

Transformation of aromatic compounds by *C. necator*

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The transformation of aromatic pollutants to aliphatic and biodegradable PHBs by *Cupriavidus necator* was accomplished. Different oxidation (alkanes to carboxylic acids) and substitution (toluene to terephthaloyl chloride) degrees of aromatics at 1 g/L concentration were used. The substitution degree appeared to be more important than oxidation one, thus only mono-substituted compounds were transformed by *C. necator*, and benzoic acid was transformed faster than benzaldehyde and toluene. Some of more substituted compounds were found to be non effective on *C. necator* growth (terephthaloyl chloride and benzene dimethanol), others – toxic (phenyl phenol, benzophenone or xylene).

Introduction

Polyhydroxyalkanoates (PHAs) in general and most particularly polyhydroxybutyrates (PHBs) are well known chiral bio-products discovered by Lemoigne in 1925. PHB is a highly crystalline thermoplastic; it is highly biodegradable and sustainable product (Sudesh et al. 2000).

We used those sustainability properties for bioremediation purpose: the transformation of aromatic pollutants to aliphatic PHBs. Previously we showed that glutamate addition to classical feeding of *Cupriavidus necator* allows the enhancement of the biomass production and maintains the PHA content in cells (Berezina et al. 2007 and Berezina et al. 2008), further we studied different nutritional media, and thus discovered that traditional mineral media induces the lowest PHA level in cells (Berezina et al. 2009). This point is of great interest for the present study as we need the PHA content in blanks as low as possible for observing the enhancement as slight as it could be in cultures containing aromatic compounds.

Results and Discussion

In order to test the action of *C. necator* on different aromatic compounds we choose to test two compounds by oxidation degree, thus we tested two alkanes (toluene and xylene), two alcohols (benzene dimethanol and phenyl phenol), two carbonylic derivatives (benzaldehyde and benzophenone) and two carboxylic acid derivatives

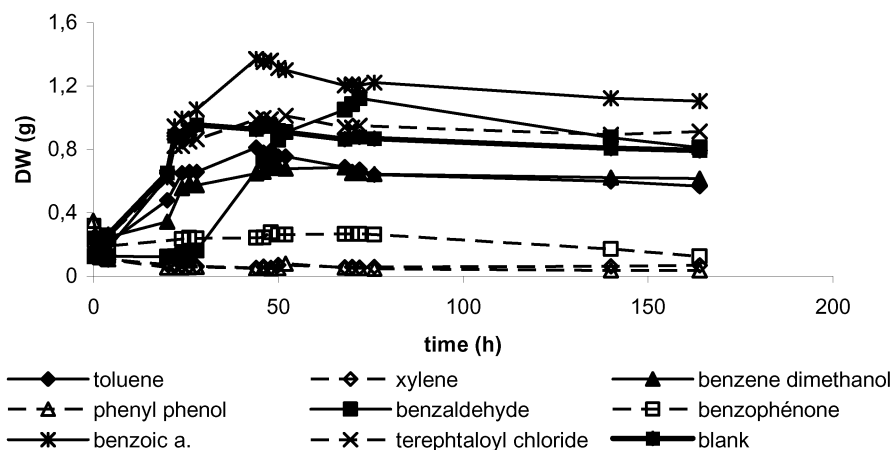


Figure 1: Time course of DW of *C. necator* cultures with different aromatic compounds compared to the blank

(benzoic acid and terephthaloyl chloride). These compounds were introduced at 1 g/L concentration in 500 mL shaking flasks containing 100 mL minimum mineral media and 5 mL of 24 hours - aged *C. necator* inoculum.

Preliminary work on evaporation advised us that for volatile alkanes, both toluene and xylene, the quantitative evaporation occurred at least at 4 hours. Thus in both these cases we added the original amount of aromatics every 4 hours during the experimentation course.

First observations were made on biomass graph (Figure 1). Thus benzophenone, phenyl phenol and xylene seemed to be toxic against *C. necator*. These observations were further confirmed by pollutants' concentration and PHA content analysis.

On the contrary toluene, benzene dimethanol, benzaldehyde, benzoic acid and terephthaloyl chloride show biomass increase at least comparable (toluene and benzene dimethanol) to this of the blank and even in some cases (benzoic acid, benzaldehyde and terephthaloyl chloride) superior to it.

When looking at pollutants' concentration¹ (Figure 2: **Time course of pollutants' concentrations during *C. necator* growth.**) we can distinguish terephthaloyl chloride. Indeed, the concentration of this pollutant still near 100 % during the whole time of the experiment, so this compound seems to be neutral to *C. necator* culture.

For other cases we compared the pollutants' concentration during the experimentation with *C. necator* to these obtained during the preliminary work for evaporation purpose (Figure 3).

¹ benzoic acid and terephthaloyl chloride analysis were performed by HPLC, those of other compounds by GC

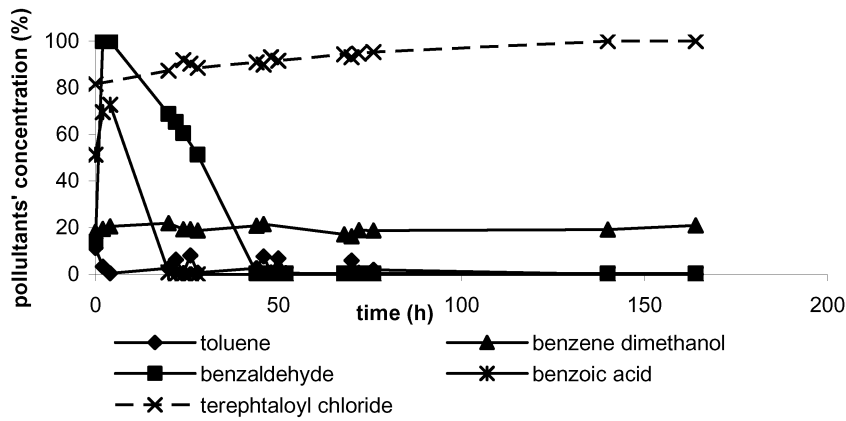


Figure 2: Time course of pollutants' concentrations during *C. necator* growth.

Here we can see that for benzene dimethanol the case seems to be similar to that of terephthaloyl chloride. Indeed, the benzene dimethanol's concentration stills similar in both evaporation and cultivation experiments, this compound presents a poor solubility in water (around 20 %) which is not affected during the cultivation experiment.

Different observations are made for benzoic acid and benzaldehyde (Figure 3). In these two cases, the consumption of pollutants is clearly indicated by concentration curves comparison, even if benzaldehyde's evaporation is far to be neglectible on experience conditions, the benzaldehyde's consumption is clearly more important in presence of *C. necator* cells.

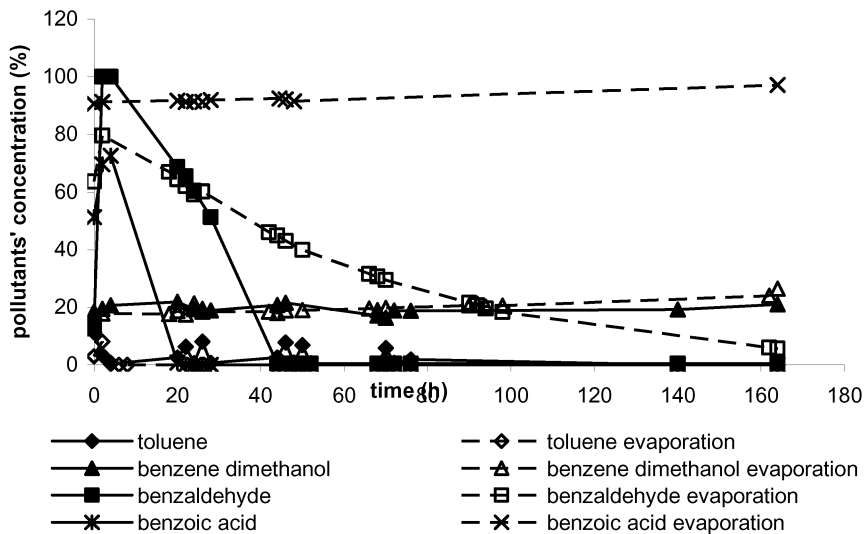


Figure 3: Comparison of pollutants' concentration during evaporation and *C. necator* growth

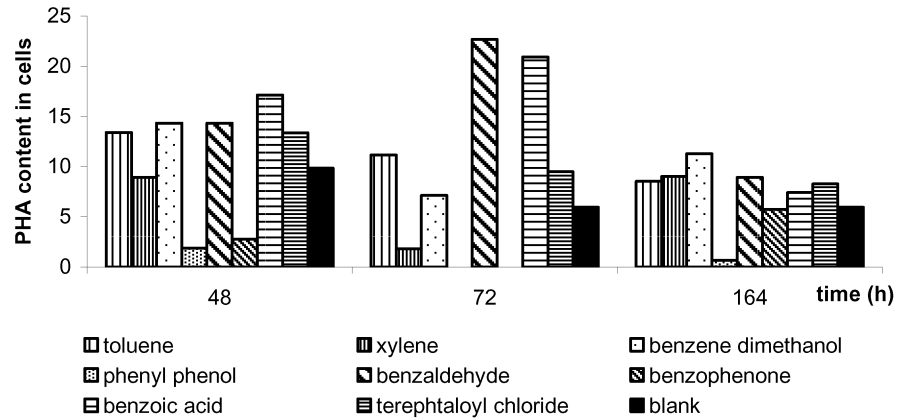


Figure 4: PHA's content analysis in *C. necator* cells during cultivation on different aromatic compounds

We can even notice that as well growth of *C. necator* as pollutant consumption is slower for benzaldehyde than for benzoic acid. This can be explained if we assume that the oxidation degree of acid is more suitable for the cells transformation than that of aldehyde. We still currently be exploring this hypothesis by HPLC analysis of benzoic acid and benzaldehyde metabolites.

In toluene case this assertion can not be assumed so far because of the extreme volatility of this compound, the concentrations' curves comparison is not effective this time (Figure 3).

Last analysis concerned PHA content in cells following TGA method described before (Talon et al. 2007). This analysis confirms the toxic effect of xylene, phenyl phenol and benzophenone; indeed for growth on these substrates we observe a very poor, if existing, content of PHA in cells. In case of benzoic acid, benzaldehyde and toluene we observe, in the contrary, an enhancement of PHA in cells, which is consistent with the hypothesis made before on the consumption of these substrates by *C. necator*.

Finally, looking at PHA content of cultures with terephthaloyl chloride and benzene dimethanol, we can notice that it is more important comparing to that of the blank. We can explain this by the stress induced by the presence of aromatics on cells. Indeed, even if these substrates are not consumed by the microorganism and are not neither inhibiting to it, they induce a stress situation which is known to enhance PHA storage (Potter et al. 2004, Mantzaris et al. 2002).

This explains the enhancement of dry weight during the *C. necator* culture on terephthaloyl chloride; no terephthaloyl chloride was consumed, nevertheless the dry weight increased, the more important PHAs' storage could be an explanation. For benzene dimethanol the situation is different, PHA content is superior to the blank but the overall dry weight is not. This can be explained by two consequences of the same effect, indeed benzene dimethanol can be partially toxic to *C. necator*, this implies partial death of cells, but on the other hand the stress created by the presence of this pollutant in the media increase the PHA production in cells stilling alive.

Conclusion

A huge amount of scientific work is actually done on bioremediation purpose. Nevertheless only few recent articles are treating on pollutant transformation in PHBs. We can outline the work on anaerobic transformation of benzoate, toluene and xylene by genetically engineered strain of *Azoarcus sp.* CIB by Zamarro et al. (2009). The accumulation of PHB, but only on 10 % of dry cell weight, was also reported by Trautwein et al. (2008) when growing *Aromatoleum aromaticum* EnN1 strain on ethyl benzene, toluene, *p*-cresol or phenol. Nair et al. (2009) submitted *Alcaligenes sp.* to phenol stress at 0.15 g/L and noticed the concomitant disappearance of the aromatic stretching in FT-IR spectroscopic analysis and the evidence for PHB presence inside the cells. Few years earlier Umweltforschungszentrum registered a patent (Babel et al. 2002) on continuous cultivation of *Variovax*, *Ralstonia* (further renamed to *Cupriavidus*) and *Comamonas* strains on aromatic compounds such as phenols, benzoic acid and benzaldehyde.

Nevertheless as far as we know, nobody was interested by systematic study of *C. necator*'s growth on different aromatic compounds. In this work we showed that the substitution degree of the aromatic compound is the most important point to be considered; indeed only mono-substituted aromatic compounds are substrates for transformation by *C. necator*. Inside this family the transformation is easier when the oxidation degree is higher (benzoic acid transformation is faster than this of benzaldehyde and benzaldehyde transformation is more efficient than this of toluene).

We also established that two other different groups of compounds are to be considered. Compounds which are definitely toxic to the microorganism (xylene, phenyl phenol and benzophenon) at least at 1 g/L concentration. But also compounds which are not consumed by *C. necator* (benzene dimethanol and terephthaloyl chloride), but which are partially toxic, just enough to create a stress situation which allows a more important PHA accumulation in cells.

Acknowledgments

Authors are thankful to Walloon Region and Europe Scientific Council for financial support.

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