

UDC 631.48:547.87  
ISSN 1330-9862*original scientific paper*

(FTB-1242)

## Enrichment and Characterization of Atrazine Degrading Bacterial Communities

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Received: April 9, 2003

Accepted: June 9, 2003

### Summary

The objective of this study was to enrich and evaluate biotransformation activity of atrazine degrading bacterial communities originating from wastewater and soil of the herbicide factory, especially to assess their effectiveness for treatment of effluents from the production of atrazine. The enrichment of atrazine degrading bacteria was carried out in continuous-flow units under the inflow of mineral salts medium containing 25 mg/L of atrazine and 50 mg/L of yeast extract (AMS). After a 2-month cultivation at a dilution rate of 0.1 h<sup>-1</sup>, the enriched communities showed similar structure (determined by plating on selective agar plates) and similar degradation activity (complete disappearance of atrazine in AMS medium, monitored by HPLC analyses) as well as substantial mineralizing activity (50–60 % of atrazine released as carbon dioxide, determined by TIC analyses).

The potential of the enriched communities for atrazine degradation in industrial wastewater was further studied in laboratory batch and continuous experiments with cells, immobilized or free in solution. Atrazine was completely degraded only in wastewater containing 25 % of mineral salts medium with cells free in the solution, while slower and incomplete degradation with accumulation of hydroxyatrazine was achieved with immobilized cells. Furthermore, there was no significant difference in the kinetics of atrazine degradation using different carrier materials for inoculum immobilization. Other *s*-triazine wastewater constituents, deethylatrazine (DEA) and deisopropylatrazine (DIA) were poorly degradable. Batch culture experiments in AMS medium amended with isopropylamine (IPA) and NaCl suggested that high salinity and high content of IPA in the industrial wastewater may be the major factors influencing the growth rate of atrazine degrading community and consequently the kinetics of atrazine degradation.

*Key words:* atrazine, atrazine-production wastewater, bacterial community, biodegradation, *s*-triazine compounds

### Introduction

The agricultural *s*-triazine herbicide atrazine (2-chloro-4-ethylamino-6-isopropylamino-1,3,5-triazine) is used extensively in many parts of the world to control a variety of weeds, primarily in the production of corn (1). Its widespread use has caused environmental con-

cern because of frequent detection of atrazine in aquatic systems such as surface waters (2) or groundwaters (3), pointing to soil contamination problems where this herbicide has been spilled. Once in aquifers, atrazine is persistent (4,5) making the development of agricultural

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management practice that would minimize atrazine pollution of surface water and groundwater resources of considerable interest.

A variety of fungi (6,7) and bacteria (8–10), which dealkylate or dechlorinate atrazine but do not mineralize it, as well as mixed communities of microorganisms (11), consortia (12) or pure strains (13,14) capable of completely mineralizing atrazine have been isolated. The capability of microorganisms to use atrazine as the sole nitrogen source (13) and/or carbon source (14,15) was clearly demonstrated. Gene encoding enzymes that are involved in atrazine biodegradation have been characterized from *Pseudomonas* sp. strain ADP (16–18). Those genes are generally widespread, highly conserved and plasmid borne (14,16,19).

Wastewaters from the manufacture of *s*-triazine-containing pesticide such as atrazine, simazine, propazine and cyanazine are especially problematic due to their high concentrations of residual chlorinated *s*-triazine compounds and other manufacturing by-products (20). The lack or insufficient amount of naturally occurring atrazine mineralizing bacteria as well as unfavorable conditions for biodegradation appeared as the major reasons for the persistence of this herbicide. Nevertheless, there are limitations to the use of acclimated microbial cultures in atrazine production wastewater treatment. Unfavorable conditions, such as extreme values of pH, temperature, salinity, toxins, predators, high concentration of substrates, *etc.* may destroy or harm the inoculum and suppress its degradation capability (21). However, some researchers have succeeded in adapting *Pseudomonas* sp. strain ADP to the biodegradation of atrazine under high salinity conditions by the addition of 1 mM of glycine betaine to the medium (20). Such osmoregulated *Pseudomonas* sp. strain ADP feeding on citrate can be used for atrazine degradation in NaCl-containing wastewater. Further, the use of immobilized stable mixed bacterial community capable of efficiently degrading atrazine from wastewater in continuous culture has been reported (22).

The purpose of this study was to enrich and isolate atrazine degrading bacteria from polluted ecosystems (wastewater and soil of herbicide factory) and to evaluate their effectiveness in the treatment of effluents from atrazine synthesis.

## Materials and Methods

### Enrichment cultures

The enrichment of atrazine degrading bacteria was carried out from the wastewater and soil within the herbicide factory Herbos, Sisak, Croatia exposed to many years of repeated spills of effluent from atrazine synthesis. The chemical composition of the wastewater is summarized in Table 1.

### Media

Atrazine mineral salts medium (AMS)

Modified Mandelbaum medium was used (23). Modification was made by adding 50 mg/L of yeast extract (Difco Laboratories, Detroit, Michigan) and by varying the amount of mineral salts. For culture growth and continuous-flow biodegradation experiments, the medium was amended with 5 mL/L of salt stock solution (23); in batch culture experiments with 2.5 mL/L and in mineralization experiment with 0.2 mL/L of salt stock solution. Furthermore, in mineralization experiment neither vitamins nor yeast extract were added. Unless otherwise noted, the concentration of atrazine was 25 mg/L and no sodium citrate was added as supplementary carbon source. After the addition of atrazine, the medium was sonicated for 1 h to reduce the particle size of atrazine crystals and to improve the solubilization.

Selective agar medium

Selective agar medium contained the same mineral salts as AMS, 1 g/L of sodium citrate, 100 or 500 ppm of atrazine and 16 g/L of Noble agar (Difco Laboratories). The medium was sterilized at 121 °C for 20 min without salt and vitamin solutions, which were added aseptically after cooling at 45 °C. The medium with 100 ppm atrazine was used for the maintenance of the mixed bacterial culture and for the isolation of individual members, while the medium with higher atrazine concentration (500 ppm) was used for checking their potential atrazine degradation activity.

### Chemicals

Technical atrazine (purity of 95.66 %) and N-isopropylamine (99 %) were donated by the herbicide factory Herbos. Sodium alginate was purchased from Fluka AG, Buchs, Switzerland. Pure atrazine (99.0 %), deethylatrazine (99.0 %), deisopropylatrazine (95.0 %) and hydroxyatrazine (99.9 %) were purchased as standards of Pestanal quality from Riedel-de Haën, Sigma-Aldrich, Seelze, Germany. Acetonitrile of gradient grade for HPLC analysis, LiChrosolv, was purchased from Merck, Darmstadt, Germany. LC-grade water was obtained by purifying water with Milli-Q water purification system, Millipore, Bedford, USA. All other chemicals were of analytical reagent grade.

Enrichment technique

The enrichment of atrazine degrading bacteria was performed in continuous-flow units (24) at the temperature of 28–30 °C during 8 weeks of cultivation in AMS medium at a dilution rate of 0.1 h<sup>-1</sup>. The units were filled with filtrates of soil sample or wastewater from atrazine synthesis and subjected to aeration. Soil filtrate was prepared by mixing 100 g of soil in AMS medium

Table 1. Chemical composition of the industrial wastewater collected from a herbicide factory

$\gamma$ (ATR)	$\gamma$ (ATOH)	$\gamma$ (DIA)	$\gamma$ (DEA)	$\gamma$ (IPA)	$\gamma$ (TOC)	$\gamma$ (N-total)	$w$ (NaCl)	pH
			(mg/L)				(%)	
27.45	4.5	25.0	120.1	768	1370	490	3.1	10.5

Abbreviations: ATR=atrazine, ATOH=hydroxyatrazine, DIA=deisopropylatrazine, DEA=deethylatrazine, IPA=N-isopropylamine, TOC=total organic carbon

(1 L). After 30 min the suspension was filtered through a coarse filter. Nonsterile conditions were used throughout.

The composition of mixed cultures during continuous cultivation and biodegradation studies was determined in the samples taken from the aeration section of the continuous-flow units by plating of appropriate dilutions on the surface of selective atrazine agar plates. The dispersion of the activated sludge particles was achieved by mixing the samples for 5 min on a vortex (Sondheim, Germany). After 7 days of incubation at 30 °C and further 7–14 days of incubation at room temperature, colonies were counted and characterized according to their macromorphological characteristics.

#### Biodegradation experiments

In all biodegradation experiments (continuous-flow or batch culture), a 1 to 2-week-old enriched mixed bacterial culture grown in AMS medium at  $D\ 0.1\ h^{-1}$  was used. Two sets of continuous-flow biodegradation experiments were carried out.

The first one was performed at a dilution rate of  $0.1\ h^{-1}$  under the inflow of wastewater from atrazine production. Three units (in duplicate) with wastewater and one with AMS medium as a control were set up. The scheme of the experiment is shown in Table 2.

Table 2. Composition of influents in continuous biodegradation experiments at  $D\ 0.1\ h^{-1}$

Units	Influent	$\gamma$ (trace elements)* (2.5 mL/L)	$\gamma$ (sodium citrate) (1 g/L)	$\gamma$ (KH <sub>2</sub> PO <sub>4</sub> ) (1.65 g/L)	pH
1	wastewater	+	–	–	10.5
2	wastewater	+	–	+	8.93
3	wastewater	+	+	+	10.5
4	AMS	+	–	+	7.25

\* The trace elements solution contained (per liter of deionized water): EDTA 2.5 g, ZnSO<sub>4</sub> 11.1 g, FeSO<sub>4</sub> 5 g, MnSO<sub>4</sub> · H<sub>2</sub>O 1.54 g, CuSO<sub>4</sub> · 5H<sub>2</sub>O 0.4 g, Co(NO<sub>3</sub>)<sub>2</sub> · 6H<sub>2</sub>O 0.25 g, Na<sub>2</sub>B<sub>4</sub>O<sub>7</sub> · 10H<sub>2</sub>O 0.18 g

The second experiment was carried out at a dilution rate of  $0.04\ h^{-1}$  under the inflow of wastewater containing 25 % of mineral salts medium and by using immobilized cells of the mixed culture. The carrier materials for cell immobilization were plastic rings, 0.8 cm in diameter (Purac, Germany), polyurethane foam pellets (Bayer, Leverkusen) and Ca-alginate beads (25). As inoculum, a 1-week-old mixed culture grown in continuous-flow unit was centrifuged (10 000 rpm for 5 min), washed with phosphate buffer (pH=7.5) and resuspended in AMS medium to the optical density at 600 nm (OD<sub>600</sub>) of 0.5. The carrier material (100 mL) was added into the units containing the prepared inoculum (100 mL), the units were filled to the overflow level with the AMS medium, and after 2 days of aeration AMS medium was introduced into the units at a dilution rate of  $0.01\ h^{-1}$ . Biodegradation experiments with wastewater from atrazine production started after 10 days of immobilization period.

Daily samples of the effluents were collected in glass bottles and the remaining *s*-triazines were determined by HPLC analysis. A sample for bacteriological

analysis was taken from the aeration section of the continuous-flow units and the bacterial number was determined by plating the appropriate dilutions on selective atrazine agar plates (atrazine 100 ppm).

Atrazine degradation batch experiments were carried out in 500-mL Erlenmeyer flasks containing 200 mL of AMS medium or industrial wastewater. To simulate wastewater composition, AMS medium was supplemented with N-isopropylamine (IPA) and NaCl, as shown in Table 3. Control experiments without the addition of N-isopropylamine and NaCl were performed as well.

Flasks were inoculated with 2-week-old mixed bacterial culture to provide a final concentration of approximately  $6 \cdot 10^6\ CFU\ mL^{-1}$ , and incubated for 9 days on the rotary shaker at 180 rpm and at 28 °C. At selected time intervals, samples (5 mL) from each flask were removed to evaluate atrazine degradation by HPLC analysis. Bacterial number at the beginning and at the end of the experiment was determined by plating on selective atrazine agar plates (atrazine 100 ppm).

#### Mineralization experiment

AMS medium with atrazine as the only carbon and nitrogen source (100 mL) was inoculated with a mixed or pure culture of atrazine degrader and incubated in

Table 3. Composition of the media in batch biodegradation experiments

Medium	$\gamma$ (IPA*) (mg/L)	$w$ (NaCl) (%)	pH
AMS	–	–	7.25
AMS	870	–	10.76
AMS	870	1	10.59
AMS	870	3	10.56
AMS	350	–	9.95
AMS	350	1	9.91
AMS	350	3	10.56
Wastewater	–	–	10.50

\*IPA= N-isopropylamine

sealed bottles (150 mL). Mineralization of atrazine to CO<sub>2</sub> was determined by measuring the increase in inorganic carbon levels over time compared with unamended blanks. Standard test procedure including all blank and control tests followed (26).

## HPLC analyses

Atrazine and its metabolites were detected and identified by reversed-phase high-performance liquid chromatography (HPLC) on a Varian ProStar system with solvent delivery module model 230 and UV photodiode array detector model 330 (Varian, Walnut Creek, CA, USA). The chromatographic column was ChromSep SS 250x4.6 mm I.D. with guard column 10x3.0 mm I.D. of 5 µm OmniSpher particle size (Varian, Walnut Creek, CA, USA). The solvents used were acetonitrile and 10 mM phosphate buffer (pH=7) with a mobile phase flow rate of 1 mL/min. The gradient elution was carried out according to the following program: 3 min at 95 % buffer and 5 % acetonitrile, from 5–100 % acetonitrile in 19 min, 3 min at 100 % acetonitrile. UV spectra of all peaks were recorded from 200 to 400 nm. The working wavelengths for quantitative analysis were 221 and 213 nm.

Atrazine and its metabolites in culture supernatant were identified by their retention times and UV spectra, which were compared to the known standards.

## Results and Discussion

Continuous-flow cultivation of soil filtrate and wastewater from herbicide factory under the conditions presented in this work (AMS medium containing atrazine 25 mg/L and yeast extract 50 mg/L;  $D = 0.1\text{h}^{-1}$ ;  $t = 20\text{--}22\text{ }^\circ\text{C}$ ) resulted in the enrichment of two bacterial communities expressing similar atrazine transformation activities (determined by HPLC analysis). The analysis of grown colonies on selective agar plates (containing 100 and 500 mg/L of atrazine) suggested that during continuous cultivation the structure of both communities changed significantly (Fig. 1). This was evidenced in contribution of the individual colony types, some of which completely disappeared and new colony types appeared. Thus, colony type 3, which was present in small proportion (1 %) at the beginning of the experiment only in industrial wastewater, increased in proportion (20 %) and represented 30 % of total population in the enriched culture originating from the soil. Furthermore, this colony type showed clearing zones on selective agar plates indicating its important role in atrazine transformation.

The results of mineralization experiment in which atrazine was the only carbon and nitrogen source (Fig. 2) suggested that both enriched communities as well as the colony type 3 (isolate Atz 1) expressed substantial atrazine degradation activity (approx. 60 % of total organic carbon released as  $\text{CO}_2$ ). These results further suggested that the isolate Atz 1 played an important role in the atrazine mineralization. More detailed characterization of this community member is currently under investigation in our laboratory.

Since both enriched communities showed similar atrazine degrading activity, they were mixed and this inoculum was used in further experiments.

In the following experiments, atrazine degradation and the changes in community structure were studied during continuous-flow cultivation in industrial wastewater and modified wastewater (amended with phos-

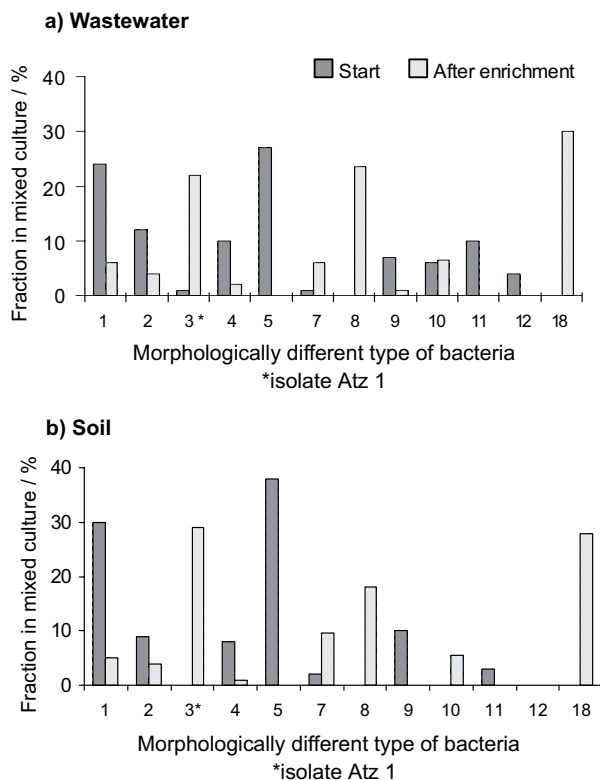


Fig. 1. Percentage composition of initial and enriched communities originating from wastewater and soil of the herbicide factory. Macromorphologically different colony types were counted on selective atrazine agar plates containing 100 ppm of atrazine

phate and citrate). As can be seen from Fig. 3, the degree of atrazine degradation was lower in industrial than in modified wastewater. Furthermore, in all the cases gradual increase of residual atrazine as well as the decrease of the estimated number of bacteria occurred. Two main reasons may be responsible for this: (i) high salinity of industrial wastewater (Table 1), which has been known to inhibit bacterial growth (20,27,28) and (ii) relatively high dilution rate ( $0.1\text{ h}^{-1}$ ), which can lead to washing out the microorganisms from the system.

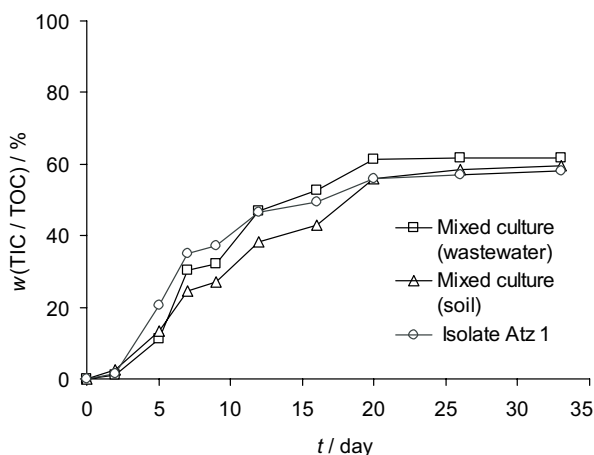
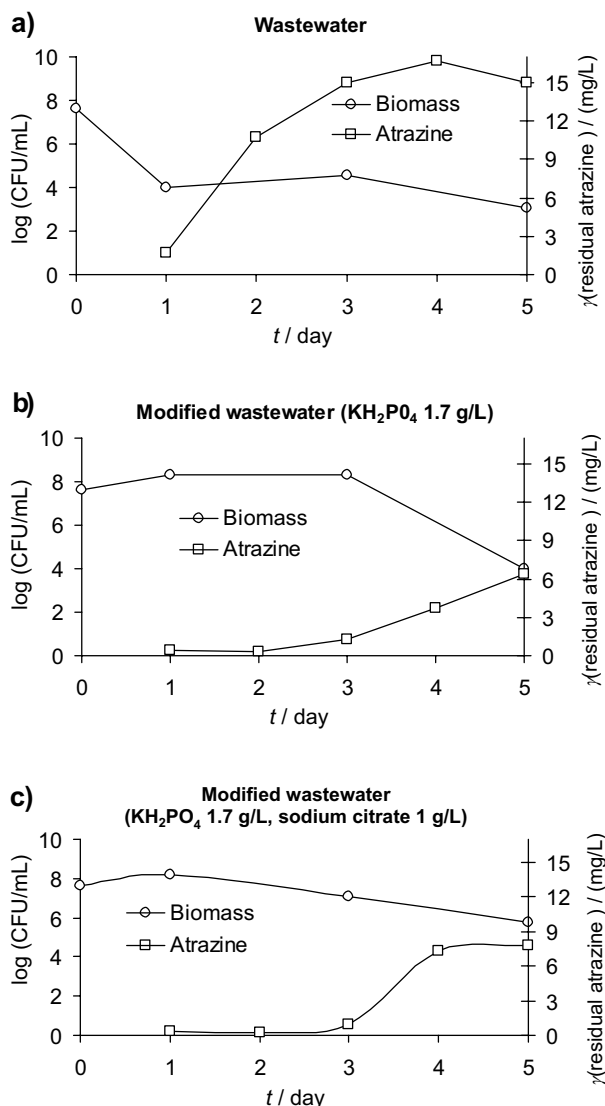


Fig. 2. Mineralization of atrazine in modified AMS medium containing atrazine (25 ppm) as the sole carbon and nitrogen source. The data are averages of duplicate samples

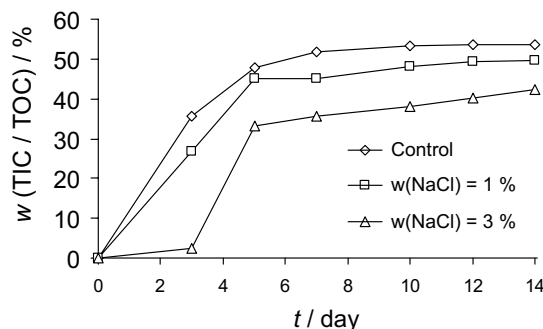


**Fig. 3.** Biodegradation of atrazine in industrial wastewater (a); wastewater amended with  $\text{KH}_2\text{PO}_4$  (1.7 g/L) (b); and wastewater amended with  $\text{KH}_2\text{PO}_4$  (1.7 g/L) and sodium citrate (1 g/L) (c). Experiments were performed in a continuous-flow unit ( $D = 0.1 \text{ h}^{-1}$ ) by using enriched bacterial community. Atrazine concentration was determined by HPLC analysis. Initial atrazine concentration was 27.45 mg/mL. Bacterial number (log CFU/mL) was determined by counting on selective atrazine agar plates

The results of the control experiment, which was conducted in AMS medium under the same experimental conditions, showed complete degradation of atrazine and a steady state of bacterial concentration (data not shown). Hence, it follows that the first mentioned reason of growth inhibition is a more probable explanation for reduced atrazine degradation in industrial wastewater.

In order to further evaluate the effect of salinity on the rate and extent of atrazine degradation, mineralization experiment was performed in modified AMS medium amended with two different concentrations of NaCl (1 and 3 %). For the control, atrazine mineralization in AMS medium without NaCl addition was also monitored (Fig. 4). It is evident that atrazine mineraliza-

tion progressively decreased with the increase in the salinity. Furthermore, at the NaCl concentration relevant to atrazine-manufacturing wastewater (3 %), a 2-day lag phase with only 40 % atrazine mineralization was observed, suggesting significant inhibition effect on the enriched community. These observations are in agreement with previously published results (20,28).

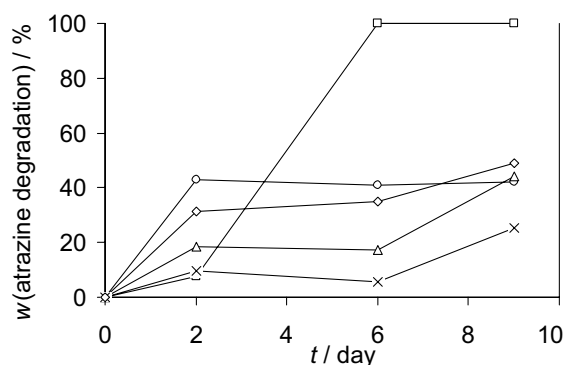


**Fig. 4.** Effect of NaCl on mineralizing activity of the enriched community. Experiments were performed in modified AMS medium (see Fig. 2) amended with NaCl (1 and 3 %)

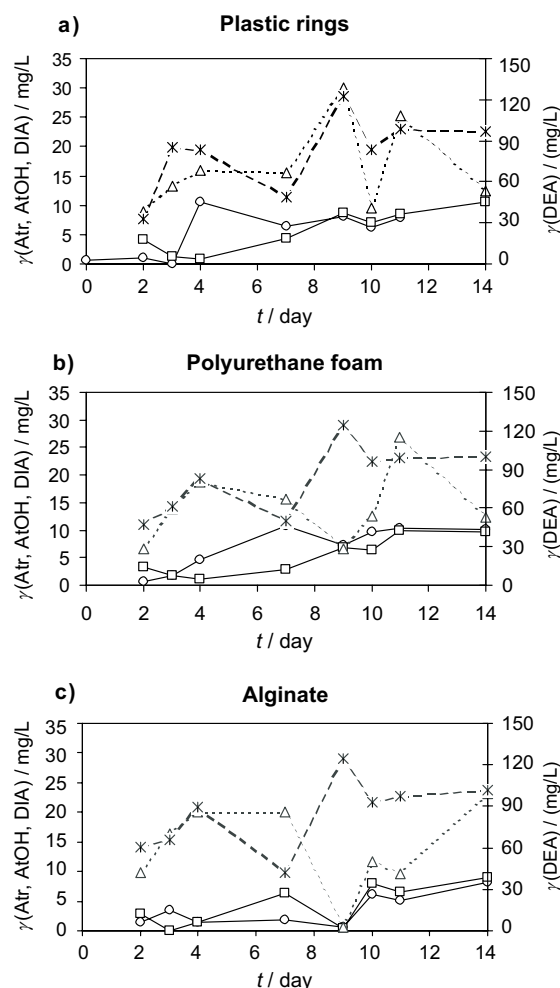
Besides high concentration of NaCl, industrial wastewater also contained high amount of N-isopropylamine (IPA) (Table 1). In order to determine the effect of this additional C and/or N source on atrazine biodegradation, IPA was added into AMS medium and the disappearance of atrazine was monitored in batch culture experiment. As presented in Fig. 5, atrazine completely disappeared in AMS medium within 6 days and bacterial concentration increased from initial  $6 \cdot 10^6$  to  $1.9 \cdot 10^7$  CFU  $\text{mL}^{-1}$  (data not shown). The addition of IPA (870 ppm) to AMS medium enhanced the initial rate of atrazine degradation (42 % of atrazine was degraded within 2 days) but no further improvement of degradation, followed by decreased bacterial concentration, was achieved ( $7 \cdot 10^3$  CFU  $\text{mL}^{-1}$ ). In the case when both IPA and NaCl were added in AMS medium, further reduction of atrazine degradation was observed followed by the growth inhibition of bacterial population. In the experiment with industrial wastewater, atrazine was degraded at a rate similar to that in IPA-amended AMS.

At lower IPA concentration (350 ppm), complete disappearance of atrazine was achieved during longer period (9 days) compared to the degradation in AMS medium without IPA addition, followed by cell growth to  $1.6 \cdot 10^9$  CFU  $\text{mL}^{-1}$  (data not presented). When this medium was further amended with NaCl (1 and 3 %), no degradation was observed during the first 6 days but complete degradation was achieved by the end of the experiment (9 days). The presented results suggested that high IPA concentration (approx. 800 mg/L) present in industrial wastewater as well as high salinity (approx. 3 %) may be the rate determining for the growth of enriched bacterial community and consequently for atrazine degradation.

In order to evaluate potential effects of other industrial wastewater constituents on atrazine degradation, another set of continuous-flow experiments was performed in which atrazine (ATR), hydroxyatrazine (ATOH), deethylatrazine (DEA) and deisopropylatrazine (DIA)



**Fig. 5.** Comparison of atrazine degradation (determined by HPLC analysis) during cultivation of the enriched community in AMS medium containing 30 ppm atrazine (□); in AMS medium with the addition of isopropylamine (IPA) (○); in AMS medium with IPA and NaCl 1% (△), NaCl 3% (×) and in the industrial wastewater (◇). The experiments were performed in Erlenmeyer flasks on a rotary shaker at 180 rpm and 28 °C. The data are averages of duplicate samples



**Fig. 6.** Biodegradation of atrazine in wastewater by the enriched bacterial community immobilized on plastic rings (a); polyurethane foam (b) and alginate beads (c). Residual atrazine, Atr (○), deisopropylatrazine, DIA (△), deethylatrazine, DEA (\*) and produced hydroxyatrazine, ATOH (□) were determined by HPLC analysis in the effluent of the continuous-flow unit. Dilution rate  $D$  was  $0.04 \text{ h}^{-1}$

were monitored by HPLC analyses. In those experiments enriched bacterial community was used, free in solution or immobilized on different types of carriers. The results of HPLC analyses of the effluents treated in continuous-flow units with immobilized inoculum (Fig. 6) indicated that more than 65 % of atrazine present in industrial wastewater was degraded. Hydroxyatrazine, as intermediate of atrazine degradation and wastewater constituent, gradually accumulated while other major *s*-triazine constituents (DEA and DIA) revealed considerable fluctuation of residual concentration in the effluents. However, although these compounds appeared to be very poorly degradable, their degradation extent may be higher than the estimated because they can be produced by the degradation of atrazine. These results point out that there was no significant difference in the rate and extent of degradation of major *s*-triazine wastewater compounds using different types of carriers for cell immobilization.

In the case when the enriched community was free in solution, quick and complete degradation of atrazine and hydroxyatrazine as well as better degradation of DIA and DEA were observed (data not shown). These results indicated that other factors than wastewater constituents (DIA and DEA) may be an obstacle to atrazine degradation. The study of those factors is a subject of our current investigations.

## Conclusions

From the presented results the following general conclusions and hypotheses can be made. Enrichment experiments from wastewater and soil of the herbicide factory resulted in the selection of two structurally similar bacterial communities expressing substantial atrazine degrading activity under batch and continuous cultivation in atrazine mineral salts (AMS) medium supplemented with yeast extract (monitored by HPLC analyses) as well as 50–60 % mineralization in AMS medium with atrazine as the only carbon and nitrogen source (determined by TIC analyses). During the enrichment the community structure changed significantly, with the appearance of morphologically new strains. One of the enriched community members (isolate Atz 1) showed clearing zones around the colonies on selective agar plates suggesting its important role in atrazine mineralization. Enriched communities were also efficient in atrazine degradation during cultivation in wastewater diluted with mineral salts medium. Batch and continuous-flow biodegradation experiments performed so far indicated that high salinity and high content of nitrogen-containing compounds (IPA) may be two major factors influencing the growth rate and atrazine degradation activity of the enriched bacterial communities.

## Acknowledgements

This work was financially supported by the Croatian Ministry of Science and Technology and the agrochemical factory Herbos, Sisak. The authors are indebted to prof. M. Glancer-Šoljan from the Faculty of Food Technology and Biotechnology (University of Zagreb) for providing the carrier materials for the immobilization of cells and to J. Vržina, V. Schiesl and Ž.

Pavlić from Zagreb Public Health Institute for their valuable engagement in mineralization experiment.

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## Uzgoj i karakterizacija bakterijskih zajednica za razgradnju atrazina

### Sažetak

Svrha je rada bila istražiti biotransformacijsku aktivnost bakterijskih zajednica podrijetlom iz otpadne vode i zemlje onečišćene herbicidom atrazinom te procijeniti mogućnost primjene tih zajednica za obradu otpadnih voda iz sinteze atrazina. Uzgoj bakterija proveden je u protočnim aparaturnama u mineralnoj podlozi s atrazinom (25 mg/L) kao jedinim izvorom ugljika i dušika, uz dodatak kvašćevog ekstrakta (50 mg/L) kao izvora vitamina (AMS podloga). Nakon dva mjeseca uzgoja (brzina razrjeđivanja  $0,1 \text{ h}^{-1}$ ) uzgojene bakterijske zajednice pokazale su sličnu biotransformacijsku aktivnost (potpunu razgradnju atrazina u AMS podlozi, određeno HPLC metodom, te 50–60 %-tnu razgradnju do ugljikovog dioksida, mjereno kao anorganski ugljik).

Biotransformacijski potencijal bakterijskih zajednica određen je također tijekom uzgoja slobodnih i imobiliziranih stanica u otpadnoj vodi iz sinteze atrazina. Potpuna razgradnja atrazina (određeno HPLC metodom) postignuta je u otpadnoj vodi razrijeđenoj s AMS podlogom (25 %), i to u pokusu sa slobodnim stanicama. U pokusu s imobiliziranim stanicama razgradnja je atrazina bila nepotpuna (prisutan je hidroksiatrazin), a brzina razgradnje podjednaka bez obzira na upotrijebljeni nosač za bakterijske stanice. Ostali sastojci s-triazinske strukture prisutni u otpadnoj vodi: deetilatriazin (DEA) i deizopropilatriazin (DIA) nisu pokazali bitniju razgradnju u eksperimentalnim uvjetima. Laboratorijskim istraživanjem utvrđeno je nadalje da su velika slanost (3 % NaCl) i prisutnost značajne količine izopropilamina (800–850 mg/L IPA) vjerojatno najvažniji čimbenici koji utječu na rast uzgojenih bakterijskih zajednica a time i na brzinu i stupanj razgradnje atrazina u otpadnoj vodi iz sinteze atrazina.