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ORIGINAL PAPER

Kinetics of growth and caffeine demethylase production of *Pseudomonas* sp. in bioreactor

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Abstract The effect of various initial caffeine concentrations on growth and caffeine demethylase production by *Pseudomonas* sp. was studied in bioreactor. At initial concentration of 6.5 g l^{-1} caffeine, *Pseudomonas* sp. showed a maximum specific growth rate of 0.2 h^{-1}, maximum degradation rate of 1.1 g h^{-1}, and caffeine demethylase activity of 18,762 U g CDW^{-1} (CDW: cell dry weight). Caffeine degradation rate was 25 times higher in bioreactor than in shake flask. For the first time, we show highest degradation of 75 g caffeine (initial concentration 20 g l^{-1}) in 120 h, suggesting that the tested strain has potential for successful bioprocess for caffeine degradation. Growth kinetics showed substrate inhibition phenomenon. Various substrate inhibition models were fitted to the kinetic data, amongst which the double-exponential ($R^2 = 0.94$), Luong ($R^2 = 0.92$), and Yano and Koga 2 ($R^2 = 0.94$) models were found to be the best. The Luedeking–Piret model showed that caffeine demethylase production kinetics was growth related. This is the first report on production of high levels of caffeine demethylase in batch bioreactor with faster degradation rate and high tolerance to caffeine, hence clearly suggesting that *Pseudomonas* sp. used in this study is a potential biocatalyst for industrial decaffeination.

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Introduction

The deleterious effect of caffeine is apparent from the numerous reports on the association of habitual caffeine intake with adverse effects on the cardiovascular system and health of women [7, 21]. In addition, effluents from coffee and tea processing industries contain high levels of caffeine (~3–10 g l^{-1}) and biological oxygen demand (BOD) and chemical oxygen demand (COD) levels, which affect the flora and fauna around water bodies [8]. Solid wastes obtained after coffee processing (coffee pulp, husk) also contain caffeine and other alkaloids, in addition to high amounts of protein and carbohydrates. Presence of caffeine and other alkaloids restricts the use of solid waste as animal fodder [27, 32, 34]. Hence, from health and environmental points of view, caffeine degradation is very important. To date, conventional methods such as solvent extraction and supercritical fluid extraction have been employed to remove caffeine [16]. Microbial and enzymatic caffeine degradation are considered more suitable than currently used conventional methods because of avoidance of toxic solvents and specificity [16]. Microbial methods can be successfully used to degrade caffeine in effluents as well as in solid wastes. However, this method cannot be used to degrade caffeine in food products because of issues arising due to direct use of microorganisms such as *Pseudomonas* sp. This can be avoided by using specific caffeine-degrading enzymes in pure form.

Several bacteria and fungi, viz. *Pseudomonas*, *Serratia marcescens*, *Klebsiella*, *Alcaligenes*, *Penicillium*, and

Aspergillus, can grow on caffeine as sole carbon and nitrogen source [4, 27, 28, 30]. However, their inability to tolerate high concentrations and degrade caffeine at faster rates necessitates identification of better strains. Studies on enzymes involved in caffeine degradation are also not explicit, with the exception of caffeine oxidase, which has been purified and characterized [4, 26, 30]. A few studies have shown that there might be separate enzymes, acting separately [4, 13] or as a complex [6, 12], which bring about specific demethylation in *Pseudomonas* sp. To date, no reports are available on production of caffeine demethylase enzyme in higher amounts in bioreactor. In order to develop successful bioprocess for caffeine degradation, we isolated *Pseudomonas* sp. from soil of coffee plant cultivation which could tolerate up to 15 g l^{-1} caffeine concentration with minimum inhibitory concentration of 20 g l^{-1} [17]. The strain can completely degrade caffeine at initial concentration of 5 g l^{-1} at rate of 0.1 g l^{-1} h^{-1}, which was further increased to 0.29 g l^{-1} h^{-1} by optimization of medium components and physical conditions [10, 11]. Induced cells of this strain could degrade caffeine at faster rate of 0.38 g l^{-1} h^{-1} [19]. Based on biochemical assays, we found that the (intracellular) enzyme involved in caffeine degradation is demethylase, which demethylates caffeine into dimethyl and monomethyl xanthine and then to xanthine [12]. Therefore, in the present study, we focused on growth kinetics and caffeine demethylase production kinetics of *Pseudomonas* sp. in batch bioreactor.

Materials and methods

Chemicals

Pure caffeine (1,3,7-trimethylxanthine) was obtained from Merck. Theobromine (3,7-dimethylxanthine), paraxanthine (1,7-dimethylxanthine), theophylline (1,3-dimethylxanthine), 7-methylxanthine, nicotinamide adenine dinucleotide (NADH), and dithiothreitol (DTT) were obtained from Sigma. All other reagents were of analytical grade and procured in India.

Microorganism

Pseudomonas sp. NCIM 5235 was maintained on caffeine associated sucrose (CAS) agar medium of composition: Na_2HPO_4, 0.12 g l^{-1}; KH_2PO_4, 1.3 g l^{-1}; $CaCl_2$, 0.3 g l^{-1}; $MgSO_4{\cdot}7H_2O$, 0.3 g l^{-1}, sucrose, 5 g l^{-1}; caffeine, 1.2 g l^{-1}; and agar, 25 g l^{-1} (pH 6.0), and was grown at 30°C in a BOD incubator and subcultured once a week.

Reactor studies

To study caffeine demethylase enzyme production and growth kinetics, experiments were performed in a stirred 7.5-L bioreactor (Bioflo 110; New Brunswick Scientific, USA) with 3.75 L optimized CAS medium as working volume. Three loops of actively grown cells from CAS agar plates were transferred to 25 ml nutrient medium containing 1 g l^{-1} beef extract, 2 g l^{-1} yeast extract, 5 g l^{-1} peptone, and 5 g l^{-1} NaCl, in 100-ml Erlenmeyer flasks. When A_{600} reached 1.5, 6% (v/v) seed culture from nutrient medium was transferred aseptically to bioreactor containing optimal CAS medium [10] with the following composition: Na_2HPO_4, 0.352 g l^{-1}; KH_2PO_4, 3.4 g l^{-1}; $CaCl_2$, 0.3 g l^{-1}; $MgSO_4{\cdot}7H_2O$, 0.3 g l^{-1}; sucrose, 5 g l^{-1}; caffeine, 6.4 g l^{-1}; and Fe^{2+}, 0.075% (w/v). The reactor was operated at the following optimal conditions: pH 7.0, temperature 30°C, aeration rate of 0.27 vvm, and agitation rate of 700 rpm [11]. Kinetic studies were carried out at various initial concentrations of caffeine of 1, 2, 3, 6.5, 10, 15, and 20 g l^{-1}, and at regular intervals of time, samples were collected and analyzed for growth, caffeine consumption, caffeine demethylase production, sucrose consumption, and dissolved oxygen (DO) profile.

Assay for caffeine demethylase activity

Pseudomonas sp. cells were harvested and washed three times with 50 mM potassium phosphate buffer (pH 8.0). The cells were then resuspended in lysis buffer [50 mM phosphate buffer (pH 8.0) containing 1 mM ethylenediamine tetraacetic acid (EDTA), 1 mM DTT, 20% glycerol, and 10% ethanol] with cell mass-to-buffer volume ratio of 1:2 (w/v). This step was carried out immediately after the last wash with 50 mM potassium phosphate buffer (pH 8.0) as mentioned above. The cells were then disrupted by sonication over ice (four cycles of 2 min each with adequate cooling between cycles). The cell debris was separated by centrifugation at 20,000 × *g* and 4°C for 30 min. The supernatant was treated as crude enzyme extract and immediately used in the assay. Enzyme activities were measured at 30°C in reaction mixture consisting of 7.5 mM caffeine in 50 mM potassium phosphate buffer (pH 8.0), and 1 mM NADH. Reaction was initiated by adding cell-free lysate containing 50 μg protein, and reaction was stopped after 10 min by addition of 10% (w/v) trichloroacetic acid (TCA). Reaction carried out with TCA addition prior to incubation served as blank for the assay. The reaction mixture was then centrifuged at 20,000 × *g* and 4°C for 15 min, and the supernatant was analyzed by high-performance liquid chromatography (HPLC). One unit of

enzyme activity (U) was defined as micromoles of substrate (caffeine) degraded per minute of reaction.

Analytical methods

Cell concentration was monitored by measuring optical density at 600 nm. Cell dry weight for *Pseudomonas* sp. was calculated from A_{600nm} values (A_{600nm} of 1 corresponds to 0.75 g l^{-1} cell dry weight). Sucrose was analyzed by 3,5-dinitrosalicylic acid (DNS) method [29]. Protein was estimated by the method described by Lowry et al. [23]. Caffeine and other methylxanthines were estimated by reversed-phase (RP)-HPLC (Jasco PU-2080 Plus equipment) using a HiQSil C-18 column with water–methanol 70:30 (v/v) as mobile phase at flow rate of 1 ml min^{-1} and at 28°C with ultraviolet (UV) detector at 254 nm. Pure caffeine (Sigma) at 2 g l^{-1} was used as a standard.

Kinetic models

Various substrate inhibition models were used to fit the growth kinetic data (Table 1). The parameters of the different growth models considered were estimated using MATLAB7 software. Since the models had nonlinear coefficients, the parameters were estimated iteratively by trust-region nonlinear least-square algorithm. The following constraints were applied to μ_m and K_S in all models while estimating the parameters

$$\mu_{max} \leq \mu_m \leq 3\mu_{max}; \quad K_S > 0.$$

Here, μ_{max} is the maximum specific growth rate that would be obtained if the growth were not substrate inhibited, calculated by fitting the data of uninhibited region to Monod's equation.

Caffeine demethylase production kinetics

Caffeine demethylase production kinetics was studied by fitting the kinetic data to Luedeking–Piret analysis:

$$q_p = \alpha\mu + \beta, \quad (1)$$

where α is the growth-associated constant, β is the non-growth-associated constant, q_p is the specific productivity, and μ is the specific growth rate. This model was originally proposed for describing product formation [24].

Statistical analysis

All experiments were performed twice, and each measurement was performed in duplicate. Hence, all reported values are means of four data points, with significance for $p < 0.05$.

Results

Caffeine *N*-demethylases seem to be an important class of enzymes that are appropriate for biodecaffeination of caffeinated food products. This paper is the first report on the production kinetics of caffeine demethylases from caffeine-degrading *Pseudomonas* sp. in batch bioreactor.

The maximum cell dry weight (X_{max}) for maximum caffeine degradation was almost the same when *Pseudomonas* sp. was grown at all concentrations of caffeine. Lag phase during growth was 4 h when isolate was grown in

Table 1 Estimated parameters of various substrate inhibition models explaining the entire data

Model	Equation	Parameter	R^2
Edwards	$\mu = \frac{\mu_m S}{K_S+S}\exp\left(-\frac{S}{K_i}\right)$	$\mu_m = 0.654$, $K_s = 3.951$, $K_i = 5.056$	0.9052
Double exponential	$\mu = \mu_m\left[\exp\left(-\frac{S}{K_i}\right) - \exp\left(-\frac{S}{K_s}\right)\right]$	$\mu_m = 0.36$, $K_s = 4.112$, $K_i = 2.134$	0.941
Haldane	$\mu = \frac{\mu_m S}{(K_S+S)(1+(S/K_i))}$	$\mu_m = 0.7982$, $K_s = 1.964$, $K_i = 1.948$	0.7068
Andrews	$\mu = \frac{\mu_m}{K_s+S+\frac{S^2}{K_i}}$	$\mu_m = 0.796$, $K_s = 4.507$, $K_i = 1.28$	0.7905
Luong	$\mu = \frac{\mu_m S}{K_S+S}\left(1-\frac{S}{S_m}\right)^n$	$\mu_m = 0.341$, $K_s = 4.216$, $S_m = 23.41$, $n = 4.12$	0.926
Yano and Koga 1	$\mu = \mu_m\frac{S}{K_s+S+\frac{S^2}{K_1}+\frac{S^3}{K_2^2}}$	$\mu_m = 0.51$, $K_s = 1.413$, $K_1 = 1{,}250$, $K_2^2 = 4.561$	0.8475
Yano and Koga 2	$\mu = \mu_m\frac{S}{K_s+S+\frac{S^3}{K_2^2}}$	$\mu_m = 0.41$, $K_s = 5.23$, $K_2^2 = 2.64$	0.946
Alagappan and Cowan	$\mu = \frac{\mu_m S}{K_s+S+\frac{S^2}{K_i}} - i(S-S_\theta)$	$\mu_m = 0.82$, $K_s = 4.366$, $K_i = 2.246$, $i = 0.004379$, $S_\theta = -2.985$	0.8907
Wayman and Tseng	$\mu = \frac{\mu_m S}{K_s+S} - i(S-S_\theta)$	$\mu_m = 0.3164$, $K_s = 0.7346$, $i = 0.01618$, $S_\theta = -0.2886$	0.7704

μ_m, maximum specific growth rate; μ, specific growth rate; S, limiting substrate concentration (caffeine in this study); K_S, Monod half-saturation constant; S_m, maximum substrate inhibitory concentration at which no growth was observed; n, constant which accounts for the relationship between μ and S; K_i, K_1, K_2, inhibition constants; S_θ, threshold substrate concentrations below which no inhibition is apparent; i, inhibition coefficient

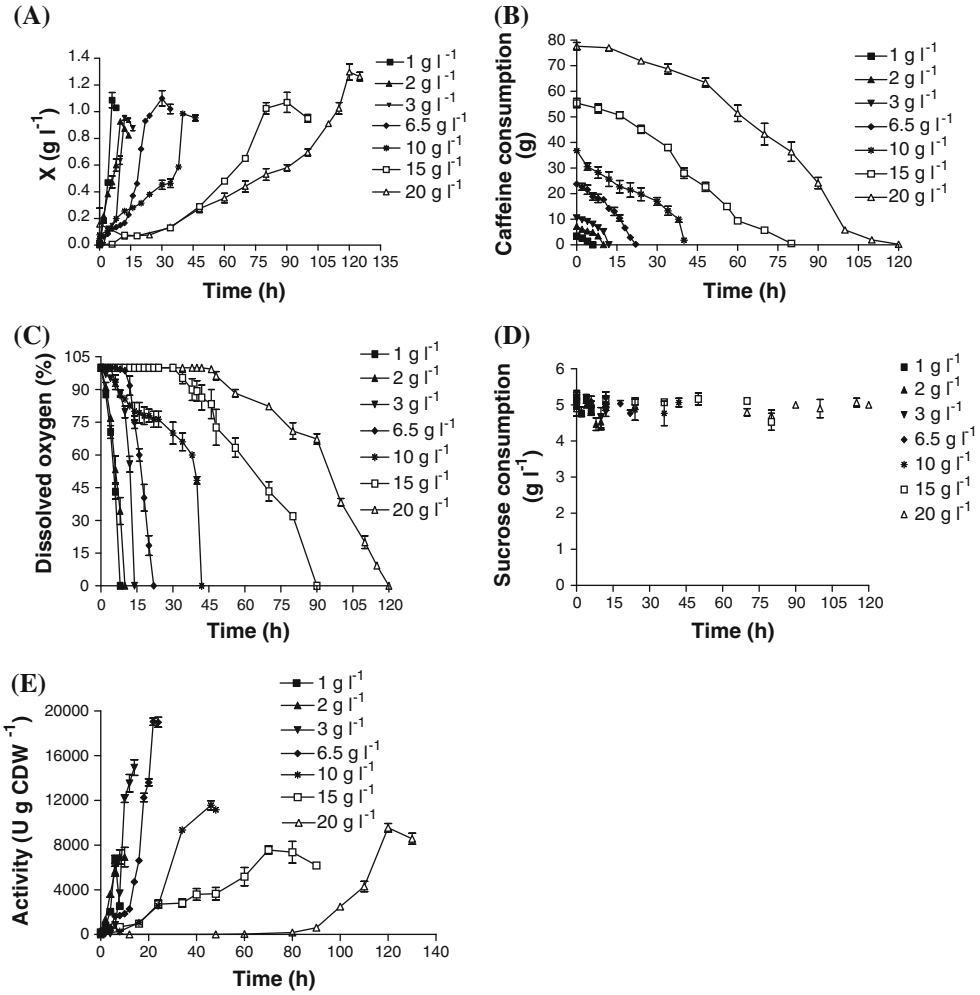

Fig. 1 Effect of initial concentrations of caffeine. **a** Cell growth, X (g l^{-1}). **b** Caffeine consumption (g). **c** Dissolved oxygen (%). **d** Sucrose consumption (g l^{-1}). **e** Time-course study on production of caffeine demethylase activity (U g CDW^{-1}) in batch bioreactor by *Pseudomonas* sp. NCIM 5235. All experiment runs were performed in triplicate. Batch bioreactor studies were carried out on 3.75 L optimized CAS medium as working volume, with agitation and aeration rates of 700 rpm and 0.27 vvm, respectively, at 30°C. Caffeine was analyzed by RP-HPLC using C-18 column and water–methanol 70:30 (v/v) as mobile phase

medium containing initial concentration of caffeine up to 3 g l^{-1}. Lag time increased to 20–30 h at higher caffeine concentration (10–20 g l^{-1}) (Fig. 1a). All concentrations of caffeine used (1–20 g l^{-1}) were completely degraded. For the first time, it was found that the isolate showed 100% caffeine degradation with initial caffeine concentration of 20 g l^{-1} (75 g) with degradation rate of 0.62 g h^{-1} (Fig. 1b). So far no reports are available on strains showing such high tolerance and degradation rates, suggesting that the strain reported in this study can be a potential strain for biodegradation of caffeine. The percentage dissolved oxygen (%DO) profile decreased with time and reached zero, which is in agreement with growth and caffeine degradation (Fig. 1c). As growth increased, %DO decreased. In all the experiments, sucrose was used at 5 g l^{-1} and was not consumed at all (Fig. 1d), which is in agreement with previous reports that sucrose enhances caffeine degradation without being utilized [20]. So far, no reports are available on study of production of enzyme involved in caffeine degradation in reactors. Production of caffeine demethylase, an intracellular enzyme involved in demethylation of caffeine into dimethyl and monomethyl xanthine and into xanthine, was maximum (18,762 U g cell dry $weight^{-1}$) when the initial caffeine concentration was 6.5 g l^{-1} (Fig. 1e). This is in agreement with our earlier study, in which we optimized the medium composition for maximum caffeine degradation and in which the optimal concentration was found to be 6.5 g l^{-1} [18]. Maximum specific growth rate of 0.28 h^{-1} was found when isolate was grown at 3 g l^{-1} initial concentration of caffeine, whereas at 1 and 2 g l^{-1}, the growth rate was 0.11 and 0.2 h^{-1}, respectively (Fig. 2a). Beyond 3 g l^{-1} caffeine concentration, the specific growth rate decreased to 0.182, 0.05, 0.032, and 0.02 h^{-1} at 6.5, 10, 15, and 20 g l^{-1}, respectively (Fig. 2a). Similar to the specific growth rate, caffeine demethylase specific production rate was maximum at 3 g l^{-1}, and specific production rate decreased with further increase in caffeine concentration (Fig. 2a). However, caffeine degradation rate was maximum (1.1 g h^{-1} or 0.3 g l^{-1} h^{-1}) when grown at 6.5 g l^{-1}

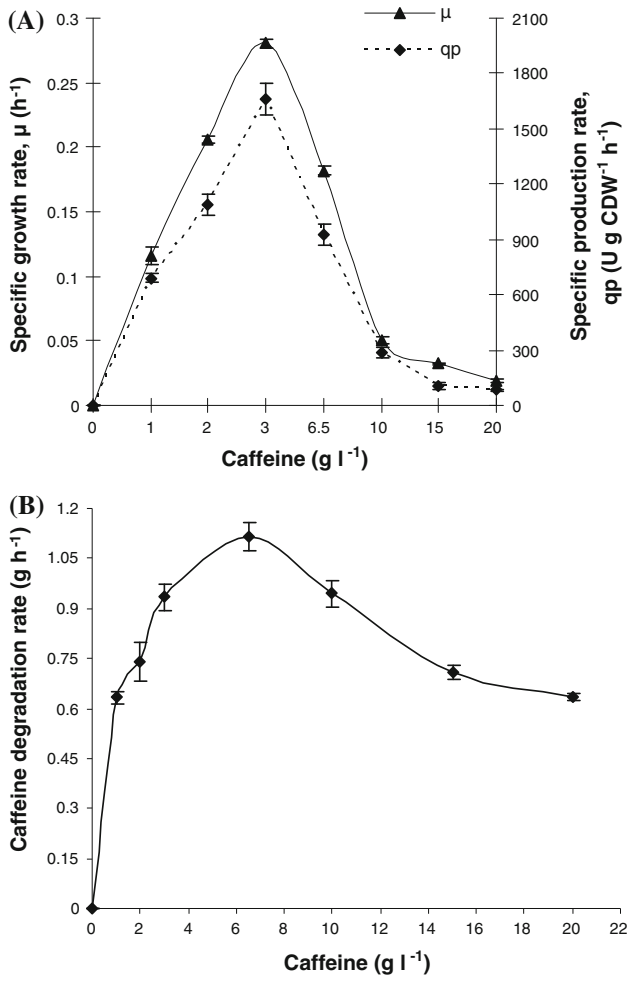

Fig. 2 Profile of caffeine degradation by *Pseudomonas* sp. in bioreactor. **a** Relationship between specific growth rate, μ (h^{-1}), and specific production rate, q_p (U g CDW^{-1} h^{-1}). **b** Relationship between degradation rate (g h^{-1}) and substrate concentration (g l^{-1}). *CDW* cell dry weight

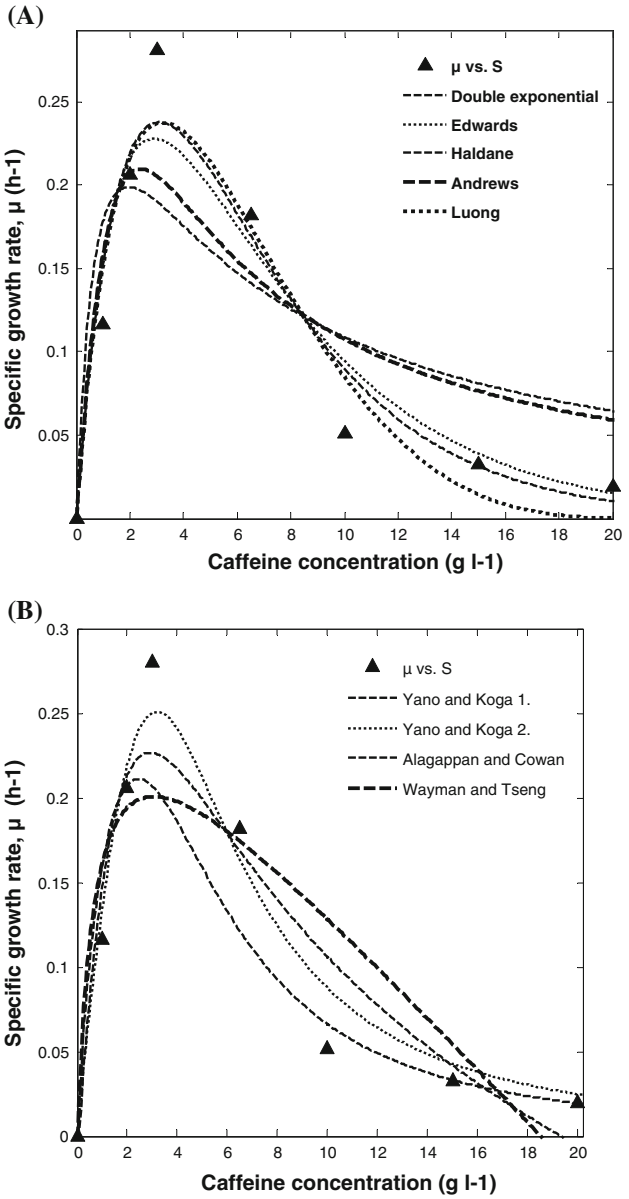

Fig. 3 Specific growth rates simulated by using various substrate inhibition models. The parameters of different growth models considered were estimated using MATLAB7 software. Parameters were estimated as described in Kinetic models. Here μ_{max} is the maximum specific growth rate that would be obtained if the growth were not substrate inhibited, calculated by fitting the data of uninhibited region to Monod's equation. Experiments were performed in triplicates under identical conditions. **a**, **b** Models fitted to entire data set

caffeine (Fig. 2b). Degradation rates obtained in bioreactor are 25 times faster than those in shake flasks [10]. Various substrate inhibition models were used to model the cell growth kinetics, and the model parameters were estimated using MATLAB version 7 (Table 1). The value of S_m of 23.41 g l^{-1} predicted by the Luong model was close to the experimentally observed value of $\sim$25 g l^{-1}. The constant n estimated by the Luong model was 4.12, which was greater than 1, suggesting a nonlinear relationship (upward concave) between μ and S during inhibition. The parameters μ_m and K_S estimated by the Luong model were quite closer to the experimental values of 0.34 h^{-1} and 4.2 g l^{-1}, respectively [17].

Similarly, the kinetic parameters estimated for double-exponential and Yano and Koga 2 models fitted well with experimental data (Table 1). The estimated kinetic parameters for all the tested models were used to simulate the specific growth and compared with experimental values (Fig. 3a, b). The simulation results also confirmed that the Luong, double-exponential, and Yano and Koga 2 models were able to simulate the experimental values better than were the other models. The relationship between biomass and products for caffeine demethylase production by *Pseudomonas putida* cultures was determined using Eq. 1. For this, each individual run's data were used to plot

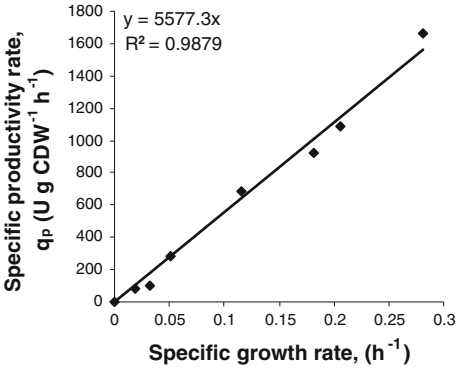

Fig. 4 Luedeking–Piret model fit between specific production rate, q_p (U g CDW^{-1} h^{-1}), and specific growth rate, μ (h^{-1})

production rate (q_p, U g CDW^{-1} h^{-1}) versus specific growth rate (μ, h^{-1}), yielding a straight line with slope of 5,577.3 and intercept of 0 (Fig. 4). Since β is zero, and very low when compared with α, caffeine demethylase production can be considered as growth associated.

Discussion

The bottleneck in the development of bioprocess for caffeine degradation is the availability of a strain that can withstand high concentration of caffeine and degrade caffeine at faster rates. There exists a report on *Pseudomonas* strains that can tolerate ~50 g l^{-1} caffeine, but the report lacks data showing condition and degradation rate at this high concentration [36]. Apart from this, there are a few important reports available on caffeine degradation. *Pseudomonas* sp., with degradation rate of 0.1 g l^{-1} h^{-1} at initial caffeine concentration of 5 g l^{-1} [35], *Serratia* sp. with degradation rate of 0.008 g l^{-1} h^{-1} at initial caffeine concentration of 0.6 g l^{-1} [28], and mixed culture of *Klebsiella* and *Rhodococcus* with degradation rate of 0.05 g l^{-1} h^{-1} at initial caffeine concentration of 0.5 g l^{-1} [25] are reported. However, the rates of degradation and resistance to high concentrations of caffeine with the existing strains do not meet demand, since the concentration of caffeine in effluents is often as high as 10 g l^{-1} [9]. Initially, we carried out kinetic studies in shake flask (before determining the optimal conditions for growth and degradation) and showed that minimum caffeine concentration required for complete inhibition of growth was 20 g l^{-1}. In addition, growth rate is very low for caffeine concentrations greater than 10 g l^{-1} and exhibits higher lag times (~24–60 h). The strain used in this study previously showed caffeine degradation rate of 0.18 g l^{-1} h^{-1}; however, after optimizing the nutrients and physical parameters (pH, agitation, and temperature), the rate of caffeine degradation was increased to 0.29 g l^{-1} h^{-1} [10, 11]. Based on these results, we performed detailed growth and enzyme production kinetics experiments in bioreactor where environmental conditions are highly controlled. Hence, kinetic results obtained in reactor are very important for scale-up and application aspects of onsite caffeine degradation. In this study, we showed similar degradation rates of 0.3 g l^{-1} h^{-1} (at 6.5 g l^{-1} caffeine) in bioreactor. In addition, for the first time, we showed complete caffeine degradation of 20 g l^{-1} at rate of 0.16 g l^{-1} h^{-1}. This implies that 75 g caffeine was degraded at rate of 0.625 g h^{-1}. So far, no reports are available on strains showing such high tolerance and degradation rates, suggesting that the strain reported in this study can be a potential strain for biodegradation of caffeine.

It has been reported earlier that sucrose enhances caffeine degradation without being utilized in shake flasks at low concentrations, which is in agreement with results obtained in this study [17]. So far, no reports are available on production of enzymes involved in degradation of caffeine using bioreactors. It has been shown that the strain used in this study produces caffeine demethylase, and the enzyme is inducible in nature [12]. It has been reported that pH and volumetric oxygen transfer coefficient (K_{La}) were the critical parameters influencing production of caffeine demethylase in bioreactors [18].

In this study, at optimal conditions of pH and K_{La}, growth and enzyme production kinetics experiments were performed. For the first time, we showed higher enzyme specific activity, degradation rate, and specific production rate of caffeine demethylase. To gain greater insight into the growth kinetics, several empirical models describing the substrate inhibition kinetics were used to fit the kinetic data (Table 1). The Andrews model has often been used to explain the inhibition growth kinetics when various toxic compounds such as phenols, thiocyanates, nitrates, ammonia, and volatile acids were used as carbon source [3]. It has been reported that growth kinetics data of *Pseudomonas* sp. using BTX (benzene, toluene, xylene) as substrate were fitted well by the Andrews inhibition model [31]. In another report it was found that simple models did not give accurate prediction of toxic compounds such as phenol, benzene, toluene, etc. Therefore, new models were used by sum kinetics with interaction parameters for *Pseudomonas putida* F1 [33]. Haldane's model is widely studied due to its mathematical simplicity and wide acceptance for representing growth kinetics of inhibitory substrates. Reports are available on phenol and catechol degradation using *Pseudomonas putida* in which Haldane's inhibitory model showed proper fit with high substrate concentration of 500 and 1,000 mg l^{-1}, respectively [1, 5]. In another report on phenol degradation using mixed culture of *Pseudomonas aeruginosa* and *Pseudomonas fluorescence*, kinetics data were fitted well by Haldane, Yano

and Koga, and Aiba and Teissier models [2]. Haldane model also fitted well for substrate inhibition kinetics for *Rhodococcus* sp. NJUST16 using picric acid (20–800 mg l^{-1}) as substrate [22]. In this study, *Pseudomonas* sp. for caffeine degradation was studied with different substrate inhibition models, amongst which the Luong, double-exponential, and Yano and Koga 2 models exhibited the best fits to experimental data for high initial caffeine concentration of 1–20 g l^{-1}. However, at these concentrations, the Edwards, Haldane, Andrews, Yano and Koga 1, Alagappan and Cowan, and Wayman and Tseng equations did not fit well (Table 1). This is due to the fact that these models are not able to predict sharp decrease in specific growth rate at high concentrations and become almost asymptote to substrate concentration (Fig. 3).

In this study also, we carried out enzyme production kinetics (Leudeking−Piret) analysis to determine whether enzyme production was growth related or not (Figs. 2a, 4). Figure 2a shows that both specific growth rate and specific production rate (caffeine demethylase) have similar profiles when grown at different concentrations of caffeine. This was further confirmed by Leudeking−Piret analysis (Fig. 4), where the relationship between specific growth rate and specific production rate is linear with high values of slope, suggesting that enzyme production kinetics is growth related. These results suggest that, in order to have higher specific productivity, the specific growth rate of the organisms has to be maintained at maximum level. In a separate study, we investigated the activity of xanthine oxidase, which is a key enzyme in caffeine catabolism in bacteria. It has been found that xanthine oxidase activity is also higher when caffeine degradation rates are maximum (unpublished results). It has been reported that xylanase production was strongly dependent on biomass in a growth-associated process with *E. coli* DH5α [14]. Similarly, it has also been reported that lipase production is strongly dependent on growth when using *Staphylococcus warneri* EX17 [15]. In conclusion, it was found that, at optimal caffeine concentration of 6.5 g l^{-1}, maximum caffeine degradation of 1.1 g h^{-1} with caffeine demethylase activity of 18,762 U g cell dry $weight^{-1}$ was observed. Among the various models considered, the Luong, double-exponential, and Yano and Koga 2 models were able to explain the cell growth kinetics and its biokinetic parameters very well. The Luedeking–Piret model indicated growth-associated dependence of product formation and biomass concentration. The important findings are: (1) increased tolerance of the strain and ability to grow in the presence of 20 g l^{-1} caffeine and utilize caffeine completely within 120 h at very high degradation rate, (2) reduced lag time when grown at higher concentration, and (3) high level of caffeine demethylase enzyme production kinetics studied for the first time in bioreactor.

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