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# Eggshell Waste Bioprocessing for Sustainable Acid Phosphatase Production and Minimizing Environmental Hazards

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**Abstract :** Background: The Environmental Protection Agency has listed eggshell waste as the 15th most significant food industry pollution hazard. The utilization of eggshell waste as a source of renewable energy has been a hot topic in recent years. Therefore, finding a sustainable solution for the recycling and valorization of eggshell waste by investigating its potential to produce acid phosphatase (ACP) and organic acids by the newly-discovered B. sonorensis was the target of the current investigation. Results: The most potent ACP-producing B. sonorensis strain ACP2 was identified as a local bacterial strain obtained from the effluent of paper and pulp industries on basis of molecular and morphological characterization. The use of consecutive statistical experimental approaches of Plackett-Burman Design (PBD), and Orthogonal Central Composite Design (OCCD), followed by pH-uncontrolled cultivation conditions in a 7 L bench-top bioreactor, revealed an innovative medium formulation that substantially improved ACP production, reaching 216 U $L^{-1}$ with ACP yield coefficient Yp/x of 18.2 and a specific growth rate (μ) of 0.1 $h^{-1}$. The metals Ag+, Sn+, and Cr+ were the most efficiently released from eggshells during the solubilization process by B. sonorensis. The uncontrolled pH culture condition is the most suited and favored setting for improving the ACP and organic acids production simultaneously. Quantitative and qualitative analyses of produced organic acids were carried out using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Lactic acid, citric acid, and hydroxybenzoic acid isomer were the most common organic acids produced throughout the cultivation process. The findings of thermogravimetric analysis (TGA), differential scan calorimeter (DSC), scanning electron microscope (SEM), energy-dispersive spectroscopy (EDS), Fourier-Transform Infrared Spectroscopy (FTIR), and X-Ray Diffraction (XRD) analysis emphasize the significant influence of organic acids and ACP activity on the solubilization of eggshells particles. Conclusions: This study emphasized robust microbial engineering approaches for the large-scale production of a newly discovered acid phosphatase accompanied by organic acids production from B. sonorensis. The biovalorization of the eggshell waste and the production of cost-effective ACP and organic acids were integrated into the current study, and this was done through the implementation of a unique and innovative medium formulation design for eggshell waste management, as well as scaling up ACP production on a bench-top scale.

**Keywords :** chicken eggshells waste, bioremediation, statistical experimental design, batch fermentation
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