

Chapter 8 Influence of pH on microbial growth: Mechanisms, adaptations, and industrial implications

Narendra Kumar¹, Keshawanand Tripathi², Shashank Upadhyay², Sarvesh Kumar Pandey³, Yashdeep Srivastava^{2*},

¹School of Biotechnology and Bioengineering, Institute of Advanced Research, Gandhinagar, Gujarat, India. ² Department of Biotechnology, Invertis University, Bareilly, Uttar Pradesh, India. ³Department of Formula Science, Invertis University, Bareilly, Uttar Pradesh, India.

³Department of Forensic Science, Invertis University, Bareilly, Uttar Pradesh, India. *Email: <u>yashbubiotech@gmail.com</u>

1. Introduction:

Microorganisms exhibit remarkable adaptability and can thrive in a wide variety of extreme environmental conditions (Reddy et al., 2019a, b; Kageyama et al., 2011). Freeliving prokaryotes exhibit remarkable pH adaptability, thriving across a broad pH spectrum, accommodating changes in $[H^+]$ spanning three pH units, equivalent to a thousand-fold difference (Padan, 2005). Understanding an organism's pH requirements for growth entails assessing three pivotal factors: the lowest pH conducive to growth, the highest supportive pH, and the pH favoring optimal growth. Bacterial growth rate patterns exhibit distinct trends as pH fluctuates.

Growth rates rise steadily from the minimum to the optimum pH, declining thereafter towards the maximum pH, reflecting the influence of changing $[H^+]$ on enzymatic reaction rates. Microorganisms inhabiting acidic environments (pH < 7.0) are termed acidophiles, while those favoring neutral pH are neutrophiles; alkaliphiles thrive in alkaline conditions (Pelczar, 1993; Tripathi et al., 2013a, b; Yadav et al., 2016). Certain microorganisms, like some *Thiobacillus* species, flourish in acidic environments due to their reliance on low pH for growth, as neutral pH exposure causes cell membrane dissolution and cell breakdown. Genera of Archaea, such as Sulfolobus and Thermoplasma, excel in highly acidic habitats (Benson, 2001; Prescott, et al., 2005; Tripathi et al., 2018).

2. Material required:

- 1. Spectrophotometer
- 2. Culture slants
- 3. Inoculating loop
- 4. Sterile pipette

- 5. YEM Broth medium of different pH
- 6. Conical flasks

3. Procedure:

- 1. Please ensure that each YEM broth flask is properly labeled with the corresponding pH of the medium (6, 7, 8, or 9) and the name of the organism that will be inoculated.
- 2. Ensure that every flask is inoculated with the test culture, while reserving one flask as a blank without any inoculation.
- 3. Measure the optical density at 0 hours for all pH ranges compared to a blank, then place the remaining flask in the incubator shaker.
- 4. Remember to measure the OD every two hours for each flask.
- 5. Create a graph by plotting the relationship between optical density and time intervals to obtain the desired curve.

4. Precautions:

- 1. Use sterile techniques to prevent contamination of cultures when adjusting pH levels.
- 2. Calibrate pH meters and use reliable pH indicators to ensure accurate measurements.
- 3. Handle acidic or alkaline solutions with care to avoid skin or eye irritation.
- 4. Maintain consistent pH conditions throughout the experiment to ensure reproducibility.
- 5. Monitor pH levels regularly during the experiment to track changes and adjust if necessary.
- 6. Use appropriate buffering agents to stabilize pH conditions and minimize fluctuations.
- 7. Choose appropriate microorganisms for the study based on their pH tolerance ranges.
- 8. Ensure pH adjustments are done gradually to prevent sudden stress to microorganisms.
- 9. Conduct experiments in controlled environmental conditions (temperature, humidity) to minimize external influences on microbial growth.

5. References:

- 1. Benson, H. J. (2001). *Microbiological applications: A laboratory manual in general microbiology* (8th ed.). McGraw-Hill.
- 2. Pelczar, M. J., Chan, E. C. S., & Krieg, N. R. (1993). *Microbiology: Concepts and applications* (5th ed.). McGraw-Hill.
- 3. Prescott, L. M., Harley, J. P., & Klein, D. A. (2005). *Microbiology* (6th ed.). McGraw-Hill.

- 4. Padan, E., Bibi, E., Ito, M., & Krulwich, T. A. (2005). Alkaline pH homeostasis in bacteria: New insights. *Biochimica et Biophysica Acta (BBA) Biomembranes,* 1717(2), 67–88.
- 5. Yadav, R. K., Thagela, P., Tripathi, K., & Abraham, G. (2016). Physiological and proteomic analysis of salinity tolerance of the halotolerant cyanobacterium *Anabaena* sp. *World Journal of Microbiology and Biotechnology*, *32*, 1–11.
- Tripathi, K., Sharma, N. K., Kageyama, H., Takabe, T., & Rai, A. K. (2013). Physiological, biochemical, and molecular responses of the halophilic cyanobacterium *Aphanothece halophytica* to Pi-deficiency. *European Journal of Phycology*, 48(4), 461–473.
- 7. Tripathi, K., Kumar, N., & Abraham, G. (Eds.). (2018). *The role of photosynthetic microbes in agriculture and industry*. Nova Science Publishers, USA.
- Tripathi, K., Sharma, N. K., Kageyama, H., Takabe, T., & Rai, A. K. (2013). Physiological, biochemical and molecular responses of the halophilic cyanobacterium Aphanothece halophytica to Pi-deficiency. *European Journal of Phycology*, 48(4), 461-473.
- 9. Reddy, Y. P., Yadav, R. K., Tripathi, K., & Abraham, G. (2019a). Isolation and characterization of high temperature tolerant mutant from the cyanobacterium *Anabaena doliolum. Journal of basic microbiology*, *59*(3), 314-322.
- Reddy, Y. P., Yadav, R. K., Tripathi, K. N., Borah, A., Jaiswal, P., & Abraham, G. (2019b). Industrially important biomolecules from cyanobacteria. A Handbook on High Value Fermentation Products-Human Welfare II. John Wiley & Sons, 147-164
- Kageyama, H., Tripathi, K., Rai, A. K., Cha-Um, S., Waditee-Sirisattha, R., & Takabe, T. (2011). An alkaline phosphatase/phosphodiesterase, PhoD, induced by salt stress and secreted out of the cells of Aphanothece halophytica, a halotolerant cyanobacterium. *Applied and Environmental Microbiology*, 77(15), 5178-5183.