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Modeling of caffeine degradation kinetics during cultivation of *Fusarium solani* using sucrose as co-substrate

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ABSTRACT

Microbial kinetics for simultaneous caffeine degradation and its biotransformation to theophylline has been investigated using *Fusarium solani*. Caffeine was utilized by the fungus as a sole nitrogen source in the presence of sucrose, which served as the primary carbon source. A reaction mechanism involving Monod kinetics with both substrate and product inhibition was formulated based on experimental evidence. A total of thirteen kinetic parameters were involved in the model formulation and these were estimated using simulated annealing algorithm. Moreover, the parameter estimation was carried out for three different experimental sets simultaneously with varying initial sucrose concentration, in order to obtain a common set. Based on sensitivity analysis, among thirteen parameters, saturation constant of biomass accumulation from sucrose utilization (K_{s1}), yield of biomass from sucrose utilization (Y_{X_2/X_1}), lumped parameter which was defined as apparent yield of biomass from caffeine degradation process (Y_{X_2/X_3}), and yield of theophylline from caffeine metabolism (Y_{X_4/X_3}), were observed to be highly sensitive. The experimental results were in good agreement with the model predictions and have also been validated for an experimental setup which was not used for calibration. The results of the present study has potential application in the development of a process for detoxification of caffeine containing wastes as well as for production of a value added product theophylline.

1. Introduction

Coffee is one of the major plantation crops in India and the world's second most tradable commodities after oil. Coffee processing generates huge amount of wastes like coffee pulp (1 ton for every 2 ton of coffee cherries processed), coffee husk (0.18 ton of husk for every ton of coffee cherries) and waste water (around 16,000–23,000 L water per ton of fruit processed) [1,2]. The generated waste is hazardous in terms of its disposal and has showed negative impact on health of humans, animals, as well as environment. The coffee pulp and husk cannot be dumped as such to the environment without processing. Even though these wastes are rich in proteins and carbohydrates, the presence of caffeine, tannins, polyphenols and organic solid residues make it undesirable [1]. The waste generated have limited use as animal feed due to their adverse effect on central nervous system (CNS) and digestive system of animals [3]. The presence of caffeine in soil can affect soil fertility and hamper growth of seedlings [4,5]. Also, channeling of these wastes into nearby water bodies like rivers and lakes affect marine ecosystem [6]. Therefore, decaffeination of coffee waste becomes highly imperative before its disposal.

Several methods like biomethanation [7], production of gibberellin (plant hormone) [8], mushroom cultivation [9], production of enzymes like tannase [10], amylase, protease and xylanase [11,12] etc., have been developed for sustainable management and value addition of coffee pulp and husk. The present investigation focuses on microbial degradation of caffeine and simultaneous synthesis of a potential bioactive molecule called theophylline.

Theophylline is one of the metabolites obtained after demethylation of caffeine by fungi [13]. This molecule has several applications in therapeutics especially as an anti-asthmatic, anti-cancer, anti-cellulite and combinatorial drug [14–16]. Theophylline available in the market today is chemically synthesized in a non-ecofriendly manner involving a number of harmful chemicals [17]. Thus, an alternate methodology involving micro-organisms would be more specific and green process for the synthesis of theophylline.

The authors have already reported an optimized process for the detoxification of caffeine with production of theophylline using *F. solani* [18]. In order to design and scale-up such processes the understanding of overall kinetics becomes inevitable. Kinetics is used to predict concentrations of reactants and products at any

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given time during the process and also to optimize and control the process accordingly. Also, development of a robust mathematical model facilitates the prediction and control of microbial processes [19].

With this background, the aim of the present investigation was to develop a comprehensive mathematical model that would closely describe the kinetics of caffeine degradation and theophylline production. To the best of our knowledge, literature reports are not available on the kinetics of cell growth and co-utilization of caffeine and sucrose for the biosynthesis of theophylline in a fungal system. In order to achieve the above objective, experimental data was generated for different initial conditions and later a mathematical model was constructed in an attempt to describe the underlying reactions. The model parameters involved were estimated using simulated annealing algorithm, and sensitivity analyses of parameters was carried out to understand the critical parameters that regulate the formulated reaction mechanism.

2. Materials and methods

2.1. Chemicals and microorganisms

Theophylline, caffeine, 3-methyl xanthine (3MX) (99.9%) procured from Sigma chemicals, St. Louis, USA. Acetonitrile (HPLC grade) was from Ranbaxy fine chemicals Ltd., New Delhi, India. The analytical grade chemicals were procured from Qualigens fine chemicals, Mumbai. *Fusarium solani* was isolated from soil samples collected from coffee pulp dump sites near coffee processing plant. Isolation and identification of the isolate have been described elsewhere [18].

2.2. Medium for caffeine degradation

Fusarium solani was grown on caffeine containing minimal media (CMM) (g/L): KH_2PO_4 (1.3), Na_2HPO_4 (0.12), $MgSO_4$ (0.3), $CaCl_2$ (0.3), Caffeine (1) at pH 5.8. For solidification, CMM media was supplemented with 15 g/L agar. For degradation and kinetic studies, fresh CMM medium containing 1 g/L of caffeine was inoculated with 4.8×10^5 spores/mL and incubated for 144 h with shaking at 150 rpm. The pH, temperature and initial inoculum size were 5.8, 24 °C and 4.8×10^5 spores/mL, respectively, which have been optimized and reported previously [18].

2.3. Degradation and microbial kinetics of caffeine utilization with sucrose supplementation

The experiments were carried out by keeping concentration of caffeine constant at 1 g/L. The pH, temperature, initial inoculum and shaking conditions were as described in the previous section. Effect of sucrose addition on growth, caffeine degradation and theophylline accumulation was studied by supplementing the medium with varying concentration of sucrose (2, 4, 6, 8 and 10 g/L). The cultivation of the fungus was carried for 144 h at 24 ± 2 °C and the samples were withdrawn at regular intervals.

2.4. Analytical methods

Caffeine, theophylline and 3MX were estimated by HPLC (LC-10A system (Shimadzu, Japan)) using a C_{18} Column (5 μm, 250 mm × 4.6 mm, Phenomenex) [20]. Water and acetonitrile 85:15 (v/v) was used as mobile phase at a flow rate of 1 mL/min. Caffeine, theophylline and 3MX were procured from Sigma (Cat no: C0750, T-1633 & 222526) and were used as standards (1 mg/mL). All experiments were carried out in triplicate under identical conditions. The analytes were detected at 273 nm. The samples were analyzed using spectrophotometer (Shimadzu UV 1800, Japan) for quantitative determination of residual sucrose using phenol sulfuric acid method [21]. The quantification was carried out using a standard curve of sucrose. All the experiments were carried out in triplicates. Biomass production was measured and expressed as dry weight in grams/L. The fungal biomass was filtered through Whatman No 1 filter paper, dried at 60 °C until it attained constant weight.

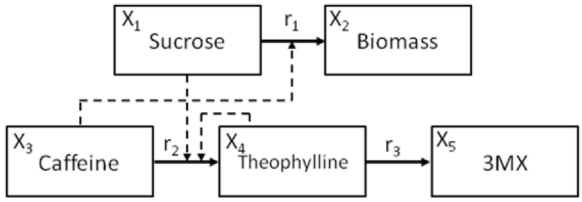

Fig. 1. Reaction scheme for caffeine degradation, theophylline and 3 MX accumulation. Solid lines represent the reaction terms and the dotted lines indicate the inhibition terms.

2.5. Model formulation

A sequential methodology was adopted to model the degradation of caffeine, accumulation of theophylline and 3MX. The reaction scheme of the caffeine degradation process to the respective metabolites in the presence of sucrose is given in Fig. 1. The model construct has two set of reactions, represented by solid lines in Fig. 1, viz., (i) sucrose to biomass formation and (ii) biodegradation of caffeine to theophylline, and theophylline to 3MX. However, these two set of reactions are interconnected with yield and inhibition parameters represented by dotted lines in Fig. 1.

The following assumptions were considered in arriving at the model structure:

1. Specific growth rate follows Monod kinetics when sucrose is the carbon source shown in Eq. (1).
2. Caffeine inhibits biomass formation and an inhibition term was included in Eq. (1).
3. Caffeine to theophylline reaction was assumed to be inhibited by sucrose and theophylline (i.e. product inhibition) as shown in Eq. (2).
4. Theophylline to 3MX was assumed to undergo simple Monod kinetics as a biotransformation reaction without any inhibition.
5. A lumped parameter which was defined as apparent yield of biomass from caffeine degradation process (Y_{X_2/X_3}) was included in the biomass formation.

The resulting kinetic equations for the above mentioned assumptions corresponding to the reaction scheme in Fig. 1 were thus:

$$r_1 = \frac{\mu_m X_1 X_2}{\left[K_{s1}\left(1 + \frac{X_3}{K_{I1}}\right) + X_1\right]} \tag{1}$$

$$r_2 = \frac{v_1 X_3 X_2}{\left[K_{p1}\left(1 + \frac{X_1}{K_{I2}} + \frac{X_4}{K_{I3}}\right) + X_3\right]} \tag{2}$$

$$r_3 = \frac{v_2 X_4 X_2}{\left[K_{p2} + X_4\right]} \tag{3}$$

$$\frac{dX_1}{dt} = -\left[\frac{1}{Y_{X_2/X_1}}\right] r_1 \tag{4}$$

$$\frac{dX_2}{dt} = r_1 + \left[\frac{1}{Y_{X_3/X_2}}\right]\left(-\frac{dX_3}{dt}\right) \tag{5}$$