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Impact of heavy metals on biodegradation of phenanthrene by *Cellulomonas hominis* strain N2

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Effects of some heavy metals (HM) namely, Cu, Mn, Ni and Zn, individually and in mixture on the biodegradation of phenanthrene (Phe) as a model for polyaromatic petroleum hydrocarbons (PAHs) using *Cellulomonas hominis* N2 previously isolated from highly heavy metals and petroleum contaminated soil (PCS) were studied.

The bacterial growth showed a decrease in the surface tension of the incubated culture in addition to good emulsification index which proves that *Cellulomonas hominis* N2 is biosurfactant producer.

***Cellulomonas hominis* N2 showed good growth and biodegradation potentials on Phe in absence and presence of heavy metals with various degrees depending on the type and concentration of the metal either being present individually or as a mixture. The biodegradation potential was found to be not linearly related to growth rates.**

Key words: Impact, Heavy metals, Biodegradation, Phenanthrene, and *Cellulomonas hominis*.

Oil pipelines leakage and accidental oil spills are common problems in petroleum industry resulting into contamination of soils. An oil spill can cause not only hydrocarbon contamination but also heavy metals contamination into the surrounding subsurface and groundwater, posing a threat to the environment and to human health[1].

Many metallic compounds occur in petroleum in extremely small concentrations, such as inorganic salts, metal soap, and organic metal-complex compounds[2].

Major metal contaminants in petroleum oil commonly include aluminum (Al), sodium (Na), iron (Fe), nickel (Ni) and vanadium (V), with frequently smaller amounts of magnesium (Mg), tin (Sn), barium (Ba). Zinc (Zn), molybdenum (Mo), calcium (Ca), copper (Cu), manganese (Mn), lead (Pb), chromium (Cr), and titanium (Ti). Vanadium and nickel are the most abundant metallic constituents of crude petroleum, sometimes reaching thousand of parts per million. They are primarily present in porphyrin complexes and other organic compounds[3].

PAHs are ubiquitous in the environment. They are known or suspected to be genotoxic or carcinogenic and have been classified as priority pollutants[4]. Persistence of PAHs in the environment linked to their general recalcitrance, binding to the soil matrix and low water solubility, make them non-bioavailable to PAH-degrading organisms[5].

Heavy metals (HM) exposure has, since the last century, been known to affect microbial growth and survival[3,6]. An extensive literature is available on the effects of HM on microbial

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populations and microbial processes, such as litter decomposition and carbon mineralization. However little is known about the effect of heavy metals on the degradation of recalcitrant hydrocarbons, such as PAHs. Some HM are thought to be essential for oil-degrading microorganisms while others are known to be toxic. Whereas some metals, such as copper, are essential for bacteria and fungi in trace amounts, high concentrations are known to be toxic. The addition of copper to the soil significantly inhibits soil respiration, nitrogen mineralization and nitrification[7]. Long-term exposure to heavy metals (Cu, Ni and Zn) has been found to alter microbial structure[8]. However, tolerance and adaptation of microorganisms to heavy metals are common phenomena, and the presence of tolerant fungi and bacteria in polluted soil has frequently been observed[9]. The negative effect of HM on soil microbes and soil microbial processes means that their presence in contaminated soils can potentially limit the bioremediation of organic pollutants. The influence of HM on PAHs degradation in polluted soils has only recently emphasized[10]. It is well documented that the presence of heavy metals can inhibit a broad range of microbial processes including methane metabolism, growth, nitrogen and sulfur conversions, dehalogenation, and reductive processes in general. Metals may inhibit pollutant biodegradation through interaction with enzymes directly involved in biodegradation (e.g., pollutants-specific oxygenases) or through interaction with enzymes involved in general metabolisms. In either case, inhibition is mediated by the ionic form of the metal[11]. Biosurfactant was reported to decrease heavy metal toxicity in polluted sites and enhance biodegradation efficiency[12].

Cellulomonas sp. has been reported for their ability to degrade cellulose, polychlorinated biphenyls, 4-chlorobenzoate and hydrocarbons[13]. *Cellulomonas* sp. has been reported to express high resistance to heavy metals[14,15]. Until now, no studies have been published that have investigated the combination effects of HM and PAHs, the impact of HM on biodegradation capabilities of *Cellulomonas* sp. or on its ability to produce biosurfactant.

The present work aims to study the impact of HM on biodegradation of Phe as a model compound for polyaromatic petroleum hydrocarbons using *Cellulomonas hominis* N2 previously isolated from highly heavy metals and petroleum polluted soil (article in press) and study its ability to produce biosurfactant.

MATERIAL AND METHODS

Basal salts medium (BSM)

BSM used in this study was prepared according to Piddington *et al*.[16] but with modifications and consists of 5.57g/l Na_2HPO_4, 2.44g/l KH_2PO_4, 2.0g/l NH4Cl, 0.2g/l $MgCl_2.6H_2O$, 0.001g/l $FeCl_3.6H_2O$, 0.001g/l $CaCl_2.2H_2O$ and 0.1g/l of yeast extract, dissolved in 1liter of deionized water. The pH was adjusted to 7 with 10% NaOH and it was sterilized by autoclaving at 121°C for 15min. Phenanthrene (Phe) dissolved in ethyl ether was added as a sole source of carbon in a final concentration of 500mg/l (BSM/Phe medium).

Luria-Bertani medium (LB)

LB used for obtaining biomass was prepared according to Kirimura *et al*.[17] and consists of 10g/l Tryptone, 5g/l Yeast extract and 10g/l NaCl, dissolved in 1liter deionized water and adjusted to pH 7 with 10% NaOH before sterilization.

Microorganism

Cellulomonas hominis strain N2 previously isolated from highly heavy metals and petroleum polluted soil (article in press) was used in this study.

Heavy metals tested

Heavy metals tested were copper, manganese, nickel and zinc. They were incorporated as soluble salts: $CuCl_2.2H_2O$, $MnCl_2.4H_2O$, $NiCl_2.6H_2O$ and $ZnCl_2$. A sterilized concentrated solution of each of the salts was prepared by dissolving a calculated quantity containing a desired concentration of the metal in deionized water to produce separate metal solutions served as stock solutions. To cover the whole concentration of the chosen predominated four heavy metals in the petroleum contaminated soil (PCS) used to isolate *Cellulomonas hominis* N2 (article in press); different concentrations of each heavy metal were prepared; for Cu and Ni (5, 10, 25, 50 and 100mg/l) and (5, 10, 25, 50,

100, 250, 500, 750 and 1000mg/l) for Mn and Zn. Aliquots of the individual four metal solutions were mixed to obtain a mixture containing concentration ranges of 12.5, 25, 50, 75, 100, 125, 150, 175, 200, 225 and 250mg/l of each metal.

Biosurfactant production test

Biosurfactant production was examined by inoculating bacteria in 20ml BSM supplemented with n-hexadecane (1% v/v) as a carbon and energy source. The cultures were incubated at 30°C for 7 days, in a shaking incubator (150rpm). Growth was monitored with total viable count (TCFU/ml) on LB/agar plates. The medium was centrifuged and the surface tension of the supernatant was measured using ring tensiometer model Kruss 8451 at 25°C and compared to the surface tension of sterilized uninoculated flask of BSM/hexadecane medium.

Emulsification Index Determination (E24)

E24 was the method used to quantify the emulsification caused by the produced biosurfactant. It was determined by the addition of 2ml of paraffin oil to 3ml of culture (bacteria/ BSM/Phe, centrifuged at 500rpm for 5min and the supernatant was used) at the end of incubation period, mixing with a vortex for 2min., and leaving to stand for 24hours. BSM/Phe uninoculated flask was used as a negative control. The E24 index is given as percentage of height of emulsified layer (mm) divided by total height of the column (mm)[18].

Impact of heavy metals on biodegradation of phenanthrene

- Seven groups of 100ml conical flasks were used where each of them contained 20ml BSM/Phe medium.
- One group amended with mixture of equal concentrations of Cu, Mn, Ni and Zn to obtain HM/BSM/Phe broth media containing different concentrations of HM ranging between (5-1000mg/l).
- One group amended with $CuCl_2.2H_2O$ to obtain Cu/BSM/Phe broth media containing different concentrations of Cu ranging between (5-100mg/l).
- One group amended with $MnCl_2.4H_2O$ to obtain Mn/BSM/Phe broth media containing different concentrations of Mn ranging between (5-1000mg/l).
- One group amended with $NiCl_2.6H_2O$ to obtain Ni/BSM/Phe broth media containing different concentrations of Ni ranging between (5-100mg/l).
- One group amended with $ZnCl_2$ to obtain Zn/BSM/Phe broth media containing different concentrations of Zn ranging between (5-1000mg/l).
- Another set of flasks were the positive control group free from any HM.
- For each group another set of flasks were prepared as the negative control group without inoculation with bacteria.

Cellulomonas hominis N2 was inoculated in LB broth media of pH 7 and incubated at 30°C for 48hours in a rotary shaking incubator (150rpm) to obtain a biomass. Cells were harvested by centrifugation at 500rpm for 15min and then washed three times with sterilized BSM free from any C-source and heavy metals. Washed cells were inoculated into 20ml BSM/Phe in 100ml Erlenmeyer conical flasks. The inocula were adjusted to have initial TCFU of about 10^5cells/ml.

The cultures were incubated at 30°C for 7 days, in a shaking incubator (150rpm). Growth was monitored with total viable count on LB/agar plates. To determine the biodegradation efficiencies of the bacterial isolates, the cultures were acidified with 1mM HCl to pH2.0 then extracted with equal volumes of ethylacetate. The extracted solutions were analyzed by HPLC. All the experiments were done in duplicates and the data listed are the average of the results obtained.

HPLC analysis

Biodegradation efficiency of bacterial isolates for Phe was followed by measuring the concentration of Phe using HPLC model Waters 600E equipped with auto sampler Waters 717 plus and dual wavelength absorbance detector Waters 2487 set at 254nm. Phe standard compound was obtained from Supelco. The conditions of operation were as follows:

- Column: Supelcosil LC-PAH, 15 cm x 4.6mm ID, 5µm particles size.
- Mobile phase: Acetonitrile/water 60:40 of HPLC grades
- Flow rate: 1.0 ml/min.
- Calibration curve for Phe (0-500mg/l) was done.

RESULTS AND DISCUSSION

Biosurfactant production and emulsification Index

Bacterial isolates that display substantial potential for production of biosurfactant enhance bioremediation of hydrocarbons in oily soil[19].

Cellulomonas hominis N2 showed good growth on hexadecane as a soul source of carbon and energy with TCFU of $1x10^{10}$ with complete degradation of the added hexadecane after 7 days of incubation starting with inoculum of $2.5x10^5$ and the surface tension was reduced by 30% using uninoculated flask as the reference surface tension.

Emulsification index is a method extensively used to identify and quantify biosurfactants in microbial cultures. *Cellulomonas hominis* N2 showed good emulsification index of 18%[18].

Biosurfactants are non-toxic, non-hazardous, biodegradable, environmentally friendly amphiphilic compounds that reduce surface and interfacial tensions by accumulating at the interface of immiscible fluids or of a fluid and a soil and increase the surface areas of insoluble compounds leading to increased mobility, bioavailability and subsequent biodegradation[20]. They are produced by many bacterial strains that can degrade or transform the components of petroleum products[21].

Impact of heavy metals on growth of *Cellulomonas hominis* N2 on phenanthrene

Effect of Cu, Mn, Ni and Zn as mixture or individually on growth of *Cellulomonas hominis* N2 on 500mg/l Phe was preformed. Distinct patterns of HM *Cellulomonas hominis* N2 resistance were vindicated at table (1) and represented in logarithmic scale of TCFU at the end of incubation period (7 days) relative to the initial TCFU, figure (1).

Phe was used in this study as a model compound for polyaromatic petroleum hydrocarbons, because of its ubiquity at hazardous waste sites and its demonstrated biodegradability[3,22].

Significant variations in the growth patterns were observed for each of the HM used in the study individually and as a mixture of HM ions.

Generally, growth of strain N2 in the presence of HM mixture Cu/Mn/Ni/Zn even at low concentrations were consistently lower than that of the reference flask without HM supplementation. Similar observations were reported by Kumar *et al*.[23] Pal *et al*.[24] and Raja *et al*.[25].

In general, there was a decrease in TCFU with the increase in HM concentrations. The growth was not particularly affected at low concentrations; in flasks containing 5mg/l of individual HM there was an increase of TCFU than the reference flasks without amending HM in the following order Cu 270%, Mn 250%, Zn 233% and Ni 220%. But in flasks containing 5mg/l of Cu/Mn/Ni/Zn mixture there was a significant decrease in TCFU to reach 35% of that in the reference flask, indicating the highest toxicity of HM mixture relative to the presence of each HM alone in the culture medium.

This is in agreement with other recent studies showing that Ni at low concentrations is non toxic and actually enhanced growth and metabolic activities of aerobic bacteria[26].

Compared to the corresponding reference flasks free from HM, the following can be observed

- At concentration of 10mg/l the growth was reduced, indicating order of toxicity: Cu > HM mixture > Mn > Zn > Ni.
- At concentration of 25mg/l the growth was furtherly reduced, indicating order of toxicity: Ni > Cu > HM mixture > Zn > Mn.
- At concentration of 50mg/l the growth was reduced, where HM mixture and Cu are nearly having the same effect of 1% and 1.2%, respectively, Ni and Mn having the same effect of 10% and Zn 11.33%. Indicating order of toxicity of HM mixture $\approx$ Cu >>> Mn = Ni > Zn.
- At concentration of 100mg/l the growth was sharply reduced in the HM mixture flask giving 0.005% growth relative to that obtained in the +ve control flask, Ni and Cu are nearly having the same effect of 0.43% and 0.6%, respectively, Mn 5% and Zn 6.67%. Indicating order of toxicity: HM mixture >>> Ni $\approx$ Cu >> Mn > Zn.
- At concentration of 250mg/l of HM

mixture, Mn and Zn, the growth was very highly reduced to be in the HM mixture 0.0005% where nearly complete inhibition occurred, Mn and Zn showed the same effect as 100mg/l of 5% and 6.67%, respectively. Indicating order of toxicity: HM mixture >>> Mn > Zn.

With increasing HM concentration to 500mg/l the growth was severely reduced in the HM mixture 0.0001% where nearly complete inhibition occurred and furtherly decreased than the start of the experiment but still there were some colonies of N2 survived. Zn 4% and Mn showed the same effect as that obtained in 250mg/l flasks of 5%. Indicating order of toxicity: HM mixture >>> Zn > Mn.

At concentration of 750mg/l the growth continued reduction relative to the reference (+ve control) flasks to be in the order HM mixture showed the same effect as in case of 500mg/l of 0.0001% where complete inhibition occurred and decreased than the start of the experiment but still there were some colonies of N2 survived. Zn showed high toxicity effect with sharp decrease of 0.4% then Mn 3%. Indicating order of toxicity: HM mixture >>> Zn >> Mn.

Finally, at concentration of 1000mg/l the growth was furtherly decreased than the start and reduced compared to the reference flask to reach to nearly complete inhibition but still there were some colonies of N2 survived to be in the order HM mixture showed 0.00005%. Zn showed reduction to be 0.007%, while Mn 0.01%. Indicating order of toxicity: HM mixture >> Zn > Mn.

In this study the degree of growth in response to metal ions varied with the type and the concentration of each heavy metal ion supplemented in the medium individually or as mixture. Similar observations were reported by Raja *et al.*[25].

Sokn *et al.*[3], Riis *et al.*[26] and Wong *et al.*[22] have reported that heavy metals, Cu, Mn, Ni and Zn are essential for bacteria in trace amounts, high concentration are known to be toxic. However, tolerance and adaptation of microorganisms to HM are common phenomenon and the presence of tolerant bacteria in polluted environments has frequently been observed.

Friis *et al.*[27] have previously reported that the reduction in growth is mainly because of the interaction between the metal cations along with phosphate, carboxyl, hydroxyl and amino groups of the cell surface.

Impact of heavy metals on phenanthrene biodegradation by *Cellulomonas hominis* N2:

Distinct patterns of HM impact on biodegradation of Phe by *Cellulomonas hominis* N2 and its resistance to HM are vindicated at Table 1 and Fig. 2.

These results show high biodegradation efficiency of *Cellulomonas hominis* N2 on Phe in BSM free of HM, reaching an average value of 96.8%.

Significant variations in the Phe biodegradation patterns in presence of each of the HM used in the study individually or as a mixture of HM ions were observed.

Generally biodegradation efficiencies of strain N2 in the presence of HM Cu, Mn, Ni and Zn supplemented individually or as mixture were consistently lower than that of the reference flask without HM supplementation. The effect of Cu/Mn/Ni/Zn mixture was generally higher than individual HM. In all cases this shows the inhibitory effect of HM on biodegradation of Phe even at low concentrations of HM.

Heavy metals are known to inhibit the degradation of xenobiotics[28].

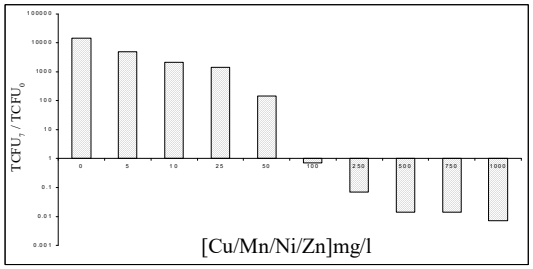
At concentration of 5mg/l HM, biodegradation of 500mg/l Phe was in the following order Cu 92.8%, Mn 90.2%, Zn and Ni nearly reached the same value 89 and 88.6, respectively. The lowest biodegradation efficiency obtained in flasks contained HM mixture 77.2%, indicating toxicity impact of 5mg/l HM on biodegradation of Phe in the following order HM mixture > Ni ≈ Zn > Mn >Cu.

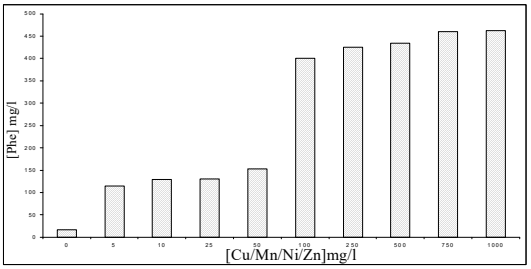
At concentration of 10mg/l HM, biodegradation of Phe was in the following order Ni 88.8%, Zn 87.4%, Mn 86.6%, and Cu 79%. The lowest biodegradation efficiency obtained in flasks contained HM mixture 74.2%, indicating toxicity impact in the following order HM mixture > Cu > Mn > Zn > Ni.

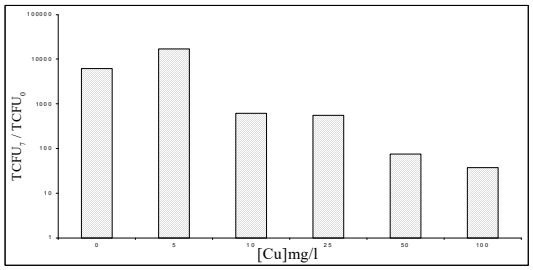
At concentration of 25mg/l HM, biodegradation of Phe was in the following order Zn 86.6%, Ni 84.6%, Mn 85.6%, and Cu 78%. The lowest biodegradation efficiency obtained in

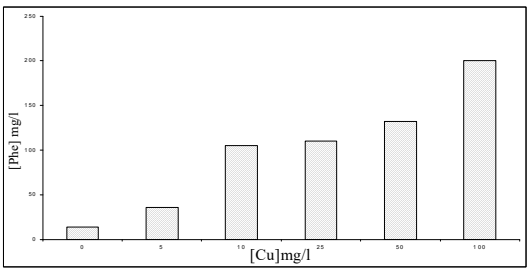
Table 1. Impact of heavy metals on growth (TCFU/ml) and biodegradation efficiency (%BD) of *Cellulomonas hominis* N2 on 500ppm phenanthrene

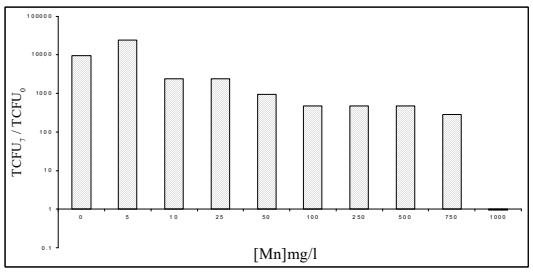
Applied metals	Concentration (mg/l)	CFU/ml Zero time	CFU/ml 7days	CFU related to the reference %	%BD
	0		$1x10^9$	-	97.2
	5		$2.7x10^9$	270	92.8
Cu	10	$1.6x10^5$	$1x10^8$	10	79
	25		$9x10^7$	9	78
	50		$1.2x10^7$	1.2	73.6
	100		$6x10^6$	0.6	60
	0		$3x10^9$	-	96.5
	5		$6.6x10^9$	220	88.6
Ni	10	$2x10^5$	$2x10^9$	66.67	88.8
	25		$2x10^8$	6.67	84.6
	50		$3x10^8$	10	83.2
	100		$1.3x10^7$	0.43	80
	0		$2x10^9$	-	96
	5		$5x10^9$	250	90.2
	10		$5x10^8$	25	86.6
	25		$5x10^8$	25	85.6
	50		$2x10^8$	10	83.6
Mn	100	$2.1x10^5$	$1x10^8$	5	80.5
	250		$1x10^8$	5	75.5
	500		$1x10^8$	5	69.8
	750		$6x10^7$	3	49.4
	1000		$2x10^5$	0.01	40.8
	0		$1.5x10^9$	-	97.5
	5		$3.5x10^9$	233.33	89
	10		$8x10^8$	53.33	87.4
	25		$2.9x10^8$	19.33	86.6
	50		$1.7x10^8$	11.33	80
Zn	100	$2.5x10^5$	$1x10^8$	6.67	80.8
	250		$1x10^8$	6.67	79.4
	500		$6x10^7$	4	76.7
	750		$6x10^6$	0.4	57.80
	1000		$1x10^5$	0.007	30.4
	0		$2x10^9$	-	96.8
	5		$7x10^8$	35	77.2
	10		$3x10^8$	15	74.2
	25		$2x10^8$	10	74
	50		$2x10^7$	1	69.4
Cu/Mn/Ni/Zn	100	$1.4x10^5$	$1x10^5$	0.005	20
mixture	250		$1x10^4$	0.0005	15
	500		$2x10^3$	0.0001	13
	750		$2x10^3$	0.0001	8
	1000		$1x10^3$	0.000005	7.6

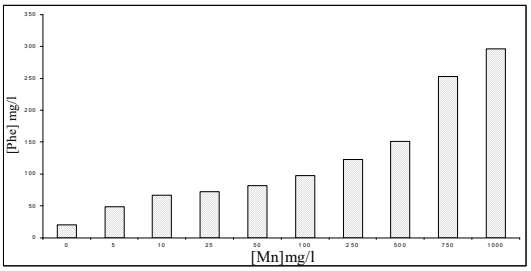


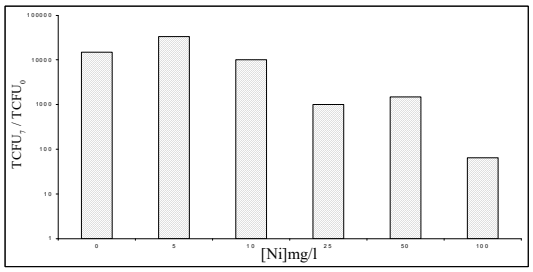


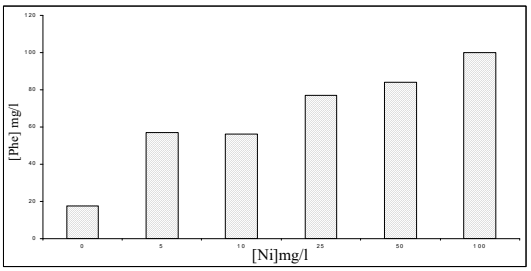


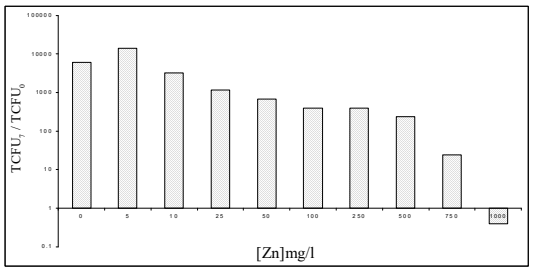












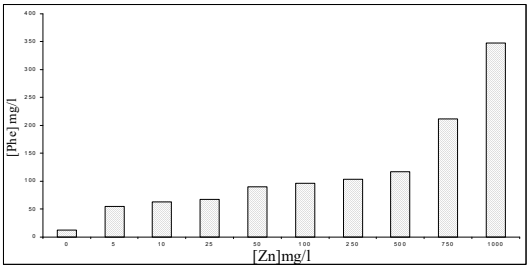


Fig. 1. Impact of HM on growth of *Cellulomonas hominis* N2 on Phe

Fig. 2. Impact of HM on biodegradation of Phe by *Cellulomonas hominis* N2.

flasks contained HM mixture 74% which was nearly the same as that obtained in 10mg/l, indicating toxicity impact in the following order HM mixture > Cu > Mn > Ni > Zn.

At concentration of 50mg/l HM, biodegradation of Phe was in the following order Mn and Ni nearly reached to the same value 83.6% and 83.2%, respectively, Zn 82%, and Cu 73.6%. The lowest biodegradation efficiency obtained in flasks contained HM mixture 69.4%, indicating toxicity impact in the following order HM mixture > Cu > Zn > Ni ≈ Mn.

At concentration of 100mg/l HM, biodegradation of Phe was in the following order Zn, Mn and Ni nearly reached the same value 80.8%, 80.5% and 80%, respectively, and Cu biodegradation efficiency was highly reduced to reach 60%. The lowest biodegradation efficiency obtained in flasks contained HM mixture which was sharply decreased to 20%, indicating toxicity impact in the following order HM mixture >> Cu > Ni ≈ Mn ≈ Zn.

At concentration of 250mg/l HM, biodegradation of Phe was in the following order Zn 79.4% and Mn 75.5%. The lowest biodegradation efficiency obtained in flasks contained HM mixture which was sharply decreased to reach 15%, indicating toxicity impact in the following order HM mixture >> Mn > Zn.

At concentration of 500mg/l HM, biodegradation of Phe was in the following order Zn 76.7% and Mn 69.8%. The lowest biodegradation efficiency obtained in flasks contained HM mixture which was furtherly decreased to reach 13%. Indicating toxicity impact in the order HM mixture >> Mn > Zn.

At concentration of 750mg/l HM, biodegradation of Phe was in the following order Zn 57.8% and Mn 49.4%. The lowest biodegradation efficiency obtained in flasks contained HM mixture which was sharply decreased to reach 8%. Indicating toxicity impact in the following order HM mixture >> Mn > Zn.

At concentration of 1000mg/l HM, biodegradation of Phe was furtherly decreased in the following order Mn 40.8% and Zn 30.4%. The lowest biodegradation efficiency obtained in flasks contained Cu/Mn/Ni/Zn mixture which was decreased to reach 7.6%, this was nearly the same as that obtained in 750mg/l. Results indicating toxicity impact in the following order HM mixture >>> Zn> Mn.

HM are often added at low concentrations in the form of trace mineral solutions in microbial cultures to maintain or enhance microbial growth. Cu, Mn, Ni and Zn are also components of several bacterial enzymes. It would therefore not be surprising to observe variable growth efficiencies and substrate removal capabilities with varying HM concentrations, which could either, present a favorable effect or an inhibitory effect depending on their concentrations.

In the present work, the HM added to the medium only at inhibitory concentrations for biodegrading enzymes. No enhancements of degradation capacities than that of the reference flasks free from HM were observed even at low concentrations tested, although enhancement of growth occurred in BSM supplemented with 5mg/l of individual HM tested, as listed in Table 3.

According to Sokhn *et al*.[3], this can be explained as high levels of HM are directly toxic to enzymes involved in the degradation of toxic intermediates formed from the degradation pathway of Phe. The outcome is incomplete mineralization of Phe and the presumed accumulation of its toxic metabolites and consequently decreases in microbial growth and biodegradation efficiencies was occured.

It was obvious from the results that *Cellulomonas hominis* N2 showed good biodegradation potentials in absence and presence of HM with various extends according to the HM ions, its concentration and being supplemented alone or as mixture. This can be attributed to its isolation form highly HM and PCS (article in press), which made N2 adapted to those sever conditions. This may be also due to the ability of *Cellulomonas hominis* N2 to produce biosurfactant, where the presence of biosurfactant generally reduces HM toxicity and enhances biodegradation of hydrocarbons[12].

Other investigators reported biodegradation of different hydrocarbons and xenobiotics in presence of HM.

Siunova *et al*.[28], reported degradation of 98% of naphthalene in presence of 100µM Ni using *Pseudomonas chloraphis* PCL1391.

Sokhn *et al*.[3], reported that Cu up to

70ppm has little effect on microbial activites during Phe degradation. However, elevated concentrations (700 and 7000ppm) showed marked reduction in microbial activities during the degradation of Phe.

Collorad *et al*.[29], reported that *Alcaligenes eutrophus* degraded polychlorinated biphenyls and 2,4-dichlorophenoxyacetic acid effectively in presence of Ni and Zn.

Sandrin and Marier[11] reported that Ni in a concentration of 200µM is known to inhibit aerobic biodegradation of naphthalene, biphenyl, xylene and a number of other xenobiotics using *Alcaligenes* and *Pseudomonas* strains.

Riis *et al*.[26], reported that Zn up to 262mg/l showed biodegradation of diesel fuel using certain microbial community and Cu showed the highest toxicity to the community used, and reported that degradation process in liquid culture with bacteria revealed significant inhibition by heavy metals.

Edgehill[30] reported that in presence of Cu at concentrations of 2 and 8mg/l *Arthrobacter* strain ATCC 33790 did not remove 89-124mg/l of pentachlorophenol.

According to Amor *et al*.[26], HM inhibit microorganisms by blocking essential functional groups or interfering with essential metal ions incorporation of biological molecules. In some cases microorganisms are resistant to some HM through different possible mechanisms.

According to Atagana[7], presence of Cu is very inhibitory to the degradation of PAHs. This could be due to the toxicity of Cu^{+} to microbial cells as these ions may directly interact with the physiological functions of the cells and possibly the membrane development in the cells. Enzyme activities, which are very important in the microbial degradation of organic substrates, could also be hindered by the presence of copper in the medium. This may explain the reduction of biodegradation efficiency in flasks amended with Cu than that amended with Ni, Mn or Zn, as listed in table (1). However the degradation in presence of Cu^{2+} occurred due to the utilization of the strongly adapted *Cellulomonas hominis* N2 previously isolated from highly HM and PCS (article in press).

Chen *et al*.[31], reported the potential toxic effect of high level of metals on microbial metabolism of petroleum hydrocarbons even though the microorganisms have been previously exposed to heavy metals accumulated over the years.

Effect of HM and PAHs together on growth and biodegradation efficiencies is complex as they both affect eachother.

Lipophilic compounds such as PAHs have a narcotic mode of toxic action and may interact with lipophilic components of cytoplasmitic membranes of bacteria, thus affecting their permeability and structure. Hence in the presence of HM and PAHs together, the penetration of HM may be more easily into microbial cells and affect their functions[32,.33]. This clearly confirms our results.

The order of toxicities obtained from the effect of HM on the bacterial growth somewhat differs from that obtained from the effect on biodegradation efficiencies. This clearly indicates that the biodegradation efficiencies are not linearly related to growth rates. There must be other factors to be involved; the enzymatic activities of the microorganisms in the tested media may be an important one of these factors. Therefore the bacterial growth is not a limiting factor in their biodegradation efficiency but the types of the enzymes involved in biodegradation process and degrees of their activities have also an important roll.

CONCLUSION

Results in this study show that heavy metal resistance of *Cellulomonas hominis* N2 is widespread. N2 shows high biodegradation potentials on phenanthrene even in presence of heavy metals, although the presence of heavy metals decreases its biodegradation potentials. *Cellulomonas hominis* N2 has the ability to produce biosurfactant which may help to overcome toxicity of heavy metals. The results show that effects of heavy metals are complex and suggest that many factors could be in operations. This may explain why in spite of the high HM concentrations used in the experiments the biodegradation of phenanthrene still takes place.

Further work is recommended to study the type of the produced biosurfactant and its effect on the kinetics of biodegradation process in presence and absence of heavy metals.

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