases from 100ppm to 300ppm** (Pescod). Higher alkalinity relates to harder water, containing greater amounts of dissolved minerals like calcium and magnesium, hence making it alkaline and raising pH (BCcampus). This additionally expresses positive direct relationship between pH and BOD of a water sample.

Therefore, my research question, **"How does pH of a water sample affect its biological oxygen demand over a course of 5 days, as found using the Winkler method?"**, is answered by **positive linear and direct relationship between pH of a water sample and its BOD**, as demonstrated in *Figure 6*. When pH of water sample (± 0.02 pH) rises from an acidic 4.10, to a neutral 7.10, and finally to an alkaline 9.90, BOD correspondingly increases from 0.349ppm (± 0.353 ppm) to 1.33ppm (± 0.534 ppm) and finally to 2.65ppm (± 0.813 ppm). BOD increases at higher pH because microbial growth is encouraged within a pH range of 6.5 to 8.5 and organic matter present in water is more at higher pH due to substances like wastewater raising pH. Hence, **my data and results analysis confirm with my hypothesis**.

This investigation helps to understand how pH of water bodies could possibly be **controlled to create an environment** where aquatic life thrives and survive well and bacteria are prevented from decomposing organic matter, thus avoiding oxygen depletion, and keeping dissolved oxygen concentration optimum and BOD minimum.

Nonetheless, the reliability of my findings through this investigation are limited by occurrence of **random** and systematic errors in the experimental procedure. The major random error would have arisen from measurement uncertainty of $(\pm 0.05 \text{ cm}^3)$ in burette readings, hence increasing random uncertainty in average titre value. These random errors went on accumulating throughout calculations. Random errors in measurement uncertainty could be reduced by appropriately reading lower meniscus of burette by keeping line of sight perpendicular to burette, thereby improving accuracy of readings. A random error was also present in pH meter, however this measurement uncertainty was quite minimal at $(\pm 0.02 \text{ pH})$. Furthermore, pH readings were more reliable by using an electronic equipment of digital pH meter. The principal systematic error would have resulted from room temperature not being constant over the course of 5 days. Despite water samples in volumetric flasks, being stored in a dark cupboard away from sunlight, temperature fluctuations could have altered microbial activity, thus giving rise to possibly misleading results for BOD. The investigation could be improvised to reduce this systematic error by using a digital thermometer to record temperature fluctuations each day and accordingly create artificial conditions to ensure temperature is kept constant.

There can be possible extensions to this investigation by **enhancing the methodology used**, hence give a more accurate understanding on actual relationship between pH of water sample and its BOD. For example, reliable electronic equipment like a **dissolved oxygen sensor** could be used to measure accurate oxygen concentration levels and compare these measurements with results found through redox titration, hence calculate percentage error. Secondly, **Winkler solutions** (Flinn Scientific, Inc) could be used instead of its alternatives like MnSO4 and KI as used in my procedure. This should provide more reliable results because **chemicals specifically tailored towards study of BOD** would be used. Lastly, different **water samples of varying pH** could be collected to understand effect of pH on BOD of water samples over a wide spectrum, such as by collecting water samples from different ponds in the same city. Impact of domestic wastewater of a particular city on BOD of ponds in the city can also be investigated by examining chemical composition of domestic wastewater. Overall, I am satisfied that cleanliness is being maintained in my city with proper wastewater disposal since BOD obtained for natural pH water sample from the pond near my home was moderate at 1.33ppm (± 0.534 ppm).

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