





Kinetics, Zymographic Analysis, and Industrial Evaluation of Alkaline Protease from *Bacillus tropicus* Isolated from Environmental Water Samples

Uba, B. O. and Umennadi, P. O.

Department of Microbiology, Chukwuemeka Odumegwu Ojukwu University Uli, Anambra State, Nigeria.

*Corresponding author e-mail address: bo.uba@coou.edu.ng

Abstract	Article History
<p>This study investigated the isolation, molecular identification, and biotechnological potential of alkaline protease-producing bacteria from fish pond (FW) and poultry farm (PW) water samples. Serial dilutions 10^{-2} and 10^{-3} were cultured on skim milk agar at 37 °C and pH 9 for 72 h to screen for proteolytic activity. Among the isolates, PW10⁻²A2 exhibited the highest protease activity with a zone of hydrolysis (ZOH) of 13 mm, compared to PW10⁻²A1 (4 mm) and PW10⁻²B2 (2 mm), despite the latter having >300 colonies. Molecular identification via 16S rRNA sequencing revealed 99.84% homology with <i>Bacillus tropicus</i> (GenBank accession MW971701.1). Zymogram analysis indicated two protease isoforms with molecular weights ranging between 36 and 116 kDa. Batch fermentation studies showed peak protease activity (142.02 U/mL) at 96 h, with a productivity of 1.48 U/h and specific growth rate of 0.0303 h⁻¹. Functional assays demonstrated complete blood stain removal when the enzyme was combined with detergent, outperforming detergent alone. Additionally, the enzyme exhibited efficient dehairing capability, with treated cow skin showing complete hair removal within 24 h compared to control. These findings highlight <i>Bacillus tropicus</i> PW10⁻²A2 as a promising candidate for industrial applications in detergent formulation and leather processing.</p> <p>Keywords: Alkaline protease; <i>Bacillus tropicus</i>; Detergent application; Enzyme kinetics; Leather processing; Proteolytic activity; Zymogram</p>	<p>Received: 18 Mar 2026 Accepted: 21 Apr 2026 Published: 08 May 2026</p> <p>Scan QR code to view*</p>  <p>License: CC BY 4.0</p>  <p>Open Access article.</p>
<p>How to cite this paper: Uba, B. O., & Umennadi, P. O. (2026). Kinetics, Zymographic Analysis, and Industrial Evaluation of Alkaline Protease from <i>Bacillus tropicus</i> Isolated from Environmental Water Samples. <i>IPS Interdisciplinary Journal of Biological Sciences</i>, 6(2), 299–309. https://doi.org/10.54117/ijbs.v6i2.161</p>	

1. Introduction

Proteases (EC 3.4.x.x) are among the most extensively utilized industrial enzymes, accounting for nearly 60% of the global enzyme market due to their broad applications in detergents, leather processing, pharmaceuticals, food processing, and waste management (Contesini *et al.*, 2018; Razzaq *et al.*, 2019; Anichebe *et al.*, 2019; Uba *et al.*, 2020a; 2020b). These enzymes catalyze the hydrolysis of peptide bonds in proteins, leading to the generation of smaller peptides and amino acids (Okoye *et al.* 2020a; 2020b; 2020c). Among the different classes of proteases, alkaline proteases are particularly significant because of their optimal activity and stability at high pH conditions (pH 8–11), making them indispensable in detergent formulations and leather industries (Sharma *et al.*, 2019; Uba, 2019a; 2019b; Okpalaunegbu *et al.*, 2025).

Microorganisms are the preferred sources of industrial proteases due to their rapid growth rates, ease of genetic manipulation, and ability to secrete large quantities of extracellular enzymes (Egurefa *et al.* 2020a; 2020b; Obiefuna *et al.* 2025; Obiefuna *et al.* 2026; Okoye *et al.*, 2026). Bacterial genera such as *Bacillus*, *Pseudomonas*, and *Streptomyces* have been widely reported as prolific producers of alkaline proteases, with *Bacillus* species being the most dominant due to their robustness and capacity to thrive under extreme environmental conditions (Sundararajan *et al.*, 2021; Uba *et al.*, 2019a; 2019b; Okolo *et al.*, 2025). In particular, *Bacillus* species are known for producing thermostable and alkali-tolerant enzymes that are highly suitable for industrial processes (Uba, 2019c; Uba *et al.* 2019c; 2019d).

Environmental niches rich in organic matter, such as fish ponds and poultry farm effluents, represent promising sources for isolating protease-producing bacteria (Uba *et al.* 2024; Mere *et al.* 2025; Enemchukwu *et al.* 2026a; 2026b; Ofunwa *et al.*, 2026a; 2026b). These environments contain high levels of proteins, nitrogenous wastes, and microbial diversity, which create selective pressure for microorganisms capable of producing extracellular proteases for nutrient acquisition (Abdel-Fattah *et al.*, 2020; Nkamigbo *et al.* 2020a; 2020b). Despite this, such ecological niches remain underexplored, especially in developing regions, highlighting the need for systematic screening and characterization of indigenous microbial strains with industrial potential (Uba *et al.* 2016; Uba *et al.*, 2017; Njoku *et al.* 2019a; 2019b).

Advancements in molecular biology techniques, particularly 16S rRNA gene sequencing, have revolutionized bacterial identification and phylogenetic analysis (Dokubo *et al.*, 2022a; 2022b; Anidu *et al.*, 2023; Uba *et al.*, 2021a; 2021b; Obiefoka *et al.*, 2023; Ubajekwe *et al.*, 2025; Uba *et al.*, 2025). This method provides high-resolution taxonomic classification and enables comparison with global databases such as GenBank, thereby facilitating the identification of novel or underreported enzyme-producing strains (Janda & Abbott, 2021; Uba *et al.*, 2020c; Dokubo and Uba, 2023; Uba and Obiefuna, 2023). Additionally, techniques such as zymogram electrophoresis allow for the detection of multiple enzyme isoforms and estimation of their molecular weights, which are crucial for understanding enzyme functionality and optimizing industrial processes (Uba *et al.* 2020d; 2020e; Ghorbel-Frikha *et al.*, 2022; Dokubo *et al.*, 2024).

Beyond isolation and identification, the evaluation of enzyme kinetics and functional performance is critical for determining industrial applicability (Uba *et al.*, 2020f; 2020g). Parameters such as specific growth rate, product yield coefficient, and enzyme productivity provide insights into fermentation efficiency and scalability (Contesini *et al.*, 2018; Ubani *et al.*, 2024a; 2024b; Ubani *et al.*, 2025; Ekwenze *et al.*, 2025). Furthermore, practical application tests, including stain removal and dehairing of animal hides, are essential for validating the enzyme's effectiveness in real-world industrial conditions. Enzymatic dehairing, for instance, offers an environmentally friendly alternative to conventional chemical methods that rely on toxic substances like sodium sulfide, thereby reducing environmental pollution and improving workplace safety (Razzaq *et al.*, 2019; Alisa *et al.*, 2020; Anukam *et al.*, 2020a; 2020b; Umeh *et al.*, 2020; 2021).

Despite the extensive research on microbial proteases, there remains a continuous demand for novel strains with enhanced catalytic efficiency, stability, and multifunctional applications. Therefore, this study aimed to isolate and screen alkaline protease-producing bacteria from fish pond and poultry farm water samples, identify the most potent strain using molecular techniques, characterize its enzyme profile through zymographic analysis, and evaluate its kinetic parameters and industrial applications in detergent formulation and leather processing.

2. Materials and Methods

2.1 Sample Site Description

The fish and poultry wastewater samples were collected from Umubazu and Umudaru Ubahudara, respectively, in Uli. Uli is a town of historic importance situated at the extreme southeastern corner of Ihiala Local Government Area (LGA) of Anambra State. The town is located between latitude 5°46'59.99"N and longitude 6°51'59.99"E. The estimated terrain elevation above sea level is 62 metres (Uba *et al.*, 2026a, 2026b, 2026c; Okwonkwo *et al.*, 2026).

2.2 Collection of Sample

One litre each of the fish and poultry wastewater are collected per point of the two designated points of the sampling sites. The sampling was done once in each of the two sampling sites in June, 2022. The wastewater sample was collected by hand dipping the 70 % ethanol sanitized clean, leak proof cylindrical shaped 500 mL plastic containers (Uba *et al.* 2018a; Uba *et al.* 2018b Uba *et al.*, 2018c). The containers with lids slightly opened were rinsed with samples twice before aseptically collecting the samples and were properly labelled with sample type, date, time and place of collection (Ibo *et al.* 2020; Okafor *et al.*, 2023; Ele *et al.*, 2025; Uba and Okonkwo, 2025; Dokubo and Uba, 2026). They were placed in a sterile bag and then transported immediately to the Microbiology laboratory, Chukwuemeka Odumegwu University, Uli Campus, Nigeria (Uba and Udaba, 2026).

2.3 Screening of Bacterial Isolates for Protease Activity

2.3.1 Serial dilution

A ten - fold serial dilution was adopted in this study. One millilitre of the fish and poultry wastewater samples was weighed using a graduated pipette and mixed with 9 mL of sterile normal saline (the 10⁻¹ labelled test tubes). Thereafter, 1 mL series of transfers were aseptically made from the 10⁻¹ tubes to the 10⁻³ test tubes, respectively and finally dispensed serially diluted (Ofunwa *et al.*, 2024; Alfred *et al.*, 2023; Alfred *et al.*, 2025; Okeke *et al.* 2025a; 2025b; Oghonim *et al.*, 2026a).

2.3.2 Primary screening of potential alkaline protease-producing bacterial isolate

Primary screening of bacterial isolates was made to screen alkaline protease directors using 1 % skim milk agar (skim milk greasepaint 2.8 g, casein enzymic hydrolysates 500 mg, yeast extract 250 mg, dextrose 100 mg, agar 1.5 g powder) with 100 mL of distilled water maintaining pH 9.0 using 0.1 N sodium hydroxide (NaOH). The medium was prepared by weighing the appropriately as stated above, dissolved by heating, and sterilized by autoclaving at 121 °C and 15 psi for 15 min. After sterilization, the medium left to cool to 45 °C and poured aseptically into the sterile Petri plates and allowed to solidify. Later, 0.1 mL aliquot of the samples stated above was inoculated into the labelled plates and were incubated at ambient temperature for 72 h. The colonies that surfaced from the plates were counted and recorded as colony forming unit (CFU/ mL). Also, a clearance zone around the invested point because of proteolytic exertion was observed after 72 h. The diameter of the clear halo- zone were measured by millimeter rule. The clear zone around the colonies was assessed as suggestion for protease activity. The colony with the topmost

clearance zone on skim milk agar was selected, that is, the isolate observed with a clearance zone of further than 10 mm was named and progressed for secondary screening (Masi *et al.*, 2021). Bacterial strains with strong protease exertion were identified by molecular techniques (Lich *et al.*, 2022; Idu *et al.*, 2026a; 2026b; Ibe *et al.*, 2023 Chukwura *et al.*, 2025).

2.4 Molecular characterization

The best isolate was carried out for molecular identification which include DNA extraction, gel electrophoresis, polymerase chain reaction, gene sequencing, blasting and phylogenetic analyses (Oghonim *et al.*, 2026b).

2.5 Zymogram

Molecular mass and enzyme activity was assessed using zymogram electrophoresis. Zymogram was performed according to Wang *et al.* (2016). Fifteen microliters of the cell-free supernatant were mixed with 2 X loading dye buffer. The mixture was subjected to SDS-PAGE with 5 % stacking gel and 12 % separating gel containing 0.8 mg/mL of casein. The gel was washed twice with Triton X-100 2.5 % (v/v) for 30 min at room temperature and three times with distilled water, then incubated in the reaction buffer (50 mmol/L Tris - HCL pH 8.3, 50 mmol/L CaCl₂) at 35 °C for 2 h. Gel was stained with Coomassie Brilliant Blue R-250 and destained in acetate methanol solution. The appearance of a clear zone on the blue background of the gel was accessed for protease activity (Lich *et al.*, 2016; Uba and Chukwura, 2016; Okafor *et al.* 2021a; 2021b).

2.6 Cell Growth Kinetic

Following the method of Enemchukwu *et al.* (2026b), the exponential growth phase can be characterized by the following first order equation which states that the rate of increase of cell mass is proportional to the quantity of viable cell mass at any instant time $dX/dt = \mu X$

Where dX/dt is the growth rate [g/L h]; X is the concentration of biomass [g/L]; μ is the specific growth rate [h^{-1}].

Equation on integration gives $\ln(X-X_0) = \mu t$ Plot of $\ln(X-X_0)$ vs time of incubation, gives specific growth rate (μ) whereat logarithmic phase of microbial growth, $\mu = (\mu)_{max}$ and amount of X_0 for protease production experiments were negligible.

2.7 Kinetic analysis

The following kinetic parameters were studied:

- 1) Maximum specific growth rate (μ_{max}) per hr. The value of μ_{max} was calculated from plot of $\ln X$ vs time of fermentation;
- 2) Product yield coefficient ($Y_{p/x} - U/g$: the amount of enzyme produced per amount of biomass), its value was determined by the equation: $Y_{p/x} = dp/dx$;
- 3) Growth yield coefficient ($Y_{x/s} - g/g$: the amount of biomass produced per amount of sugar consumed): Its value was determined by the equation: $Y_{x/s} = dx/ds$;
- 4) Specific product yield coefficient ($qp - U/g/h$: the amount of enzyme produced per amount of biomass per h): the value of qp was determined by the equation $qp = Y_{p/x} \cdot \mu_{max}$;

5) Specific growth yield coefficient ($qx - g/g/h$: amount of biomass produced per amount of sugars consumed per h): Its value of qx was determined by the equation: $qx = Y_{p/x} \cdot \mu_{max}$; and

6) Productivity ($P - U/h$ the amount of enzyme produced per total time of fermentation): Its value was determined by the equation: $P = \Delta P/\Delta t$

2.8 Destaining of Cotton Fabrics

Following the method of Kotb *et al.* (2023), white cotton fabrics of 4×4 cm² dimension were stained with 100 μ L of whole blood sample. After the dryness of stains at 60 °C for 60 min, they were immersed in a solution of one set of the experiments at pH 9.0. The sets were (A) Whole blood sample (B) Distilled water as a control, (C) Detergent solution at 7 mg/mL concentration, (D) Protease enzyme preparation at a concentration of 150 U/mL and (E) denatured detergent solution plus enzyme preparation at a concentration of 150 U/mL. The incubation was allowed at room temperature (25 °C) for 1 h with a shaking speed of 150 rpm. By the end of incubation, fabrics were air-dried after being rinsed with tap water, and the stain elimination effectiveness was determined by visual inspection of the color intensity of cloth.

2.9 Dehairing Activity

Following the method of Kotb *et al.* (2023), a skin piece of cow was obtained from a local slaughterhouse then washed with distilled water to eliminate impurities and dried for 30 min at 50 °C. It was cut into smaller pieces of 4.5×4.5 cm². The pieces were then immersed in 150 U/mL of protease enzyme at pH 9.0 and 55 °C for 24 h with a shaking speed of 150 rpm (B) while the other piece was immersed in water control (A). Dehairing activity was checked after 60 min of exposure by shedding the hair using sterile forcep.

2.10 Statistical Analysis

The data obtained was analyzed using one way analysis of variance (ANOVA) in GraphPad Prism Version 8.0.2. statistical package and Microsoft Excel package (Uba *et al.*, 2020h; Afulukwe *et al.*, 2025; 2026).

3. Result

3.1 Isolation, Primary and Secondary Screening of Potential Alkaline Protease-producing Bacterial from Fish Pond and Poultry Farm Water Samples

The both water samples from fish pond (FW) and poultry farm (PW), were serially diluted and tube 10^{-2} and 10^{-3} was pour plated on skim milk agar at 37 °C and pH 9 for 3 days in duplicates, for the determination of proteolytic activity. After incubation period was over, PW 10^{-2} A2 was observed to have more discreet clear zone around the colonies (13mm) and was sub cultured to get more discreet colony (Appendix I) which revealed the production of protease most predominantly, PW 10^{-2} B2 had many colonies (>300) but little diameter zone of hydrolysis (2mm), followed by PW 10^{-2} A1 with 22 colonies and 4 mm ZOH. Table 1 revealed the screening of the samples for protease producing organisms. The isolate was sub cultured and maintained in NB media for future tests.

Table 1: Description of colonies grown from both the water samples from fish bond (FW) and poultry farm (PW)

Sample codes	No. Of colonies that showed zone of hydrolysis (ZOH)	Diameter of zones (mm)	Colony description
PW10 ⁻² A1	22	4	Milky tiny circular colonies with little ZOH
PW10 ⁻² A2	3	13	Milky large colonies with ZOH
PW10 ⁻³ B1	-	-	-
PW 10 ⁻³ B2	>300	2	Milky tiny circular colonies with little ZOH
FW10 ⁻² A1	2	Insignificant	Milky tiny circular colonies with little ZOH
FW10 ⁻² A2	-	-	-
FW10 ⁻³ B1	2	-	Milky circular colonies with no ZOH
FW10 ⁻³ B2	4	-	Milky circular colonies with no ZOH

3.2 Molecular Phylogeny and Identification of the Strain of Microorganism

Genomic DNA of the selected bacterial isolate PW10⁻²A2 was extracted and the sequence was aligned and compared with the bacterial sequences available in the GenBank database. The Blast result showed that the isolate is 99.84 % homologous to the nucleotide of *Bacillus tropicus* strains MW971701.1. A phylogenetic tree was constructed as shown in Figure 1 based on

16S rRNA gene sequences of isolate (*Bacillus tropicus* strains MW971701.1) Table 2. This was completed in the National centre for biotechnology information (NCBI) website. Homologies of the gene sequences were checked and compared with the sequences of the NCBI. NCBI accession number were also assigned to the bacterial strain as seen in Table 2.

Table 2: Blasting profile of the alkaline proteolytic bacterial isolate

Parameter	Description
ID NO	5_785F_D11_11.ab1
Description	<i>Bacillus tropicus</i> strain INV FIR211 16S ribosomal RNA gene partial sequence
Scientific name	<i>Bacillus tropicus</i>
Max score	1147
Total score	1147
Query cover	100%
E value	0.0
% ID	99.84%
Accession LENGTH	1419
Accession NO from GenBank	MW971701.1



Figure 1: Phylogenetic tree constructed based on 16S rRNA gene sequences of *Bacillus tropicus* (MW971701.1) obtained from the GenBank database

3.3 Zymogram

Molecular mass of the enzyme activity was assessed using zymogram electrophoresis. The cell-free supernatant of the selected bacterial strain *Bacillus tropicus* PW10⁻²A2 was subjected to polyacrylamide gel electrophoresis to separate

and determine the molecular weight of the extracellular alkaline protease. The zymogram showed two distinct casein-resolution luminous areas on the gel with molecular masses of between 36 and 116 kDa (Figure 2).

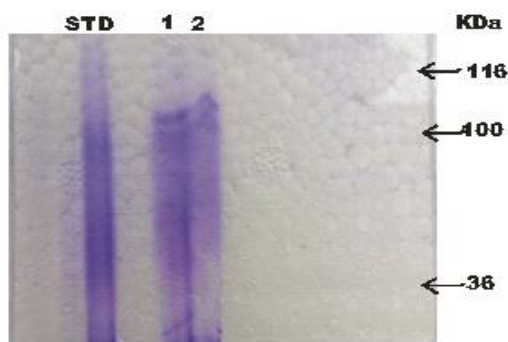


Figure 2: Proteolytic zymogram of *Bacillus tropicus* PW10⁻²A2 cell-free supernatant.

Key: Lane STD: SigmaMarker™ (Sigma-Aldrich, St. Louis); Lane 1, 2: *Bacillus tropicus* cell-free supernatant

3.4 Kinetics Profile on Growth of Protease production by *Bacillus tropicus* PW10⁻²A2

A kinetic study of protease production by *Bacillus tropicus* PW10⁻²A2 was carried out, providing fairly good quantitative information regarding the behavior of a system (Table 3), which is essential for the study of fermentation process. The utilization of sugar by Dinitro Salicylic Acid (DNS) method of the system was determined. Specific growth rate, product yield coefficient, growth yield coefficient, specific product yield, specific growth yield coefficient and productivity was determined at every incubation time as shown in Table 3. Exploitation of such kinetics analysis would be useful in marketing microbial enzyme production.

Table 3: Kinetic parameters of growth and protease production for batch fermentation by parameters of protease production by *Bacillus tropicus* PW10²A2 at optimized condition

Incubation time (h)	Specific growth rate (μ_{max})	Product yield coefficient	Growth yield coefficient g^{-1}	Specific product yield (U/h)	Specific growth yield coefficient $g^{-1} h^{-1}$	Protease activity (U/ml)	Productivity (U/h)
24	0.1001	100.2436	25.0392	4.1768	1.0433	115.21	4.8004
48	0.0539	81.7943	27.42	2.1914	0.5713	105.1875	2.1914
72	0.0389	61.8337	36.7585	1.4774	0.5105	106.3725	1.4774
96	0.0303	74.2769	40.6549	1.4793	0.4235	142.0175	1.4793
120	0.0206	65.3806	42.7569	1.0951	0.3563	131.415	1.0951

3.5 Application of Alkaline Protease Produced by *Bacillus tropicus* PW10⁻²A2

Blood was stained on white cotton materials and washed with different solution, which the alkaline protease produced by the above-mentioned isolate was one of them. The result revealed that, E - the combination of protease solution with detergent resulted in complete stain removal, seconded by C- detergent alone and then D, B (Figure 3)

Cow skins were soaked in alkaline protease produced by *Bacillus tropicus* PW10⁻²A2 after 96 hours and sterilized water of the same value (100 ml) and condition, after 24 h the cow skin in alkaline protease was seen to peel off effortlessly than that of sterilized water as seen in Figure 4A - B. Alkaline protease from *Bacillus tropicus* PW10⁻²A2 was efficient in unhairing cow skins after 24 h more than the counterpart.



Figure 3: Washing performance analysis test of alkaline protease produced by *Bacillus tropicus* PW10⁻²A2

Key: (A) Control: untreated stained cloth pieces or stained cloth pieces washed with, (B) distilled water (50 ml), (C) Detergent (0.05 g/50 mL), (D) alkaline protease produced by *Bacillus tropicus* PW10⁻²A2 (50ml), (E) alkaline protease produced by *Bacillus tropicus* MW971701.1 (50 mL) + detergent (0.5g)



Figure 4A - B: Dehairing ability of alkaline protease produced by *Bacillus tropicus* PW10⁻²A2 on cow skin against the negative control. Key: A- Alkaline protease produced by *Bacillus tropicus* PW10⁻²A2; B - in sterilized water.

4. Discussion

The successful isolation of protease-producing bacteria from poultry farm water (PW) and fish pond water (FW) confirms these environments as rich reservoirs of industrially relevant microorganisms. The superior performance of isolate PW10⁻²A2, with a ZOH of 13 mm, indicates high extracellular protease secretion, as larger hydrolysis zones correlate with increased enzymatic activity (Sharma *et al.*, 2019; Umennadi *et al.* 2026a; 2026b). Interestingly, isolate PW10⁻²B2 exhibited dense growth (>300 colonies) but low proteolytic activity (2 mm ZOH), suggesting that microbial abundance does not necessarily equate to enzyme productivity, a trend also reported by some researchers (Iheukwumere *et al.*, 2012a; 2012b; Mundi *et al.*, 2013; 2014; Okoye *et al.* 2013; Okoye *et al.* 2014; Okoye *et al.*, 2016a; 2016b; Razzaq *et al.*, 2019; Alisa *et al.*, 2020).

Molecular identification revealed 99.84% homology with *Bacillus tropicus*, a species increasingly recognized for its enzymatic capabilities. The use of 16S rRNA sequencing and phylogenetic analysis aligns with current best practices in microbial taxonomy and ensures accurate strain identification (Janda & Abbott, 2021; Anameze *et al.* 2023; Ezeamama *et al.* 2025a; 2025b; Umezulora *et al.*, 2026a; 2026b).

Zymogram analysis revealed two protease isoforms (36 – 116 kDa), indicating the presence of multiple proteolytic enzymes. This multiplicity may enhance substrate versatility and stability under varying industrial conditions (Ghorbel-Frikha *et al.*, 2022; Enemchukwu *et al.* 2026b). Similar findings have been reported for *Bacillus* spp., where multiple proteases contribute to improved catalytic efficiency.

Kinetic analysis demonstrated that maximum protease activity (142.02 U/mL) occurred at 96 h, coinciding with the late exponential or early stationary phase of bacterial growth. This observation is consistent with previous reports that protease production in *Bacillus* species is growth-associated but peaks during nutrient limitation (Contesini *et al.*, 2018). The decline in activity at 120 h suggests possible enzyme degradation or feedback inhibition.

The enzyme's effectiveness in stain removal, particularly when combined with detergent, highlights its compatibility with commercial formulations. Proteases enhance detergent performance by hydrolyzing protein-based stains such as blood, a property widely exploited in laundry industries (Sundararajan *et al.*, 2021; Kotb *et al.*, 2023).

Furthermore, the efficient dehairing of cow skin within 24 h demonstrates the enzyme's potential in eco-friendly leather processing. Traditional chemical methods involve harsh chemicals like sodium sulfide, which pose environmental and health risks. Enzymatic dehairing offers a sustainable alternative, reducing pollution and improving leather quality (Abdel-Fattah *et al.*, 2020; Kotb *et al.*, 2023).

5. Conclusion

This study successfully isolated and characterized a potent alkaline protease-producing bacterium identified as *Bacillus tropicus* PW10⁻²A2 from poultry farm water. The isolate exhibited high proteolytic activity, optimal enzyme production at 96 h (142.02 U/mL), and multiple protease isoforms (36 – 116 kDa). Its demonstrated efficiency in detergent-assisted stain removal and enzymatic dehairing highlights its strong industrial potential. These findings support the application of *Bacillus tropicus* PW10⁻²A2 as a sustainable and cost-effective source of alkaline protease for detergent and leather industries.

References

- Abdel-Fattah, Y. R., Saeed, H. M., Gohar, Y. M., & El-Baz, M. A. (2020). Improved production of alkaline protease by *Bacillus licheniformis* using fed-batch fermentation. *Biocatalysis and Agricultural Biotechnology*, 25, 101601. <https://doi.org/10.1016/j.bcab.2020.101601>.
- Afulukwe, S. C., Emmy-Egbe, I. O., Anyaegbulam, L. C., Uba, B. O., Obi-Ezeani, C. N., Akulue, J. C., Egbe, P. A., & Nnoruka, O. E. (2026). Evaluation of Biochemical Indices of Liver and Kidney Tissues of Albino Wistar Rats Treated with Anthelmintic Drug (Albendazole). *IPS Interdisciplinary Journal of Biological Sciences*, 6 (1): 211 – 220. <https://doi.org/10.54117/ijjbs.v6i1.120>.
- Afulukwe, S. C., Uba, B. O., Okemadu, O. C., Akulue, J. C., Akudu, L. S., & Anaebonam, E. C. (2025). Histopathological Examination of Liver and Kidney Tissues of Albino Wistar Rats Treated with Albendazole Drug. *Health Science Research International*, 2(1): 10 – 22. <https://doi.org/10.54117/hsri.v2i1.54>.
- Alfred, P.N., Mbachu, I.A.C. and Uba, B.O. (2023). Water Quality Indices and Potability Assessment of Three Streams in Akwa North and South Local Government Areas, Anambra State, Nigeria. *Journal of Applied Sciences and Environmental Management* 27 (2):223 – 228. <https://dx.doi.org/10.4314/jasem.v27i2.6>.
- Alfred, P. N., Mbachu, I. A. C., Uba, B. O., Iweriolor, S.N. and Okemadu, O.C. (2025). Bacterial Pathogen Community Profiling of Three Freshwater Bodies in Akwa North and South Local Government Areas, Anambra State, Nigeria. *IPS Journal of Public Health*, 5 (3): 302 - 309. <https://doi.org/10.54117/rrrmk019>.
- Alisa, O. C., Anukam, N. B., Ogukwe, N. C., Chinwuba, J. A. and Uba, B. O. (2020). Determination of compost humification and other constituents that can be used as stability index. *International Research Journal of Modernization in Engineering Technology and Science*, 2 (12): 139 – 147. <https://www.irjmet.com/paperdetail.php?paperId=47fd0a18a604b911124f7def72690f1c>
- Anameze, C.I., Emmy-Egbe, I.O., Anyaegbulam, L.C., Ogomaka, I.J., Uba, B.O., Odumodu, O.A., Ezeigwe, C., Kamalu, N.L., Chukwubude, C.B., Akogu, O., Ezekweme, E., Emmy-Egbe, C.C., Obiefoka, O.S., Ezenwata, S.I. and Ilechukwu, C.C. (2023). Qualitative and quantitative phytochemical analysis of *Gongronema latifolium* leaf extract. *IPS Journal of Applied Microbiology and Biotechnology* 2 (1): 16 – 19. <https://doi.org/10.54117/ijamb.v2i1.10>.
- Anichebe, C.O., Uba, B. O., Okoye, E. L. and Onochie, C.C. (2019). Comparative study on single cell protein (SCP) production by *Trichoderma viride* from pineapple wastes and banana peels. *International Journal of Research Publications*, 23 (1): DOI: 10023122019517.
- Anidu, F.N., Uba, B.O., Ezemba, C.C., Okoye, E.L. and Dokubo, C.U. (2023). Study on optimization, degreasing and destaining potentials of glycopospholipid biosurfactant produced by *Bacillus anthracis* S62A. *Dutse Journal of Pure and Applied*

- Sciences 9 (1a): 29 – 43. <https://dx.doi.org/10.4314/dujopas.v9i1a.4>.
- Anukam, N. B., Alisa, O. C., Ogukwe, N. C., Chinwuba, J. A., Uba, B. O. and Ogukwe, E. C. (2020a). Phyto – toxicity evaluation of agro – waste formulated compost on five different plant seeds. *The International Journal of Engineering and Science*, 9 (12): 21 -26. [C0912012126.pdf](https://doi.org/10.54117/ijjbs.v6i2.161) Or <https://dx.doi.org/10.9790/1813-0912012126>.
- Anukam, N. B., Alisa, O. C., Ogukwe, N. C., Chinwuba, J. A., Uba, B. O. and Ogukwe, E. C. (2020b). Physico–chemical evaluation of agro–waste formulated compost from five different waste source. *American Journal of Applied Chemistry*, 8 (6): 130 – 134. doi: <https://dx.doi.org/10.11648/j.ajac.20200806.11>.
- Chukwura, E. I., Uba, B. O., Dibua, N. A., Chude, C. O., Okoye, E. C. S., Ubajekwe, C.C., Eleanya, L. C., Agbo, B. C. and Nwajiobi, F. O. (2025). Physicochemical and bacteriological quality assessment of Ogbunike abattoir wastewater Anambra State, Nigeria for irrigation purpose. *Journal of Global Ecology and Environment* 21 (3): 378 – 385. doi: [10.56557/jogee/2025/v21i39625](https://doi.org/10.56557/jogee/2025/v21i39625).
- Contesini, F. J., Melo, R. R., & Sato, H. H. (2018). An overview of Bacillus proteases: From production to application. *Critical Reviews in Biotechnology*, 38(3), 321–334. <https://doi.org/10.1080/07388551.2017.1354354>
- Dokubo, C. U., Uba B. O., Nnubia, C.P. and Akaun, I.P. (2022a). Evaluation of toxicity and resistant effects of heavy metals and antibiotics on the growth of marine bioluminescent bacteria. *International Journal of Frontline Research in Science and Technology* 01 (02): 030 – 037. <https://doi.org/10.56355/ijfrst.2022.1.2.0041>.
- Dokubo, C. U., Uba B. O. and Nnaji, I. G. (2022b). Combined coagulation and disinfection efficiencies of *Mangifera indica*, *Carica papaya* and solar disinfection on synthetic agro - waste water. *International Journal of Advanced Multidisciplinary Research and Studies* 2 (4):789 - 793. <https://www.multiresearchjournal.com/arclist/list-2022.2.4/id-437>.
- Dokubo, C.U. and Uba, B.O. (2023). Assessment of the decontamination and disinfecting potentials of *Ocimum gratissimum* synthesized silver nanoparticles on water and wastewater samples. *IPS Journal of Public Health* 3 (2): 58 – 65. <https://doi.org/10.54117/ijph.v3i2.20>.
- Dokubo, C.U., Mbachui, I.A.C., Umeaku, C.N. and Uba, B.O. (2024). Isolation, screening and identification of multi – metal resistant fungi isolated from biogas slurry sample. *Tropical Journal of Applied Natural Sciences*, 2 (2): 140 – 159.
- Dokubo, C.U. and Uba, B.O. (2026). Green synthesis of Calcium oxide Nanoparticles by Endophytic Fungi for Sustainable Textile and Leather Wastewater Remediation. *IPS Journal of Applied Microbiology and Biotechnology*, 6(1), 378 - 396. <https://doi.org/10.54117/ijamb.v6i1.14>.
- Egurefa, S.O., Orji, M.U. and Uba, B.O. (2020a). Toxic effect of refinery industrial effluent using three toxicity bioassays. *South Asian Journal of Research in Microbiology*, 6 (2): 10 – 23.
- Egurefa, S.O., Orji, M.U. and Uba, B.O. (2020b). Toxicological evaluation of two Nigerian refinery effluents using natural biomarkers. *Research & Reviews: A Journal of Toxicology*, 10 (2): 22 – 31.
- Ekwenze, T. N., Uba, B. O., Dibua, N. A., Ike, V. E., Mere, C. A., & Chikwendu, J. C. (2025). Effect of Biosynthesized Nanoparticles on the Germination Profile of Zea mays Under Salinity Stress. *IPS Journal of Agriculture, Food Technology and Security*, 2(1), 53–59. <https://doi.org/10.54117/ijafst.v2i1.72>.
- Ele, E.E., Okoye, E.L., Uba, B.O., Aniekwu, C.C., Iheukwumere, C.M., Obumseli, H. and Okoye, P.A. (2024). Antibacterial effects of phytofabricated silver nanoparticles against some selected bacteria. *International Journal of Research and Innovation in Applied Science* 9 (10): 460 – 467. <https://doi.org/10.51584/IJRIAS>.
- Enemchukwu, C. N., Lukong, C. B., Nwaka, A. C., Uba, B. O., Ifemeje, J. C., Mere, C. A., & Igrid, V. C. (2026a). Green synthesis of eco-friendly potassium nanoparticles immobilized lipase enzyme and its potentials in biodiesel production. *International Journal of Global Trends and Research*, 3 (1): 66 – 76. <https://doi.org/10.54117/n3bqr651>.
- Enemchukwu, C. N., Lukong, C. B., Nwaka, A. C., & Uba, B. O. (2026b). Isolation of Lipase from Soybean Seeds and Its Immobilization in Calcium Alginate Beads. *IPS Journal of Biotechnology and Applied Biochemistry*, 2(1), 93–100. <https://doi.org/10.54117/ijbab.v2i1.118>.
- Ezeamama, M. M. C., Chukwura, E. I., Uba, B. O., Chikwendu, J. C., Ubajekwe, C. C., Ike, V. E., & Egbe, P. A. (2025a). Evaluation of the Urease Inhibitory, Antiulcer and Acute Toxicity Effects of Ethanolic Seed Extracts of *Garcinia Kola* against Chemically Induced Ulcers. *IPS Journal of Phytochemistry and Medicinal Plant Research*, 1(2): 20 – 26. <https://doi.org/10.54117/ijpmpr.v1i2.4>.
- Ezeamama, M. M. C., Chukwura, E. I., Uba, B. O., Iheukwumere, I. H., Awari, V. G., Ike, V. E., & Agu, K. C. (2025b). Assessment of the Phytochemical and Antibacterial Profiles of Aqueous and Ethanolic Extracts of *Garcinia Kola* Seed. *IPS Journal of Drug Discovery Research and Reviews*, 3(2): 51 – 56. <https://doi.org/10.54117/ijddr.v3i2.39>.
- Ghorbel-Frikha, B., Sellami-Kamoun, A., & Haddar, A. (2022). Production and characterization of proteases from *Bacillus* species: A review. *International Journal of Biological Macromolecules*, 194, 1023–1035. <https://doi.org/10.1016/j.ijbiomac.2021.11.145>.
- Ibe, C.O., Mbachui, I.A.C. and Uba, B.O. (2023). Analysis and characterization of untreated greywater obtained from Enugu Metropolitan. *Tropical Journal of Applied Natural Sciences* 1 (1): 1 – 17. <https://tjansonline.org/view-paper.php?id=20>.
- Ibo, E.M., Umeh, O.R., Uba, B.O. and Egwuatu, P.I. (2020). Bacteriological assessment of some borehole water samples in Mile 50, Abakaliki, Ebonyi State, Nigeria. *Archives of Agriculture and Environmental Science* 5 (2): 179 – 189. <https://doi.org/10.26832/24566632.2020.0502015>.
- Idu, P. N., Chukwura, E. I., Uba, B. O., Okoli, F. A., & Oghonim, P. A. N. (2026a). Assessment of the bacteriological quality of selected surface water resources in Anambra Central Senatorial Zone, Anambra State. *Journal of Public Health, Policy, and Society*, 3(1), 87–100. <https://doi.org/10.54117/b8kkjp54>.
- Idu, P. N., Chukwura, E. I., Okonkwo, I. F., Uba, B. O., & Oghonim, P. A. N. (2026b). Microbial Diversity Metrics: An Insight into the Ecological Status of Different Water Resources in Anambra State Central Senatorial Zone, Nigeria. *IPS Interdisciplinary Journal of Biological Sciences*, 6(1), 257–271. <https://doi.org/10.54117/ijjbs.v6i1.143>.
- Ifediegwu, M. C., Uba, B.O., Awari, V., Chukwujekwu, A. G. and Akaun, I. P. (2023a). Post-reclamation evaluation of residual hydrocarbons in crude oil contaminated soil using gas chromatographic techniques and plant growth indices. *Journal of Pollution Monitoring, Evaluation Studies and Control*, 2 (1): 15 – 29.
- Ifediegwu, M. C., Onuora, S. C., Uba, B.O., Okoye, E. L., Egurefa, S. O. and Awari, V. G. (2023b). Assessment of the plasmid mediated biodegradation of crude oil under optimal growth conditions. *IPS Interdisciplinary Journal of Biological Sciences*, 2(1): 32 – 44.
- Ifediegwu, M.C., Uba, B.O., Awari, V.G. and Okongwu, D.J. (2024a). Biodegradation of bonny light crude oil by plasmid and non-plasmid borne soil bacterial strains using biostimulation and bioaugmentation techniques. *Science World Journal*, 19 (1): 178 – 188.
- Ifediegwu, M.C., Orji, M.U., Onuora, S.C. and Uba, B.O. (2024b). Evaluation of the degrading potentials of plasmid and non-

- plasmid borne soil bacterial strains on Bonny light crude oil. *Archives of Agriculture and Environmental Science* 9(1): 14 – 22.
- Ifediegwu, M.C., Orji, M.U., Onuorah, S.C. and Uba, B.O. (2024c). Exploration of the catabolic plasmid genes profile of crude oil degrading bacteria isolated from aged oil contaminated soils of Anambra State. *Scientia Africana*, 23 (1): 11 – 30.
- Ihekumwere, I., Uba, B.O. and Ubajekwe, C.C (2012). Antibacterial activity of *Annoria muricata jmmmn* and *Persca americana* leaves extracts against ampicillin resistant *S. aureus*. *Journal of Science, Engineering Technology*, 19(2): 10786-10798.
- Ihekumwere, I., Uba, B.O. and Ubajekwe, C.C (2012). Anti-fungal, haematological and wound healing activity of *Mucuna pruriens* leaves extracts. *Journal of Applied Science*, 15(2): 10541 - 10550.
- Janda, J. M., & Abbott, S. L. (2021). 16S rRNA gene sequencing for bacterial identification in clinical microbiology. *Journal of Clinical Microbiology*, 59(1), e01228-20. <https://doi.org/10.1128/JCM.01228-20>
- Kotb, E.; Alabdallal, A.H.; Alsayed, M.A.; Alghamdi, A.I.; Alkhalidi, E.; AbdulAzeez, S.; Borgio, J.F. Isolation, Screening, and Identification of Alkaline Protease-Producing Bacteria and Application of the Most Potent Enzyme from *Bacillus* sp. Mar64. *Fermentation* 2023, 9, 637. <https://doi.org/10.3390/fermentation9070637>
- Mere, C. A., Uba, B. O., Dim, C. N. (2025). Reducing Potentials of *Pennisetum Glaucum* and *Sorghum bicolor*. *Tropical Journal of Applied Natural Sciences*, 3 (1): 9.
- Mundi, K.S., Okoye, E.L., Uba, B.O., Esimone, C.O. and Attama, A.A. (2013). Evaluation of the antibacterial activity of some commercial disinfectants against methicillin-resistant *Staphylococcus aureus*. *International Journal of Applied Science and Engineering* 1 (1): 19 – 22. <http://dx.doi.org/10.2139/ssrn.3448993>.
- Mundi, S.K; Okoye, E.L., Uba, B.O., Esimone, C.O, and Attama, A.A. (2014). The combined antibacterial activity of face cleaning agent and *Psidium guajava* leaf extract on methicillin resistant *Staphylococcus aureus*. *International Journal of Agriculture and Biosciences* 3 (2): 77 – 81. <https://www.ijagbio.com/pdf-files/volume-3-no-2-2014/77-81.pdf>
- Njoku, N.O., Mbachu, I.A.C. and Uba, B.O. (2019a). Impact of cow dung on the physicochemical and metabolic indicators during composting of agro wastes. *Tropical Journal of Applied Natural Sciences*, 2 (3): 59 – 70.
- Njoku, N.O., Mbachu, I.A.C. and Uba, B.O. (2019b). Influence of physicochemical and microbiological properties on the composting of agro wastes using cow dung as a booster. *Animal Research International*, 16 (1): 3238 – 3246.
- Nkamigbo, P.N., Mbachu, I.A.C. and Uba, B.O. (2020a). Investigation of the toxic effects of herbicides on some selected microbial populations from soil. *World Journal of Advanced Research and Reviews*, 06 (01): 40 – 49.
- Nkamigbo, P.N., Machu, I.A.C. and Uba, B.O. (2020b). Influence of glyphosate and 2, 4 - D amine herbicides on soil metabolic processes. *Research & Reviews: A Journal of Biotechnology*, 10 (1): 1 – 11.
- Nnaka, O. B., Umeaku, C.N., Uba, B.O., Anyene, C. C. and Nkachukwu, M. B. (2024). Determination of the effect of mycoremediation on the physicochemical properties of hydrocarbon polluted soils of the Niger Delta region of Nigeria. *Tropical Journal of Applied Natural Sciences*, 2 (1): 1 – 18.
- Nwigwe, V.N. and Uba, B.O. (2022). Role of electrochemically active bacteria in the treatment of piggery and poultry wastewaters from Umuagwo in Ohaji Egbema Local Government Area of Imo State, Nigeria. *Journal Applied Science and Environmental Management* 26 (12): 2085 – 2093. <https://dx.doi.org/10.4314/jasem.v26i12.24>.
- Nwigwe, V. N., Nwigwe, H. C., Okereke, J. N., Uba, B.O. and Dokubo, C.U. (2023). Potential of agro-based industrial wastewater as an alternative substrate for bioelectricity. *Animal Research International* 20 (1): 4741 – 4747. <https://www.ajol.info/index.php/ari/article/view/246974>.
- Obiefoka, S.O., Emmy-Egbe, I.O., Anyaegbunam, L.C., Uba, B.O., Anameze, C.I., Ogoamaka, I.J., Kamala, N.L., Ezeigwe, C., Akaogu, O., Odumodu, O.A., Emmy-Egbe, C.C., Ezenwata, O.S. and Chukwubude, C.B. (2023). The Prevalence of Lymphatic Filariasis Infection among Primary School Children (5-9 Years) of Infected Adults in Ihiala Local Government Area of Anambra State, Nigeria. *IPS Journal of Public Health*, 3 (2): 66 - 72.
- Obiefuna, O. H., Nzekwe, C. M., Onuorah, S. C., Uba, B. O., Ubajekwe, C. C., Okey-Ndeche, N. F., and Ike, V. E. (2025). Assessment of the seasonal impact on physicochemical quality of borehole water samples in Emene, Enugu State, Nigeria. *IPS Journal of Public Health* 5 (4): 422 – 430. <https://doi.org/10.54117/8rr3ms81>.
- Obiefuna, O. H., Onwuofor, E. C., Nduka, A. C., Uba, B. O., Ebenebe, I. N., Ngozika, F. O. N., Mere, C.A. and Egbe, P.A. (2026). Molecular Analysis and *In Vitro* Pathogenicity Evaluation of Bacteria Isolated from Frozen Chicken. *IPS Journal of Nutrition and Food Science*, 6(1): 755 - 763.
- Ofunwa, J.O., Mbachu, I.A.C., Umeaku, C.N. and Uba, B.O. (2024). Impact of composting on the physical factors of municipal solid waste materials with organic additives in Ihiala Anambra State. *Tropical Journal of Applied Natural Sciences*, 2 (2): 94 – 112.
- Ofunwa, J. O., Anyanwieze, B. U., Uba, B. O., Anaebonam, E. C., Mere, C. A., Okongwu, D. J., & Onwudinjo, U. F. (2026a). Monitoring Compost Maturity Using Enzymatic Indicators in Microbial and Nanocomposite-Treated Systems. *IPS Journal of Biotechnology and Applied Biochemistry*, 2(1), 142–153. <https://doi.org/10.54117/ijbab.v2i1.154>.
- Ofunwa, J. O., Anyanwieze, B. U., & Uba, B. O. (2026b). Comparative assessment of microbial and nanocomposite amendments on compost maturity and decomposition kinetics. *International Journal of Global Trends and Research*, 3(2), 224–237. <https://doi.org/10.54117/bn8kqt92>.
- Oghonim, P. AN., Uba, B. O., Afulukwe, S.C., Dokubo, C.U., Okongwu, D.J., Mere, C.A. and Anaebonam, E.C. (2026a). Molecular Identification, Phylogenetic Analysis, and Antibiotic Susceptibility Profiles of Bacteria Isolated from Borehole Water across Seasonal Variations. *Interdisciplinary Journal of Biological Sciences*, 6(2): 272 - 283.
- Oghonim, P. AN., Onuorah, S. C., Onyima, C. S., Idu, P. N., Uba, B. O., Onyekpeze, C. E., Agbakhamon, E., Alari, E. J., Osunde, G., & Ottah, F. (2026b). Public health risk assessment of heavy metal contamination in abattoir wastewater-irrigated soil and edible crops in Agbor, Delta State, Nigeria. *INOSR Applied Sciences*, 14(1): 9 – 17.
- Okafor, F.N., Orji, M.U., Onuorah, S.C., Uba, B.O., Dokubo, C.U. and Ofunnwa, J.O. (2021a). *In vitro* Interactive Toxicity of Binary Mixtures of Selected Herbicides on *Lysinibacillus fusiformis*. *Asian Journal of Biology* 12(3): 30-41. <https://dx.doi.org/10.9734/AJOB/2021/v12i330165>.
- Okafor, F.N., Orji, M.U., Nweke, C. O., Onuorah, S.C., Uba, B.O. and Dokubo, C.U. (2021b). Toxicity of Quaternary Mixture of Formulated Glyphosate and Phenols on *Providencia vermicola* Dehydrogenase Activity. *Archives of Current Research International* 21(4): 1 – 10. <https://dx.doi.org/10.9734/ACRI/2021/v21i430239>.
- Okafor, C. A., Uba, B.O. and Dokubo, C.U. (2023). Application of myco-fabricated silver nanoparticle in the adsorption malachite green and trypan blue from aqueous solution. *Nigerian Journal of Life Sciences* 12 (2): 8 – 15. <https://doi.org/10.52417/njls.v12i2.354>.
- Okeke, M. I., Okpalla, J., and Uba, B. O. (2025a). Antibiotic Resistant Profile Of The Bacterial Strains Isolated From Goat And Rabbit Meat Obtained From Local Meat Vendors. *Tropical Journal of Applied Natural Sciences*, 3 (1), 8.

- Okeke, M. I., Okpalla, J., and Uba, B.O. (2025b). Bacterial Load, Haemolytic and Enzymatic Activity Profile of Bacterial Strains in Goat And Rabbit Meat Samples Obtained From Local Meat Vendors. *Tropical Journal of Applied Natural Sciences*, 3 (1): 7.
- Okolo, O.C., Uba, B. O. and Ike, V.O. (2025). Influence of untreated noodle wastewater on physicochemical, enzymatic and bacteriological dynamics of soil. (2025). *Journal of Pollution Monitoring, Evaluation Studies and Control* 4 (2): 110 –119. <https://doi.org/10.54117/jpmesc.v4i2.20.2025>.
- Okonkwo, O. P., Uba, B. O., Ifemeje, J. C., Ozochi, C. A., Okongwu, D. J., & Anaebonam, E. C. (2026). Green Synthesis of Silver Nanoparticles from Aqueous Seed Extract of C. papaya and its Application in Surface Water Resources Decontamination. *IPS Journal of Plant, Animal, and Environmental Sciences*, 2(1): 22–31. <https://doi.org/10.54117/ijpae.v2i1.121>.
- Okoye, E.L., Uba, B.O. and Obiukah, O. (2013). Microbial quality of some commercial yoghurt samples in Awka metropolis, Anambra State, Nigeria. *Journal of Pharmaceutical and Allied Sciences*, 10 (1): 1677 – 1683.
- Okoye, E.L., Uba, B.O., Uhobo, P.C., Oli, A.N. and Ikegbunam, M.N. (2014). Evaluation of the antibacterial activity of methanol and chloroform extracts of *Alchornea cordifolia* leaves. *Journal of Scientific Research and Report* 3 (1):255 – 262. <https://journaljsrr.com/index.php/JSRR/article/view/1692/3353>.
- Okoye, E.L., Obiweluzor, C.J., Uba, B.O. and Odunukwe, F.N. (2016a). Epidemiological survey of tonsillitis caused by *Streptococcus pyogenes* among children in Awka Metropolis (A case study of hospitals in Awka Community, Anambra State). *IOSR Journal of Pharmacy and Biological Sciences*, 11 (3): 54 – 58.
- Okoye, E.L., Ozumba, A.I., Uba, B.O. and Odunukwe, F.N. (2016b). Prevalence of Hepatitis B Virus among immunocompromised individuals attending Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi. *Journal of Pharmaceutical and Allied Sciences*, 13 (2):2407 - 2413.
- Okoye, E. L., Uba, B. O., Dike, U. C. and Eziefule, U. J. (2020a). Growth rate and antifungal activities of acetone extracts of *Ocimum gratissimum* (Scent Leaf) and *Allium sativum* (Garlic) on cassava and banana peels formulated media. *Journal of Advances in Microbiology*, 20 (4): 19 – 29.
- Okoye, E. L., Uba, B. O. and Ugwuoke, C. J. (2020b). Determination of the growth rate and susceptibility pattern of fungi using agro-waste formulated media. *Nigerian Journal of Microbiology*, 34(2): - 5258 – 5268.
- Okoye, E. L., Uba, B. O. and Onwunyili, C. E. (2020c). Antibacterial activity and protein sequences of actinomycetes isolated from coastal area of Niger Delta against human and fish pathogens. *International Journal of Biosciences and Technology*, 13 (1): 1 – 17.
- Okoye, C. P., Mbachu, I. A. C., Uba, B. O., Okongwu, D. J., Mere, C. A., Anaebonam, E. C. and Dokubo, C. U. (2026). Chemical Oxygen Demand Reduction Potential of Halotolerant Bacterial Consortia in Saline Wastewater Treatment System. *International Journal of Global Trends and Research*, 3 (2): 199 - 203.
- Okpalaunegbu, C.A., Chinweuba, A.J., Ojiako, E.N., Uba, B.O. and Okafaonyali, J.O. (2025). Physicochemical properties and heavy metal analysis of sewage and leachate wastewater collected from the Sewage Tank at the University of Nigeria, Nsukka and the First Market Municipal Dumpsite, Ifite-Awka, Anambra State. *Journal of Global Ecology and Environment* 21 (3): 320 – 332. <https://doi.org/10.56557/jogee/2025/v21i39583>.
- Prabhavathy, G., Rajan, S., & Sundararajan, M. (2013). Production and characterization of alkaline protease by *Bacillus subtilis* isolated from soil. *Journal of Microbiology and Biotechnology Research*, 3(1), 102–108
- Razzaq, A., Shamsi, S., Ali, A., Ali, Q., Sajjad, M., Malik, A., & Ashraf, M. (2019). Microbial proteases applications. *Frontiers in Bioengineering and Biotechnology*, 7, 110. <https://doi.org/10.3389/fbioe.2019.00110>
- Sharma, K. M., Kumar, R., Panwar, S., & Kumar, A. (2019). Microbial alkaline proteases: Optimization of production parameters and their properties. *Journal of Genetic Engineering and Biotechnology*, 17(1), 1–13. <https://doi.org/10.1186/s43141-019-0008-4>
- Sundararajan, S., Kannan, C. N., & Chittibabu, S. (2021). Alkaline protease from *Bacillus* species: Applications in detergent industry. *Biotechnology Reports*, 30, e00658. <https://doi.org/10.1016/j.btre.2021.e00658>
- Uba, B.O., Okoye, E.L. and Chukwura, E.I. (2016). Bioremediating potentials of marine mercury-resistant bacteria on polyaromatic hydrocarbons components of Bonny light crude oil. *Journal of Advances in Biology and Biotechnology*, 7 (4): 1- 12.
- Uba, B. O., Okoye, E. L., Ekwueme, C., Azubike, T. C. and Ugoma, J.C. (2017). Heavy metals and antibiotics resistance pattern of bacteria isolated from brewery and plastic industries effluent waste. *African Journal of Education, Sciences and Technology*, 3(3): 43 – 50.
- Uba, B. O. (2018a). Effect of aromatic hydrocarbons and marine sediments from Niger Delta on the growth of microalga *Phaeodactylum tricorutum*. *Biotechnology Journal International*, 22 (4): 1 – 18.
- Uba, B. O. (2018b). Growth profile and catabolic pathways involved in degradation of aromatic hydrocarbons by marine bacteria isolated from Niger Delta. *Microbiology Research Journal International*, 26 (5): 1 - 18.
- Uba, B. O., Chukwura, E. I., Okoye, E. L., Ubani, O., Irabor, M. I., Onyekwuluje, N. V., Ajeh, J. E., Muogbo, C. S., Nwafor, M. C., Igboesorom, C. C., Nwodo, C. J., Okafor, J. C. and Nwachukwu, C. J. (2018a). Multiple degradation and resistant capabilities of marine bacteria isolated from Niger Delta, Nigeria on petroleum pollutants and heavy metals. *Journal of Advances in Biology and Biotechnology*, 20 (1): 1 -17.
- Uba, B. O., Okoye, E. L., Dokubo, C.U., Azuanichie, T. and Nworah, O.M. (2018b). Biostimulatory effect of organic and inorganic nutrients on soil biological indicators in diesel contaminated soil. *Journal of Bioscience and Biotechnology*, 3(6): 121 – 135.
- Uba, B. O., Chukwura, E. I., Okoye, E. L., Umehosi, A.A., Agbapulonwu, U. F., Muogbo, O. C., Okoye, C. L., Oranta, L.O., Odunukwe, A.M., Ndurue, C. P. and Ehirim, O. S. (2018c). Biofilm and biosurfactant mediated aromatic hydrocarbons degradation by marine bacteria isolated from contaminated marine environments of Niger Delta. *Journal of Applied Life Sciences International*, 19 (4): 1 -17.
- Uba, B.O. (2019a). Aromatic hydrocarbons degradation and plasmid profile of marine bacterial isolates obtained from petroleum contaminated marine environments of Niger Delta, Nigeria. *Microbiology Research Journal International*, 27 (1): 1 – 20.
- Uba, B.O. (2019b). Effects of aromatic hydrocarbons and marine water from Niger Delta on the β -galactosidase activity of mutant *Escherichia coli*. *Archives of Current Research International*, 16 (3): 1 – 16.
- Uba, B.O. (2019c). Phylogenetic framework and metabolic genes expression analysis of bacteria isolated from contaminated marine environments of Niger Delta. *Annual Research & Review in Biology*, 30 (5): 1 – 16.
- Uba, B. O., Okoye, E. L., Anyaeji, O.J. and Ogbonnaya, O.C. (2019a). Antagonistic Potentials of actinomycetes isolated from coastal area of Niger Delta against *Citrus sinensis* (Sweet Orange) and *Lycopersicon esculentum* (Tomato) fungal pathogens. *Research and Reviews: A Journal of Biotechnology*, 8 (3): 4 – 15.
- Uba, B.O., Akunna, M.C., Okemadu, O. C. and Umeh, C. J. (2019b). Kinetics of Biodegradation of total petroleum hydrocarbon in diesel contaminated soil as mediated by organic and inorganic nutrients. *Animal Research International*, 16 (2): 3295 – 3307.

- Uba, B. O., Chukwura, E. I., Okoye, E. L., Ubani, O., Chude, C.O. and Akabueze, U. C. (2019c). *In vitro* degradation and reduction of aromatic hydrocarbons by marine bacteria isolated from contaminated marine environments of Niger Delta. *Advances in Research*, 18 (3): 1 - 17.
- Uba, B.O., Okoye, E.L., Ebodi-Henry, J.N. and Okoye, W.K. (2019d). Organic and inorganic nutrients mediated enhanced bioremediation of diesel contaminated soil. *Tropical Journal of Applied Natural Sciences*, 2 (3): 39-51.
- Uba, B.O., Okoye, E.L., Chude, C.O. and Ogamba, J.O. (2020a). Assessment of the toxicity potentials of spent laptop battery wastes on essential soil microbes and plant bioindicators. *Asian Journal of Biology*, 9(2): 33 - 46. <https://doi.org/10.9734/AJOB/2020/v9i230085>.
- Uba, B.O., Okoye, E.L., Nweke, B.G. and Ibeneme, C.P. (2020b). Evaluation of the ecotoxicity potentials of e-waste using *Selenastrum capricornutum* (microalga), *Eisenia fetida* (earth worm) and *Allium cepa* (onion bulb) as bioindicators. *Asian Journal of Biotechnology and Genetic Engineering*, 3(2): 20 - 31. <https://journalajbge.com/index.php/AJBGE/article/view/24>.
- Uba, B.O., Egbujor, J.C. and Umeh, O.R. (2020c). *Selenastrum capricornutum* Prinz, *Zea mays* L. and *Phaseolus vulgaris* L. biomonitor: Natural monitors of spent phone battery toxicity. *Asian Journal of Advanced Research and Reports*, 13 (1): 31 - 41. <https://doi.org/10.9734/AJARR/2020/v13i130300>.
- Uba, B.O., Okonkwo, C.J. and Umeh, O.R. (2020d). Experimental assessment of the toxicity effects of phone battery wastes on aquatic and terrestrial bioindicators. *Asian Journal of Biochemistry, Genetics and Molecular Biology*, 5(1): 17 - 27. <https://doi.org/10.9734/AJBGM/2020/v5i130117>.
- Uba, B. O., Udeh, C.A., Nduneri, C. F. and Akaun, I. P. (2020e). Potentials of carrot (*Daucus carota*) and cocoyam (*Colocasia esculenta*) peels as suitable mycological culture media. *Research & Reviews: A Journal of Life Sciences*, 10 (3): 22 - 29.
- Uba, B. O., Chukwura, E. I., Iheukwumere, I.H., Okeke, J.J. and Akaun, I.P. (2020f). Evaluation of marine waste water and aromatic hydrocarbons toxicity using a battery of assays. *Research & Reviews: A Journal of Toxicology*, 10 (2): 1 - 13.
- Uba, B. O., Obidike, K.N., Dokubo, C.U. and Nnaodi, I.D. (2020g). Bioelectricity generation using marine sediment and cow dung. *EC Microbiology*, 16 (10): 1 - 12.
- Uba, B. O., Okoye, E. L., Nnanna, O. E., Dibua, N. A., Vivian, N. Anakwenze, V.N. and Ifediegwu, M. C. (2020h). Testing for the environmental fate and safety of e-waste using *Nitrobacter* and mice model. *International Journal of Environment, Agriculture and Biotechnology*, 5(6): 1 - 8.
- Uba, B. O., Chukwura, E. I., Okoye, E. L., Ubani, O. and Odibo, F.J.C. (2021a). Toxicological evaluation of aromatic hydrocarbons using toxi-chromo test and mice model. *Indian Journal of Ecology*, 48 (5): 1533 - 1541. <https://indianecologicalsociety.com/wp-content/themes/ecology/fullpdfs/1635504109.pdf>.
- Uba, B. O., Chukwura, E. I., Okoye, E. L., Emmy-Egbe, I. O. and Ubani, O. (2021b). Assessment of Toxicity of Marine Sediment and Aromatic Hydrocarbon Samples using Marine Algal Toxicity and Phytotoxicity Tests. *Indian Journal of Environmental Protection*, 41 (2): 123
- Uba, B. O. and Anidu, F. N. (2023). Evaluation of the characterization and heavy metals remediation potential of biosurfactant produced by *Aeromonas hydrophila* S62A. *Archives of Agriculture and Environmental Science*, 8 (2): 116 - 124.
- Uba, B. O. and Obiefuna, G. O. (2023). Aerobically enhanced nanobioremediation of diesel oil contaminated soil and water using mycosynthesized silver nanoparticle as biostimulating agent. *Science World Journal* 18 (1): 75 - 82. <https://scienceworldjournal.org/article/view/23510>.
- Uba, B.O., Okoye, E.L., Anyichie, J.C., Dokubo, C.U. and Ugwuoji, E.T. (2024). Synthesis, characterization and application of biogenic silver nanoparticles as antibacterial and antifungal agents. *Journal of Advances in Microbiology* 24 (3): 65 - 78. <https://doi.org/10.9734/JAMB/2024/v24i3809>.
- Uba, B. O., Alfred, P. N., Ukpai, E. G., Ike, V. E. & Chikwendu, J. C. (2025). Diversity Of The Bacterial Communities Of Three Selected Streams In Anambra State, Nigeria. *Open Journals of Environmental Research*, 6 (2): 59 - 72. DOI: <https://doi.org/10.52417/ojer.v6i2.453>.
- Uba, B.O. and Okonkwo, O.P. (2025). Surface water treatment potentials of silver nanoparticles biosynthesized from *Moringa oleifera* seed extract. *African Journal of Health, Safety and Environment*, 6(2): 01 - 18. <https://doi.org/10.52417/ajhse.v6i2.622>.
- Uba, B. O. and Udaba, P.I. (2026). Evaluation of the Production of Biosurfactant by Yeast Strains Isolated from Fruit Pastes and their Biodegradative Potential on Waste Engine Oil. *Journal of Pollution Monitoring, Evaluation Studies and Control*, 5 (1): 147 - 157. <https://doi.org/10.54117/eimptp50>.
- Uba, B. O., Okonkwo, O. P., Idigo, M. A., Igiri, V.C., Okongwu, D. J., Okemadu, O.C. & Anaebonam, E. C. (2026a). Disinfecting Potentials and Eco-Safety Evaluation of Nano-treated Surface Water Resources by Biogenic Silver Nanoparticles Using Bacterial and Phytotoxicity Indices. (2026). *African Journal of Applied Research & Sustainable Development*, 4(1): 47-58. <https://doi.org/10.54117/j2qae873>.
- Uba, B. O., Udaba, P.I., Dibua, N.A., Ubajakwe, C.C., Igiri, V.C., Okongwu, D. J., & Anaebonam, E. C. (2026b). Toxicity and Safety Evaluation of Glycolipid Biosurfactant Produced by Yeast Strains Isolated from Fruit Pastes. *IPS Journal of Toxicology*, 4(1): 100 - 109. <https://doi.org/10.54117/axm1gf40>.
- Uba, B.O., Dokubo, C.U., Okongwu, D.J., Okemadu, O.C., Mere, C.A., Anaebonam, E.C., Oghonim, P. AN., & Agbata, E.F. (2026c). Potentials of *Aspergillus terreus* and Wistar Mice Bioassays as Tools for Monitoring the Environmental Health Concern of E-Waste Disposal. *Health Science Research International*, 3 (1): 82 - 92. <https://doi.org/10.54117/hsri.v3i1.69>.
- Ubajakwe, C. C., Chukwura, E. N., Dimejesi, S. N., Uba, B. O., Eleanya, L., Ezendianafo, J. N., & Dibua, N. A. (2025). Screening for Lipase Enzyme Producing Potentials of *Bacillus* Species Isolated from Different Automobile Workshops in Anambra State. *IPS Journal of Advanced and Applied Biochemistry*, 1(2), 51-56. <https://doi.org/10.54117/ijaab.v1i2.71>.
- Ubani, O., Obiefuna, G.O., Uba, B.O., Dokubo, C.U., Mere, C. A. and Akaun, I.P. (2024a). Kinetic modelling and half-life study on bioremediation of diesel oil contaminated soil and water using nano - remediation strategy: kinetic modelling and half-life study on bioremediation of diesel oil. *Multidisciplinary Science Journal* 7: e2025182. <https://doi.org/10.31893/multiscience.2025182>.
- Ubani, O., Uba, B.O., Modise, S. J., Okoye, E. L., Omeazu, S. C., Ndibe, C.R., Umeh, O. R. and Dokubo, C. U. (2024b). Responses of *Selenastrum capricornutum*, *Eisenia fetida*, *Brassica nigra* and *Sorghum bicolor* to spent phone battery toxicity. *Multidisciplinary Science Journal*, 6 (7): 2024107 - 2024107.
- Ubani, O., Uba, B. O., Modise, S. J., Egburefa, S. O., Orji, M. U. and Dokubo, C. U. (2025). A characterization and evaluation of the ecotoxicity of petroleum refinery effluents using a battery of bioassays. *Multidisciplinary Science Journal* 8 (3): 2026159. <https://doi.org/10.31893/multiscience.2026159>.
- Ukalska-Jaruga, A., Debaene, G. and Smreczak, B. (2018). Particle and structure characterization of fulvic acids from agricultural soils. - *Journal of Soils and Sediments*, 8 (2): 1 - 11.
- Umeh, O.R., Chukwura, E.I., Ibo, E.M. and Uba, B.O. (2020). Evaluation of physicochemical, bacteriological and parasitological quality of selected well water samples in Awka and its environment, Anambra State, Nigeria. *Archives of Agriculture and Environmental Science* 5 (2): 73 - 88. <https://doi.org/10.26832/24566632.2020.050201>.

