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# Aerobic Degradation of Formaldehyde in Wastewater from the Production of Melamine Resins

*Margareta Glancer-Šoljan[1*], Vice Šoljan[2], Tibela Landeka Dragičević[1] and Ljiljana Čačić[3]*

[1]Faculty of Food Technology and Biotechnology, University of Zagreb, Pierottijeva 6, P.O.B. 625, HR-10 000 Zagreb, Croatia

[2]»Ekološki inženjering« d.o.o. Poreč, Ive Andrića 76, HR-52 000 Poreč, Croatia

[3]»Ekolaboratorij«, Vodovod d.o.o. Osijek, Poljski put 1, HR-31 000 Osijek, Croatia

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*Dedicated to the memory of Professor Vera Johanides*

**Summary**

Selected strains of *Pseudomonas putida, Pseudomonas cepacia*, *Trichosporon penicillatum* yeast and the mixed culture of these three strains were used for aerobic degradation of formaldehyde and formic acid in the synthetic medium and wastewater generated by melamine resins production. It has been shown that the mixed culture in the synthetic medium degrades 1000 mg/L of formaldehyde over 18–24 hours and 500 mg/L of formic acid over 12–18 hours. Aerobic degradation of wastewater from the production of melamine resins with the use of mixed bacterial and yeast culture was achieved in 24 hours with COD reduction of over 90 % and complete degradation of formaldehyde, methanol and butanol. The role of *Trichosporon penicillatum* yeast in the mixed culture, during aerobic degradation of formaldehyde in the synthetic medium and wastewater, was to form flocculent biomass that is self-precipitating.

*Key words*: mixed culture of bacteria and yeast, aerobic degradation of formaldehyde, wastewater from production of melamine resins

## Introduction

Formaldehyde is used in industrial processing of wood and in the production of paper, leather, resins and glue (*1*). It occurs in wastewaters of various origins and due to its biocidal action it is toxic to many microorganisms (*2*). It has been shown that formaldehyde is the product of biodegradation of $C_1$-compounds, *e.g.* methane and methanol, and of nitrogen compounds, where it is a key intermediate of degradation (*3*).

According to scarce literature data the following microorganisms are capable to degrade formaldehyde: *Pseudomonas* spp. generum (*4*), *Halomonas* spp. (*5*) and various strains of methylotropha (*6*); *Debariomyces* spp. and *Trichosporon* spp. yeast genera (*7*), *Hansenula* spp. (*8*), *Candida* spp. (*9*) and *Gliocladium* spp. fungi (*10*).

Formic acid is the basic intermediate in formaldehyde biodegradation (*3–6*). *Pseudomonas* spp. degrade

* Corresponding author; E-mail: mglancer@pbf.hr; Phone: ++385 (0)1 4605 033; Fax: ++385 (0)1 4605 072

formaldehyde with formaldehyde dismutase enzyme (*11*) while a yeast, *e.g. Hansenula* spp. and *Candida* spp. genera, achieves it using the enzymes formaldehyde- and formate dehydrogenase (*9,10*). The reactions are:

$$2\ HCHO \xrightarrow[\text{formaldehyde dismutase}]{\textit{Pseudomonas putida}} CH_3OH + HCOOH$$

$$HCHO \xrightarrow[\text{formaldehyde hydrogenase}]{\textit{Hansemula polymorfa or Candida boidinii}} HCOOH \xrightarrow[\text{formate dehydrogenase}]{} CO_2$$

Not many microorganisms can degrade formaldehyde, which is attributed to its toxic effect on various parts of bacterial cells such as spores, cell wall and compounds with amino-group.

It has been shown that formaldehyde undergoes biodegradation in synthetic media. There are, however, scarce reports on its degradation in wastewaters. This can be explained by the fact that, in addition to formaldehyde, wastewaters contain other substances that may affect formaldehyde degradation. For example, wastewaters from the production of melamine resins do not contain only formaldehyde (70–2700 mg/L), but also methanol (up to 300 mg/L), butanol (up to 200 mg/L) and melamine (700–5000 mg/L) (*13*); wastewaters from the production of urea formaldehyde resins contain formaldehyde (200–4000 mg/L), urea (100–800 mg/L) and ammonium (up to 400 mg/L) (*14*).

The subject of this study was an aerobic degradation of formaldehyde in the synthetic medium, as well as of wastewater from the production of melamine resins, using the mixed culture of two bacteria of *Pseudomonas* spp. genus and *Trichosporon* spp. yeast genus. The role of each species is discussed on the basis of its action in the mixed culture.

## Material and Methods

### *Mixed culture of microorganisms*

The mixed culture used in the experiments on formaldehyde degradation contained two bacteria of *Pseudomonas* generum – *Pseudomonas putida* LOV 400 and *Pseudomonas cepacia* LOV 401, as well as of *Trichosporon* generum – *Trichosporon penicillatum* LOV 200. (LOV = Collection of microorganisms at the Laboratory for biological wastewater treatment, Faculty of Food Science and Biotechnology, University of Zagreb).

### *Synthetic medium and wastewater*

The synthetic medium was aqueous solution of formaldehyde (concentration of 1000 mg/L) with addition of 100–250 mg/L of $(NH_4)_2HPO_4$ as the source of nitrogen and phosphorus.

To test the activity of each component of the mixed culture used for degradation of formic acid as an intermediate of formaledhyde degradation, 500 mg/L of formic acid was added to the synthetic medium, instead of formaldehyde, and adjusted pH by the addition of 2 M NaOH. Table 1 shows the composition of the wastewater used in this work. The analyses of methanol, butanol and formic acid were performed in the Laboratory of »Melamin«-Kočevje, Slovenia.

Table 1. Composition of wastewater from the production of melamine resins

| Ingredients | *Wastewater |
|---|---|
| γ(formladehyde)/mg L-1 | 500 – 1000 |
| γ(methanol)/mg L-1 | 350 – 800 |
| γ(butanol)/mg L-1 | 150 – 400 |
| γ(resins)/mg L-1 | 800 – 1500 |
| γ(COD)/mg L-1 | 1500 – 3500 |
| pH | 7.7 – 8.0 |

*Data refer to the sample of wastewater prepared by the treatment of the original wastewater with lime and $FeCl_3$ 30 % solution to remove insoluble resins

Resins in wastewater were melamins that are known to be hardly biodegradable (12,15). Nitrogen from melamine is unavailable to microorganisms, therefore $(NH_4)_2HPO_4$ had been added to wastewater as the source of nitrogen and phosphorus. Wastewater had been kept refrigerated at +4 °C.

### *Preparation of inoculum*

*P. putida*, *P. cepacia* and yeast *T. penicillatum* strains had been kept refrigerated. Prior to the experiment, each culture had been inoculated on the agar plates with the culture medium for bacterial growth containing (g/L): meat extract 3, bacto peptone 5, NaCl 5 and agar 25, and the medium for yeast growth was prepared (g/L) of glucose 20, bacto peptone 10, yeast extract 5 and agar 25. Incubation was carried out at 28 °C over 48–72 hours.

Each culture was transferred from the plate to 1 L serum bottle. Volume of the synthetic medium was 0.7 L with formaldehyde concentration of 50 mg/L. Similar procedure was performed concomitantly with the synthetic medium containing 50 mg/L of formic acid. Serum bottles were supplied with the aeration system. Growth of each culture had been monitored through the change of absorbance (optical density, OD) at 610 nm (*4*) for bacteria and at 660 nm (*7*) for the yeast. Concentration of formaldehyde and formic acid was measured at stabilised levels of optical density.

After adaption of each bacterial culture in the mineral medium with formaldehyde or formic acid degradation of formaldehyde and formic acid took place. Then the original volume of the medium with formaldehyde or formic acid were re-established in the serum bottles. After five consecutive adaptations, when equal formaldehyde and formic acid degradation time with two re-adaptations had been achieved, the inoculum of each culture was completed. The inoculum of mixed culture was also prepared by combining every bacterial strain and yeast in almost equal biomass concentrations. The inocula were tested for degradation of 1000 mg/L formaldehyde, and 500 mg/L formic acid, as a potential intermediate of formaldehyde degradation.

### *Aerobic degradation of formaldehyde and formic acid in the synthetic medium with single strains and the mixed culture*

The experiments on aerobic degradation of formaldehyde and formic acid in the synthetic medium were carried out in 2 L serum bottles filled with 1.5 L of culture medium. Both experiments were performed in five serum bottles, 3 for each bacterial culture, 1 for the mixed culture and 1 for the comparative study in which the mineral medium containing formaldehyde or formic acid was aerated but not inoculated. The bottles were closed with rubber stoppers with an opening for aeration system, sampling and degasification. The experiments were carried out at 19–20 °C and dissolved oxygen concentration was maintained in the range of 2–4 mg/L.

During the experiment the concentrations of formaldehyde and formic acid in the synthetic medium were gradually increased up to 1000 mg/L and 500 mg/L, respectively. The media in which pH dropped below 6.5 were adjusted to 7.7 by the addition of 2 M NaOH.

After experiments in the synthetic medium, degradation kinetics of formaldehyde and other substances in wastewater was examined using the mixed culture. The experiments were done in one 2 L serum bottle, with 1.5 L of wastewater at 19–20 °C and 2–4 mg/L of dissolved oxygen. Degradation experiments with the mixed culture were performed with the original wastewater and the wastewater diluted in the ratio 2:1 and 1:1 with tap water. The degradation kinetics had been monitored through the changes of total organic matter expressed as COD and the change in the concentration of formaldehyde, methanol, butanol, biomass and pH.

## Analytical methods

Biomass of each bacterial strain and yeast was determined by measuring optical density for the bacteria at 610 nm (*4*) and yeast at 660 nm (*7*).

Biomass of the mixed culture was determined gravimetrically by passing the sample through 0.45 μm filter and drying it over 8–10 hours at 110 °C.

Chemical oxygen demand (COD) of the samples was determined by APHA dichromate method (*16*).

Formaldehyde concentration was determined by spectrophotometry (*17*) and iodometry (*18*).

Formic acid, methanol and butanol were analysed by gas chromatography, GC 3300 Varian using capillar column DB-WAX with nitrogen (T column=100 °C, T injector=220 °C, T detector=250 °C).

## Results and Discussion

The aim of this study was preparation of the mixed culture of bacteria and yeast that would be highly efficient in degrading formaldehyde from wastewater generated by melamine resins production. In addition to formaldehyde, the wastewater (Table 1) contained other substances: methanol, butanol and melamine resins (*13,15*).

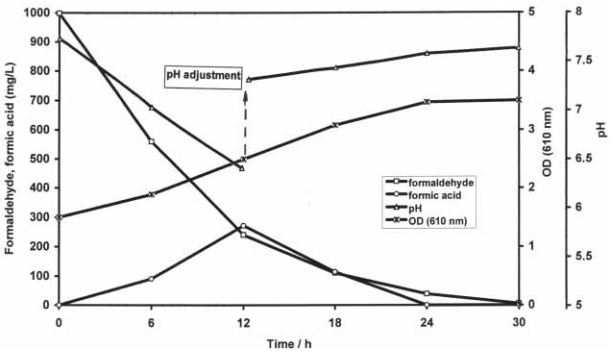


**Fig. 1. Aerobic degradation of formaldehyde with *P. putida*** in the synthetic medium – changes in pH and OD at 610 nm

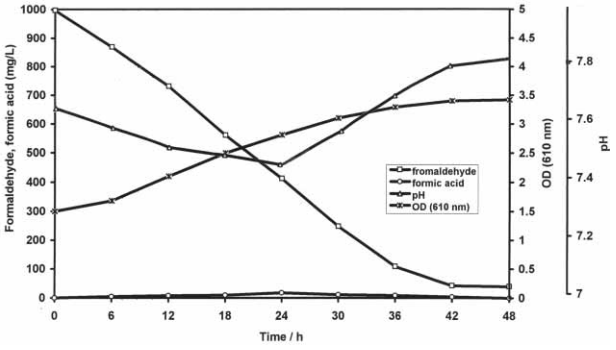


**Fig. 2. Aerobic degradation of formaldehyde with *P. cepacia*** in the synthetic medium – changes in pH and OD at 610 nm

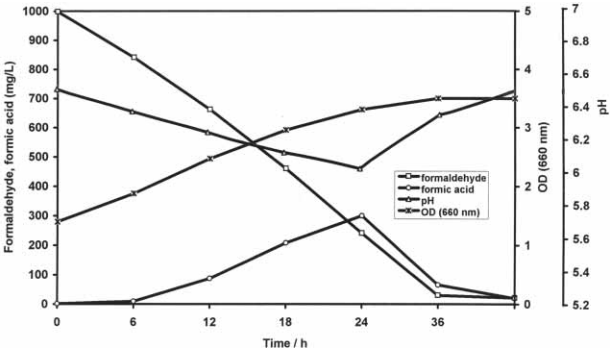


**Fig. 3. Aerobic degradation of formaldehyde with *T. penicillatum*** in the synthetic medium – changes in pH and OD at 660 nm

Degradation kinetics of formaldehyde (1000 mg/L) with *P. putida* is shown in Fig. 1, *P. cepacia* in Fig. 2, and yeast *T. penicillatum* in Fig. 3. Degradation kinetics of formaldehyde and biomass growth of the mixed culture are given in Fig. 4.

Over the first 12 (of 30) hours of formaldehyde aerobic degradation with *P. putida*, formic acid developed as an intermediate and affected pH. From 12th to 30th hour formaldehyde and formic acid degraded concomitantly and were used up in the synthesis of bacterial biomass after pH adjustment with 2 M NaOH (Fig. 1). It may be assumed that the action of *P. putida* in formalde-

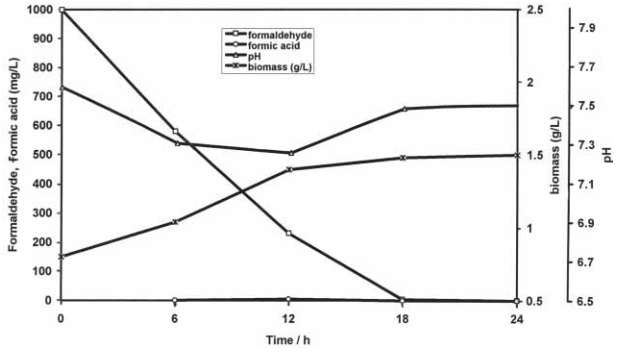


**Fig. 4. Aerobic degradation of formaldehyde with the mixed culture of *P. putida*, *P. cepacia* and *T. penicillatum*** in the synthetic medium – biomass growth and changes in pH

hyde degradation, *i.e.* in accumulation of formic acid, was relevant to enzymatic activity, or that unfavourable pH affected generation of formic acid, as confirmed by other authors (*11*).

Over 48 hours of formaldehyde aerobic degradation, *P. cepacia* (Fig. 2) accumulated significantly lower levels of formic acid than *P. putida* (Fig. 1). For that reason pH was not been affected significantly. *P. cepacia* was capable of degrading formaldehyde over 48 hours without accumulation of formic acid. Therefore it may be assumed that its enzymatic system was superior to that of *P. putida*. The increase of optical density (Figs. 1 and 2) confirmed that formaldehyde and the accumulated formic acid were used as carbon sources for the growth of *P. putida* and *P. cepacia* biomass.

The selected *T. penicillatum* yeast strain behaved similarly to *P. putida.* Formaldehyde was degraded in 36 hours, formic acid was generated up to 24th hour of degradation and pH=6.0 was found to facilitate the use of formic acid and formaldehyde for synthesis of yeast biomass (Fig. 3).

With the use of the mixed culture, aerobic degradation of formaldehyde was completed in 18 hours (Fig. 4). That was significantly faster than with the use of individual strains. During degradation, it was shown that formic acid developed as the intermediate product, but in much lower concentrations than with individual strains. Changes in pH from 7.6 to 7.2 were favourable for maintaining enzymatic activity of both bacterial strains. That resulted in complete degradation of formaldehyde, without accumulation of formic acid, accompanied by biomass synthesis.

In a separate experiment the mixed culture of *P. putida* and *P. cepacia* showed equal pattern of formaldehyde aerobic degradation as the mixed culture of *P. putida*, *P. cepacia* and *T. penicillatum.* (Fig. 4). Therefore, the addition of *T. penicillatum* contributed to the formation of a flocculent mixed culture biomass that could be easily separated from the medium (Figs. 5 and 6).

The possibility for achieving high degree of formaldehyde degradation with the use of individual bacterial strains and yeast have been confirmed (*4,7,11*). There are no literature data, however, on aerobic degradation of formaldehyde with the use of mixed cultures containing bacteria and yeast.

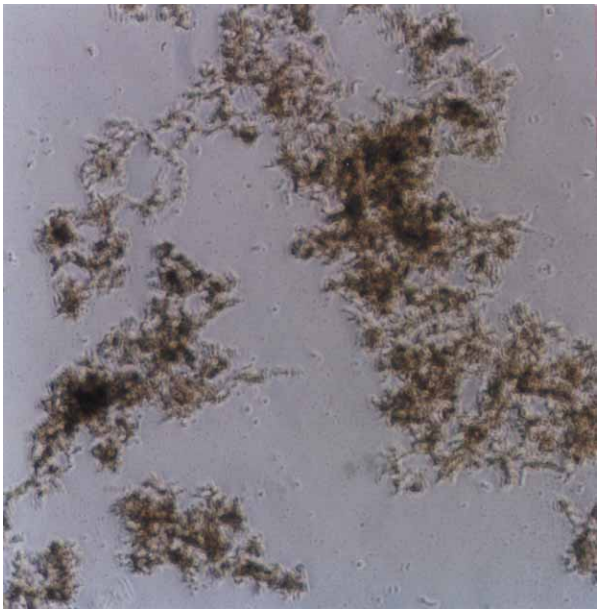

**Fig. 5. Microscopic view of the mixed culture biomass containing *P. putida* and *P. cepacia*** developed during aerobic degradation (enlarged 16x40)

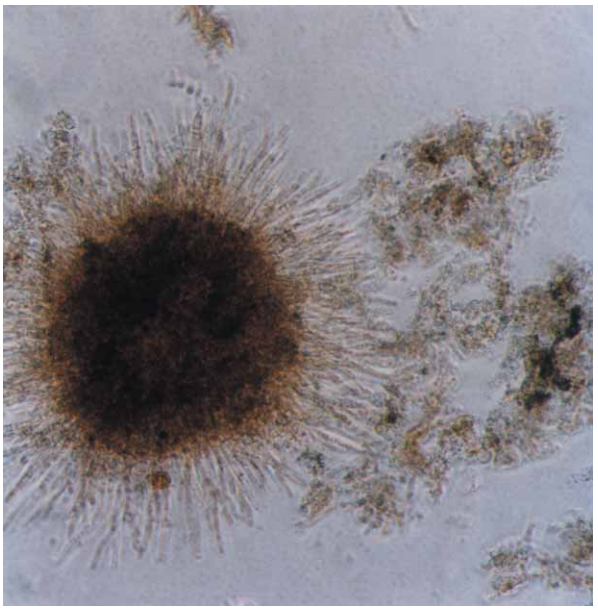

**Fig. 6. Microscopic view of the mixed culture biomass containing *P. putida, P. cepacia* and *T. penicillatum*** in the flocculare form during aerobic degradation (enlarged 16x40)

Regarding formic acid degradation individual strains and the mixed culture performed efficiency as shown in the figures: *P. putida* – Fig. 7, *P. cepacia* – Fig. 8, *T. penicillatum* – Fig. 9, and the mixed culture – Fig. 10.

All individual strains, and particularly the mixed culture, were capable of degrading formic acid within 18–24 hours, with the accompanying growth of biomass and the increase in pH.

*P. putida* degraded formic acid (500 mg/L) over 24 hours (Fig. 7) and *P. cepacia* over 12–18 hours (Fig. 8)

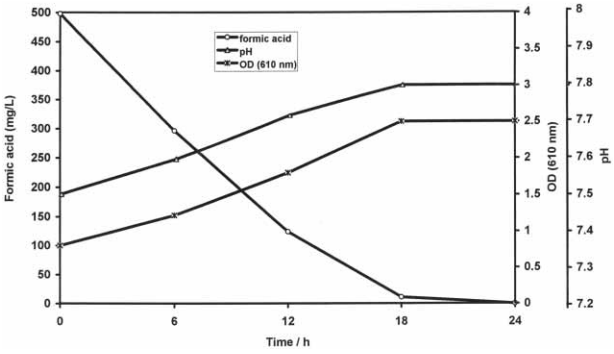


**Fig. 7. Aerobic degradation of formic acid with *P. putida*** in the synthetic medium – changes in pH and OD at 610 nm

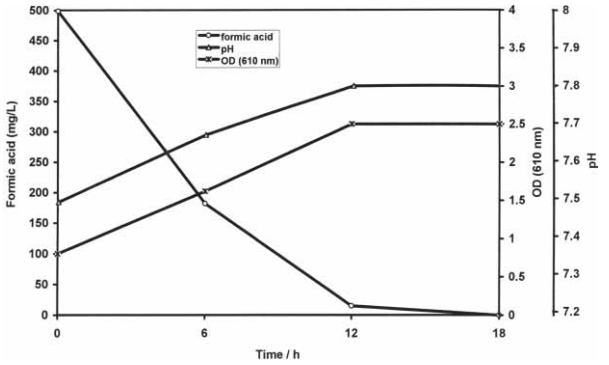


**Fig. 8. Aerobic degradation of formic acid with *P. cepacia*** in the synthetic medium – changes in pH and OD at 610 nm

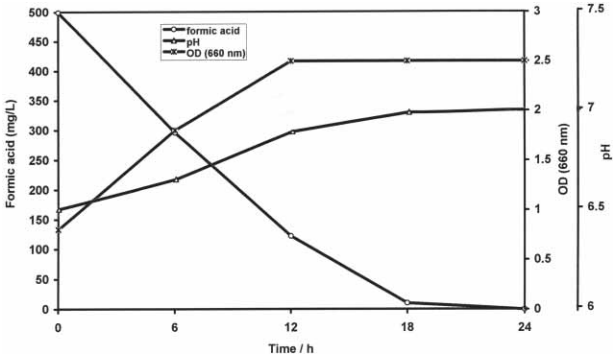


**Fig. 9. Aerobic degradation of formic acid with *T. penicillatum*** in the synthetic medium – changes in pH and OD at 660 nm

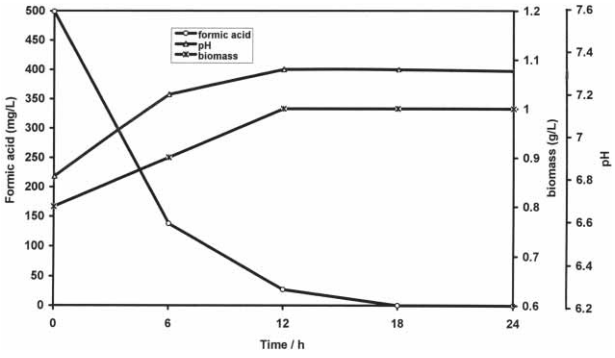


**Fig. 10. Aerobic degradation of formic acid with the mixed culture of *P. putida*, *P. cepacia* and *T. penicillatum*** in the synthetic medium – biomass growth and changes in pH

under concomitant increase of biomass. Optical density had been monitored at the same time. *P. cepacia* was markedly more efficient in degrading formic acid when the medium contained only formic acid (Fig. 8) without formaldehyde. Literature data (*11*) state similar duration of degradation (18–24 hours) and pH (6.9–7.2) for complete degradation of the same concentration of formic acid with *Pseudomonas*.

*T. penicillatum* also showed enzymatic potential for degradation of formic acid, but at lower pH (pH= 6.5–7.0, Fig. 9) compared to the bacteria (Figs. 7 and 8). Formic acid degradation with *T. penicillatum* over 18–24 hours was accompanied by growth in biomass (Fig. 9). The literature data (*7,9,10*) state that the enzymes contained in various types of yeast (formaldehyde- and formate-dehydrogenase enzymes) can be found in *Trichosporon* genus too during the growth on formaldehyde.

With the mixed culture of the selected individual strains, formic acid degradation (Fig. 10) accompanied by the growth of biomass, occurred within approximately the same time as with *P. cepacia* (Fig. 8).

In separate experiments the mixed culture of *P. putida* and *P. cepacia* showed equal kinetics of formic acid degradation as the mixed culture of bacteria and yeast (Fig. 10). Again the contribution of *T. penicillatum* yeast in the mixed culture was not in the improvement of degradation kinetics of formic acid, but rather in the formation of the flocculated biomass of mixed culture (Figs. 5 and 6).

After the study of degradation of formaldehyde and formic acid on the synthetic medium, we used mixed cultures to study the kinetics of aerobic degradation of formaldehyde and other substances contained in wastewater from melamine resins production. Composition of wastewater (Table 1) shows that in addition to formaldehyde, it contained methanol and butanol. It is known that their aerobic degradation may generate formaldehyde and contribute to increasing its levels (*6,9,10*). Over three consecutive adaptations of the mixed culture (in diluted wastewater), it was entirely adapted to the

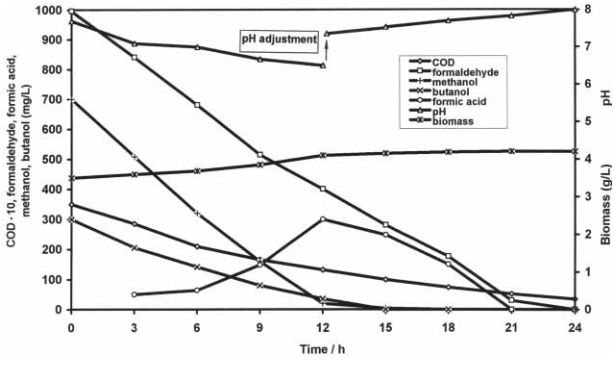


**Fig. 11. Aerobic degradation of organic matter (COD), formaldehyde, methanol, butanol and formic acid, growth of biomass and changes in pH of wastewater** from melamine resins production achieved with the mixed culture of *P. putida*, *P. cepacia* and *T. penicillatum*

ingredients of the original wastewater. Their degradation is shown in Fig. 11.

Over 24 hours of aerobic degradation of wastewater, the mixed culture exhibited its capability of degrading all substances contained in wastewater (formaldehyde, methanol and butanol) thus decreasing their concentration as well as the concentration of total organic matter expressed as COD.

Taking into consideration the experiments on degradation of formaldehyde and formic acid in the synthetic medium it was expected, that formaldehyde degradation in wastewater would not generate significant amount of formic acid. However, the experimental 12-hour aerobic degradation of wastewater caused accumulation of formic acid, probably due to methanol and butanol degradation; formic acid was degraded after pH adjustment. Biomass growth of the mixed culture and 90 % COD reduction over 24 hours evidenced that the wastewater components were used during aerobic degradation as the sources of carbon.

Just a few literature data on aerobic degradation of wastewater from melamine resins production (*13*,*15*) report that such wastewaters can be biodegraded (COD reduced by 87 %) in 5 days with the use of the adapted activated sludge. Results of this work suggested that this could be done more efficiently.

## Conclusions

The selected mixed culture containing two bacterial strains of *Pseudomonas* (*P. putida* and *P. cepacia*) and *Trichosporon* yeast genera (*T. peicillatum*) has exhibited high efficiency of degradation of formaldehyde and formic acid in the synthetic medium.

The mixed culture also degraded formaldehyde, methanol and butanol contained in wastewater from melamine resins production.

The selected mixed culture can be bio-augmented to increase the efficiency of the current bio-systems using activated sludge to treat wastewaters containing formaldehyde, methanol and butanol.

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# Aerobna razgradnja formaldehida u otpadnoj vodi iz proizvodnje melaminskih smola


### Sažetak

Odabrani sojevi *Pseudomonas putida*, *Pseudomonas cepacia* i kvasca *Trichosporon penicillatum* te miješana kultura ova tri soja upotrijebljeni su za aerobnu razgradnju formaldehida i mravlje kiseline u sintetskoj podlozi, te u otpadnoj vodi dobivenoj pri proizvodnji melaminskih smola. Pokazalo se da mješovita kultura u sintetskoj podlozi razgrađuje 1000 mg/L formaldehida tijekom 18–24 sata i 500 mg/L mravlje kiseline za 12–18 sati. Aerobna degradacija otpadne vode iz proizvodnje melaminskih smola, primjenom miješane bakterijske i kvaščeve kulture, postignuta je za 24 sata snizivanjem KPK-vrijednosti preko 90 % uz potpunu razgradnju formaldehida, metanola i butanola. Dodatak kvasca *Trichosporon penicillatum* u miješanoj kulturi tijekom aerobne razgradnje formaldehida u sintetskoj podlozi i u otpadnoj vodi utjecao je na stvaranje flokula biomase koje se talože.