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Biodegradation kinetics of caffeine by *Leifsonia* sp. strain siu

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ABSTRACT

Caffeine is an important naturally occurring compound that can be degraded by bacteria. Excessive caffeine consumption can leads to some adverse effects. The biodegradation kinetic of caffeine by a pure culture of new bacterial strain *Leifsonia*sp. strain SIU was studied. In this study various caffeine concentrations ranging from 300 to 1500 mg/L were used. Five kinetic models (Monod, Haldane, Luong, Aiba and Teissier) were investigated. Luong model were fitted to the experimental degradation kinetics data and gave a very good fitting R^2 value of 0.9843 compared to other models. Luong model also predicted the significant substrate concentration (S_m) value, at which specific substrate degradation rate falls to zero (1.695 g/L). The values of μ_{max}, K_i, and K_s were 0.0754h-1, 0 g/L, and 1.598 g/L, respectively. Out of all the kinetic models, monod gave a poor R^2 of 0.4796 and μ_{max} of 0.009 h^-

Keywords: **Biodegradation, Caffeine concentration, Kinetic models, *Leifsonia*sp.,**

INTRODUCTION

Caffeine is a white crystalline alkaloid of xanthine, which is bitter, odourless and amorphous in its pure state that acts as a drug stimulant with an empirical formula of $C_8H_{10}N_4O_2$, half-life of 5 hours and molecular weight of 194.2 g/mol(Ibrahim et al., 2014). It is found in more than 60 plant species, with significant levels in coffee beans, tea and cocoa (Mazzafera, 1991; Suzuki et al., 1992). Caffeine is considered to be the stimulatory effect of the central nervous system and it is the most widely consumed beverages in the world without any legal restrictions (Gummadi et al., 2012). Apart from its medical significance, caffeine is also commercially important in terms of its consumption in the form of caffeinated beverages. Researches has shown that excess intake of caffeine are associated to many health implications in humans such as mutation; it is teratogenic, causes DNA repair mechanism inhibition, inhibition of seed germination and cyclic AMP phosphodiesterase activity (Friedman and Waller, 1983a, 1983b). It is the major cause of cardiovascular disease, cancer, osteoporosis, irregular muscular activity, adrenal stimulation, apathy, fatigue and headache, complications in aging and pregnant women as it increases the spontaneous risk of abortion and affects fetal growth thus causing malfunction in the fetus (Dlugosz and Bracken, 1992; Fenster et al., 1991; Green and Suls, 1996; Lorist and Tops, 2003; Smith, 2002; Weng et al., 2008).

At high concentration, caffeine is toxic to saprophytic organisms that are concerned in important biotransformation in the environment, which cause a disorder in environmental stability. Caffeine being one of the most important poisonous compounds produced by solid wastes in tea and coffee manufacturing industries, i.e. tea waste, husks and coffee pulp. Even though these waste are enriched in proteins and carbohydrates, but due to the presence of anti-nutritional factors such as caffeine, tannins, polyphenols and other harmful substances limited, it cannot be used as animal feeds (Gummadi et al., 2012; Ibrahim, 2014; Mazzafera, 2002; Pandey et al., 2000). The caffeine in liquid effluents of tea and coffee manufacturing industries cannot be allowed to be channeled into lakes and rivers as it would affect the marine organisms in their environment (Gibson et al., 2009; White and Rasmussen, 1998). For this reason, caffeine degradation becomes essential (Dash and Gummadi, 2012). Hence, there is a strong need for caffeine degradation from products and waste streams by alternative route other than conventional extraction techniques (Gokulakrishnanet al., 2005).

Quite a few bacterial strains were reported to degrade caffeine (Brandet al., 2000; Gokulakrishnan et al., 2006; Hakilet al., 1999; Mazzaferaet al., 1996; Yamoka-Yano and Mazzafera, 1998) Strains with high degradation ability and efficiency to withstand high caffeine concentrations are required for efficient degradation. In spite of the fact that several works has been reported on caffeine biodegradation processes, astoundingly, no work has been published on biodegradation kinetics of caffeine based on specific substrate consumption. This works present the degradation ability of *Leifsonia* sp. strain SIU locally isolated from an agricultural soil in Malaysia and the effect of initial substrate (caffeine) concentration on its degradation.

MATERIALS AND METHODS

Chemicals: Caffeine anhydrous >99% was purchased from Sigma, Aldrich USA. Other chemicals used are analytical grade that were obtained from recognized chemicals suppliers, Fisher (Malaysia) and Merck (Darmstadt, Germany).

Media: *Leifsonia*sp. strain SIU previously isolated from agricultural soil, was cultured at 30°C in sterilised caffeine liquid medium (CLM) containing the following (g/L): 0.4 K_2HPO_4, 0.2 KH_2PO_4, 0.1 NaCl, 0.1 $MgS0_4$, 0.01 $MnSO_4.H_2O$, 0.01 $Fe_2(SO_4)_3$, 0.01 $NaMoO_4.2H_2O$, 0.4 NH_4Cl. The media contains 0.3 g/L of caffeine in addition to the above compositions. Carbon sources if any added to the medium were sterilized separately and then mixed to the medium under aseptic conditions. For solid medium, (25 g/L) agar was added to the caffeine medium. The isolates were maintained and sub-cultured in the caffeine agar medium.

Analytical methods: Degradation of caffeine was estimated by HPLC (Agilent 1100 series from Agilent technologies, Waldbronn, Germany, Product No. G2170AA) equipment using a ZORBAX® SB-C18 column (USA, Product No. 880975-902, Batch No. B03024) with 10 mM ammonium phosphate buffer (pH 2.5)/ acetonitrile (4:1, v/v) as mobile phase (with reference to specifications in the HPLC user manual). Pure caffeine at 2 mg/mL was used as the standard. Retention time of caffeine was found to be 2.1 min at a flow rate of 1 mL/min and at 30°C. Detection of caffeine was done at 254 nm (detector sensitivity: 1×10^{-14} absorbance unit).

Flask culture experiments: A single colony of the strain from caffeine agar plates was transferred to 5 mL sterile caffeine medium. The tubes with cotton plugs were aerated on a rotary shaker at 150 rpm and incubated for 24 h at room temperature. About 4% (v/v) of the culture was transferred to 50 mL of the caffeine medium in 250 mL Erlenmeyer flasks and incubated on a rotary shaker at 150 rpm for 48 h at 30°C. Samples were collected after 48 h and caffeine degradation were measured.

Kinetics modelling experiment: Batch experiment was carried out using a shake flask studies in an optimum conditions for caffeine degradation by strain SIU. The flask was incubated for 48 h at room temperature and 150 rpm. About 4% (v/v) of the seed culture was transferred to 25 mL of caffeine liquid media containing various initial caffeine concentrations ranging from 300 to 15000 mg/L in 100 mL Erlenmeyer flasks and incubated on a rotary shaker at 150 rpm and at room temperature. Samples were collected at different time intervals and measured for cell growth and caffeine degradation (Agarwal et al., 2009; Gokulakrishnan and Gummadi, 2006). In this study, the kinetic models as listed in Table 1 were used to represent the kinetics of caffeine. All the kinetic models were fitted to the experimental data by using a curve fitting toolbox available from MATLAB R2012a based on Windows vista (Singh et al., 2008).

The rate of bacterial degradation can be represented as cell production rate. The formula for various kinetics models is as shown in Table 1 where S, S_m, μ, μ_{max}, K_s, and K_i,are specific substrate concentration (g/L), the above critical substrate concentration above which the cell degradation of caffeine completely stops (g/L), cell degradation rate (hr^{-1}), maximum cell degradation rate (hr^{-1}), saturation constant or half velocity constant (g/L), and the inhibition constant (g/L) respectively.

Table.1.Various kinetic models for effect of substrate on caffeine cell degradation.

Author	μ (rate)	References
Monod	$\mu_{max}\frac{S}{K_s+S}$	(Monod, 1949)
Haldane	$\mu_{max}\frac{S}{S+K_s+\left(\frac{S^2}{K_i}\right)}$	(Haldane, 1930)
Luong	$\mu_{max}\frac{S}{K_s+S}(1-\frac{S}{S_m})^n$	(Luong, 1987)
Aiba	$\mu_{max}\frac{S}{K_s+S}exp^{(-K_pP)}$	(Aiba *et al.*, 1968)
Teissier	$\mu_{max}\left(1-\exp\left(-\frac{S}{K_s}\right)\right)$	(Teissier, 1942)

RESULTS AND DISCUSSION

Biodegradation kinetics: The most efficient technique for absolute degradation of organic contaminants is biodegradation (Alexander, 1999). Microorganisms play a significant role in bioremediation of contaminated substances in the environment. Thus, the fundamental theory of biodegradation kinetics is that substrates are consumed through enzymes catalysed reactions taking place by the microorganism. Hence, substrate degradation rates are directly proportional to the organisms' concentration capable to degrade the substrates (catalyst

concentration) and are dependent on concentration of substrate saturation kinetics characteristic. Saturation kinetics proposed that at high substrate concentrations, rates are independent of substrate concentration, while at low substrate concentrations, rates are proportional to the substrates concentrations (Okpokwasili and Nweke, 2005). As such, the substrate contributes to the growth of the *Leifsonia* sp. strain SIU, substrate degradation rates are linked to growth rates.

Modelling degradation kinetics: The batch cultivation experiments were carried out using caffeine as single limiting substrate for *Leifsonia*sp. strain SIU. Different initial caffeine concentrations ranging from 300 to 1500 mg/L were used. Caffeine degradation amount using these different initial concentrations of caffeine was examined for numerous batch residence times by irregular sampling.Figure 1 shows the caffeine biodegradation for *Leifsonia*sp. strain SIUin degrading caffeine in the concentration range of 100 to 1500 mg/L. It has been shown that at higher concentration caffeine has an inhibitory effect (Waller et al., 1986). Ever since caffeine degradation ensues with cell growth, the figure also illustrates the typical cell degradation curve.

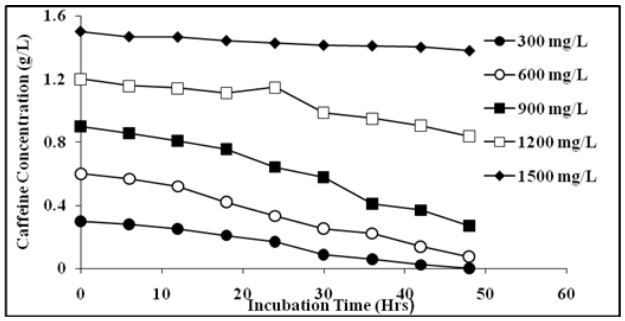

Figure.1. Effect of different caffeine concentration on *Leifsonia* sp. strain SIU caffeine degradation. Data represents mean ± STDEV, n = 3.

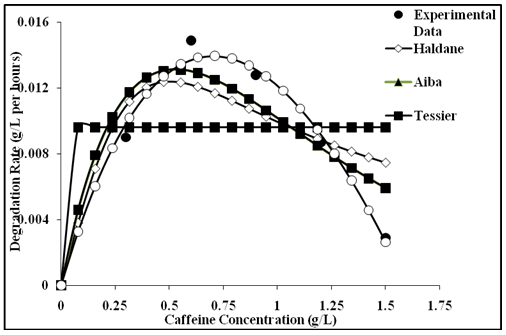

Figure.2. Comparison of caffeine degradation kinetics experimental values with different kinetic models from literature.

Figure 2shows the comparison of the time course for caffeine degradation of all the batches, it is obvious that the caffeine degradation rate decreased with an increase in the initial caffeine concentration. Ruiz-ordaz et al. (2001) and Agarry and Solomon, (2008) reported an analogous observation on *Candida tropicalis*and *Pseudomonas fluorescence* grown on phenol, respectively. Hence, it is shown that the duration of the lag phase increased as the initial concentration increased; and thus, prolonging the degradation time as a result of decrease in the degradation rate. Microbial performance was suggested to be the most important measure for substrate degradation rate (Prpich and Daugulis, 2005). Relatively, there were very few reports in the literature on growth and degradation kinetics of caffeine. Most of the reports accessible concerns specific growth rate of phenol as there are few data on degradation kinetics. On this basis, the caffeine degradation rate was calculated and plotted against caffeine degradation as shown inFigure 2. As shown on the graph, the rate of caffeine degradation increased with an increase in caffeine (substrate) concentration until it reaches it stationary point where the rate of degradation decreased as caffeine concentration increased. Agarry and Solomon (2008) reported similar observation. Hence, Posten proposed that the microbial growth can be illustrated by the most commonly used kinetic models that can be based on specific substrate consumption rate (Solomonet al., 1994). Among numerous models used to fit the present experimental data of specific caffeine degradation rates versus different initial caffeine concentrations was fitted to all the inhibition.

The result in Table 2 shows that Luong gave a good correlation coefficient (R^2) of 0.9843 compared to other models, and thus, signifying a very good fit to the batch experimental data. The table also reports the values of μ_{max} and *Ks* as per Aiba model by nonlinear regression method. Haldane model predicts marginal differences in both $\mu_{max}$and *Ks* values, but varies greatly in correlation coefficient (R^2) value and also interms of the degradation rates. This could be due to the fact that Haldane model takes care of the inhibition constant value K_I, that is an essential parameter in understanding the microbial kinetics in the living organism. Luong model also predicted the significant substrate concentration (S_m) value, at which specific substrate degradation rate falls to zero (1695 mg/L).

Table.2.Parameter estimation for different substrate-inhibition models for degradation.

Model	μ_{max}(hr^{-1})	K_s (g/L)	K_i (g/L)	K (g/L)	S_m (g/L)	R^2
Haldane	0.2	1.5	0.1	-	-	0.744
Teissier	0.09965	0.8215	0.814	-	-	0.6835
Monod	0.009	-	-	-	-	0.4796
Luong	0.0754	1.598	-	-	1.695	0.9843
Aiba	0.005	0.7	16	-	-	0.777

CONCLUSION

Caffeine biodegradation kinetics was studied using a newly isolated bacterium *Leifsonia* sp. strain SIU. The biodegradation kinetics studies experiment was done with a fixed inoculum size of 4% and different caffeine concentration ranging from 300 to 700 mg/L. The kinetic models were fitted to the experimental data and kinetic parameters were determined. Luong gave the most suitable kinetics model with an R^2 of 0.9843 for caffeine biodegradation by *Leifsonia* sp. strain SIU, the values of μ_{max}, K_i, K_s and S_m were 0.0754 h^{-1}, 0 g/L, 1.598 g/L, and 1.695 g/L respectively. Out of all the kinetic models, monod gave a poor R^2 of 0.4796 and μ_{max} of 0.009 h^{-1}. It can be concluded that biodegradation kinetic parameters calculated using the models showed better tolerance, and as such a complete caffeine substrate degradation.

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