MINIMIZING HEAVY METAL IN CRAFT-SETTLEMENT WASTEWATER BY SULFATE-REDUCING BACTERIA-DESULFOVIBRIO DESSULFURICANS

Lai Thuy Hien[1], Tran Dinh Man[1], Le Thi Lai[1], Pham Hung Viet[2]
[1] *Institute of Biotechnology, Hanoi, Vietnam*
[2] *Research Centre for Environmental Technology and Sustainable Development, HUS*

ABSTRACT

Environment pollution caused by heavy metal wastewater impacts badly on environment and human health. Some heavy metals are toxic at any dose (Hg, Cd, as...) especially Cr 6+ is the cause of human diseases: nervous, abnormal baby borning, liver cancer...

Many methods for heavy metal wastewater treatment have been used widely in the world. The methods mainly concentrated in physicochemical such as: absorption, ion exchange, and precipitation... Despite effective treatment, those methods are expensive with high cost of equipment, chemicals and manpower. Moreover, its products influence on water quality. So using microbial treatment method to remove metals contaminants has been considerable interested now. Some American, European, Asian experts (Wildeman, Sucha, Sottnik,) were successful in application SRB to remove heavy metal from acid mine drainage, from wastewater of plating industry. The basic activity of microbial treatment is returning mobile and toxic contaminants to their stable immobile mineral forms. In anaerobic condition SRB reduce SO42- to H2S and form sulfide precipitation. This method has advantages of low running cost for large a mounts of wastewater and simplicity of its operation.

In Vietnam, there are many manufacturing- craft-settlements, which produce metal equipment, especially concentrated in Namdinh province up to 84 settlements. Because of using old technology, lack of treatment technical planning knowledge of environment protection. Almost wastewater was discharged directly into the environment without treatment. The polluted environment influenced seriously on human health. The portion of people has got the cancer disease, nervous disease and borning abnormal baby in these settlements was very high. Average longevity of people here is only 55-57 years old, lower than state average 10-12 years.

In order to solve heavy metal wastewater pollution of craft-settlements. We isolated some native bacteria capable of heavy metal minimizing. In number of them, Desulfovibrio desulfuricans showed good ability to minimize heavy metal concentration in wastewater with experimental scale, reduced from 60 to 90%. We set up some treatment trial models (50l, 100l) for heavy metal removal at Vanchang craft-settlement by using isolated SRB. The results showed that concentration heavy metal in wastewater after treatment were very low, reached the Vietnamese standard for industrial wastewater B level (TCVN 5945-1995).

Introduction

SRB Desulfovibrio was found by Beijerincke from 1895 but their ability of metal removal just known in recent decades. In 1980, Hammer and Brodie studied process of heavy metal removal by iron-oxidizing bacteria. After several year later Wildeman and co-worker at Colorado University informed the important role of SRB in Pb removal in underground water and Cr removal in plating wastewater. In 1999, Sucha and Sottnik expanded large scale of heavy metal treatment by using SRB in Sobov mining exploitation (Slovakia). That role of SRB was confirmed by results of Zaluski et al in 2000, Pb reduced from 700mg/l to 1mg/l after 8 months of experiments. Recently, in Korea Ji Won Yang (2002) arranged successful experimental model for Cu treatment in laboratory scale. Concentration of Cu decreased from 600 mg/l to 0.1 mg/l after 10 month-operation.

In Vietnam, there are many manufacturing- craft-settlements, which produce metal equipment, especially concentrated in Namdinh province up to 84 settlements. Because of using old technology, lack of treatment technical planning and knowledge of environment protection. Almost wastewater was discharged directly into the environment without treatment. The polluted environment influenced seriously on human health. The portion of people has got the cancer disease, nervous disease and borning abnormal baby in these settlements was very high. Average longevity of people here is only 55-57 years old, lower than state average 10-12 years.

In order to solve heavy metal wastewater pollution of craft-settlements. We carried out some studying on native heavy metal removal bacteria in Vanchang settlement.

Materials and methods

Materials

* Metal contaminated water and mud samples such as Cr, Ni, Al and Fe were collected from the different places in Vanchang village, Namdinh province, Vietnam.
* The composition and role of substrates in experimental modeling include:
- Straw, sawdust, cobbles, mineral constitute stable supporting material for bacterial growth
- Cow manure served as organic carbon for bacterial growth.
- Crushed limestone provides buffering capacity to increase the pH

* Media for inoculation of sulfate-reducing bacteria:
+ *Desulfobulbus* sp.: 3g of Na_2SO_4; 0.2g of KH_2PO_4; 0.3g of NH_4Cl; 1g of NaCl; 0.5g of KCl; 0.4g of $MgCl_2$; 0.15g of $CaCl_2.2H_2O$; 0.5g of $FeSO_4$. $7H_20$; 1g of yeast extract; 0.1g of vitamin; 0.1g of mineral; 0.1g of ascorbic acid; 0.1g of thioglycolate acid; 1 liter of distilled-water; pH: 7.2-7.4
+ *Desulfovibrio* sp.: 0.5g of KH_2PO_4; 1g of NH_4Cl; 1g of NaCl; 1g of $CaSO_4$; 1g of $MgSO_4$. $7H_2O$; 3.5g of sodium lactate; 0.5g of $FeSO_4$. $7H_20$; 1g of yeast extract; 0.1g of vitamin; 0.1g of mineral; 0.1g of ascorbic acid; 0.1g of thioglycolate acid; 1 liter of distilled-water; pH: 7.2-7.4.

* Media for inoculation of *Thiobacillus*:
+ *Thiobacillus ferooxidans*: 0.15g of $(NH_4)_2SO_4$; 0.05g of KCl; 0.5 of MgSO4; 0.1g of KH_2P0_4; 0.01g of Ca $(NO_3)_2$; 1 liter of distilled-water; 10ml of $FeSO_4$ (10%); adjust pH to 4.
+ *Thiobacillus thiooxidans*: 5g of $Na_2S_2O_3$. $5H_2O$; 0.1g of NH_4Cl; 0.25g of $CaCl_2$; 0.1g of $MgCl_2$; 3.0g of KH_2PO_4; 1 liter of distilled-water; adjust pH to 3.
+ *Thiobacillus thioparus* 5g of $Na_2S_2O_3$. $5H_2O$; 1g of $NaHCO_3$; 0.2g of $NaHPO_4$; 0.1g of $MgCl_2$; 0.1g of NH_4Cl; trace of $FeSO_4$; 1 liter of distilled-water; adjust pH to 7.8.

* Media for inoculation of denitrifying bacteria:
2.0g of KNO_3; 1.0g of asparagines; 5.0g of sodium citrate; 1.0g of KH_2PO_4; 1.0g $MgSO_4$; 0.2g of $CaCl_2$; trace of $FeCl_3$; 1liter of distilled-water; adjust pH 6.8-7.2 (by Na_2CO_3)

Methods

Morphological property of bacteria

* Morphology of bacteria *and the heavy metal presence inside the cells* was observed by optical microscope **Olympus** and Electronmicroscope **JEM 1010**

Electron microscope procedure: The culture incubated for 7 days were harvested by centrifugation at 5000 rpm for 15 min, washed with PBS buffer (0.1 M, pH 7.4). Washed cells were fixed in glutaraldehyde (2.5%) for 20 min. Washed twice by centrifugation. The specimens were mounted on collodion coated 200 mesh copper grids and steam covered with Cr and then observed under JEM 1010 instrument. The instrument was operated at 100 kV. Thin section were cut with LKB 8800 Ultramicrotome and mounted on carbon-coated 200-mesh copper grids. Thin sections then were stained according to Renold. The specimens were examined by JEM 1010 instrument. The instrument was operated at 100 KV.

Classification of bacteria by biochemical kits API 20 NE, 50 CHB and 16S rDNA analysis

Assessment the bacteria growing in heavy metal wastewater

Set up trial model for heavy metal removal in Vanchang settlement

We set up large-scale model (100 liters) for heavy metal wastewater treatment according to principle of up flow-anaerobic.

Methods for heavy metal analysis

Analyzing the content of heavy metal and measuring pH and Eh value during the experiment by atomic absorption spectrometry (AAS), paleography, UV-V13 (ultraviolet) and pH-Eh measuring instrument.

Mechanism of heavy metal removal by SRB

In anaerobic condition SRB can decompose organic compound using SO_4^{2-} as terminal electron acceptor producing H_2S. It reacts with desolved metal to form sulfide precipitation (1), (2)

$$2CH_2O + SO_4^{2-} \rightarrow H_2S + 2HCO_3^- \quad (1)$$

$$H_2S + M^{2+} \rightarrow MS\downarrow + 2H^+ \text{ (M: metal)} \quad (2)$$

Zaluski and Canty demonstrated the ability of sulfate-reducing bacteria to reduce Cr (VI) to Cr (III), a much less toxic form of chromium by a series of experiments. Hydrogen sulfide produced by the sulfate-reducing bacteria reacts with Cr (VI) according to the following chemical reaction:

$$3\,HS^- + 6\,FeSO_4 + 4\,CrO_4^{2-} + 13\,H_2O + OH^- \rightarrow 3S^o + 6\,Fe(OH)_3 + 4\,Cr(OH)_3 + 6\,SO_4^{2-} \quad (3)$$

Results and Discussion

Isolation and classification of heavy metal removal bacteria

a/ The result of heavy metal removal bacteria isolation in Vanchang settlement

From mud and water samples were taken from different places in Vanchang village, we isolated and collected some strains, which were capable of heavy metal removal (Table 1). The number of metal-oxidizing bacteria in aerobic condition presence both of mud and water with highest number up to 10^3 CFU/ml (gram of mud). SRB appeared in all samples was estimated from 10^2-10^5 CFU/ml; the amounts of them in mud sample which were taken from the middle of Vanchang river is highest with 10^7 CFU/ml.

Table 1. Number of bacteria in mud and water in Vanchang village

Sampling places	*Sign*	*Bacteria number (CFU/ml); CFU/g*				
		Thiobacilus			*SRB*	
		Tf	*Tt*	*Tp*	*Dv*	*Db*
Water	M1	$2x10^2$	10^2	$3x10^1$	$3x10^5$	10^4
	M2	$2x10^2$	$3x10^2$	10^3	10^6	10^6
	M3	10^1	10^1	0	10^2	10^2
	M4	0	0	10^1	10^2	10^1
	M5	10^2	10^2	10^2	10^5	10^5
Mud	M1B	$2x10^2$	10^2	0	10^6	10^6
	M2B	$3x10^3$	$2x10^3$	0	$5x10^6$	$4x10^6$
	M3B	$4x10^1$	10^2	0	10^4	10^4
	M4B	0	0	0	10^3	10^3
	M5B	10^3	$6x10^2$	10^3	10^7	$2x10^6$

Notice:

M1, M1B - $N_0$4 line; M2, M2B – Kindergarten;
M3, M3B – Dongthinh; M4, M4B – artificial pond;
M5, M5B- Vanchang river

b/ Identification of some selected bacteria strains

We carried out cell morphology and their physiological, biochemical properties and analysis of the16s rDNA. Results presented in Table 2

Table 2. Morphological properties of some strains isolated form Vanchang settlement.

Sign strain	*Sampling places*	*colony*	*cell*	*Species*
VCI	- Pond water - Pond mud	Opaque, white, rough, serrate edge, 5 –6 mm	Gram -, oval	*Alcaligenes defragans*
VC2	- River water	Dark yellow, serrate edge, 2 –3 mm	Gram -, circle, oval, single	*Pseudomonas chlororaphis*
VC3	- Deposit mixture modeling	Red, circle, convex, 2-3mm	Gram -, rod, single or in pairs	*Sphingomonas spec*
VC4	- Deposit Ni-50	Translucent, circle, convex, glossy, 1 mm	Gram -, oval, short rods	*Comamonas testosteroni*
VC5	- Outlet Cr-50 HP	Circle, entire, shiny, 1-2mm	Gram +, oval	*Rhodococcus equi*
VC6	- Deposit mixture modeling	Light orange, circle, convex, smooth shiny, 2mm	Gram +,rod, single or in pairs	*Rhodococcus facians*
VC7	- Pond mud - Ni - 100 - Mixture 100	Opaque white, serrated edge, smooth, shiny, 3 – 4 mm	Gram +, short rods	*Bacillus pumilus*
VC8	- Pond mud - Ni - 100 - Mixture 100	White, flatened, shiny, 3-4mm	Gram +, big short rods or chains	*Bacillus cereus*
VC9	- Outlet mixture -50	Black, circle, entire edge, 1– 2 mm	Gram -, small rods, single polar flagella	*Desulfovibrio desulfuricans (1)*
VC10	- Outlet Cr -50	Black, egg shape, entire edge, 5 – 6 mm	Gram -, rod or vibrio, single polar flagella	*Desulfovibrio desulfuricans (2)*

Combine the cell morphology, physiological, biochemical properties and the 16s DNA analysis, the selected studied bacteria were determined as species: *Desulfovibrio desulfuricans, Alcaligenes defragans, Pseudomonas chlororaphis, Sphingomonas spec., Comamonas testosteroni, Rhodococcus equi, Rhodococcus facians, Bacillus pumilus, Bacillus cereus.*

Assessment of heavy metal removal ability of some collected strains

Among of identified species, Comamonas testosteroni, Rhodococcus equi and Desulfovibrio desulfuricans dominated in Vanchang wastewater. Their ability of heavy metal removal was appeared in Table 3, 4.

Table 3: Effect of heavy metal to aerobic bacteria growing

Bacteria	***Number of bacteria (CFU/ml)***		***Concentration of Cr (mg/l)***
	Before exp.	***After 7 days exp.***	
Control	0	0	40.5
Comamonas testosteroni	10^3	3×10^6	40.5
Rhodococcus equi	2×10^3	10^7	40

The data in table 3 shows those two aerobic bacteria can grow in wastewater but they had not played any role in heavy metal removing.

We selected two species of SRB isolated from deposit of trial model for assessment of heavy metal removal (Table 4). Activity of these strains was determinate d by number of bacteria, Cr and Ni concentration before and after treatment. In anaerobic condition two studied strains grew well. Number of bacteria increased from 10^4-10^8 CFU/ml. And concentration of Cr decreased to 99%, concentration of Ni reduced 60% in compression with the control.

Table 4: Heavy metal removal ability of *Desulfovibrio desulfuricans*

Bacteria	***Number of bacteria (CFU/ml)***		***Concentration of metal (mg/l)***			
			Cr		***Ni***	
	Before exp.	***After 7 days exp.***	***0 day***	***7 days***	***0 day***	***7 days***
Control 1	0	0	30	30		
D. desulfuricans (1)	104	2x108	30	0.32		
Control 2	0	0			97	97
D. desulfuricans (2)	104	108			97	37

Above result showed that SRB could minimize heavy metal concentration of wastewater in Vanchang village. So these native bacteria could be applied to heavy metal treatment model in craft-settlements.

Trial model for heavy metal treatment in Vanchang settlement (100 liters)

We set up three mixtures modelling for heavy metal treatment (Cr, Ni, Fe, Zn and Cd...) in Vanchang village. The models were operated according to up-flow anaerobic principle

* *Composition of substrates in the model includes:*

- Cowmanure and bacteria: 3%
- Straw : 1.5%
- Sawdust: 1.5%
- Crushed limestone: 1-2%

* *Operating process for heavy metal wastewater treatment trial model (100 liters)*

- First two weeks keep static stage for bacteria growing.
- After two weeks start to add wastewater effluent with 1/10 volume and take out the same volume.
- Following closely the experiment time: after 4 weeks adding cow manure. After 6 weeks adding supporting material in order to maintain stable activity of the model.

Results of 100 liters scale in table 5 and 6 showed that heavy metal concentration significantly reduced: Cr 82 $\rightarrow$ 1.02 mg/l; Ni 84 $\rightarrow$ 0.175 mg/l; Fe 76 $\rightarrow$1.5 mg/l. Number of SRB was stable at $10^7 \rightarrow 10^9$ CFU/ml after 4 weeks of treatment. The result appeared more clearly in the figure.

As the law of nature: Where occurs pollution there appear cleaning factors. One of important factors is microorganism. The results showed that in Vanchang craft-settlement wastewater is available heavy metal removing bacteria. In number of them *D. desulfuricans showed* high activity on Cr, Ni and Fe removal. It is a good possibility to use them to treat wastewater in craft-settlement.

Table 5. Charge of bacteria number and pH, Eh in mix-100l model

Date	*Exp. time (week)*	*Bacteria*						*pH*	*Eh (mV)*
		Dv	*Db*	*NO_3*	*Tp*	*Tt*	*Tf*		
10/8/02	0	10^4	10^5	10^5	0	0	0	7,35	217
26/8/02	2	10^8	10^7	10^6	3.10^1	$<10^1$	$<10^1$	6,1	-190
10/9/02	4	10^9	10^8	10^7	3.10^1	3.10^1	$<10^1$	6,30	-79
26/9/02	6	10^7	10^6	10^6	$<10^1$	$<10^1$	$<10^1$	6,39	-50
7/11/02	8	10^7	10^8	10^6	0	$<10^1$	0	6,47	-125

Table 6. Concentration of heavy metal before and after treatment in mixture modelling 100 liters

Tank	*Effluent*	*Outlet*			*Standard TCVN 1945- 1995 B level*
Week	0	2	4	8	
Fe (mg/l)	76	17	4,2	1.5	5
Cr (mg/l)	82	7.0	5.8	1.025	1.1
Ni (mg/l)	84.4	2.8	4.0	0.175	1
Zn (mg/l)	1.85	0.065			2
Cu (mg/l)	3.8	0.15			1
Mn (mg/l)	4.0	0.5			1
NO_3^- (mg/l)	4.96	0.62			
SO_4^{2-} (mg/l)	202.13	4.92	0.1		

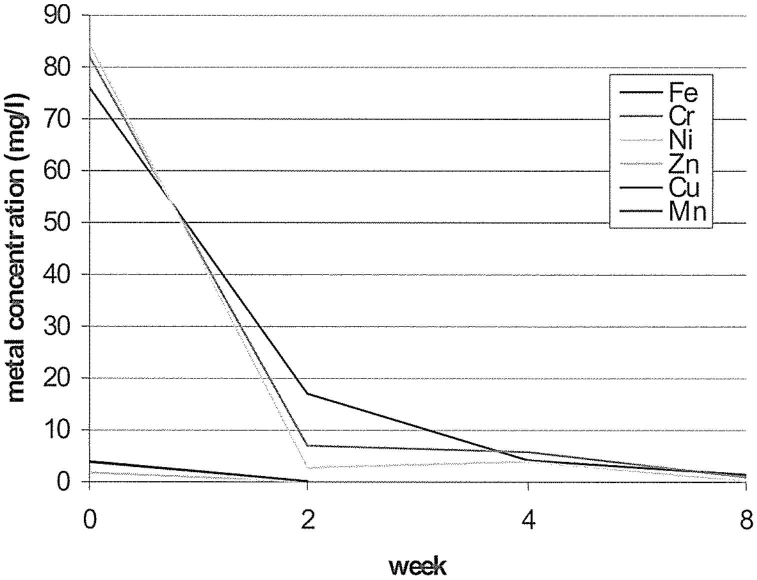

Figure: Concentration of heavy metal before and after treatment

Conclusions

From mud and wastewater of Vanchang craft-settlement, Namdinh province, we isolated different species of bacteria (*Alcaligenes defragans, Pseudomonas chlororaphis, Sphingomonas spec, Comamonas testosteroni, Rhodococcus equi, Rhodococcus facians, Bacillus pumilus, Bacillus cereus, Desulfovibrio desulfuricans (1), Desulfovibrio desulfuricans (2)).* In number of them *Desulfovibrio*, *Desulfobulbus*, *Thiobacillus* were capable of heavy metal removal.

Experiment of single strain at laboratory scale showed that *Desulfovibrio desulfuricans* grew well in Cr, Ni, Fe wastewater and It could reduce Cr from 30→ 0.32 mg/l (99%); Ni 97 → 37 mg/l (60%).
The results of trial mixture modelling-100 liters in Vanchang village confirmed the heavy metal removal ability of SRB. After 4 week-operation, concentration of heavy metals were great reduced: Cr 82→1.02mg/l; 84→0.175 mg/l; Fe 76→1.5 mg/l; Mn 4.0→ 0.5 mg/l; Cu 3.8→ 0.15 mg/l. That result reached Vietnamese standard for industrial waster water B level (TCVN 5945- 1995).

References

1. Anne O. S., *The hard stuff: metals in bioremediation*. University of Georgia, Athens, USA, 3: 271-276, (1992)
2. Bigham J.M., Schwertmann U., Pfab G., *Influence of pH on mineral special in bioreactor stimulating acid - mine drainage*. Applied Geochemistry, 11, 845 – 849, (1996).
3. Brierley CL.,. *Bioremediation of Metal-contaminated Surface and Groundwater*. Geomicrobiol J., 8: 201-223, (1990)
4. Brock, M.T. Madigan, J.M. Martinko, J. Parker, *Biology of microorganisms*. Ninth Edition, pp: 608-609. (2000)
5. Clayton L.D, Bolis J.L, Wildeman T.R, et al.. *A case study on the aerobic and anaerobic removal of manganese by wetland processes*. Department of Chemistry and Geochemistry, Colorado School of Mines, pp: 1-10.
6. Drovak D.H., Medin R.S., Edenborn H.M., and Mantire R.E.,. *Treatment of metal contaminated water using bacteria Sulfate-reducing: results from pilot-scale reactors*. In: Proceeding of the 1991 National Meeting of the American Society of Sulfate Mining and Reclamation, Princeton, pp: 241-248. (1991)
7. Eaton A. D., L. S. Clesseri, and A. E. Greenberg (ed.),. *Standard methods for the examination of water and wastewater*. American Public Health Association, Washington, D.C., p. 368-370, (1995)
8. Hien L.T, K.Q. Hoa, L.T. Binh, L.T. Lai, Preliminary results on isolation and characterization of *Thiobacillus* from Vanchang wastewater. International workshop on environment protection community health for sustainable development of craft-settlements in Namdinh, pp: 87-94, (2000)
9. Lai L. T., J. Kasbohm, J. Eidam,. *Assessment of environmental pollution of metal processing villages of Namgiang (Namdinh)*. Journal of Sciences in the earth, 22(2): 140-145, (2000)
10. Zaluski M., M. Canty, J. Trudnowski. *Application of Sulfate-reducing bacteria for passive remediation of water contaminated with metals*. MSE Technology Applications, Inc. 200 Technology Way, Butte, Montana 59701, USA.
11. Machemer S.D., and Wildeman T.R., 1992. *Adsorption compared with sulfide precipitation as metal removal processes from acid mine drainage in a constructed wetland*. Journal of contaminant hydrology, 9: 115-131
12. Plumer S.M., Updegraff D.M., and Wildeman I.R., *Sulfate reduction versus methanogenesis in substrates designed to treat acid mine drainage*: 201 St National meeting, American Chemical Society. (1991)
13. Sucha V., Kraus I., Zlocha M., Stresko V., Gasparovicova M., Lintneriva. Uhlik P.,. *Prejavy a priciny acidiifikacie v obkasti Sobova (Stiavnicke vrchy)*, Mineralia Slovaca, 29, 407 - 416. (1997)
14. Wildeman T.R., *Biogeochemical principles. Dept. of chemistry and Geochemistry Colorado School of Mine*, pp: 1-14. (1998)
15. Wildeman T.R., Updegraff D. M., *Passive Bioremediation of Metals and Inorganic Contaminants*. In Perspectives in Environmental Chemistry, Oxford, 473 - 495. (1997)
16. Wildeman T.R., Updergraff D. M., Reynolds J.S., and Bolis S.L., *Passive bioremediation of metals from water using reactors or constructed wetlands*. Emerging Technology for Bioremediation of metals, Lewis Publishers, Bocaraton, Florida, (1994.)
17. Yang J.W., Kim S.J., *Application of Brewery UASB Granules to Treatment of Acidic Industrial Wastewater containing Heavy Metals*. First ASEM Conference on Bioremediation, Hanoi, Vietnam, Sept. 24-27, (2002)