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Formaldehyde and methanol biodegradation with the methylotrophic yeast *Hansenula polymorpha*. An application to real wastewater treatment

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Abstract

The application of methylotrophic yeast *Hansenula polymorpha* to the treatment of methanol and formaldehyde-containing wastewater was experimentally verified. A variety of real wastewater samples originating from chemical industry effluent were examined. The yeast cell culture could grow in the wastewater environment, revealing low trophic requirements and a very high adaptation potential to poor cultivation conditions. The proliferation of cells was accompanied by a concomitant xenobiotic biodegradation. Grown, preadapted cellular suspension at a density of about 1×10^7 cells/ml proved to be able to utilize formaldehyde present in wastewater at concentrations up to 1750 mg/l, levels toxic to most microorganisms. The biological waste treatment method presented shows the enhanced potential by means of specific enzymatic activities of monocarbonic compound oxidations through methylotrophic pathway reactions. The need to obtain mutants highly resistant to formaldehyde has also been rationalized.

Introduction

Methylotrophic yeasts are primitive eukaryotic microorganisms able to metabolize monocarbonic compounds such as methanol and formaldehyde (FD) (Gleeson & Sudbery 1988a; Sibirny et al. 1988). The metabolism of single-carbonic compounds in these organisms is performed by means of the unique and complex biochemical reactions pathway (Harder et al. 1987; Gleeson & Sudbery 1988a; Sibirny et al. 1988; Jones & Bellion 1991), schematically given in Figure 1.

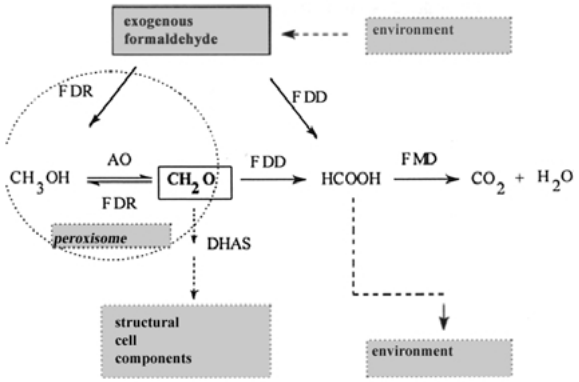

Figure 1. Metabolic pathway of methanol and formaldehyde oxidation in methylotrophic yeasts. The enzyme abbreviations are: FDR – formaldehyde reductase, AO – alcohol oxidase, FDD – formaldehyde dehydrogenase, FMD – formate dehydrogenase, DHAS – dihydroxyacetone synthase.

According to the simplified enzymatic pathway, formaldehyde appears in a cell as a result of peroxisomal alcohol oxidase (AO) activity. In a contaminated environment, however, FD also enters the cell diffusionally as exogenously supplied formaldehyde and can reach toxic levels in a cytoplasm. Then, it can be directly neutralized by FD reductase (FDR) yielding methanol, but the subsequent AO activity would produce yet another pool of FD. Therefore, the ultimate way to utilize formaldehyde is to as-

similate it into cellular carbon via dihydroxyacetone synthase (DHAS) pathway and/or to oxidize it in energy-yielding process into carbon dioxide and water first by FD dehydrogenase (FDD) and then by formate dehydrogenase (FMD).

The content of the wastewater generated by chemical plants is strictly correlated with the specificity of the technological process applied. Even at high chemical oxygen demand (COD) levels, the total load of waste results from elevated concentrations of single chemical compounds. These high concentrations make industrial wastewater a poor medium for the development of activated sludge in biological treatment plants (Buraczewski 1994). For the same reason, the biomass of such sludge is usually less heterogeneous than that found in municipal wastewater treatment plants. In addition, the management of the chemical waste is made even more difficult due to high toxicity of particular compounds and to a very high range of concentration changes of certain constituents, which can yield additional risk of weakening the physiological state of the activated sludge.

Formaldehyde present in wastewaters can be found at concentrations up to 10 000 mg/l in FD industry such as technical formalin synthesis, artificial manure, synthetic materials and resins production (Bożko & Grabińska-Łoniewska 1973). It is often accompanied by methanol and by chemical FD derivatives which are difficult to biodegrade. Although new alternative industrial technologies tend to reduce the risk of FD contamination, the use of this chemical is still widespread in developing countries and it causes severe environmental problems. Formaldehyde itself is a highly reactive compound, toxic to living organisms (Walker 1964; Bardana & Montanaro 1991; Chang & Gershwin 1992). According to our observations (data not shown) and to other authors (Bożko & Grabińska-Łoniewska 1973), at concentrations of approx. 1200 mg/l or more, FD exerts a strong negative effect on the activated sludge, causing biocenosis degradation, fragmentation of flocs and, finally, loss of biochemical activity.

In the previous study (Kaszycki & Kołoczek 2000) we have shown that in a very poor medium of synthetic wastewater, the methylotrophic organism *Hansenula polymorpha* can grow on and utilize formaldehyde present in the environment at toxic concentrations. In this paper we present experimental evidence that the methylotrophic yeast *H. polymorpha* can be employed in the treatment of real wastewaters, rich in FD and methanol, originating from the chemical industry.

Materials and methods

All of the chemicals used were of analytical grade. The solutions and buffers were made using redistilled water. Whenever required, fully sterile conditions were applied.

Wastewater samples

Wastewater types originating from various technological fluxes of chemical syntheses plants were collected before group biological treatment stations and they are listed and characterized in Table 1. The samples were cooled to 4 °C and analyzed as soon as possible after transportation. In several cases, when needed, they were kept frozen at −20 °C before biodegradation experiments. Sample freezing was checked experimentally to have no impact on COD, methanol and formaldehyde levels determined.

Yeast cell culture cultivation and preadaptation

The yeast strain used in investigations was a prototrophic revertant of a *Hansenula polymorpha* NCYC 2309 (*Leu*$^-$) parental strain, obtained from the National Yeast Culture Collection, Norwich, U.K. Cells were always grown on 2% methanol in the absence of glucose in order to keep methylotrophic pathway enzymes induced. The optimal growth media as well as cell cultivation conditions were described in detail previously (Kaszycki & Kołoczek 2000). At about 8–12 hours before experiments in wastewater environment the cell culture was preadapted to formaldehyde by adding 300 mg/l of this compound to the suspension. Then, the optimal medium was removed by centrifuging the yeast culture (5000 rpm for 5 min) and the cells were added to a sample at a desired biomass. Yeast biomass was measured turbidimetrically as optical density at 540 nm (OD_{540}) assuming OD_{540} =1 to be equal to 9.2×10^6 cells/ml. Cell survival tests and direct population determination was performed using the technique of surface plating of appropriate suspension dilution onto Petri dishes with a yeast-selective Sabouraud/agar solid medium and then by counting culturable colonies.

Cell growth in wastewater media and xenobiotic biodegradation observations

In a typical growth experiment, a 50 ml wastewater specimen in a 250 ml flask was inoculated with approx. 4.5×10^5 cells/ml (OD_{540} = 0.05). The aeration

of the culture was provided by vigorous rotary shaking of the flasks (250 rpm) in a water bath at a desired temperature. During culture growth, at each time interval, the OD_{540} of the culture was measured to observe the biomass increase and whenever needed some of the suspension was used for surface plating to check for cell survival. At the same time, a 1 ml sample was preserved for further determination of methanol and/or formaldehyde. In fast biodegradation kinetic observations a higher density of the cell suspension, i.e., $\approx 1.1 \times 10^7$ cells/ml (OD_{540} = 1.2), was added to 15–20 ml of wastewater in a 250 ml flask. Respective control samples, that is uninoculated wastewater, were always observed at identical experimental conditions.

Specimens for the determination of methanol and formaldehyde content were usually prepared by collecting in Eppendorf tubes of about 0.5–1 ml of a given wastewater suspension at each time interval during the course of an experiment. After centrifuging (10000 rpm for 3 min), the supernatants were kept frozen until the whole set was collected for analyses. For COD level measurements, larger sample volumes (about 5–10 ml) were preserved in a similar way.

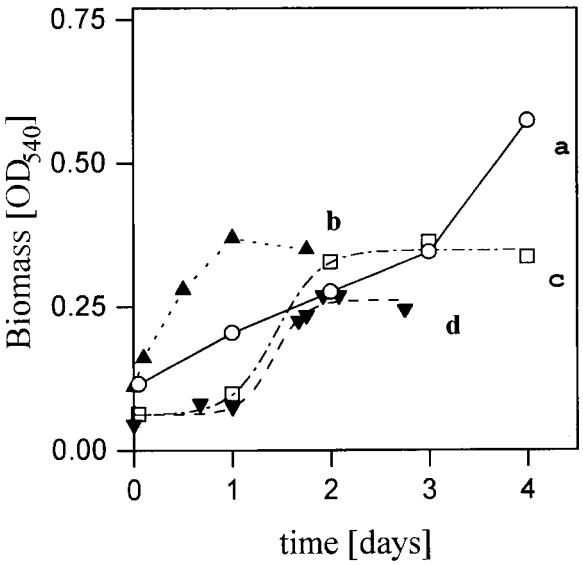

Figure 2. Typical growth kinetics of *Hansenula polymorpha* cell culture cultivated at 25 °C in wastewater samples from chemical industry plants (as described in Table 1). (a) Wastewater sample no. 4; (b) no. 2; (c) no. 3, 10 times diluted, and (d) no. 1. The OD_{540} increase of the control samples (the absence of yeast cells) was negligible and never reached the minimum OD value of inoculated wastewater. Further details are given in the text.

Analytical methods

Methanol was determined by a colorimetric enzymatic method as described in detail elsewhere (Kaszycki & Kołoczek 2000). In several cases the wastewater content of methanol was verified by a gas-chromatography analysis, yielding similar results.

Formaldehyde level in wastewater samples was measured with two independent colorimetric techniques. The concentration of "free" (i.e., unbound), monomeric form was determined by means of a Nash reagent (Nash 1953), as described earlier (Kaszycki & Kołoczek 2000). In order to determine the total FD load, i.e., the sum of free and chemically bound fractions, a simplified chromotropic method was applied, as based on standard procedures (Polish Norms 1977 and 1985). In brief, 0.54 ml of appropriately diluted specimen was treated with 0.045 ml of 2% aqueous solution of chromotropic acid (1,8-dihydroxynaphthalene-3,6-disulfonic acid, disodium salt) and 0.45 ml of concentrated H_2SO_4, and then immediately mixed thoroughly. After incubation in a boiling water bath for 30 min the absorbance at 570 nm was measured in a 1 cm cell with a reagent blank. For both FD determination methods standard curves were generated using defined FD solutions after hydrolysis of paraformaldehyde.

COD analyses were performed using a titrimetric standard method (Polish Norm 1987) with closed reflux and sodium dichromate digestion in sealed tubes.

Results and discussion

Growth of cells in wastewater samples

In all of the cases studied, except for the wastewater type no. 3 (see Table 1), where some apparently toxic components were present, the yeast cells were able to grow in the wastewater medium, provided they were fed with a low dose (≈0.005–0.01%) of yeast extract. The addition of this nutrient, however, did not cause a significant increase in the original COD value of the wastewater; in fact, it led to further rapid decrease in COD of the sample due to yeast cell culture proliferation and stimulation of biochemical activity. The growth kinetics, as presented in Figures 2 b-d and 3A, were similar to that observed in a model wastewater containing formaldehyde and methanol (Kaszycki & Kołoczek 2000) and in optimal media. Typically, the initial preparatory lag phase at low biomass was observed after inoculation of the sample. Then, a rapid

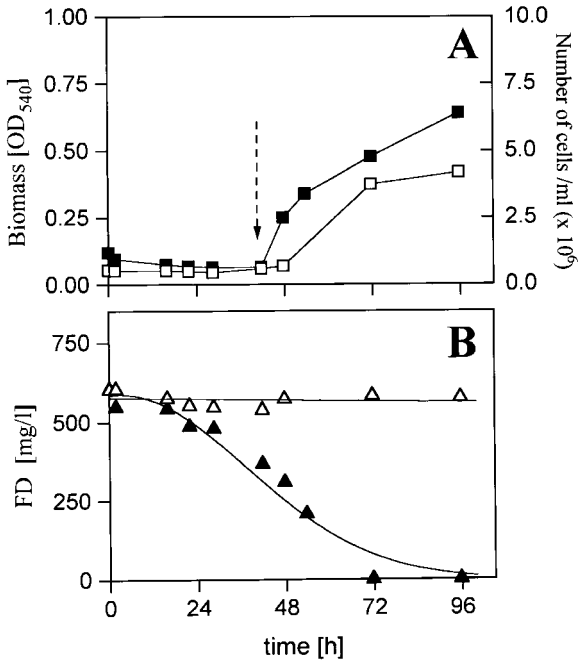

Figure 3. The development of *H. polymorpha* cells (section A) and growth-associated formaldehyde biodegradation kinetics (section B, ▲) during yeast cultivation at 25 °C in a sample of wastewater type no. 5, containing 600 mg/l formaldehyde and 230 mg/l methanol. After prolonged incubation, cell growth was stimulated by adding 0.02% of yeast extract (indicated by an arrow). In section A biomass increase was monitored either by optical density at 540 nm (left scale, □) or by the method of individual cell colony counts on plates with Sabouraud/agar medium (right scale, ■). Open triangles in (B) represent the FD level during incubation of uninoculated wastewater sample (control).

logarithmic biomass increase preceded the final stationary phase, characteristic of a mature, grown cell culture. The final amount of biomass formed depended on the cell-accessible carbon source concentration and it correlated with the COD value of a given wastewater sample (compare Figure 2a and d, and the respective average COD values measured for wastewater types 4 and 1 in Table 1). The morphology of the cell culture grown in nontoxic wastewater media, e.g., in wastewater no.4 as shown in Figure 4a, resembled that of the yeast cells proliferating in optimal media conditions.

The time required by the growing culture to achieve the final growth stage was dependent mainly on the chemical content of the medium; the more toxic the environment, the longer was the lag phase usually needed for physiological adaptation. In order to shorten the adaptive lag phase in toxic media, the appropriate dilution of a particular sample enabled the cells to grow and effectively biodegrade FD and methanol. For example, the typical sigmoidal cell growth curve in Figure 2c was obtained in a 10 times diluted wastewater type 3. Note that the toxicity of this sample was not due to the elevated FD content but to other chemically aggressive components. In highly toxic media (e.g., undiluted wastewater no. 3) it was observed that some part of the cell culture initially died out. The main reason responsible for the growth inhibition, however, was not the ultimate lethal activity against all the cells but rather the strong environmental stress response of the culture. This stress reaction was very similar to that described as a "nutritional stress" (Gleeson & Sudbery 1988a, b). In such cases the cells take multiple morphological forms and shapes such as "mating figures" exemplified by conjugation tubes seen in Figure 4b. These figures are typical of the stress-induced generative phase which leads to intense genetic recombination and sporulation, and finally to new clones of better adapted progeny. Thus, in the cases described above it was often observed that after prolonged incubation (2–3 days) the cells were still active and able to proliferate afterwards. This observation supports the idea of a very high adaptation potential of eukaryotic organisms to even extreme environmental conditions which are lethal to most bacterial species. It should be stressed that the observed yeast's capabilities to physiologically adapt to conditions far from growth optima is of great advantage in terms of a possible use of these organisms in the process of wastewater treatment. This is especially important for the case of chemical industry-originated wastewater whose content can vary greatly due to specific technological processes performed at a given time as well as to season production activity changes. For example, in various samples of wastewater no. 5 examined within one month, FD and methanol concentration ranged from 752 to 3122 mg/l and 222 to 1074 mg/l, respectively.

The FD degradation, accompanied by a parallel growth of the cell culture, is presented in Figure 3. It should be noted that the viability of the cells was kept high throughout the whole experiment, however, the additional fertilization of the medium by 0.02% of yeast extract (indicated by an arrow), led to immediate dramatic growth stimulation. In Figure 3A two independent ways of monitoring biomass growth are shown: turbidimetric (OD_{540}) as well as a direct method of culturable cell counts after plating the cell suspension. The latter method has been used to confirm the OD data since it only gives the dir-

Table 1. A list of wastewater types used in the study. All of the parameters given are mean values of at least three separate samples examined. Standard deviations calculated for COD and FD determination were not greater than 3% and for methanol less than 7% of the original values

Wastewater sample no.	Source	pH	COD ($mg\ O_2\ l^{-1}$)	FD (total) ($mg\ l^{-1}$)	FD (monomer) ($mg\ l^{-1}$)	Methanol ($mg\ l^{-1}$)	Other major constituents ($mg\ l^{-1}$)
1.	Synthetic material chemical works: Buffer wastewater tank	8.1	366	9.5	1.5	nd	phenol (total): 4.6
2.	Synthetic material chemical works: Formaldehyde department	7.3	958	60.1	45.0	nd	phenol: <2
3.	Chemical synthesis plant: Trioxane flux	6.2	2223	767	195	217	heterogeneous*
4.	Chemical synthesis plant: Urotropine manufacturing department	8.1	9864	1580	629	367	heterogeneous*
5.	Nitrogen chemical plant	7.7	975	1873	1922	579	ammonium nitrogen: 261

* As verified by HPLC and gas chromatography analysis.

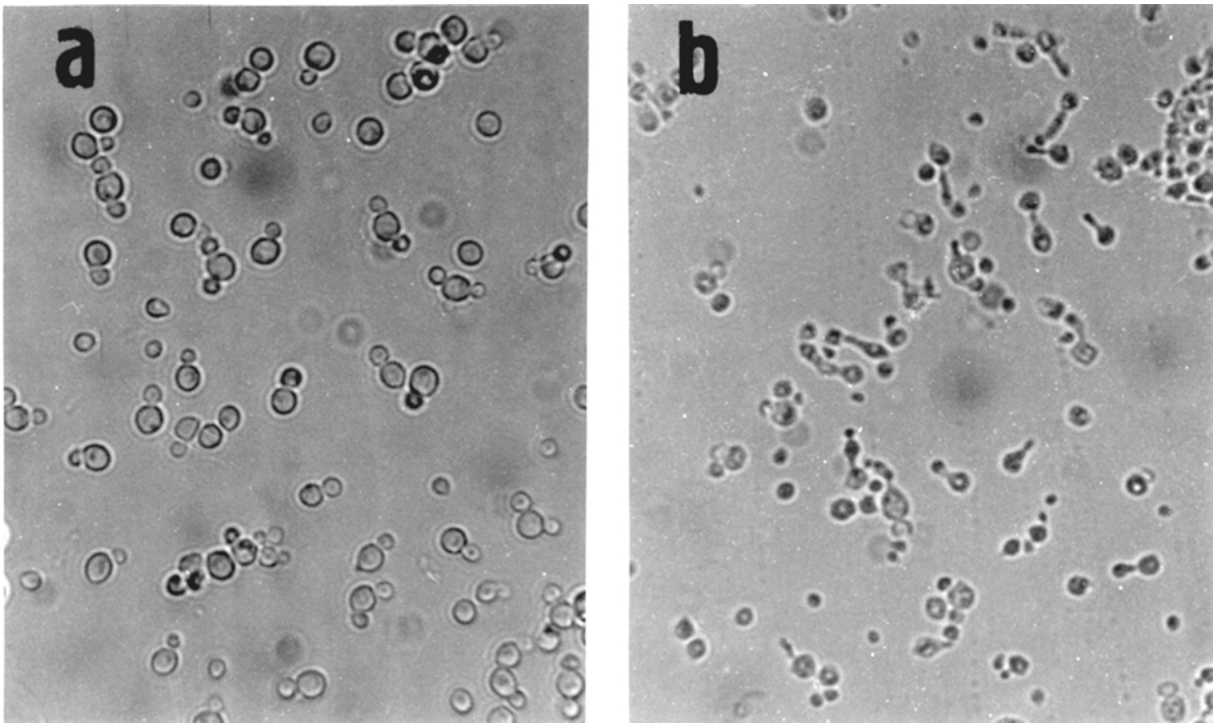

Figure 4. Light microscopic images (magnification 400×) of *Hansenula polymorpha* cells cultivated for 48 h in wastewater samples: (a) sample of type no. 5, containing 600 mg/l monomeric formaldehyde and 230 mg/l methanol; (b) sample no. 3 (undiluted), containing chemically bound formaldehyde at concentration 500 mg/l (mostly trioxane and trioxane polymers), free FD monomer at 250 mg/l, methanol at 220 mg/l, and other – apparently toxic – components.

ect information on the number of viable cells in the medium.

Biodegradation of formaldehyde

When wastewater was inoculated with a *H. polymorpha* culture grown up to the late exponential phase, formaldehyde present in the sample decreased rapidly at initial concentrations up to 1750 mg/l of FD monomer. In a typical experiment, the cells were previously grown on 2% methanol and then further preadapted to formaldehyde by adding 300 mg/l of this compound to the cultivation medium. After total FD degradation in the optimal medium the cell culture was regarded as fully active and induced toward xenobiotic utilization. Biodegradation kinetic measurements were performed at high biomass content (OD_{540} = 1.2, i.e., about 1.1×10^7 cells/ml). No additional nutrients such as traces of yeast extract were found necessary to keep the cells active. Figures 5 and 6 show the kinetics of FD decay in various wastewater types which contained formaldehyde at low (Figure 5 ●), medium (Figure 6 ●) and high (Figure 5 ■) initial concentrations. Note that sometimes a very rapid drop of the initial FD level was observed right after (in a matter of minutes) inoculation (cf. Figures 5 and 6) which was followed by slower kinetics represented by the fitted solid lines. This biphasic decay can be accounted for by direct sorption of formaldehyde by cell structures and then by a slower effect of cellular metabolism. The initial content of FD monomer in a wastewater, equal to ≈1750 mg/l was the highest concentration in which a dense, grown and preadapted cell suspension could effectively biodegrade this xenobiotic without a significant negative impact on the cell culture (Figure 5). However, after the experiments with FD levels above 1000 mg/l, we have observed about 30–40% decrease in cell survival. So, in the planned future studies with simulated continuous wastewater flow at high FD load, an additional fresh biomass supply during biodegradation should be considered. At higher FD concentrations all the wastewater samples became toxic and caused the inhibition of activity and then a dramatic loss of cell survival after 12 hours of incubation. It should be noted here that the degradation of 1750 mg/l formaldehyde in the real industrial wastewater revealed the yeast's biodegradation capabilities which were similar to that found in optimal growth media and much higher than in model media (Kaszycki & Kołoczek 2000) where the cells were able to utilize up to 850 mg/l FD. The above experiments imply that it would be of great advantage to select mutant strains of methylotrophic yeasts resistant to high FD levels, often present in real wastewaters. The attempts to obtain such mutants are already being carried out by our group and we are now testing *H. polymorpha* mutants which in optimal conditions can utilize formaldehyde even at 2400 mg/l initial concentration.

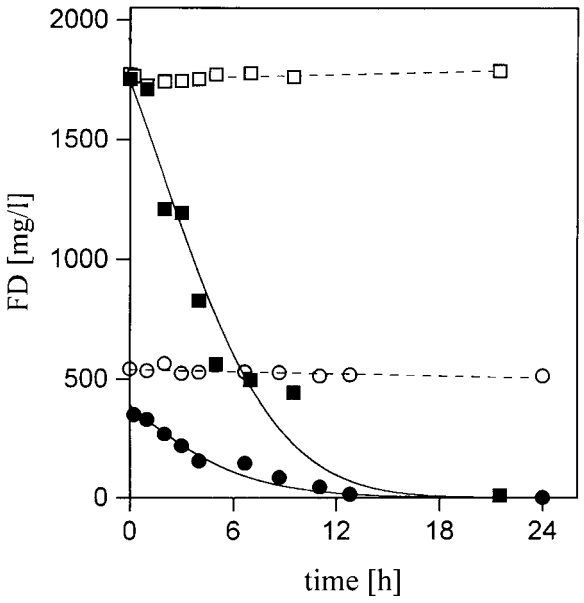

Figure 5. Formaldehyde biodegradation kinetics (filled symbols) obtained at 25 °C in two independent wastewater samples (no. 5), inoculated with approx. 1.1×10^7 *H. polymorpha* cells/ ml (OD_{540} = 1.2). The samples contained monomeric formaldehyde at initial concentrations of 1750 mg/l (■) and 530 mg/l (●) and methanol at 440 mg/l and 230 mg/l, respectively. Open symbols (dashed lines) represent the respective FD control levels (uninoculated wastewater).

In the experiment presented in Figure 5, the wastewater-contained formaldehyde was present totally as a monomeric form and was accompanied by methanol (440 and 230 mg/l, respectively). The presence of methanol may have some moderating effect by influencing the cell physiological adaptation to high FD levels. Methanol may also stimulate enzymatic activities in the methylotrophic pathway; yet, the above suggestions need further verification and experimental proof. The striking result was that, even though methanol was present in the described samples, an elevated formaldehyde concentration (i.e., 1750 mg/l) was found toxic to the specially preadapted activated sludge from the wastewater treatment plant of the same chemical factory. After suspending the activated sludge in the wastewater environment the

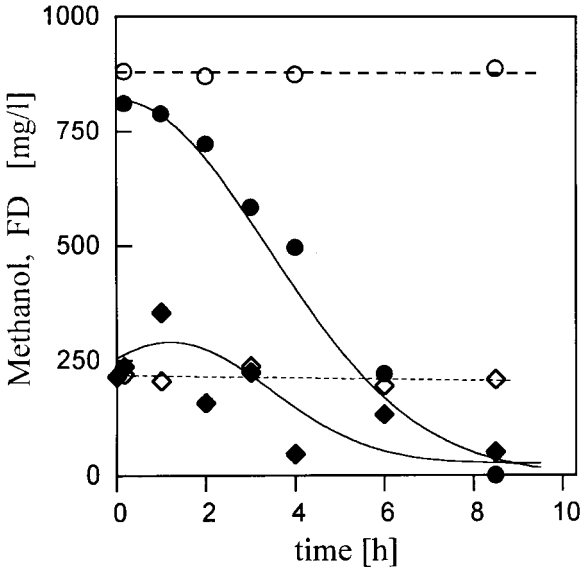

Figure 6. Kinetics of methanol (♦) and formaldehyde (●) biodegradation at 25 °C in a wastewater sample no. 5. containing FD and methanol at initial concentrations of 875 and 240 mg/l, respectively, inoculated with *H. polymorpha* preadapted cell culture (1.1×10^7 cells/ml). Open symbols represent the respective control levels of both compounds determined in the sample in the absence of cells.

floc structure became impaired and the biological activity was totally hampered. This particular activated sludge in earlier experiments was found to be resistant to formaldehyde concentrations up to about 750 mg/l.

Biodegradation of methanol

Methanol can serve as an easily accessible carbon source for methylotrophic yeasts and it is much less toxic than formaldehyde (Gleeson & Sudbery 1988a; Sibirny et al. 1988). It was present in all wastewater samples used in this work and – together with FD – contributed much to the total COD value determined in any particular specimen. According to our previous observations (Kaszycki & Kołoczek 2000), the concomitant presence of both compounds may result in the preferential detoxication action against formaldehyde xenobiotic at the expense of temporary methanol utilization delay or even some concentration increase in the medium. We suggest that this mechanism, due to FD toxicity, should be significant at higher exogenous formaldehyde concentrations. Accordingly, at low FD and methanol load in wastewater (of about 20 mg/l) methanol was being degraded in a manner very similar to the monomeric FD (data not shown). When concentrations of both xenobiotics were much higher (see Figure 6, 875 and 240 mg/l, respectively), methanol decreased more slowly than FD, reaching the zero level after complete removal of formaldehyde. Moreover, at the beginning of the biodegradation test, as presented in Figure 6, the utilization of methanol was preceded, typically, by some lag period in which sometimes a slight concentration increase above control could be observed. Even at high loads of formaldehyde, however, *H. polymorpha* was able to ultimately utilize all the methanol. As indicated earlier, the presence of methanol in the medium might also have some positive effect on FD biodegradation capabilities of the yeast.

The data on biodegradation efficiency of both formaldehyde and methanol in various wastewater types are summarized in Table 2.

Chemical oxygen demand (COD) determinations

All the biodegradation experiments were supported by parallel measurements of COD in a given wastewater specimen. In many cases the observed decrease of a COD value in a wastewater treated with methylotrophic yeast (see Table 2) was greater than that resulting from calculations of sole oxidation of FD and methanol (assuming roughly 1 mg O_2 required for oxidation of 1 mg of a tested compound). The above fact indicates that *H. polymorpha* cells were also involved in oxidation reactions of other compounds which contributed to the total COD value of a given wastewater, such as bound FD, urea, phenols, etc. This is consistent with the information on broad range of nutrients used as a carbon source for the yeast's growth (NCYC Strain data, 1997). Such results additionally prove the potential of the yeast to biodegrade various chemicals present in heterogeneous wastewater environments.

In most of the control experiments, the extensive shaking of uninoculated wastewater led to a significant COD decrease which seems to be a result of direct oxidation due to intense aeration. However, independent determinations of the FD and methanol content in control samples indicate that the observed COD decrease was not related to the spontaneous oxidation and/or evaporation of these compounds.

Environmental conditions for effective biodegradation

Similarly to previous studies in model systems (Kaszycki & Kołoczek 2000), biodegradation experiments were performed at various pH (3–9), temperatures (14–39 °C), yeast cell densities (1×10^5–2×10^7 cells/ml) and at several dilutions of toxic wastewater samples. Our research demonstrates the great ability

Table 2. Comparison of biodegradation efficiency of monomeric formaldehyde and methanol, COD decrease and cell biomass growth in wastewater samples treated with *Hansenula polymorpha* at 25 °C. Biodegradation tests were performed for 24 h at yeast cell culture densities of 1.1×10^7 cells/ml

Wastewater sample no.	Biodegradation tests			Cell culture growth test[a]
	FD degradation efficiency (%)	Methanol degradation efficiency (%)	COD decrease (% of the initial value)	Biomass increase in 72 h (% of the initial inoculum density)
1.	100[b]	nd	50.0	475
2.	98.1	nd	47.6	231
3.[c]	100	100	58.0	352
4.	100	100	35.9	480
5.	100	100	92.6	820

[a]Cell culture growth is shown as OD_{540} increase after inoculating samples with approx. 4.5×10^5 cells/ml.
[b]Levels below detectable values using the analytical method. [c]Results obtained for a 10-fold dilution of the sample.

of eukaryotic methylotrophs to physiologically adapt over a broad range of conditions in which biochemical activities of xenobiotic utilization are still retained. Although optimal growth conditions for *H. polymorpha* (pH 5.0, temperature 37 °C) often differ from that present in real industrial wastewaters, the cells could effectively oxidize methanol and formaldehyde at temperatures as low as 14 °C and were able to adapt to basic environment with pH up to 9.0. In the case of toxic waste levels, the chemically active compounds acted as stressful agents, inducing a typical stress reaction of the yeast cell culture (Figure 3b) and led to further adaptation afterwards.

Finally, we should mention that in all biodegradation experiments the original yeast inoculum was neither reused in consecutive wastewater treatment tests nor introduced to the activated sludge biocenosis. In order to further confirm the practical validity of the approach presented in this work it would be worth to verify whether the yeast culture can adapt and survive in competition (or in symbiosis) with other species of the activated sludge and whether it can remain biochemically active in prolonged treatment processes. It also should be stressed that only the monomeric form of formaldehyde was monitored and that it was successfully biodegraded in all of the wastewater samples tested, including those which contained high loads of a chemically bound FD (see Table 1). However, the activity of *H. polymorpha* against FD derivatives as well as the problem of the yeast integration with the activated sludge are the subject of a separate study (Kaszycki et al., manuscript in preparation).

Conclusions

The experimental work presented in this paper leads to several final inferences:

1. Methylotrophic yeast *Hansenula polymorpha* can be introduced into real industrial wastewater environments, containing high levels of methanol and formaldehyde, and can effectively biodegrade these compounds. For the case of FD monomer, the preadapted yeast cell suspension could easily utilize this xenobiotic at initial concentrations reaching 1750 mg/l, that is at levels exceeding the observed toxicity threshold against organisms of the activated sludge (1200 mg/l). The presence of methanol in the medium had a positive effect on yeast's resistance to formaldehyde.
2. The yeast species has proved to be active in various types of wastewater. It reveals high physiological adaptation potential to changeable environmental conditions characteristic of industrial wastewater.
3. Methylotrophic yeast monocultures, grown in separate aerated chambers, may be applied to treat formaldehyde- and methanol-containing wastewater as biological filters specialized in biodegradation of single-carbonic compounds. In such a case the cell biomass formed on assimilated compounds might be further utilized in a variety of agricultural applications as a source of vitamins and pro-

tein. However, the presented approach should be verified by appropriate, full-scale technical tests.

4. The yeast *Hansenula polymorpha* applied in this work can be regarded as a maternal strain for further selection of mutants with stimulated biochemical activity and high resistance to elevated formaldehyde concentrations.

Acknowledgment

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