BIODEGRADATION AND DEGRADATION PATHWAY OF DIISOPROPYL ETHER BY *MYCOLICIBACTERIUM* SP. STRAIN CH28

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Abstract

A diisopropyl ether (DIPE) degrader bacterium, *Mycolicibacterium* sp. strain CH28 was isolated from a pharmaceutical groundwater. Based on our results strain CH28 is capable of the complete mineralization of DIPE with a maximum degradation rate of 1.63 ± 0.03 nmol min^{-1} mg $biomass^{-1}$. We proposed the metabolic pathway of microbial DIPE degradation in our isolate based on the detection of 2-propanol, acetone, and acetate as degradation intermediates. Our results revealed that strain CH28 holds great potential in the bioremediation of sites contaminated with fuel oxygenate ethers (e.g.: DIPE).

Introduction

Fuel oxygenate ethers, like methyl *tert*-butyl ether (MTBE), ethyl *tert*-butyl ether (ETBE), *tert*-amyl methyl ether (TAME), and diisopropyl ether (DIPE) have been increasingly used since the 1970s as octane enhancers to replace lead and induce complete fuel combustion [1]. There are various treatment methods for degrading ether oxygenates from the contaminated medium including air stripping, absorption [2], adsorption [3], chemical oxidation [4], additionally several combined physical/chemical and biological processes are also spreading [5]. Despite these compounds are usually poorly biodegradable because of their highly stable ether bonds and branched carbon structure [1], biological treatment is the most common method of remediating ether-polluted wastewaters [6].

DIPE is widely used as an industrial solvent for oils, waxes, dyes, and resins [7], and for the extraction and purification of many compounds [8]. To date, only three bacterial isolates have the ability to degrade DIPE have been described: *Rhodococcus ruber* IFP 2001 [9], *Pseudonocardia* sp. strain ENV478 [10], and *Aquincola tertiaricarbonis* L108 [11].

In this abstract, we describe the kinetics and some intermediates of DIPE biodegradation observed regarding *Mycolicibacterium* sp. strain CH28, furthermore, we propose a part of the degradation pathway of aerobic DIPE mineralization as well.

Results and discussion

Resting cell experiments with Mycolicibacterium sp. strain CH28

To measure the DIPE-degrading capacity of strain CH28 resting cell, experiments were carried out with 200 mg l^{-1} DIPE. The total amount of DIPE was efficiently mineralized in less than 11 hours, the maximum degradation rate was 1.63 ± 0.03 nmol min^{-1} mg $biomass^{-1}$. The rate of DIPE degradation was seemed to be of the same magnitude as for the previously described strains [9-11]. During the experiment, acetone and acetate were detected as intermediates (Figure 1). This was the first time that acetate had been identified as a degradation intermediate of DIPE biodegradation.

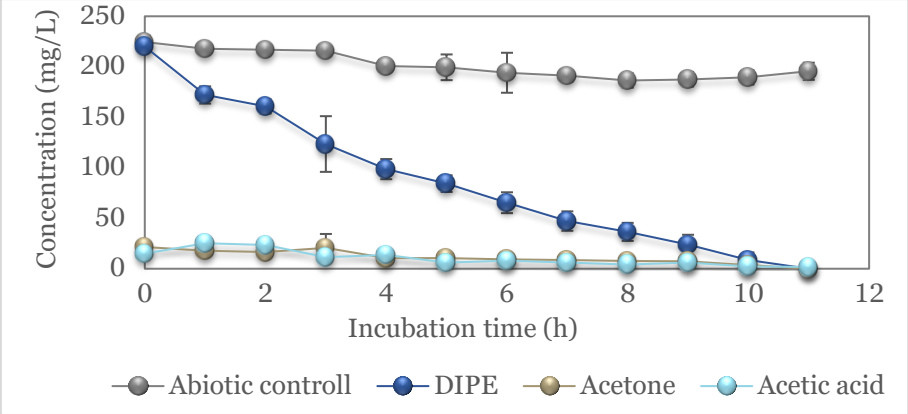

Figure 1. Degradation of DIPE by *Mycolicibacterium* sp. strain CH28 resting cells. Cells were pre-grown in SMM supplemented with 200 mg l^{-1} DIPE. The concentrations of DIPE (abiotic controls (□); bioaugmented systems (▲)), acetone (●), and acetate (◊) were monitored. Error bars indicate the standard deviations of three parallel measurements.

Biodegradation pathway of DIPE in Mycolicibacterium sp. strain CH28

Biodegradation of ether oxygenates such as MTBE, ETBE, dimethyl ether, diethyl ether, and aralkyl ethers was described to proceed through an *O*-dealkylation reaction [12]. Generally, this process is catalyzed by monooxygenases, during the degradation an oxygen atom incorporates to the alpha carbon atom of the ether bond. This results the formation of an unstable hemiacetal structure which spontaneously decomposes to an alcohol and a carbonyl compound [1]. *O*-dealkylation of DIPE generates to the simultaneous formation of 2-propanol and acetone. Most likely, 2-propanol is converted to acetone by a secondary alcohol dehydrogenase. These results are in good agreement with our experiments since we have managed to detect 2-propanol (in microcosm studies, data not shown) and acetone as degradation intermediates (Figure 2). Considering our and the above showed results we propose the upper pathway of DIPE degradation by strain CH28 (Figure 3) resulting in the formation of 2-propanol and acetone as the major intermediates.

The lower pathway of DIPE biodegradation includes the degradation of acetone producing CO_2 and bacterial biomass. Microbial metabolism of acetone has been well-studied in general, five major pathways were revealed [13]. Two of these involve the formation of formaldehyde but we could not detect this intermediate in any of our samples. Strain CH28 is not capable of the biodegradation of formaldehyde, so if it had been formed, it would have accumulated. During the biodegradation of DIPE, acetate was generated by strain CH28. Accordingly, it could be formed virtually in all the reported acetone degradation pathways. However, further research is required to clarify the actual biodegradation pathway of acetone in strain CH28 (Figure 3).

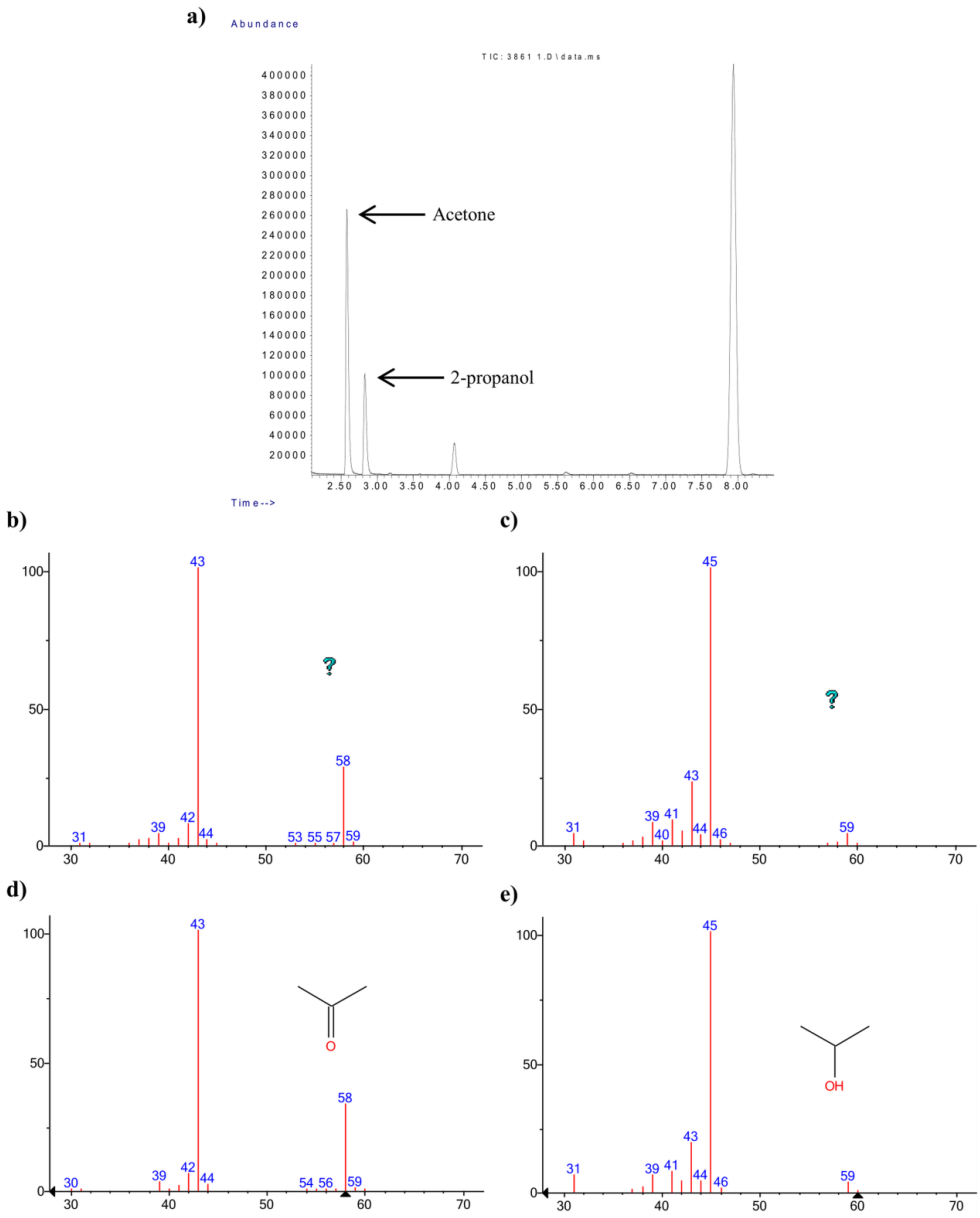

Figure 2. Acetone (at t_R=2.578 min) and 2-propanol (at t_R=2.829 min) as identified intermediates of DIPE degradation by *Mycolicibacterium* sp. strain CH28 in our experiments (**a**), with mass spectra of acetone and 2-propanol from the microcosm samples (**b** and **c**, respectively) and from the NIST/EPA/NIH Mass Spectral Library (Version 2.0f, NIST, Gaithersburg, MD, USA) (**d** and **e**, respectively).

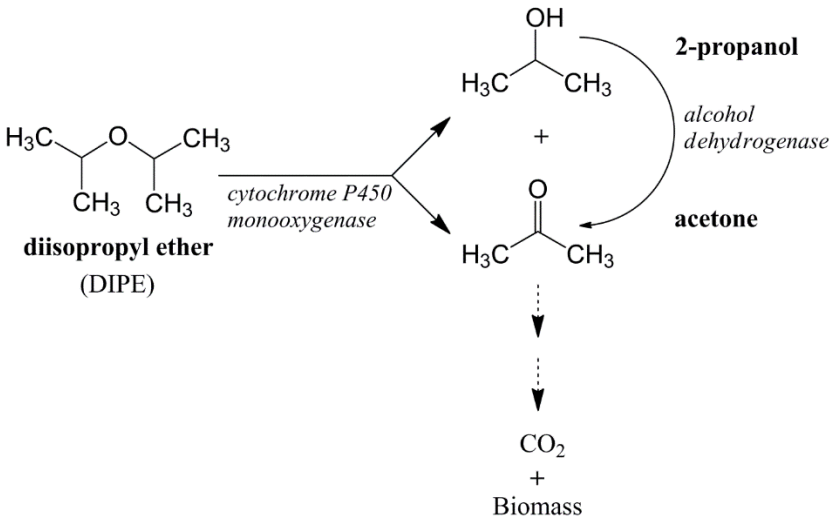

Figure 3. Upper pathway of DIPE biodegradation by *Mycolicibacterium* sp. strain CH28.

Conclusions

Mycolicibacterium sp. CH28, isolated from a pharmaceutical groundwater, is a powerful DIPE-degrader. Regarding strain CH28, major part of upper DIPE-degradation pathway was revealed, however, further studies are needed to clarify the exact lower degradation pathway as well.

Acknowledgements

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References

[1] White, G. F., N. J. Russell, and E. C. Tidswell. 1996. Bacterial scission of ether bonds. Microbiological Reviews 60 (1):216–32.
[2] Cortez, J. S. A., B. I. Kharisov, T. E. S. Quezada, and T. C. H. García. 2017. Micro- and nanoporous materials capable of absorbing solvents and oils reversibly: The state of the art. Petroleum Science 14 (1):84–104.
[3] Vakili, M., M. Rafatullah, B. Salamatinia, M. H. Ibrahim, N. Ismail, and A. Z. Abdullah. 2017. Adsorption studies of methyl tert-butyl ether from environment. Separation & Purification Reviews 46 (4):273–90.
[4] Kashir, M., and R. McGregor. 2018. Stabilized hydrogen peroxide for the remediation of hydrocarbons and MTBE in high temperature and saline groundwater. Remediation Journal 29 (1):27–36.
[5] Liang, J., S. Tang, J. Gong, G. Zeng, W. Tang, B. Song, P. Zhang, Z. Yang, and Y. Luo. 2020. Responses of enzymatic activity and microbial communities to biochar/compost amendment in sulfamethoxazole polluted wetland soil. Journal of Hazardous Materials 385: 121533.
[6] Borisov, I., I. Podtynnikov, E. Grushevenko, O. Scharova, T. Anokhina, S. Makaev, A. Volkov, and V. Volkov. 2020. High Selective Composite Polyalkylmethylsiloxane Membranes for Pervaporative Removal of MTBE from Water. Polymers 12: 1213.

[7] Patnaik, P. 2007. A comprehensive guide to the hazardous properties of chemical substances. 3rd ed. Hoboken, NJ: Wiley-Interscience
[8] Harrington, P.J. 2011. Pharmaceutical Process Chemistry for Synthesis: Rethinking the Routes to Scale-Up. 1st ed. Hoboken, N.J: Wiley.
[9] Hernandez-Perez, G., F. Fayolle, and J.-P. Vandecasteele. 2001. Biodegradation of ethyl *t*-butyl ether (ETBE), methyl *t*-butyl ether (MTBE) and *t*-amyl methyl ether (TAME) by Gordonia terrae. Applied Microbiology and Biotechnology 55: 117–121.
[10] Vainberg, S., K. McClay, H. Masuda, D. Root, C. Condee, G.J. Zylstra, and R.J. Steffan. 2006. Biodegradation of ether pollutants by *Pseudonocardia* sp. strain ENV478. Applied and Environmental Microbiology 72: 5218–5224.
[11] Lechner, U., D. Brodkorb, R. Geyer, G. Hause, C. Härtig, G. Auling, F. Fayolle-Guichard, P. Piveteau, R.H. Müller, and T. Rohwerder. 2007. *Aquincola tertiaricarbonis* gen. nov., sp. nov., a *tertiary* butyl moiety-degrading bacterium. International Journal of Systematic and Evolutionary Microbiology 57: 1295–1303
[12] Kim, Y.-H., and K.-H. Engesser. 2005. Inhibition of diethyl ether degradation in *Rhodococcus* sp. strain DEE5151 by glutaraldehyde and ethyl vinyl ether. FEMS Microbiology Letters 243: 317–322.
[13] Hausinger, R.P. 2007. New insights into acetone metabolism. Journal of Bacteriology 189: 671–673.