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Kinetic Studies of Alkaline Protease from *Bacillus licheniformis* NCIM-2042

Bhunia, Biswanath[1], Bikram Basak[1], Pinaki Bhattacharya[2], and Apurba Dey[1]*

[1]*Department of Biotechnology, National Institute of Technology, Durgapur, Mahatma Gandhi Avenue, Durgapur-713209, India*
[2]*Department of Chemical Engineering, Heritage Institute of Technology, Kolkata-700107, India*

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An extensive investigation was carried out to describe the kinetics of cell growth, substrate consumption, and product formation in the batch fermentation using starch as substrate. Evaluation of intrinsic kinetic parameters was carried out using a best-fit unstructured model. A nonlinear regression technique was applied for computational purpose. The Andrew's model showed a comparatively better R^2 value among all tested models. The values of specific growth rate (μ_{max}), saturation constant (K_S), inhibition constant (K_I), and $Y_{X/S}$ were found to be 0.109 h^{-1}, 11.1 g/l, 0.012 g/l, and 1.003, respectively. The Leudeking–Piret model was used to study the product formation kinetics and the process was found to be growth-associated. The growth-associated constant (α) for protease production was sensitive to substrate concentration. Its value was fairly constant up to a substrate concentration of 30.8 g/l, and then decreased.

Keywords: Cell growth, fermentation, kinetics, modeling, simulation

Proteases are obtained through a wide variety of sources such as plants, animals, and microorganisms. Alkaline protease is one of the most important groups of industrial enzymes, being extensively used in leather, food, pharmaceutical, textile, organic chemical synthesis, waste water treatment, and other industries [4, 29]. They hold a major share of the enzyme market, with two-third share alone in the detergent industry [2, 6, 14]. For use in detergent formulations, proteases must have a high level of activity over a broad range of pH and temperature. Alkaline proteases from high yielding strains have been studied extensively. One of the major drawbacks of the proteases obtained from these strains is their instability in alkaline pH and higher temperature [12]. It is desirable to overcome this problem by searching for new proteases with novel properties from different sources. Although there are many microbial sources available for producing proteases, only few are recognized as commercial producers. Of these, Species of *Bacillus* is considered an important commercial protease producer [25].

Large-scale production of proteases can only fulfil the demand and usefulness of the proteases in the industry. In industry, microbial enzyme production is carried out through the fermentative process, which offers a great deal of advantage in terms of reducing the process cost and the raw material utility [33]. In the fermentation process, the metabolism and product production pattern of each microorganism depend mainly on their fermentative, nutritional, physiological, and genetic nature [9, 23, 24]. Exploitation of such microbial metabolism by regulating the critical fermentation parameters helps in commercial economic production of the required enzyme [32]. Hence, careful kinetic studies are required to monitor the growth of microorganisms on various levels of substrates and their role in the overall productivity in the fermentation process. A kinetic study provides huge quantitative information regarding the behavior of a system, which is essential for study of the fermentation process. The kinetic study also describes the biological significance of each parameter and their levels with statistical reliability [11]. In general, structured or unstructured models are used in a fermentation process. Structured models are used in intracellular metabolic pathways and unstructured models are most frequently employed for modeling microbial systems based on the simplicity and technical robustness. Both models are equally useful in bioprocesses [18, 34]. In most of the biotechnological processes, high concentration of substrates or products often lead to inhibitory effects. So substrate utilization reduces decreasing both the product yields and fermentation rates [17].

In this work, various unstructured kinetic models were used to characterize the fermentation process using *Bacillus*

**Corresponding author*
Phone: +91-343-2755209; Fax: +91-343-2547375;
E-mail: apurbadey.bt@gmail.com