Effect of glucose and chloramphenicol on ABS biodegradation by a bacterial consortium

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Abstract:
A variety of environmental inocula were tested for the development of 2-aminobenzenesulfonate (2-ABS) degrading bacterial enrichment. A bacterial consortium (BC) consisting of two strains, which could utilize 2-ABS as the sole carbon and energy sources under aerobic conditions, could only be developed from the sludge derived from wastewater treatment facility of a large organic chemical industry manufacturing nitro and amino aromatics. These strains have been identified, to be belonging to the genus, *Acinetobacter* and *Flavobacterium,* by 16S rDNA gene sequence analysis. 2-ABS removal pattern in the presence of glucose was significantly influenced by acclimation characteristics of the culture. Consortium adapted to 2-ABS/glucose demonstrated the concomitant removal of both substrates, whereas initial glucose utilization and diauxic growth pattern were observed with glucose adapted culture. These results along with the effect of chloramphenicol showed that the 2-ABS degrading enzymes are inducible in nature.

Keywords: 2-Aminobenzenesulfonate, Biodegradation, Mineralization, Bacterial Consortium.

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Introduction:
Aminobenzenesulfonates, which represent xenobiotic compounds, exhibit many useful properties and are used extensively in the production of azo dyes, pharmaceuticals and pesticides (Lindner, 1985). Thus they are synthesized in large quantities and released into the environment through wastewaters emanating from these industries. Xenobiotic character as well as polar nature of ABS renders these compounds resistant to degradation by unadapted activated sludge and bacterial species utilizing normal aromatics (Laskin and Lechevalier, 1984; Fiegel and Knackmuss, 1993). During last two decades, few mixed as well as pure bacterial cultures, which can utilize ABS as sole carbon and energy sources, have been isolated (Nortemann et al., 1986; Thurnheer et al., 1986; Locher, et al., 1989; Rozgaj and Glancer, 1992; Stolz, 1999).

Materials and Methods:
Source of inoculum
Activated sludge from domestic and industrial wastewater treatment units as well as dye contaminated soils (taken from the vicinity of dyeing industries) were used as inocula for the development of enrichment culture.

A defined mineral medium (MM), consisting of following constituents, was used for the enrichment as well as the growth of 2-ABS degrading culture: 12g Na_2HPO_4, 2g KH_2PO_4, 0.5g NH_4Cl, 0.1g $MgCl_2$. $6H_2O$, and 0.05g $CaCl_2$.2 H_2O per liter of distilled water (Nortemann et al., 1986). 1 ml filter-sterilized trace element solution (Kneimeyer et al., 1999) and required volume from the stock 2-ABS solution (5 g/l, neutralized to pH 7.0 using 1 N NaOH) were added to the growth medium after sterilization. Final pH of the medium was 7± 0.2. Liquid cultures were grown in 100 ml medium taken in 250 ml Erlenmeyer flask, which were kept on orbital shaking incubator (120 rev min^{-1}) maintained for 35 ± 2^0 C.

Enrichment and development of 2-ABS degrading culture
Either 10 ml of 1:10 diluted sludge suspension or 1 gram soil (suspended in sterilized medium) was added to 90 ml portions of MM containing 200 mg l^{-1} 2-ABS. 2-ABS removal and turbidity were monitored periodically. When over 80% 2-ABS was degraded, further enrichment was carried out by transferring 10 ml (v/v) culture to fresh medium. 2-ABS concentration in the medium was increased gradually in subsequent transfers up to 800 mg l^{-1}. After many transfers, the culture, thus obtained, was serially diluted and appropriate dilutions were spread on either nutrient agar or MM agar (20 g l^{-1}) supplemented

with 1000 mg l^{-1} 2-ABS. Two morphologically distinct colonies appeared on nutrient medium during all isolation procedures, after 24 h of incubation at 35± 2^0C. The composition of this culture was periodically checked by plating on nutrient agar. All studies were carried out with this stable two-member culture.

Growth on 2-ABS

2-ABS degradation was monitored at different initial concentrations ranging from 100-800 mg l^{-1}. Aliquots were withdrawn periodically. Bacterial growth was determined by measuring the turbidity at 555 nm. Samples were then centrifuged at 1100 xg and 2-ABS was estimated in the supernatant.

Effect of glucose on 2-ABS degradation

Cells of BC, which, were adapted to 2-ABS, 2-ABS + glucose and only glucose, were used as inocula for studying the effect of glucose on 2-ABS degradation. For adapting the culture, they were grown for three cycles on respective substrates prior to their use as inocula. Glucose and 2-ABS removal as well as bacterial growth were monitored periodically. A control flask, where the inoculum was derived from 2-ABS grown culture, was also included.

Characterization and identification of bacterial isolates

Isolates from 2-ABS degrading consortium were characterized using gram- staining and few biochemical tests (Cappuccino and Sherman, 1999). Identification of the isolated bacterial strains was done by sequencing of the 16S rDNA gene.

Genomic DNA extraction and purification as well as the PCR-mediated amplification of the 16S rDNA were carried out as described by Bhattacharya et al. (2003). Sequencing of 16S rDNA gene was carried out using the Microseq 16S DNA sequencing Kit^{TM} (Applied Biosystems, Foster City, Calif.). The sequences were analyzed with an ABI Prism 310 Genetic analyzer (Applied Biosystems, Foster City, Calif.) as per manufacturer's instructions. The sequences of 16S rDNA genes were subjected to BLAST searches of the NCBI GenBank database for identification. The sequence has been deposited in the NCBI GenBank PubMed database (http://www.ncbi.nlm.nih.gov/) with accession numbers EF449505 and EF470567.

Analytical procedures

Biomass growth was monitored by measuring the absorbance at 555 nm against distilled water in a UV-Visible spectrophotometer (Shimadzu, Japan, model-160A). 2-ABS was estimated by measuring the absorbance at 237 nm in UV-Visible spectrophotometer.

Results and Discussion:

Development of 2-ABS degrading consortium

Many source inocula were used in this study to develop bacterial culture, which can utilize 2-ABS as the sole carbon and energy source. No enrichments could be obtained when the sludge, from Kanpur city domestic wastewater and a local dyeing wastewater treatment units, were used as the inocula. Similar observations were made with few dye-contaminated soils. A sustainable 2- ABS degrading culture could only be developed from the sludge derived from the aerobic biological unit treating effluents generated in a large organic chemical manufacturing industry located at Rasayani, Maharashtra, India. This industry manufactures a variety of organic chemicals, which include nitro and amino aromatics. Thurnheer et al. (1986) have reported that the enrichments for the degradation of benzenesulfonates could only be developed from the inoculum derived from either large domestic or sulfonate chemicals wastewater treatment units. Recently Tan et al. (2005) showed that aerobic degradation of 2-ABS was observed only with two of the inoculum sources that were historically polluted with sulfonated aromatic amines. These observations indicate that 2-ABS degrading organisms are still very rare in the environment.

Degradation of 2-ABS in the presence of glucose

2-ABS and glucose degradation and growth of 2-ABS adapted bacterial consortium on these mixed substrates is shown in Fig. 1a. Glucose and 2-ABS were utilized simultaneously and the degradation rates of both these substrates were similar. Fig. 1b presents the observations with only 2-ABS as growth substrate.

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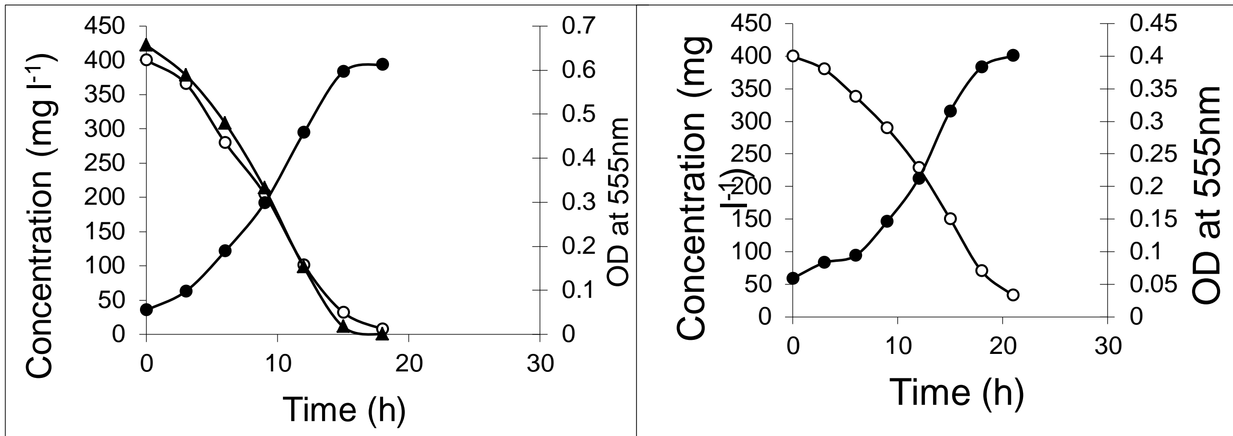

Figure 1a

Figure 1b

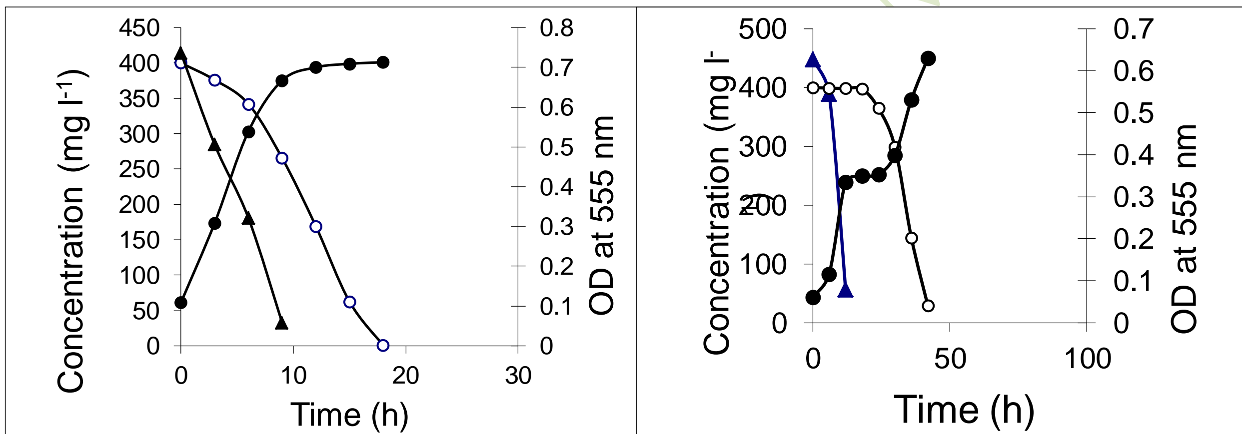

Figure 2a

Figure 2b

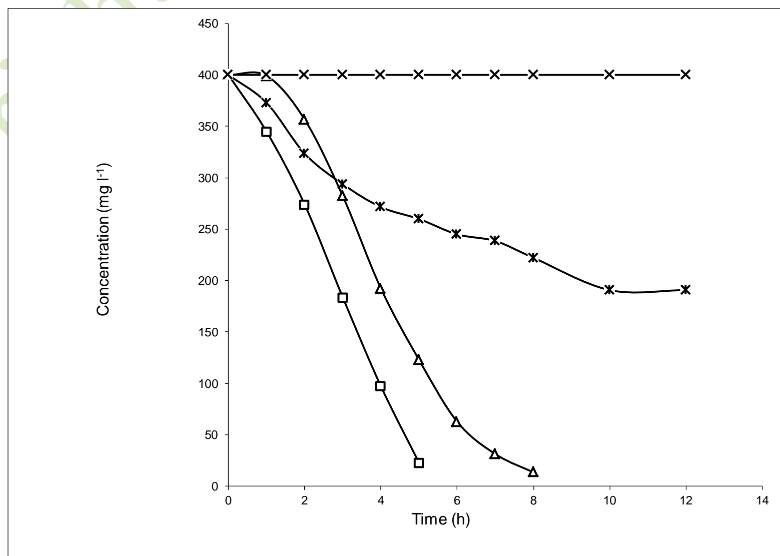

Figure 3

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Fig. 1a. Glucose and 2-ABS removal and growth of 2-ABS acclimated culture, (▲) Glucose, (o) 2-ABS and (●) Growth.

Fig. 1b. 2-ABS removal and growth of 2-ABS acclimated culture. (o) 2-ABS and (●) Growth.

Fig. 2a. Kinetics of substrate removal and growth of 2-ABS glucose acclimated culture. (▲) Glucose , (o) 2-ABS and (●) Growth.

Fig. 2b. Glucose and 2-ABS removal and growth of glucose acclimated culture, (▲) Glucose, (o) 2-ABS and (●) Growth.

Fig. 3. Effect of chloramphenicol on 2-ABS degradation. (△) Succinate precultured cells, (x) Succinate precultured cells+chloramphenicol (100 mg l^{-1}), (□) 2-ABS precultured cells, (*) 2-ABS precultured cells+chloramphenicol (100 mg l^{-1}).

When the culture was grown either only in the presence of glucose or glucose and 2-ABS for three growth cycles prior to its use as the inoculum for kinetic studies, substrate removal pattern was significantly different. Glucose and 2-ABS were utilized simultaneously during the growth of the culture, when the inoculum was derived from the culture grown on both these substrates. Glucose utilization rate was, however, higher than that of 2-ABS (Fig. 2a). As expected with simultaneous utilization of growth substrates, diauxic growth was not observed. Another interesting observation was that the degradation rate of 2-ABS was similar to that observed in a control flask with only 2-ABS (Fig. 1b).

In contrast, 2-ABS degradation was observed only after the complete utilization of glucose when inoculum was derived from glucose grown cultures (Fig. 2b). Typical diauxic growth pattern was observed. Initial growth phase was associated with glucose utilization. This was followed by a long lag phase of 18 h and a second exponential growth phase was associated with 2-ABS utilization. Even when only 2-ABS was used as the carbon source, inoculum derived from glucose grown culture exhibited a lag phase of 18 h, whereas more than 90% was degraded within 21 h in the control flask (Fig. 1b).

Biological destruction of xenobiotic compound in wastewater treatment facilities is one of the major concerns. In these situations, such compounds are found in mixtures with nontoxic or "conventional" wastes. Thus the effect of the presence of easily degradable alternate carbon source on the biodegradation of xenobiotic compounds is of practical importance. Present observation has shown that the substrate removal pattern exhibited by 2-ABS degrading consortium is significantly influenced by the acclimation characteristics of the culture. Consortium adapted to mixed 2-ABS/glucose substrates demonstrated rapid glucose removal with concomitant utilization of 2-ABS. On the other hand, omitting 2-ABS in the medium, for only three growth cycles, resulted in significant changes in substrate utilization pattern. The cell generally synthesizes enzymes required for the degradation of toxic and xenobiotic compounds only when these compounds are present into medium. Results clearly indicate that the presence of 2-ABS is highly essential for the maintenance of the degradation activity in the microbial consortium.

Effect of chloramphenicol on 2-ABS degradation

2-ABS degrading culture was grown on succinate. The cells were collected washed and resuspended in fresh medium containing 400 mg l^{-1} 2-ABS in the presence and absence of chloramphenicol (100 mg l^{-1}). Results are presented in Fig.4. When precultured on 2-ABS, cells degraded 2-ABS immediately, whereas preculturing on succinate led to a short lag phase of 2 hours. The addition of chloramphenicol to cells precultured on succinate resulted in a total inhibition of 2-ABS degradation, whereas partial degradation was observed with 2-ABS grown cells. This incomplete degradation could be due to cell inactivation by chloramphenicol. Chloramphenicol inhibits nascent protein synthesis in bacterial cells. Inability of succinate grown cells to degrade 2-ABS further indicates that enzymes required for its degradation are not constitutive, but are induced.

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Conclusions:

Studies were carried on the degradation of 2-ABS by a two-member bacterial consortium. 2-ABS could serve as the sole carbon and energy source for the bacterial growth. Removal of upto 800 mg l^{-1} 2-ABS was observed within 24 h. Substrate removal pattern in the presence of glucose was dependent on the acclimation characteristic of the consortium. With 2-ABS adapted culture, both glucose and 2-ABS were concomitantly utilized at similar rates. However with 2-ABS and glucose adapted culture, glucose was rapidly degraded even though both substrates were utilized concomitantly. In contrast, glucose adapted consortium exhibited sequential substrate removal and a typical diauxic growth pattern.

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