Effect of substrate concentration on the transient dynamics of specific cell growth during bioconversion of Cr\(^{+6}\) to Cr\(^{+3}\) using polyculture consortia

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The transient dynamics of specific cell growth rate during bioconversion of hexavalent chromium (Cr\(^{+6}\)) to trivalent chromium (Cr\(^{+3}\)) has been studied covering both Cr\(^{+6}\) uninhibited and Cr\(^{+6}\) inhibited conditions in a batch contacting system. Suitable polyculture consortia necessary for this bioconversion have been isolated and purified from tannery waste. It is observed that above the Cr\(^{+6}\) concentration of 33 mg/dm\(^3\), effect of Cr\(^{+6}\) inhibition on the specific cell growth rate is appreciably high. Detailed simulation and modeling work indicate that while Monod model equation is capable of describing the cell growth dynamics under Cr\(^{+6}\) uninhibited condition, the same can well be represented by Briggs-Haldane uncompetitive type model equation under Cr\(^{+6}\) inhibited condition. Since for the present system the difference in magnitudes of two intrinsic kinetic parameters, namely, Monod constant, \(K_S\) and the inhibition constant, \(K_i\) is not appreciably large, a unified transient specific cell growth dynamic equation has been presented.

Keywords: Transient growth dynamics, Cr\(^{+6}\) inhibition, Briggs-Haldane equation, Biological switch, Unified rate equation

Hexavalent chromium (Cr\(^{+6}\)) is regarded as one of the major hazardous chemicals due to its toxic\(^1\),\(^2\), carcinogenic\(^3\),\(^4\) and mutagenic\(^5\),\(^6\) effects on living body. Conversion of Cr\(^{+6}\) to its trivalent form by chemical route is economically feasible when the former is present in an appreciable concentration. However, if the initial concentration of hexavalent chromium is low or very low, as is the case in related industrial effluent but still sufficiently hazardous to cause health hazard\(^7\),\(^8\), use of bioconversion of hexavalent form of chromium to its harmless trivalent form may be an important route to abate environmental pollution threat from hexavalent chromium compounds. A considerable quantum of investigation has been reported on isolation and purification of Cr\(^{+6}\) reducing microorganisms from native source as well as on their identification with the help of classical microbiological and biochemical tests or even 16S rRNA identification method\(^11\),\(^13\). Notably, Xu \textit{et al.}\(^14\) and Cheung and Gu\(^15\) have recently successfully applied the bioreduction technique for converting toxic hexavalent chromium compound to its non-toxic trivalent form. These classical investigations, however, are more related to physicochemical studies of the system and a detailed bioprocess study with respect to specific cell growth response under the forcing function of change in initial Cr\(^{+6}\) concentration is still awaited. As such the understanding of such cell growth response is probably the first important step in bioprocess study of any bioremediation system.

In the present investigation the transient dynamics of specific cell growth, perturbed by the change in initial Cr\(^{+6}\) concentration during bioconversion of hexavalent chromium to its trivalent form using mixed culture consortia has been studied. The main interest of the present investigation was to identify the inhibitory effect of Cr\(^{+6}\) concentration on the specific cell growth rate and subsequently to correlate the response of cell growth as a function of initial inhibitor, Cr\(^{+6}\), concentration encompassing both inhibition free and inhibited regimes through judicious simulation and modeling work. The unified response equation so obtained is expected to be useful in predicting the cell growth rate in both uninhibited and inhibited regimes of bioconversion of Cr\(^{+6}\). Such prediction is considered as an \textit{apriori} requirement for understanding of any biochemical system.

Microorganisms used for this study were isolated from tannery waste. These were purified and characterized by standard microbiological tests\(^16\),\(^18\) as well as scanning electron microscope (SEM). The mixed culture consortia, so obtained, consisted of \textit{Pseudomonas} sp., \textit{Bacillus} sp. and \textit{Micrococcus} sp. The chromium resistant nature of the above
microorganisms has also been observed by previous investigators\textsuperscript{11,19-21}.

**Experimental Procedure**

**Bacterial strains**

Microorganisms isolated and purified from polyculture, collected from the liquid effluent of local tanneries, situated in Kolkata, India, has been used for the present investigation. The genus of the isolated microorganisms have been identified as *Pseudomonas* sp., *Bacillus* sp. and *Micrococcus* sp. through 16S rDNA technique and Scanning Electron Microscope (SEM).

**Chemicals**

\(\text{NH}_4\text{Cl (Ranbaxy), MgSO}_4\cdot7\text{H}_2\text{O (E. Merck), } \text{FeSO}_4\cdot7\text{H}_2\text{O (E. Merck), CaCl}_2\cdot2\text{H}_2\text{O, (E. Merck), CH}_3\text{COONa}\cdot3\text{H}_2\text{O (E. Merck), K}_2\text{HPO}_4 \text{ (E. Merck), yeast extract powder (LOBA chemie), SD}-\text{diphenylcarbazide (E. Merck), Acetone, AR (E. Merck), H}_2\text{SO}_4 \text{ (E. Merck), MOPS-NaOH buffer (Sigma) and K}_2\text{Cr}_2\text{O}_7 \text{ (E. Merck) have been used.}}\)

**Bacterial enrichment**

The polyculture consortia were grown and maintained in Luria broth\textsuperscript{11,12,22}. This acetate-minimal medium was composed of (per litre) \(\text{NH}_4\text{Cl (1 g), MgSO}_4\cdot7\text{H}_2\text{O (0.2 g), FeSO}_4\cdot7\text{H}_2\text{O (0.001 g), CaCl}_2\cdot2\text{H}_2\text{O (0.001 g), CH}_3\text{COONa}\cdot3\text{H}_2\text{O (5 g), K}_2\text{HPO}_4 \text{ (0.5 g) and yeast extract powder (0.5 g). The phosphate source was autoclaved separately in 10 mL distilled water and added to the rest of the medium when cool. Initially the effluent sample with trimmings (1 mL) was inoculated in 20 mL of sterile \(0.2 \text{ M.Pa., 121°C, 15 min} \) selective media. The effluent-medium suspension was supplemented with \(\text{K}_2\text{Cr}_2\text{O}_7 \text{ solution at a concentration of 1 mg/dm}^3 \text{ of Cr}^{6+}\). To adapt to the new laboratory environment, consortia were incubated at 28°C in a rotary shaker at 150 rpm for seven days. Enriched culture was obtained by repeated inoculation of preceding bacterial culture in fresh selective medium mentioned earlier along with \(\text{K}_2\text{Cr}_2\text{O}_7 \text{ solution. The potassium dichromate solution was added to a final concentration of 80.29 mg/dm}^3 \text{ of Cr}^{6+} \).

**Batch experiments**

To determine the growth kinetics of the microorganisms with respect to different initial \(\text{Cr}^{6+} \) concentration in culture medium, batch experiments were conducted in Erlenmeyer flasks. For each initial reactant concentration a set of experiments was performed for a period of 48 h. The initial concentration of \(\text{Cr}^{6+} \) was varied from 5.25 to 80.29 mg/dm\(^3\). For each set, biomass and the residual hexavalent chromium concentration of the reaction broth were determined at an interval of 4 h.

**Determination of biomass concentration**

The concentration of bacterial mass in the reaction broth was determined both spectrophotometrically\textsuperscript{23,24} and by dry weight method\textsuperscript{25}. In this method 20 mL nutrient broth, enriched with bacterial strains, was centrifuged at the rate of 10,000 rpm for 15 min and the bacterial mass was washed with buffer solution. The washed wet cell mass was then transferred to a pre-weighed aluminum cup and dried at 80°C for 24 h. Cell concentration of bacterial suspensions withdrawn at different time intervals were determined at 600 nm, spectrophotometrically.

**Hexavalent chromium analysis**

After centrifugation, the supernatant was collected to measure the residual \(\text{Cr}^{6+} \) concentration. As \(\text{Cr}^{6+} \) concentration decreased with the reaction time, the reactant activity in supernatant was determined by \(\text{Cr}^{6+} \) specific colorimetric reagent SD-diphenylcarbazide\textsuperscript{11,12}. SD-diphenylcarbazide (0.025 g) was dissolved in 9.67 mL acetone (AR) to prepare SD-diphenylcarbazide solution. The reaction mixture was prepared in the test tube containing 200 \(\mu\)L sample, 400 \(\mu\)L 20 mM MOPS-NaOH buffer, 33 \(\mu\)L 3M H\(_2\)SO\(_4\), 40 \(\mu\)L SD-diphenylcarbazide solution and 327 \(\mu\)L distilled water. Spectrophotometric measurements were done at 540 nm.

**Scanning electron microscopy**

Scanning electron microscopy (SEM) study of individual monoculture, isolated from the mixed culture by petridish colony forming technique was carried out at Indian Association for the Cultivation of Science. The genus of the isolated microorganisms, were identified from the SEM pictures [Fig. 1(a-c)]. These strains were also identified by 16S rDNA technique.

**Results and Discussion**

Biodegradation of \(\text{Cr}^{6+} \) to \(\text{Cr}^{3+} \) is the result of a combination of biotic and abiotic processes as indicated by Daulton and his coworkers\textsuperscript{26}. Although \(\text{Cr}^{6+} \) in true sense is not a substrate of concerned
microorganisms but in reality there exists a linear proportionality between the consumption of carbon source (true substrate) and the conversion of Cr$^{+6}$ to Cr$^{+3}$. Thus, from the reaction engineering point of view monitoring of Cr$^{+6}$ conversion can lead to the estimation of kinetic parameters. In fact all the characteristics of substrate are present in Cr$^{+6}$ conversion including the inhibition effect. In the present investigation thus monitoring of Cr$^{+6}$ concentration has been made and used for kinetic analysis in place of the conventional substrate.

The progress curves of cell growth as a function of initial Cr$^{+6}$ concentration are shown in Fig. 2. The figure clearly indicates the presence of an exponential phase followed by a stationary phase for all the reactant concentration studied. However, the lag phase is not clearly visible possibly due to prior adaptation of the cells during its purification stage. Figure 3 shows the progress curves of Cr$^{+6}$ depletion with its initial concentration as parameter. It is apparent from the figure that at lower reactant concentration an exponential decay results whereas at higher concentration such trend is not followed. This behaviour is an indication of inhibition of cell growth by Cr$^{+6}$ at its higher initial concentration. The observation is in accordance with the findings of previous workers$^{27}$ who have reported that due to the presence of some enzymes called chromium reductases, different microorganisms belonging particularly the genus, *Pseudomonas* can reduce Cr$^{+6}$ to Cr$^{+3}$. It is also expected that an abiotic reduction of Cr$^{+6}$ is also possible in presence of Fe$^{+2}$ present in the system$^{28}$. The transformation capacity of Cr$^{+6}$ by microorganisms at higher initial concentration of Cr$^{+6}$ observed by other researchers$^{22}$ was re-established by the observation of inhibitory effect of Cr$^{+6}$ at high concentration under the present investigation. In order

Fig. 1—Scanning electron microscopic photograph of (a) isolated *Bacillus* sp., (b) isolated *Pseudomonas* sp. and (c) isolated *Micrococcus* sp., in colony and individual form.

Fig. 2—Time history of biomass concentration with varying initial Cr$^{+6}$ concentration.

(-4.25 mg/dm$^3$; ■ 11.05 mg/dm$^3$; ▲ 15.47 mg/dm$^3$; × 33 mg/dm$^3$; *45.31 mg/dm$^3$; ● 55.86 mg/dm$^3$; + 80.29 mg/dm$^3$)

Fig. 3—Time history of Cr$^{+6}$ concentration with varying initial Cr$^{+6}$ concentration.

(-4.25 mg/dm$^3$; ■ 11.05 mg/dm$^3$; ▲ 15.47 mg/dm$^3$; × 33 mg/dm$^3$; *45.31 mg/dm$^3$; ● 55.86 mg/dm$^3$; + 80.29 mg/dm$^3$)
to study the reaction engineering behaviour of the present system, the initial specific cell growth rate (μ) has been computed for each initial Cr\textsuperscript{+6} concentration and subsequently these have been plotted against the corresponding initial reactant concentration as shown in Fig. 4. From the figure it follows that the specific cell growth rate increases up to a Cr\textsuperscript{+6} concentration of 33 mg/dm\textsuperscript{3}, beyond which the initial specific cell growth rate sharply declines with the increasing initial Cr\textsuperscript{+6} concentration. This indicates inhibition on the specific growth rate by cell. The appearance of two different reaction mechanisms as evident from Fig. 4 necessitates the bifurcation of the entire reaction engineering domain into two distinct zones, namely, inhibited and uninhibited regimes for their reaction engineering analysis.

**Analysis of Cr\textsuperscript{+6} uninhibited domain**

The nature of the cell growth progress curve and the exponential decay of Cr\textsuperscript{+6} concentration with time (t) (Figs 2 and 3) at lower concentration region indicate that the system may follow classical Monod equation. Thus, in the present investigation simulation has been carried out using the classical Monod equation as the model equation. The Monod equation in terms of cell growth (r\textsubscript{C}) and substrate depletion (r\textsubscript{S}) are given by

\[
r\textsubscript{C} = \frac{\mu_{\text{max}} \cdot S \cdot C}{(K\textsubscript{S} + S)} \quad \cdots (1)
\]

\[
(-r\textsubscript{S}) = \frac{1}{Y_{\text{CS}}} \cdot \frac{\mu_{\text{max}} \cdot S \cdot C}{(K\textsubscript{S} + S)} \quad \cdots (2)
\]

where, C and S are the biomass and Cr\textsuperscript{+6} concentration respectively, \(\mu_{\text{max}}\) is the maximum specific cell growth rate and \(Y_{\text{CS}}\) is the yield coefficient of cell mass with respect to Cr\textsuperscript{+6} concentration.

Verification of the validity of Monod equation can be done by two different techniques, namely, utilization of rate versus concentration data and direct experimental data from batch plug flow or mixed flow reactor. Since good interpretable data can only come from the above mentioned reactors it is best to use the direct fit of raw data from these reactors rather than evaluate the rate at each point and then fit the rate versus concentration data\textsuperscript{29}.

In the present investigation kinetic study has been carried out using batch mode. The performance equation of a batch reactor carrying out a fermentative reaction is obtained by integrating the Monod equation assuming yield coefficient \((Y_{\text{CS}})\) to be constant. Such assumption is reasonably valid if experimental data are picked up during the exponential cell growth phase since it represents the balance as discussed earlier. The performance equation can be obtained by integrating Eq. (2) and utilizing the relation between the Cr\textsuperscript{+6} concentration and cell concentration given by

\[
y_{\text{CS}} = \frac{dC}{dS}
\]

or, \(C - C_0 = Y_{\text{CS}} \cdot (S_0 - S)\) \quad \cdots (3)

where, \(C_0\) and \(S_0\) are the initial biomass and initial Cr\textsuperscript{+6} concentration respectively. Integrating Eq. (3) and on rearrangement one gets

\[
\frac{t}{\ln \frac{C}{C_0}} = \frac{1 + M}{\mu_{\text{max}}} + \frac{M}{\mu_{\text{max}}} \cdot \frac{\ln \frac{S_0}{S}}{\ln \frac{C}{C_0}} \quad \cdots (4)
\]

where \(M = \frac{K\textsubscript{S}}{Y_{\text{SC}} \cdot C_0 + S_0}\)

Eq. (4) predicts that if the system follows uninhibited Monod model equation a plot of \(\frac{t}{\ln \frac{C}{C_0}}\) versus \(\frac{\ln \frac{S_0}{S}}{\ln \frac{C}{C_0}}\) should yield a straight line having slope \(\frac{M}{\mu_{\text{max}}}\) and an intercept on the ordinate \(\frac{1 + M}{\mu_{\text{max}}}\).
In order to test the validity of Monod’s equation the experimental data within the Cr\(^{+6}\) uninhibited region (shown in Fig. 4) have been plotted for three different initial Cr\(^{+6}\) concentration with \(\frac{t}{C_0}\) as ordinate and \(\frac{S}{C_0}\) as abscissa. This is shown in Fig. 5. It is evident from the plots that a reasonably good straight line can be drawn for the case of each initial Cr\(^{+6}\) concentration. Thus, the present simulation work leads to the conclusion that the reaction engineering behaviour of the present system on the Cr\(^{+6}\) uninhibited condition can be effectively described by the Monod model. In order to evaluate the intrinsic kinetic parameters, the maximum specific cell growth rate, \(\mu_{\text{max}}\) and the Monod constant, \(K_S\) present in Monod equation, the slope of each line and the corresponding intercept on the ordinate have been evaluated by regression analysis and the average numerical values of \(\mu_{\text{max}}\) and \(K_S\) are found to be 0.0318 h\(^{-1}\) and 32.388 mg/dm\(^3\) respectively. The rate equation for cell growth under chromium poison free condition may, therefore, be written as

\[
r_C = \frac{0.0318SC}{(32.388 + S)}
\]

The numerical value of \(Y_{CS}\) has been computed by taking the ratio of magnitude of cell growth to the corresponding substrate depletion at a definite time interval in the exponential phase of growth. When the yield coefficient at several time intervals within the exponential growth phase have been evaluated, it is observed that the numerical value of \(Y_{CS}\) in each case remains almost constant. In the present investigation the average of four values of \(Y_{CS}\) taken at four different time intervals has been used. Computation of \(Y_{SC}\) has been done by taking the inverse of the value of \(Y_{CS}\).

**Analysis of Cr\(^{+6}\) inhibited domain**

In analyzing the chromium inhibited cell growth region as shown in Fig. 4. Briggs-Haldane model has been used. The model equation is given by

\[
\mu = \frac{\mu_{\text{max}}S}{K_S + S + \frac{S^2}{K_i}}
\]

It is evident from Eq. (6) that when \(K_S/S<1\), the specific growth rate experiences Cr\(^{+6}\) inhibition. Moreover when \(K_S<<1\), the Eq. (6) becomes

\[
\mu = \frac{\mu_{\text{max}}S}{1 + \frac{S}{K_i}}
\]

which may be arranged in linear form as follows

\[
\frac{1}{\mu} = \frac{1}{\mu_{\text{max}}} + \frac{S}{K_i} \frac{1}{\mu_{\text{max}}}
\]

From Eq. (8), it is evident that a plot of \(\frac{1}{\mu}\) versus \(S\) should give a straight line having slope \(\frac{1}{\mu_{\text{max}}}K_i\) and an intercept on the ordinate as \(\frac{1}{\mu_{\text{max}}}\), if Briggs-Haldane type model mechanism is followed by the system.

In the present investigation, the experimental data on cell growth progress curve (Fig. 2) have been utilized to compute the initial specific cell growth rate, \(\mu\) by measuring the slope of the tangent drawn at the initial points and dividing the magnitude so obtained by the initial cell concentration. The
computed data of initial specific cell growth rate for different initial Cr\textsuperscript{6+} concentration have been used to construct a plot of reciprocal of specific cell growth rate against corresponding initial Cr\textsuperscript{6+} concentration. This is shown in Fig. 6. A reasonably good straight line so obtained, indicates that the dynamics response of the specific cell growth rate may be explained by Briggs-Haldane type uncompetitive substrate inhibition model equation. Calculating the slope and intercept of the straight line shown in Fig. 6 by regression analysis, the magnitudes of the first order rate constant $\mu_{\text{max}}$ and the inhibition constant $K_i$ are found to be 0.0328 h\textsuperscript{-1} and 35.92 mg/dm\textsuperscript{3} respectively. It is interesting to note that the magnitude of $\mu_{\text{max}}$ is slightly different from that obtained using Monod equation. This is, however, expected since an error (however small it may be) has been introduced in developing Eq. (6) by neglecting $K_S$ with respect to $K_i$. This error can easily be visualized by noting $K_S/S$ ratio which for the case of maximum Cr\textsuperscript{6+} concentration (80.29 mg/dm\textsuperscript{3}) has the magnitude of 0.404. Although this magnitude does not represent a situation, which is much less than unity, the system is observed to follow modified Briggs-Haldane equation reasonably well as indicated by Fig. 6. The rate equation under substrate inhibited domain may therefore be written as

$$\mu = \frac{0.0328.S}{32.34 + S + \frac{S^2}{35.92}}$$ \hspace{1cm} \ldots (9)$$

**Overall dynamics of the cell growth**

The Briggs-Haldane model equation is general in the sense that when $K_i$ is much greater than $K_S$, the equation automatically turns into the Monod equation at low Cr\textsuperscript{6+} concentration. On the other hand at high Cr\textsuperscript{6+} concentration the Briggs-Haldane equation readily transforms into Eq. (9), which describes the system dynamics under Cr\textsuperscript{6+} inhibited condition. However, in the present case, the magnitude of $K_i$ is marginally greater than $K_S$, indicating that the present system dynamics cannot be described by a single model equation i.e. by Briggs-Haldane equation alone. This necessitates the addition of two biological switches, which will be active in a given preset condition. A unified equation capable of describing the dynamics of the overall cell growth may therefore be compounded as follows:

$$\mu = \sigma \cdot \frac{0.0318.S}{32.388 + S} + \delta \cdot \frac{0.0328.S}{32.34 + S + \frac{S^2}{35.92}}; \hspace{1cm} \ldots (10)$$

where, $\sigma$ and $\delta$ are dynamic biological switches.

$\sigma = 1$ and $\delta = 0$ when $S \leq 33$ mg/dm\textsuperscript{3}

$\sigma = 0$ and $\delta = 1$ when $S \geq 33$ mg/dm\textsuperscript{3}

Where the critical value of Cr\textsuperscript{6+} concentration is identified as 33 mg/dm\textsuperscript{3} for the present investigation.

**Conclusion**

Inhibition effect of hazardous hexavalent form chromium on the specific cell growth rate is quite appreciable above its concentration level of 33 mg/dm\textsuperscript{3}. A thorough investigation on how the systematic cell growth rate is affected by the Cr\textsuperscript{6+} concentration, reveals two distinctly different regimes, the first one is characterized by substrate uninhibited situation (below a concentration level by 33 mg/dm\textsuperscript{3}) where dynamic response may be described by classical Monod model equation. The second domain (Cr\textsuperscript{6+} concentration level above 33 mg/dm\textsuperscript{3}) is evidently a situation where appreciably Cr\textsuperscript{6+} inhibition is observed. The response of this regime has been successfully simulated by Briggs-Haldane type model equation. A unified bioconversion rate equation of the entire region (4.25 mg/dm\textsuperscript{3} to 80.29 mg/dm\textsuperscript{3}) covering both Cr\textsuperscript{6+} uninhibited and inhibited has been proposed which is expected to be useful in bioreactor design for this system.

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