

Ability of a Local *Bacillus cereus* DS1 Isolate of Dematallization of Heavy Metals

Eman H Qatie, Nibras M Al- hasani, Alia F Hachim, Ameena G Alsudani and Sanaa Kh Maied
Miistry of Science and Technology-Baghdad-Iraq

Abstract

Bacillus cereus DS1 isolate obtained from oil contaminated soil sample, has been isolated in the previous experiments. It was selected for its ability to degrade Nickel protoporphyrin disodium as a model of a heavy metal organic compound. In this research we report capability of the bacterial isolate of growing on Vanadium oxide octaethyl porphyrin with concentration of 20 mg / l as a carbon source. Result showed less efficiency to grow with existence of this substrate. Effect of other compounds used as a chloride metals was investigated. Bacterial strain exhibited a deferent pattern of growth with addition of this metals. The results shown an obvious growth inhibition in state of using Zn metal, when added Cu stimulated the growth activity. Such isolate can be interesting in improving oil quality.

Keywords: bacteria, protoporphyrins, heavy metals, dematallization and crud oil

Introduction

Oil is a mixture of hydrocarbons containing a variety of organic compounds. Most often determined trace elements in crude oils are nickel and vanadium, which are usually the most abundant. Most crude oils contain traces of vanadium, which cause significant detrimental impact during catalytic conversion and combustion. The concentrations in bitumen and vacuum residue are generally much higher, which poses a problem for the economical upgrading of these feedstocks. These problems are relevant since the world reserves of conventional light oils are dwindling and being replaced by an increasing amount of heavier feedstocks. Heavy oil residua contain large amounts of hetero-atoms, such as sulfur, nitrogen, oxygen and metals (Reynolds, 2001). The metals, mainly vanadium and nickel, are removed by catalytic hydro treatment. Sulfur, nitrogen however, as many as 45 elements in crude oils have been reported (Premuzic and Lin 1999). Knowledge of trace elements in crude oil is important because they can have an adverse effect on petroleum refining and product quality. These effects can include catalyst poisoning in the refinery and excessive atmospheric emission in combustion of fuels, (Eckemann and Vogelpohl, 1990; Mogollen *et al.*, 1998). The presence of vanadium and nickel in crude oil has an adverse effect on the refinery processes and acts as a poison on catalysts used in catalytic cracking, hydrogenation and hydro-desulphurization processes. This leads to a significant decrease in the yield of cracked products. Elements such as iron, arsenic, and lead are catalyst poisons. Vanadium compounds can cause refractory damage in furnaces, and sodium compounds have been found to cause superficial fusion on fire brick. Some organometallic compounds are volatile which can lead to the contamination of distillate fractions and a reduction in their stability or malfunctions of equipment when they are combusted (Premuzic *et al.* 1997). The value of crude oil can be determined, in part, by the concentrations of nickel, vanadium, and iron (Arellano *et al.*, 2003). When fuels are combusted, vanadium present in the fuel can form corrosive compounds. The value of crude oils can be determined, in part, by the concentrations of nickel, vanadium, and iron. Nickel and vanadium, present at trace levels in petroleum fractions, can deactivate catalysts during processing (Xu *et al.* 1998). These test methods provide a means of determining the concentrations of nickel, vanadium, and iron (Kukes and Aldag 1985, Xu *et al.* 1992). Among strategies for removing metals from crude oil, chemical processes have been commonly used because of their effectiveness. Many chemical processes such as metal removal with solvent (Savastano, 1991), and the hydrodemetallizing process (Bartholdy and Hannerup, 1990) are used for this purpose. These are often expensive and produce secondary pollution in the environment (Hernandez *et al.*, 1998). Because of these concerns, the use of microbial methods is expected to increase. Although some researchers have already reported microorganisms with the ability to degrade nickel protoporphyrins (Dedeles *et al.*, 2000), research is currently being carried out on the demetallization of VOOEP as a model of metallic petroporphyrins in the crude oil (Fig. 1). This research reports the activity of a local *Bacillus cereus* isolate obtained from oil polluted soil with a good ability to degrade metallic porphyrin rings such as NiPPDS, to resist different concentration of vanadium, as a parameter of the microbial demetallization of vanadium.

Material and methods

Bacterial purification and activation

Bacillus cereus isolate selected for its ability degrade 75% of NiPPDS, was purified and activated by growing in L-Broth medium at 30°C with 150 rpm for 48 hr, then maintained in same cultural media with addition of agar.

Growth measurement

Incubation was carried out at 30° C, with shaking at 150 rpm for 7 days. The culturing medium (pH 7.2)

included KH_2PO_4 , 2.44 g; Na_2HPO_4 , 2.57 g; NH_4Cl , 2 g; $\text{MgCl}_2 \cdot 2\text{H}_2\text{O}$, 0.2 g; $\text{CaCl}_2 \cdot 6\text{H}_2\text{O}$, 0.001 g; $\text{FeCl}_2 \cdot 6\text{H}_2\text{O}$, 0.001 g; $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.004 g, and VOOEP, 2 mg/l or NiPPDS. VOOEP was synthesized in Hazardous Department laboratory. The cell growth was monitored by measuring the optical density of the culture broth at 660 nm (Fedorak et al., 1993).

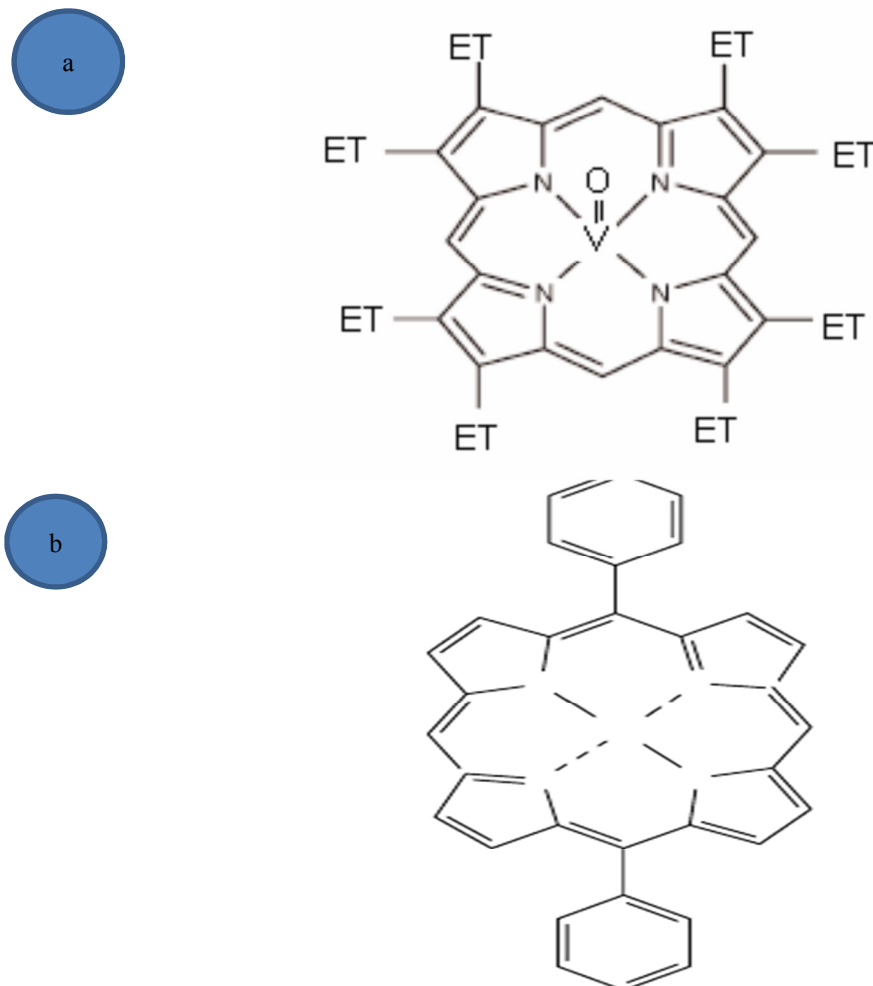


Figure (1) Molecular structure of (a) vanadium oxide octaethyl porphyrin (VOOEP) and (b) NiPPDS (Flynn and Freeman 1987; S A Krasnikov et al,2007).

Analytical methods:

In order to estimate the degradation activity of the isolate, culture broth was centrifuged and NiPPDS residual was extracted from the supernatant using methylene chloride as solvent. The organic phase was separated from the aqueous phase by centrifugation at 3000 \times g for 15 min. The aqueous phase obtained was then examined for the presence of the released Nickel, Inductively coupled plasma optical emission spectroscopy Instrument was used.

Metal ions effect

CaCl_2 , CuCl_2 , MnCl_2 , NaCl_2 and ZnCl_2 (1.0 mg/l) for each were separately added to the liquid culturing medium supplemented with NiPPDS or VOOPE as carbon source. The colonies in pure culture were inoculated into the broth medium. The growth was observed after 5 days of incubation at 30° C.

Results and discussion

Demetallization of crude oil efficiency of *bacillus cereus* DS1 bacterial isolate fig (2) was studied. As shown in fig (3) the consumption of NiPPDS evaluated by optical density (OD) at 660nm and determination of releasing Nickel in aqueous medium. It was observed that isolate capable of releasing about 1.4ppm of initial concentration of substrate accomplished with 1.6 rate of growth after 5 days of incubation at 30°C and 150 rpm.

Obtained results compared with ability to grow on VOOPE which represent another type of protopurperenic compounds containing vanadium. There were a significant differences on the growth rate of

the bacterial isolate between the two types of complexes as shown in figure (4). It was observed that the higher recorded value (1.4) with growing in NiPPDS supplemented medium while on VOOPE was only 1.0. The effect of metal ions on the activity of the bacterial isolate to consume organic matter after incubation at 30°C after 5 days, was established by measuring the change in turbidity. Result shown that the addition of CuCl_2 raise the growth to 2.4, 1.8 on Nickel and Vanadium respectively. MnCl_2 also stimulated the growth rate table (1) while the addition of ZnCl_2 and NaCl_2 effect was inhibitors. It seems to that some metals used to stimulate the consumption of protoporpheric compounds due to its relation with the active site of prophyrinase enzyme or by competition between substrate and these ions as electron donor. This could be attributed to the fact that these metals differs in there effect depending on final concentration because it may be act as inhibitor in high or low levels.



Bacillus cereus DS1 growing on L-agar

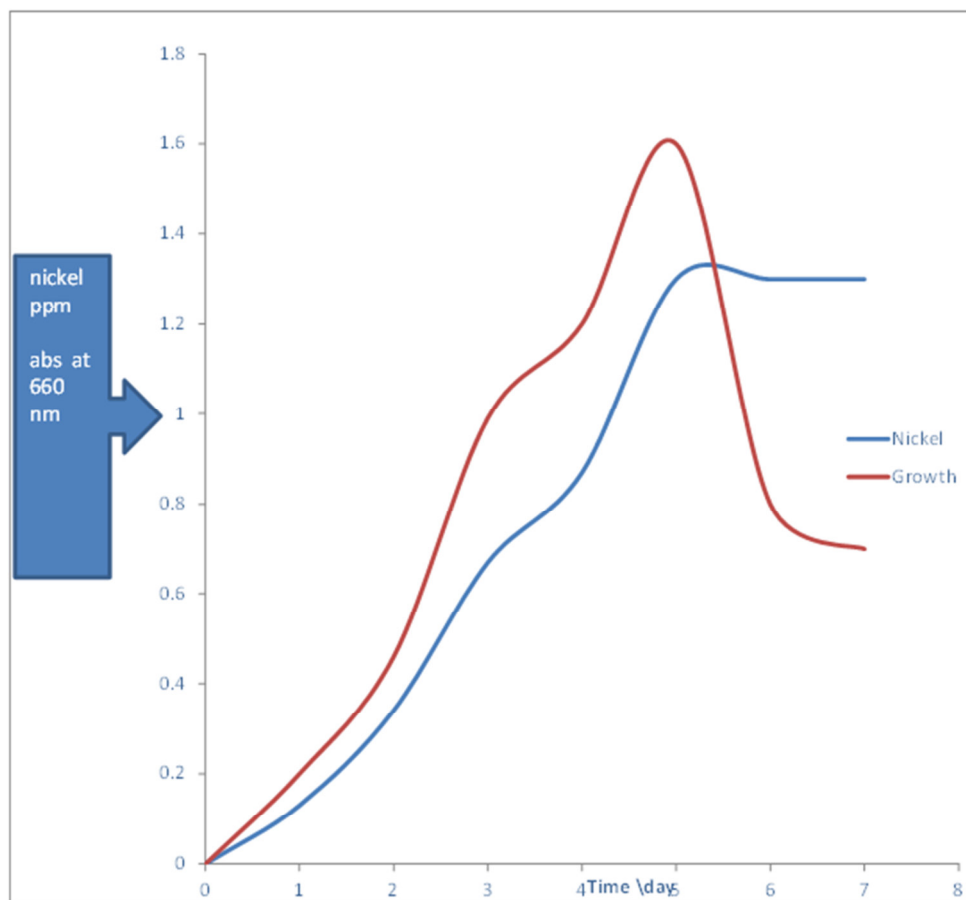
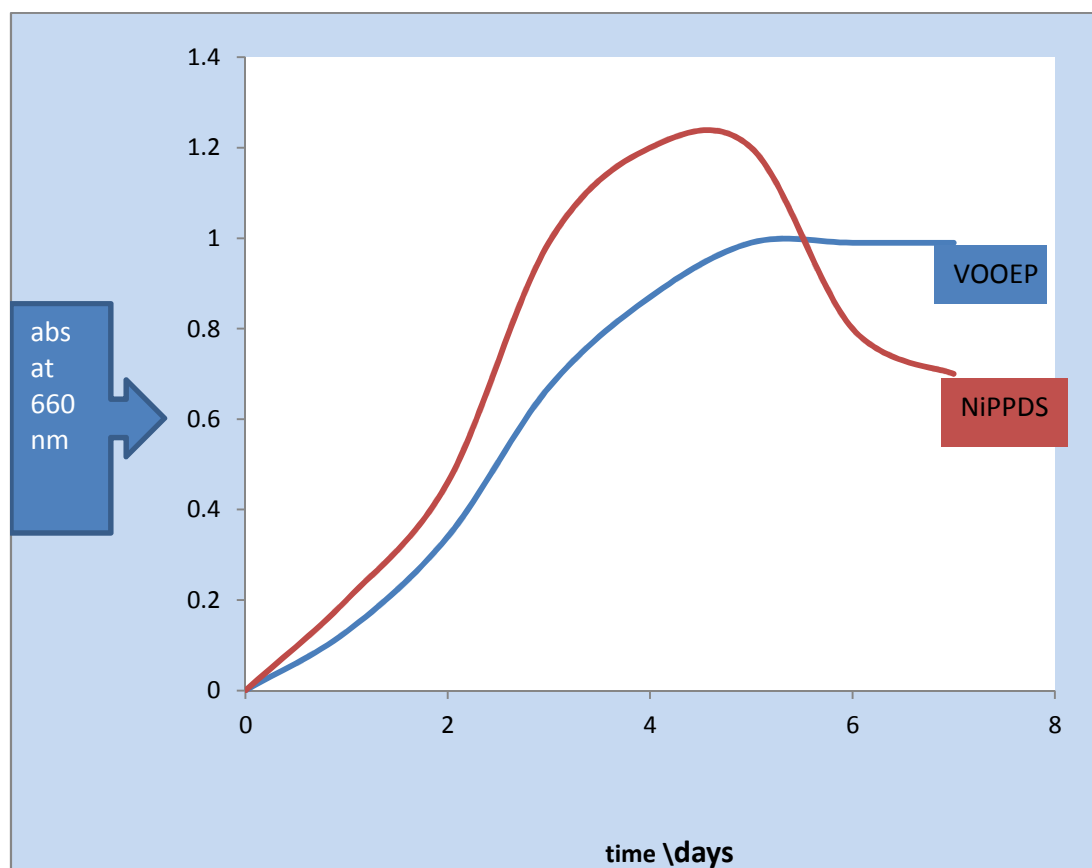


Fig (3) Growth rate and releasing Nickel of *Bacillus cereus* after activation

Table (1) Effects of metal on the bacterial ability to grow in culture medium containing NiPPDS or VOOPE as a carbon source

Metal chloride 0.1mg\l	Absorbance at 660nm with Nickel	Absorbance at 660nm with Vanadium
CaCl ₂	1.8	1.6
CuCl ₂	2.4	1.8
MnCl ₂	1.9	1.8
NaCl ₂	1.2	1.0
ZnCl ₂	0.6	0.4



. Figure (4) Growth rate of bacillus cereus on two types of compounds

References

- Arellano HG, Gonzalez EB, Vazquez-Duhalt R (2003). Biocatalyst transformation of petroporphyrins by chemical modified cytochrome C. *Biotech Bioeng.* 85: 790-798.
- Bartholdy J, Hannerup PN (1990). Hydrodemetallation in residue hydro processing. *Amer Chem Soc.* 35: 619-625 hydro processing. *Amer Chem Soc.* 35: 619-625
- Dedeles GR, Abe A, Siato K, Asano K, Siato K, Yokota A, Tomita F (2000). Microbial demetallization of crude oil: nickel protoporphyrin disodium as a model organo- metallic substrate. *Biosci Bioeng.* 90: 515-521.
- Eckemann B, Vogelpohl A (1990). Deasphaltization and demetallizing of heavy crude oils and distillation residues with CO₂. *Chem Eng Technol.* 13: 258-264.
- Fedorak PM, Semple KM, Vazquez-Duhalt R, Westlake DWS(1993). Chloroperoxidase-mediated modifications of petroporphyrins and asphaltenes. *Enz Microb Technol.* 15: 429-438.
- Flynn J, Freeman D (1987). Mobile phase selectivity mapping of nickel and vanadyl metalloporphyrins in reversed-phase liquid chromatography. *Chromatogr.* 64: 4377-4320.
- Greg P. Dechaine* and Murray R. Gray .Chemistry and Association of Vanadium Compounds in Heavy Oil and Bitumen, and Implications for Their Selective Removal *Energy Fuels*, 2010, 24 (5), pp 2795–2808.
- Hernandez A, Mellado R, Martinez J, (1998). Metal accumulation and vanadium induced multidrug resistance by environmental isolates of *Escherichia hermani* and *Enterobacter cloacae* *Appl Environ Microbiol.* 64: 4377-4320.
- Kukes SG, Aldag AW (1985). Chemical demetallization of heavy oils. *Amer Chem Soc.* 30: 119-129.
- Mogollon L, Rodriguez R, Larrota W, Ortiz C, Torres R (1998). Biocatalytic removal of nickel and vanadium from petroporphyrins and asphaltenes. *Appl Biochem Biotechnol.* 70: 765-777.
- Premuzic ET, Lin MS, Lian H, Zhon WM, Yablon J (1997). The use of chemical markers in the evaluation of crude oil bioconversion products. *Fuel Processing Technol.* 52: 207-223.
- Premuzic ET, Lin MS (1999). Induced biochemical conversions of heavy crude oils. *Petroleum Sci Eng.* 22: 171-180.
- Rapper KB, Fenell DJ (1977). *The genus Aspergillus*. Krieger Publishing Company, New York.
- Reynolds JG (2001). Understanding metals in fossil fuels. In: Yen TF and Chilingarian GV (eds), *Asphaltenes and asphalts*. Vol.2, Elsevier Science, Amsterdam, PP. 233-248.
- S.A. Krasnikov , A.B. Preobrajenski , N.N. Sergeeva ,M.M. Brzhezinskaya A.A. Cafolla,M.A. Nesterov M.O.

- Senge A.S. Vinogradov Electronic structure of Ni(II) porphyrins and phthalocyanine studied by soft X-ray absorption spectroscopy *Chemical Physics* 332 (2007) 318–324.
- Savastano CA (1991). Solvent extraction approach to petroleum demetallation. *Fuel Sci Technol.* 9: 833-871.
- Xu GW, Mitchell KW, Monticello DJ (1992). Process for demetallizing a fossil fuel. *US Patent* NO. 5624844.
- Xu GW, Mitchell KW, Monticello DJ (1998). Fuel product produced by demetallizing a fossil fuel with an enzyme. *US Patent* NO. 5726056.