Polish J. Environ. Stud. Vol. 15, No. 2 (2006), 355-360

Original Research

Biotransformation of Phosphogypsum in Petroleum-Refining Wastewaters

D. Wolicka*, W. Kowalski

Institute of Geochemistry, Mineralogy and Petrology, Faculty of Geology, Warsaw University, Żwirki i Wigury 93, 02-089 Warsaw, Poland

Received: January 11, 2005
Accepted: September 29, 2005

Abstract

Eleven isolates of sulphate-reducing bacteria (SRB) were isolated from soil contaminated with crude oil derivatives and petroleum-refining wastewaters and used to investigate the effectiveness of carbon reduction and biotransformation of phosphogypsum. One of the isolates (culture no. 10) was found to be very effective with 90% carbon reduction (measured as COD) and the simultaneous biotransformation of approximately 2.65g phosphogypsum/L in industrial petroleum-refining wastewaters.

Keywords: biotransformation of phosphogypsum, petroleum-refining wastewaters, SRB.

Introduction

Sulphate-reducing bacteria (SRB) are usually encountered in anaerobic environments containing sulphates and accessible organic compounds. They have been isolated from sediments, thermal springs and associated with iron oxides [1] and are the most frequently found group at crude oil-contaminated sites [2-6]. Many reports on the anaerobic degradation of crude oil have suggested that SRB are important in the biodegradation of crude oil components, and may therefore play a role in the protection of the environment [7].

Hydrated calcium sulphate, commonly referred to as phosphogypsum, is a side product formed during the production of phosphoric acid from apatites or phosphorites according to the reaction: $3H_2SO_4 + Ca_3(PO_4)_3 + 6\ H_2O \rightarrow$ $\rightarrow 2H_3PO_4 + 3CaSO_4*2H_2O$. The production of 1 ton of phosphoric acid is accompanied by the formation of approximately 5 tons of phosphogypsum [8].

Recently a waste stream containing phosphogypsum is a rich source of sulphate ions [9]. Wastewaters such as petroleum-refining wastewaters are a valuable source of organic compounds. A new direction in environmental biotechnology is an attempt at the simultaneous biodegradation of mixed industrial wastewaters containing phosphogypsum [10-15] for growth that can then be used. Petroleum-refining wastewaters contain hydrocarbons, alcohols, aldehydes and phenols. However, phenols are not characteristic of crude oil and their presence is a result of solvent use or by-products of the industry [16]. The amount of wastewaters formed in refineries depends on the quality of the crude oil and the extent to which it is processed and averages 10-18 m^3 per ton of processed crude oil.

The mixture of the petroleum refinery wastewaters with industrial wastewaters containing phosphogypsum could theoretically support the growth of SRB.

The aim of thise study was to determine the biotransformation of phosphogypsum in cultures of SRB, with simultaneous purification of petroleum-refining wastewaters.

*Corresponding author; e-mail: d.wolicka@uw.edu.pl

Table 1. Composition of phosphogypsum.

Determinations (% by weight)	Own studies
SO_3	42.2
CaO	29.6
P_2O_5	2.2
SrO	1.6
SiO_2	0.65
F	0.5
Al_2O_3	0.2
Na_2O	0.4
Fe_2O_3	0.14
K_2O	0.1
BaO	0.03
MgO	0.05
H_2O	20.2

Materials and Methods

Phosphogypsum

Phosphogypsum samples were collected from mounds located in Wizów near Bolesławiec, Lower Silesia. The composition of phosphogypsum is given in Table 1.

Isolation of Microorganisms

Microorganisms were isolated from soil that had been collected in the vicinity of a gas station, a military range car depot, a petroleum refinery, from wastewaters of a petroleum-refinery – "slops" (petroleum products collected from mechanical treatment plant), crude wastewaters and wastewaters purified using the activated sludge method.

Propagation of Microorganisms

Soil microorganisms were cultured using the method of *"microcosms"* and those from the petroleum-refining wastewaters were cultured using the agar shake culture method.

a) The method of *"microcosms"* – Soils from the study sites were placed in 100mL containers together with 5g/L of phosphogypsum and covered with appropriatly. Three kinds of medium were used: Postgate and minimal media with various carbon sources: ethanol, lactate, phenol, lactose, sodium caseinate and Emerson medium supplemented with fuel oil or modified Emerson medium, in which fuel oil was replaced with phenol (0.5g/L) [17]. The containers were tightly closed and incubated for 6 weeks at 30 or 55°C to select for sulphidogenic communities of microorganisms capable of carrying out the biotransformation of phosphogypsum.
b) Enrichment cultures – soil was placed in 300mL glass bottles, which after the addition of appropriate medium were tightly closed. The ratio of soil to medium was 1:10.
c) Agar shake culture method from petroleum-refining wastewaters - medium solidified with agar: Postgate medium, minimal with phenol, Emerson medium with fuel oil and modified Emerson medium, in which fuel oil was replaced with phenol, was poured into tall test tubes. All the media contained Na_2SO_4 in concentrations equal to the content of sulphates in 5g phosphogypsum/L (about 2.5g SO_4/L) . The media were then inoculated with slops and wastewater drawn up into sterile glass capillary tubes.

Culture Conditions

Cultures were set up in 100, 300, 500 or 1000mL bottles closed tightly with rubber stoppers punctured with a needle with a syringe fitted on top of it. The syringes served to introduce the inoculum and to withdraw samples. The ratio of inoculum to medium was 1:10. The cultures were incubated at 30°C or at 55°C, without access of light.

Media:

a) Postgate medium C [18] or modified Postgate medium, in which Na_2SO_4 (4.5g/L) was replaced with 5.0g/L of phosphogypsum
b) Minimal medium (NH_4Cl - 1.0 g/L, Na_2SO_4 - 4.5 g/L) or modified minimal medium, in which Na_2SO_4 (4.5g/L) was replaced with 5.0g/L of phosphogypsum. Both types of medium were supplemented with lactate (2.64 g/L, 3.5 g/L or 7.92g/L), ethanol (3mL/L), acetate (3.8g/L), caseinate (2.7g/L), lactose (9g/L or 3g/L) or phenol (0.5g/L or 1.468g/L) as the sole carbon source.
c) Emerson medium: K_2HPO_4 (0.07g/L), $MgSO_4$ (0.5g/L), yeast extract (4g/L) distilled water (750mL), tap water (250 mL), Tween 80 (0.3mL/L), fuel oil (5mL/L) or modified Emerson medium with phenol (0.5g/L). To all cultures resazurin in concentration 0.001g/L was added as an indicator of redox conditions in the medium.
d) petroleum-refining wastewaters (the composition of the wastewaters is given in Table 2).

Determinations

Sulphides in the cultures were determined using the iodometric method, sulphates with the hot barium method, chemical oxygen demand (COD) by the dichromate method, $N\text{-}NO_3$, $N\text{-}NO_2$, $N\text{-}NH_4$, $P\text{-}PO_4$ using established

Table 2. Composition of petroleum-refining wastewaters.

Determinations	Petroleum-refining wastewaters	
	Literature data	Own studies
pH	6.2-10	6.2
COD (mg O_2/L)	140-3300	1860
Sulphides (mg S/L)	0-38	40
Sulphates (mg SO_4/L)	0-180	180
Phenols (mg/L)	0.3-155	120
Alkalinity (mval/L)	1.53-3.6	1.4
Oils (mg/L)	23-200	np*
Phosphates (mgP-PO_4/L)	0-100	10
Ammonium nitrogen (mg N/L)	0-120	5
Nitrite nitrogen (mg N/L)	0-12	0
Nitrate nitrogen (mg N/L)	0-6	0
Chlorides mg Cl/L	20-1100	30

*np – not present

colorimetric methods [19], chlorides with Merck indicator strips, salinity was determined with the use of inoLab apparatus, pH was determined using a pH-meter or with bromothymol indicator and color scale. The reaction of the culture was corrected with 0.1 N HCl or 0.1 N NaOH to pH about 7.

Determinations involving post-culture sediments and fluids were made using the following analytical procedures: IPC emission spectrometry with induced excitation in the medium and X-ray analysis of post-culture sediments using a DRON-2 X-ray diffractometer.

Results and Discussion

Communities of anaerobic microorganisms capable of carrying out the biotransformation of phosphogypsum at 30 or 55^{0}C were selected from environments contaminated with petroleum derivatives. Altogether, 70 cultures (35 at each of the temperatures used) were set up. Selection was carried out using one of three methods: *"microcosms"* (28 cultures), growth cultures (18 cultures), and in culture on medium solidified with agar (24 cultures on agar stabs). After a 6-week incubation blackening and the characteristic smell of hydrogen sulphide was observed in 11 cultures (10 mesophilic and 1 thermophilic). The maximal activity of SRB in cultures of sulphidogenic bacterial communities is presented in Table 3.

The most active community of microorganisms was isolated from soil contaminated with car fuel (culture no. 1). In this culture, set up in Postgate medium with lactate, the highest activity of SRB manifested by effective biotransformation of 1460mg phosphogypsum/L/day was observed. In the remaining cultures the maximal values were somewhat lower (in medium with ethanol - 1272mg, casein – 1055, lactose – 536 and for phenol - 750mg phosphogypsum/L/day).

The obtained communities of microorganisms were then transfered to media that were used for propagation, *i.e*. Postgate medium with phosphogypsum as sole source of sulphates and different carbon sources (lactate or ethanol, lactose, caseinate or phenol). The cultures were passaged many times. The biotransformation of phosphogypsum, calculated on the basis of the amount of hydrogen sulphide in the cultures, is presented in Fig.1.

For cultures of microorganisms isolated from environments contaminated with petroleum-derived compounds, the highest concentration of 838 mg HS^-/L was obtained in the case of culture no. 3 in Postgate medium with ethanol as sole carbon source. This corresponded to the reduc-

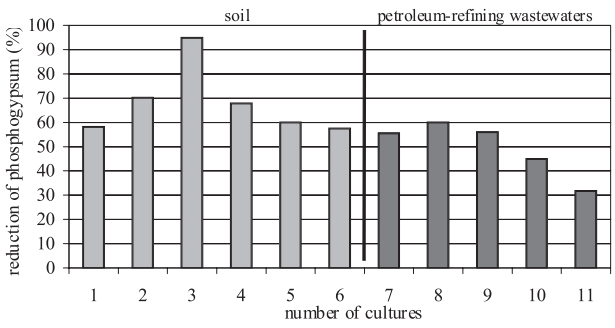

Fig. 1. Biotransformation of phosphogypsum in cultures of the isolated bacterial communities (100% corresponds to 5g phosphogypsum introduced into the culture).

Table 3. Maximal activity of SRB in cultures of sulphfidogenic bacterial communities.

No of cultures	Isolation	Method of isolation	Temp. of incubation °C	Medium	Max activity mg/L/day		
					HS^-	SO_4^{2-}	fg
1	soil in the vicinity of a gas station	„microcosms"	30	P_m +fg	259	730	1460
2	soil in the vicinity of a gas station	„microcosms"	30	P_k+ fg	187	527	1055
3	soil from military range car depot	„microcosms"	30	P_e+ fg	225	636	1272
4	soil from a petroleum refinery	enrichment cultures	30	P_m+ fg	104	294	586
5	soil from a petroleum refinery	enrichment cultures	30	P_k+ fg	153	431	863
6	soil from a petroleum refinery	enrichment cultures	30	P_l+ fg	95	268	536
7	petroleum-refining crude wastewaters	depth inoculation	30	P_f+ fg	77	217	434
8	petroleum-refining wastewaters purified	enrichment cultures	30	P_f+ fg	110	310	620
9	petroleum-refining wastewaters purified	enrichment cultures	30	P_f+ fg	77	217	434
10	"slops"	depth inoculation	30	P_f+ fg	133	375	750
11	"slops"	depth inoculation	55	P_f+ fg	120	338	677

The capital letter P indicates Postgate medium, the small letters the carbon source m – lactate, k - casein, e - ethanol, l - lactose, f – phenol, fg – phosphogypsum.

tion of 2365 mg SO_4/L and 95% reduction of phosphogypsum introduced into the medium.

In the case of cultures of microorganisms isolated from soil (cultures no. 1-6) a higher reduction of sulphates (average about 67%) was recorded than in cultures of microorganisms isolated from industrial wastes when the value was about 50% (cultures no. 7-11).

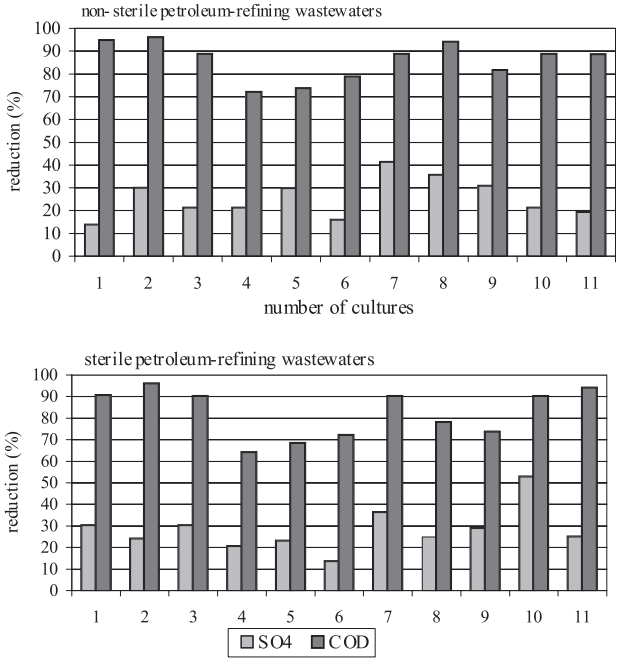

Fig. 2. Reduction of sulphates and COD in cultures of the isolated microorganisms.

The toxicity of sulphides and free H_2S produced in the course of the microbiological reduction of sulphates and competition for electron donor are the two main factors responsible for the inhibitory effect. In SRB populations growing in medium with lactate, the maximal content of sulphides was obtained at the pH of the culture of 6.7, whereas the amount of HS^- of 547mg/L (16.1mM) was regarded as fully inhibiting growth [20]. Studies on the toxicity of hydrogen sulphide were carried out in culture of *Desulfovibrio sp.* The HS^- values in studies on mixed communities are lower and, for instance, Hilton and Oleskiewicz [21] mention 400 mgS/L, McCartney and Oleszkiewicz [22] - 448 mgS/L, and Shimada [23] - 471 mg mgS/L.

In cultures of communities of microorganisms isolated from sites contaminated with products derived from petroleum, the obtained HS^- concentrations were higher, which reflects the adaptation of microorganisms to high H_2S concentrations.

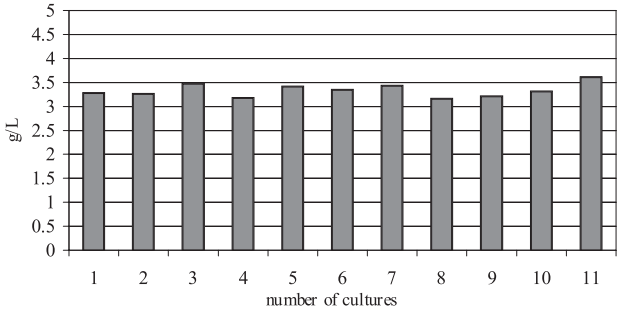

Fig. 3. Weight of sediments in SRB cultures in sterile petroleum-refining wastewaters.

The obtained communities of microorganisms served as inocula for setting up cultures in non-sterile and sterile petroleum-refining wastewaters, supplemented with phosphogypsum (5g/L). The characteristics of petroleum-refining wastewaters are presented in Table 2.

Twenty-two cultures of microorganisms were set up and passaged twice. The incubation period for each passage was 14 days. Reduction of sulphates and COD of the wastewaters in the cultures of the microorganisms are presented in Fig. 2.

In evaluating the effectiveness of biotransformation in the environment of petroleum-refining wastewaters, two parameters should be considered, namely the purification of the above-mentioned wastewaters and the active biotransformation of phosphogypsum. The most active community of microorganisms in non-sterile wastewaters was community no. 7 (microorganisms isolated from petroleum-refining wastewaters). In this culture 90% COD reduction, with simultaneous biotransformation of 40% sulphates, was recorded. In cultures in sterile wastewaters the highest simultaneous reduction of COD and sulphates was observed for microorganisms isolated from "slops" (culture no. 10) as well as from petroleum-refining wastewaters (culture no. 7). These microorganisms purified the wastewaters by about 90%, with simultaneous biotransformation of 53 and 38% phosphogypsum/L, respectively.

Knowing the solubility of phosphogypsum - about 2g/L, as well as the content of sulphates in petroleum-refining wastewaters, it can be assumed that the total amount of sulphates in the cultures was about 1180mg/L. The value of the above-mentioned ratio calculated on this basis is 1.57, which points to the creation of optimal conditions for the selection of SRB [24, 25, 26].

In order to eliminate the effect of accompanying microflora on the biotransformation of phosphogypsum, in further studies only cultures in sterile wastewaters were employed. After completing the second passage of the culture the post-culture sediments were separated and weighed. The mass of these sediments in the cultures is presented in Fig. 3.

In the case of all SRB cultures in petroleum-refining wastewaters an approx. 35% loss in the mass of phosphogypsum was observed. In turn, all 11 sediments were subjected to X-ray studies. No diffractogram revealed chemical or mineral components other than gypsum and celestine, with a drop in the total mass of phosphogypsum. It is thus highly likely that part of the phosphogypsum did not undergo biotransformation and remained in the post-culture fluid in the form of Ca^{2+} and SO_4^{2-} ions, as confirmed by ICP studies.

Telang *et al.* [27] in their analysis of mixed bacterial communities isolated from oil fields found that in oil-contaminated environments consortia embracing bacteria other than SRB are formed. They can use sulphides, which are the product of the reduction of sulphates, as an energy source, oxidizing them to elementary sulphur or even sulphates.

Recently several papers have been published whose authors describe the purification of wastewaters with high sulphate content under conditions favoring the selection of SRB [28]. Deswaef [29] and Kaufman [30] describe the purification of wastewaters using the method of sulphidogenesis, following their earlier enrichment in industrial gypsum, which is a solid waste. The aim of these studies was to attempt the simultaneous biodegradation of two arduous industrial wastes – wastewaters and gypsum.

The presence of hydrogen sulphide in the cultures and reduced mass of the sediments at the same time point to the occurrence of the process of phosphogypsum biotransformation in petroleum-refining wastewaters, whose character is that of liquid organic wastes.

Acknowledgements

Work has been executed in cooperation with the Department of General Microbiology, Warsaw University in Poland.

The authors would like to thanks Prof. Roman Mycielski for many technical contributions and insightful discussions made in this area of microbiology.

References

1. CHI MING SO, YOUNG L.Y. Isolation and characterization of a sulfate-reducing bacterium that anaerobically degrades alkanes. Appl.Environ.Microbiol. **65,** 2969, **1999.**
2. VOORDOUW G., VOORDOUW J.K., JACK T.R., FOGHT J., FEDORAK P.M., WESTLAKE W.S. Identification of district communities of sulfate-reducing bacteria in oil fields by reserve sample genome probing. Appl.Environ.Microbiol. **58,** 3542, **1992.**
3. MUELLER R.F., NIELSEN P.H.Characterization of thermophilic consortia from two souring oil reservoirs. Appl. Environ.Microbiol.**62,** 3083, **1996.**
4. JENNEMAN G. E., GEVERTZ D. Identification, characterization and application of sulfide-oxidizing bacteria in oil fields In: Microbial Biosystems: New Frontiers. Proceedings of the 8 th International Symposium on Microbial Ecology Bell CR, Brylinsky M, Johnson-Green P (ed) Atlantic Canada Society for Microbial Ecology, Halifax, Canada, **1999.**
5. STETTER K. O., HUBER R. The role of hyperthermophilic prokaryotes in oil fields. In: Microbial Biosystems: New Frontiers. Proceedings of the 8 th International Symposium on Microbial Ecology Bell CR, Brylinsky M, Johnson-Green P (ed) Atlantic Canada Society for Microbial Ecology, Halifax, Canada, **1999.**
6. MAGOT M., OLLIVIER B., PATEL B. K. C. Microbiology of petroleum reservoirs. *Antonie van Leeuwenhoec* **77,** 103, **2000.**
7. SCHROTH M. H, KLEIKEMPER J., BOLLIGER C., ZAYER J. Quantification of microbial sulphate reduction in a petroleum aquifer. Land Contamination & Reclamation. **9,** 293, **2001.**

8. ZIJLSTRA J. J. P. Geochemical engineering of phosphogipsum tailings Raport of Geochem. Reasearch BV; Groen van Prinstererweg 15-17; 3731 HA De Bilt; The Netherlands **2001.**
9. KOWALSKI W., PRZYTOCKA–JUSIAK M., WOLICKA D., HOŁUB W. Biotransformation of phosphogypsum in medium including organic compounds presenting main contaminate different liquid waste organic.(in Polish). Materiały I Krajowego Kongresu Biotechnologii, Wrocław. **1999.**
10. KOWALSKI W., BŁASZCZYK M., MYCIELSKI R., PRZYTOCKA – JUSIAK M., RZECZYCKA M. Microbiological recovery of lanthanides from phosphogypsum waste. Appl. Mineralogy Proceedings of the 5th International Congress on Applied Mineralogy in the Minerals Industry. Warsaw University of Technology, Warsaw.**1996.**
11. PRZYTOCKA-JUSIAK M., KOWALSKI W., RZECZYCKA M., BŁASZCZYK M., MYCIELSKI R. Products of microbial biotransformation of phosphogypsum in anaerobes cultures of thermophilic bacteria. (in Polish). Biotechnologia. **29,** 102, **1995.**
12. RZECZYCKA M., MYCIELSKI R., KOWALSKI W., GAŁĄZKA M. Biotransformation of phosphogypsum in media containing different forms of nitrogen. Acta Microbiol. Polon. **50,** (3/4), 281, **2001**
13. KOWALSKI W., WOLICKA D., HOLUB W., PRZYTOCKA-JUSIAK M. Biotransformation of phosphogypsum in anaerobes cultures of microflora, isolated from different environmental, in medium with ethanol. (in Polish). Materiały II Ogólnopolskiego Sympozjum Naukowo-Technicznego „Bioremediacja Gruntów", Wydawnictwo Politechniki Śląskiej, Wisła Jarzębata. **2000**.
14. PRZYTOCKA-JUSIAK M., KOWALSKI W., WOLICKA D., HOŁUB W., GRZYBOWSKA M. Biotransformation of phosphogypsum in wastewater dairy.(in Polish) Materiały VII Ogólnopolskiego Sympozjum Naukowo-Technicznego „Biotechnologia Środowiskowa", Wisła-Jarzębata. **2001.**
15. KOWALSKI W., PRZYTOCKA-JUSIAK M., BŁASZCZYK M., HOŁUB W., WOLICKA D., WESOŁOWSKA I. Effect of nitrate on biotransformation of phosphogypsum and phenol uptake in cultures of autochthonous sludge microflora from petroleum refining wastewaters. Acta Microbiol. Polon. **51,** 47, **2002.**
16. BABICH H., DAVIS D. Phenol: A review of environmental and health risiks, Regulatory Toxicol.Pharmacol.**1,** 90, **1981.**
17. WOLICKA D., KOWALSKI W., BOSZCZYK-MALESZAK H. Biotransformation of phosphogypsum by bacteria isolated from petroleum-refining wastewaters. Polish Journal of Microbiology **2,** 54, **2005.**
18. POSTGATE J. R. The sulphate reducing bacteria. 2nd ed., Cambridge University Press, Cambridge **1984**.
19. HERMANOWICZ W., DOŻAŃSKA W., SIKOROWSKA C., KELUS J. Physical and chemical research of wastewater. Warszawa **1972**.
20. REIS M. A. M., ALMEIDA J. S., LEMOS P. C., CARRONDO M. J. T. Effect of hydrogen sulfide on growth of sulfate reducing bacteria. Biotechnology and Bioengineering **40,** 593, **1992.**
21. HILTON B.L, OLESKIEWICZ J.A. Sulfide-induced inhibition of anaerobic digestion. J.Environ.Engng. **144,** 1377, **1989**.
22. Mc CARTNEY D.M., OLESZKIEWICZ J.A. Sulfide inhibition of anaerobic degradation of lactate and acetate. Water Res. **25,** 203, **1991.**
23. SHIMADA K. Removal of heavy metals from mine wastewater using sulfate-reducing bacteria. J.Wat.Waste, **31,** 52, **1987**.
24. HAO O. J., CHEN J. M., HUANG L., BUGLASS R. L. Sulfate-reducing bacteria. Crit.Rev. in Environm.Sc. and Technol. **26,** 155, **1996.**
25. CLANCY P. C., VENKATARAMAN N., LYND L. R. Biochemical inhibition of sulfate reduction in batch and continouus anaerobic digesrters. Water Sci. Technol., **25,** 51, **1992.**
26. OUDE ELFERINCK S. J. W. H., VORSTMAN W. J. C., SOPJES A., STAMS A. J. M. *Desulforhabdus amnigenus gen.sp.nov.,* a sulfate reducers isolated from anaerobic granular sludge. Arch.Microbiol. **164,** 119, **1998.**
27. TELANG A. J, JENNEMAN G. E., VOORDOUW G. Sulfur cycling in mixed cultures of sulfide-oxidizing and sulfate- or sulfur- reducing oil field bacteria. Can J. Microbiol. **45**, 905, **1999**.
28. LENS P.N.L., SIPMA J., HULSHOFF POL L.W., LETTINGA G. Effect of nitrate on acetate degradation in a sulfidogenic staged reactor. Wat. Res. **34**, 31,**2000.**
29. DESWAEF S., SALMON T., HILIGSMANN S., TAILLIEU X., MILANDE N., THONART PN, CRUINE M. Treatment of gypsum waste in a two stage anaerobic reactor. Water Sci. Tech. **34**, 367, **1996.**
30. KAUFMAN E.N., LITTLE M.H., SELVARAJ P.T. Recycling FGD gypsum to calcium carbonate and elemental sulfur using mixed sulfate reducing bacteria with sewage digest as a carbon source. J.Chem.Technol.Biotechnol. **66**, 365, **1996.**