Biodegradation kinetics of benzoic and anthranilic acids by *Micrococcus* sp.

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This study presents biodegradation of benzoic acid (BA) and anthranilic acid (AA) by *Micrococcus* sp. under aerobic conditions. Initial concentrations of BA and AA were varied (500-2500 mg/l) and almost complete biodegradation of BA and AA was observed within 30 h. Andrews kinetic model for single substrate was fitted to obtain maximum specific growth rates, half saturation and substrate inhibition constants. Cell growth with degrading BA ($\mu_{m,BA} = 0.645 \text{ h}^{-1}$) was faster than with degrading AA ($\mu_{m,AA} = 0.63 \text{ h}^{-1}$). Under biodegradation of BA and AA, first order rate constant values decreased with increase in initial concentration.

**Keywords:** Andrews model, Anthranilic acid, Benzoic acid, Biodegradation, *Micrococcus* sp.

**Introduction**

Biological degradation of toxic organic compounds offer advantages than other methods that are cost intensive and end up with secondary pollutants. Wastewater from some industries consists of benzoic acid (BA) and its derivatives. Benzoate is an intermediate during biodegradation of aromatics and recently has been reported as an intermediate of anaerobic biodegradation of benzene. Biodegradation of benzoate by *Pseudomonas putida* and its degradation pathway has been reported. Nelson *et al.* reported biodegradation of benzoate by *Burkholderia cepacia* G4. Biodegradation of benzoate using *P. cepacia* occurred through ortho and meta pathways, induced simultaneously. Genetically modified microorganism also employed for degradation of chloro and methyl substituted BAs and compounds were metabolized by ortho cleavage route. Degradation of BA in a three phase fluidized bed reactor has been reported. Chlorobenzoates were degraded effectively by *Burkholderia* sp. and *Bradyrhizobium* sp. Ajithkumar & Kunhi reported that *P. aeruginosa* 3mT degrade high concentrations of 3-chlorobenzoate and 4-chlorobenzoate and degraded 4-CBA (> 99%) through formation of respective chlorocatechol, via a modified ortho-pathway. Miguez *et al.* reported mechanism of uptake of BA and 2,4-dichlorobenzoic acid by *Alcaligenes denitrificans* BRI 3010, BRI 6011 and *Pseudomonas* sp. strain B13 and these organisms were capable of degrading various isomers of chlorinated benzoic acids. David *et al.* reported that *Enterobacter aerogenes*, *Raoultella ornithinolytica*, *Stenotrophomonas maltophilia*, *Xanthomonas campestris* and *Stenotrophomonas acidaminophila* degrade BA (up to 9 mM) under anoxic conditions with nitrate as electron acceptor.

Many of nitro- and amino aromatics, produced in large amounts by chemical industries, are toxic to environment. Anthranilic acid (AA) is reported as an intermediate during biodegradation of o-nitrobenzoate and carbazole. Mineralization of AA as a sole carbon and energy source using methanogenic granular sludge was studied and treatment was carried out in batch and in an anaerobic expanded granular sludge bed reactor at low concentrations under anaerobic conditions. AA has been completely oxidized to $\text{CO}_2$ and $\text{NH}_4^+$ by anaerobic degradation with two strains of denitrifying bacteria of *Pseudomonas**. Sludge isolated from wastewater treatment plant has been successfully used for biodegradation of 2, 3 and 4-iodobenzoic acids. Biodegradation of halogenated BAs has also been reported.

This study presents feasibility of BA and AA biodegradation at higher initial concentrations and studies growth kinetics using Andrew’s model.
Materials and Methods

Culture Media Preparation

Mineral salt medium (MSM)\(^{21}\) (pH 7) contained: \(\text{NH}_4\text{NO}_3\), 1; \((\text{NH}_4)_2\text{SO}_4\), 0.5; \(\text{NaCl}\), 0.5; \(\text{MgSO}_4\), 0.5; \(\text{K}_2\text{HPO}_4\), 1.5; \(\text{KH}_2\text{PO}_4\), 0.5; \(\text{FeSO}_4\), 0.01; and \(\text{CaCl}_2\), 0.01 g/l.

Microorganism Growth Conditions

Microorganism utilizing BA and AA as a sole source of carbon was isolated from petroleum refinery environment by enrichment culture in MSM containing 200 mg/l of BA and AA. Enrichments were incubated for 5 days at 30\(^\circ\)C on rotary shaker operated at 180 rpm, and portions of enrichments were then transferred to fresh medium. After five 58 h interval transfers, culture was streaked on plates containing solidified agar containing MSM. A pure culture of isolate was maintained in refrigerator at 4\(^\circ\)C. Isolated bacterium, identified as \textit{Micrococcus} sp., degraded BA and AA effectively.

Degradation of Benzoic Acid (BA) and Anthranilic Acid (AA)

Free cell suspension (3 ml) was inoculated into an Erlenmeyer flask (250 ml) containing sterile MSM (97 ml) and agitated and aerated in a rotary shaker at 30\(^\circ\)C and 180 rpm. Initial BA and AA concentrations (200-2500 mg/l) were used in batch experiments carried out at pH 7.

Cell Density Measurements and COD Determination

Bacteria concentrations were determined by optical density measurement at 610 nm using a UV-VIS digital spectrophotometer and correlated to biomass concentration. BA and AA concentrations were assessed in terms of chemical oxygen demand (COD), which was determined by APHA method.

Results and Discussion

Biodegradation of Benzoic Acid (BA) and Anthranilic Acid (AA)

With increase in incubation time, COD decreased (Fig. 1a) and complete destruction of BA (500 mg/l) was achieved within 10 h, whereas 24 h required for destruction of BA at 2500 mg/l. \textit{Micrococcus} sp. utilized BA as a sole source of carbon with faster degradation rate. As initial concentration increases, time required for complete mineralization also increases due to increase in organic load.

Initial COD increased with an increase in initial concentration of AA and decreased with an increase in incubation time (Fig. 1b). Complete destruction of AA (500 mg/l) was in 12.5 h, whereas AA (2500 mg/l) required 29 h. \textit{Micrococcus} sp. was found more efficient in degrading BA than AA.

Kinetic Studies

First order kinetics generally has following form:

\[
\ln C = -Kt + A. \quad \text{...(1)}
\]

where \(C\), initial concentration of BA or AA in terms of COD (mg/l); \(K\), first order kinetic constant, h\(^{-1}\); \(t\), time in s; and \(A\), constant. Half-life of first order reaction of BA and AA by \textit{Micrococcus} sp. can be obtained as

\[
t_{1/2} = \frac{\ln 2}{K}. \quad \text{...(2)}
\]

Experimental data were fitted with Eqs (1) and (2) and kinetic equations obtained for BA and AA biodegradation (Table 1) gave a better fit for first order model. Increase in initial concentrations of BA and AA significantly decreased first-order rate coefficient (K), indicating dependence of biodegradation rate on initial concentration of substrate employed.
Growth Kinetics

Among substrate inhibition models, Andrews kinetic model is most widely used\textsuperscript{22,23}. Gouder \textit{et al}\textsuperscript{24} reported that Andrews model also acts as a good representation of experimental data. Andrews model\textsuperscript{25} describing substrate inhibition kinetics of a single substrate is given as

\[
\mu = \frac{1}{X} \frac{dX}{dt} = \frac{\mu_m S}{K_s + S + S^2/K_i} \quad \ldots(3)
\]

where $S$ is substrate concentration (mg/l); $t$, time (s); $\mu$, specific growth rate (h$^{-1}$); $X$, biomass concentration (g); $\mu_m$, maximum specific growth rate (h$^{-1}$); $K_s$, half saturation constant (mg/l) and $K_i$ is substrate inhibition constant (mg/l). Constants were evaluated by using nonlinear least squares technique. Biomass concentrations were measured with time for different substrate concentrations (200-2500 mg/l) of BA and AA. Specific growth rate, $\mu$, for various initial concentrations of BA and AA was determined using semi logarithmic plots of biomass versus time. Using specific growth rates obtained, Andrews kinetic model for single substrate was fitted and relevant plots drawn (Fig. 2). Model equations and their regression coefficients are given as

For BA

\[
\mu = \frac{0.65 S}{11 + S + S^2/12500} \quad (R^2 = 0.99)
\]

For AA

\[
\mu = \frac{0.63 S}{5 + S + S^2/4000} \quad (R^2 = 0.98)
\]

Fig. 2 — Specific growth rate of Micrococcus sp. on: a) BA; and b) AA

<table>
<thead>
<tr>
<th>Compound name</th>
<th>Initial concentration mg/l</th>
<th>Kinetic equations</th>
<th>Half life $t_{1/2}$ h</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzoic acid</td>
<td>500</td>
<td>ln C = -0.2472t + 6.458</td>
<td>0.357</td>
<td>0.978</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>ln C = -0.2335t + 7.481</td>
<td>0.337</td>
<td>0.964</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>ln C = -0.1353t + 7.871</td>
<td>0.195</td>
<td>0.965</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>ln C = -0.1259t + 8.380</td>
<td>0.182</td>
<td>0.989</td>
</tr>
<tr>
<td></td>
<td>2500</td>
<td>ln C = -0.1023t + 8.616</td>
<td>0.148</td>
<td>0.95</td>
</tr>
<tr>
<td>Anthranilic acid</td>
<td>500</td>
<td>ln C = -0.1917t + 6.575</td>
<td>0.276</td>
<td>0.966</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>ln C = -0.1105t + 7.355</td>
<td>0.159</td>
<td>0.966</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>ln C = -0.1027t + 8.022</td>
<td>0.148</td>
<td>0.961</td>
</tr>
<tr>
<td></td>
<td>2000</td>
<td>ln C = -0.0979t + 8.282</td>
<td>0.141</td>
<td>0.978</td>
</tr>
<tr>
<td></td>
<td>2500</td>
<td>ln C = -0.0863t + 8.456</td>
<td>0.125</td>
<td>0.97</td>
</tr>
</tbody>
</table>
Maximum ¼ values obtained for BA (0.65 h⁻¹) and AA (0.63 h⁻¹) were higher than those reported²⁶ for μ_max (0.27 h⁻¹) of Pseudomonas sp. during aerobic biodegradation of phenol at 30°C. Aerobic shake flask biodegradation of phenol twenty-fourth by microorganism gave μ_max at 0.251 h⁻¹. K₉ values (11 mg/l for BA and 5 mg/l for AA) indicate that microorganism has ability to grow at lower concentration of substrates. K₁ values for BA (12500 mg/l) and AA (4000 mg/l) illustrate that inhibition is significant only at higher concentrations.

Conclusions

BA and AA can be degraded effectively by Micrococcus sp. Increase in initial concentration increased time required for complete degradation. Also, biodegradation kinetics described by first-order reaction model indicated that rate of degradation depends on initial concentration of substrate. Microorganism growth kinetics was described by Andrews kinetics model and predicted microorganism growth well on BA and AA. Also, BA was found better substrate for Micrococcus sp. than AA.

References