Mobilization of metals from metal-containing solids using cyanogenic microorganisms

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Introduction

Car catalysts contain a metal covered ceramic honeycomb. The metals catalyze reactions with the poisonous exhaust. The metals used for these reactions are platinum, palladium and rhodium. Nowadays, these metals are recycled with hydrometallurgical methods.[5]

The goal of our project was to mobilize platinum like metals from the ceramic surface by cyanogenic microorganisms. Cyanide interacts with many metals and forms water soluble complexes with them, also with platinum like metals [4,6]. Cyanide is e.g. used to leach gold from ores. SC Campbell et al from Montana State University investigated the application of biogenic produced cyanide in gold recovery. They used *Chromobacterium violaceum* as cyanogenic organism. The leaching was possible, but a close association between the gold surface and the cyanide was required to solute the gold [2].

We tried to solute Pd, Pt and Rh by means of *Chromobacterium violaceum*, a well described cyanogenic bacterium which produces violacein as pigment, and of *Pleurotus ostreatus*, a basidiomycet. For cyanide production both organisms need glycine [2].

Figure 1
The old car catalyst with the honeycombs which are used in our experiment.

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Material and methods

Organisms and culture conditions

1 ml inoculum of *Chromobacterium violaceum* was grown aerobic at 30°C in shake flasks containing 100 ml of complex medium. The complex medium consisted of 4.413 g/l L-glutamate, 1.36 g/l KH_2PO_4, 2.13 g/l $NaHPO_4*7H_2O$, 0.2 g/l $MgSO_4*7H_2O$, 0.005 g/l $FeCl_3*6H_2O$, 0.75 g/l glycine and 1.5 g/l L-methionine. It was autoclaved for 15 min at 121°C.

2.5 ml *Pleurotus ostreatus* inoculum was grown aerobic at 30°C in shake flasks containing 100 ml of the following medium: 5 g/l malt extract, 15 g/l yeast extract, 15 g/l D-glucose and 0.15 g/l glycine (autoclaved for 15 min at 121°C).

To two of the shake flasks containing *Chromobacterium violaceum* and *Pleurotus ostreatus* respectively we added 0.05 g of Pt-wire (not sterilized), to two shake flasks we added 5 g of the old catalyst honeycomb (not sterilized).

For the violacein identification *Chromobacterium violaceum* was grown aerobic at 30°C in shake flasks containing 100 ml of minimal medium containing 5 g/l peptone and 3 g/l meat-extract.

For growth monitoring both *Chromobacterium violaceum* and *Pleurotus ostreatus* were plated out an a agar plate by the thirteen line method [1]

Growth monitoring

Growth of the bacteria was monitored by absorbance at 600 nm and by pH change at 22°C.
Growth of the fungi was monitored by pH change at 22°C and by eye.
We measured growth 25 h, 29 h and 6 d after incubation.

Violacein extraction

Violacein was extracted with 96% ethyl acetate. The watery phase was threw away. The spectrum was made in 50 mm quartz cuvettes from 200 nm to 800 nm.

Cyanide detection

For qualitative detection of free cyanide we used the spot-test described by Feigl and Anger (1966) [3]: 10 mg copper(II) ethyl acetoacetate, 10 mg 4,4'-methylenebis (N,N-dimethylalinine) in 2 ml chloroform. We covered the samples with a chromatography paper soaked in the spot-reagent. Incubation at 30°C.

Cyanide-metal complex detection

For the detection of the cyanide-metal complexes we used HPLC. The stationary phase consisted of a hydrophobic C_{18} column. The mobile phase contained 60 mM tetrabutylammonium hydroxide (TBAOH), 150 mM phosphoric acid, 25% acetonitrile and 2.34 mM sodium perchlorate*H_2O (pH 7). The samples were centrifuged and filtrated and 20 µl were injected. The flow-rate was 1 ml/min at 40°C and the pressure was 180 bar. We took the following samples: *Chromobacterium violaceum* with catalyst, 29 h old; *Chromobacterium violaceum* with catalyst, 15 d old; *Pleurotus ostreatus* with catalyst, 29 h

old; *Pleurotus ostreatus* with catalyst, 15 d old; 3.5 g catalyst with 10 ml cyanide solution containing 100 mg/l CN^-.

Detection was at 230 nm.

As standard a mixture of AgCl, Au, $FeCl_3$, $CuSO_4$ and Ni was used.

Results

Chromobacterium violaceum was able to grow in all shake flasks. The platinum wire did not have any influence on bacterial growth. In the shake flasks with car catalyst the pH did not sink as deep as in the other flasks. Lowest pH and highest absorbance were observed 25 h after incubation for all shake flasks.
Pleurotus ostreatus was also able to grow in all shake flasks. Best growth was observed in the shake flasks neither platinum wire nor catalyst. Lowest pH was detected 6 d after incubation.

Chromobacterium violaceum cultivated on minimal media showed a typical violacein spectrum.

The first spot-test showed a change of color, but it was a little bit blurred. The second spot-test showed cyanide more clearly.

Cyanide-platinum like metal complexes were detected in the sample of *Chromobacterium violaceum* with catalyst 29 h after incubation and in the sample with catalyst and the cyanide solution (Figure 2 and 3). No cyanide complexes were detected in the 15 d old sample of Chromobacterium violaceum.
In the samples with Pleurotus ostreatus no cyanide-platinum complexes were detected.
In all samples tested cyanide-copper complexes were present.

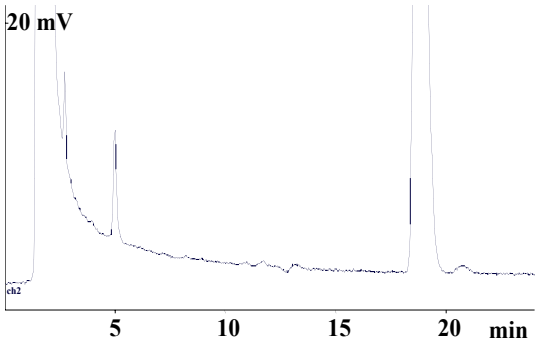

Figure 2

HPLC of 3.5 g catalyst with 10 ml cyanide solution (100 mg/l). After 5 min. cyanide-copper complexes are detected. After 19 min. a large cyanide-platinum or cyanide-palladium complex peak appears (stationary phase: hydrophobic C_{18} column; mobile phase: TBAOH; detection at 230 nm).

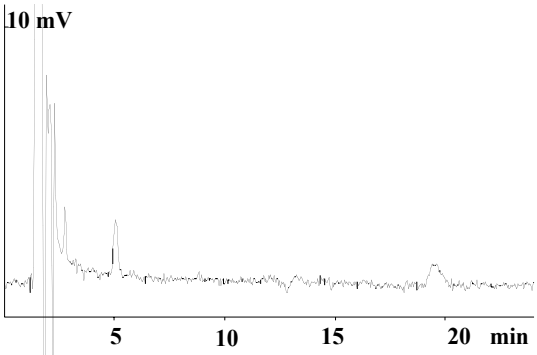

Figure 3

HPLC of *Chromobacterium violaceum* with catalyst, 29 h old. Beside of a cyanide-copper peak after 5 min. there is a clear peak visible which represents cyanide-platinum or cyanide-palladium complexes (stationary phase: hydrophobic C_{18} column; mobile phase: TBAOH; detection at 230 nm).

Discussion

Our findings are in general agreement with our expectations. *Chromobacterium violaceum* was able to leach platinum like metals out of the piece of car catalyst. With *Pleurotus ostreatus* solubilization of platinum like metals was not detected, but we only had measured twice. The cyanide complexes are not very stable. They may all have disappeared.

In our spot-test the chromatography paper dried up to fast, probably because of the high incubation temperature. This effect may have caused the blurred shape which made it impossible to say which sample had the highest cyanide concentration.

Growth in presence of car catalyst was not as good as in its absence. The catalyst may be contaminated with poisonous substances. We did not clean it at all before adding it to the cultures.
The piece of catalyst was partially pulverized by the shaking of the cultures. This may also have had a negative effect on growth of the organisms.
After one day *Chromobacterium violaceum* was in stationary phase. From then on the population decreased. *Pleurotus ostreatus* grows much slower. For fast mobilization of the metals *Chromobacterium violaceum* would be more suitable.

HPLC we made could not separate cyanide-platinum complexes from cyanide-palladium complexes. For more exact determination there should be taken an other mobile and stationary phase.
Apparently car catalysts contain copper. Copper may be used as carrier material for the platinum like metals. Copper competes with platinum like metals for complexation. This may be a problem in commercial use of this recycling method.

Lack of time made it not possible to measure free cyanide quantitatively nor to work off the mobilized metals. If there had been more time the percentage of the leached metals would have been determined, too.

Acknowledgements

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