Degradation of di-2-ethylhexyl phthalate (DEHP) by indigenous isolate *Acinetobacter* sp.

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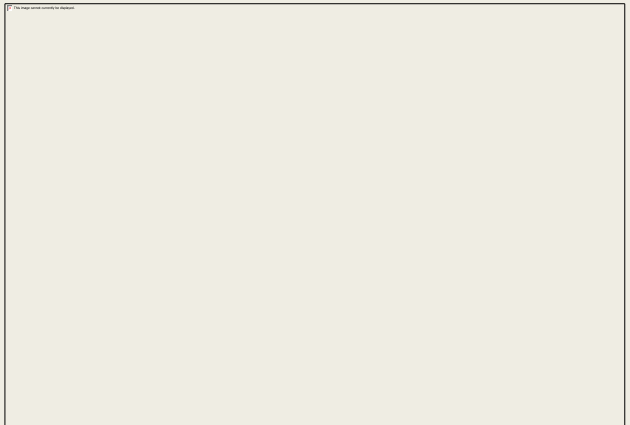
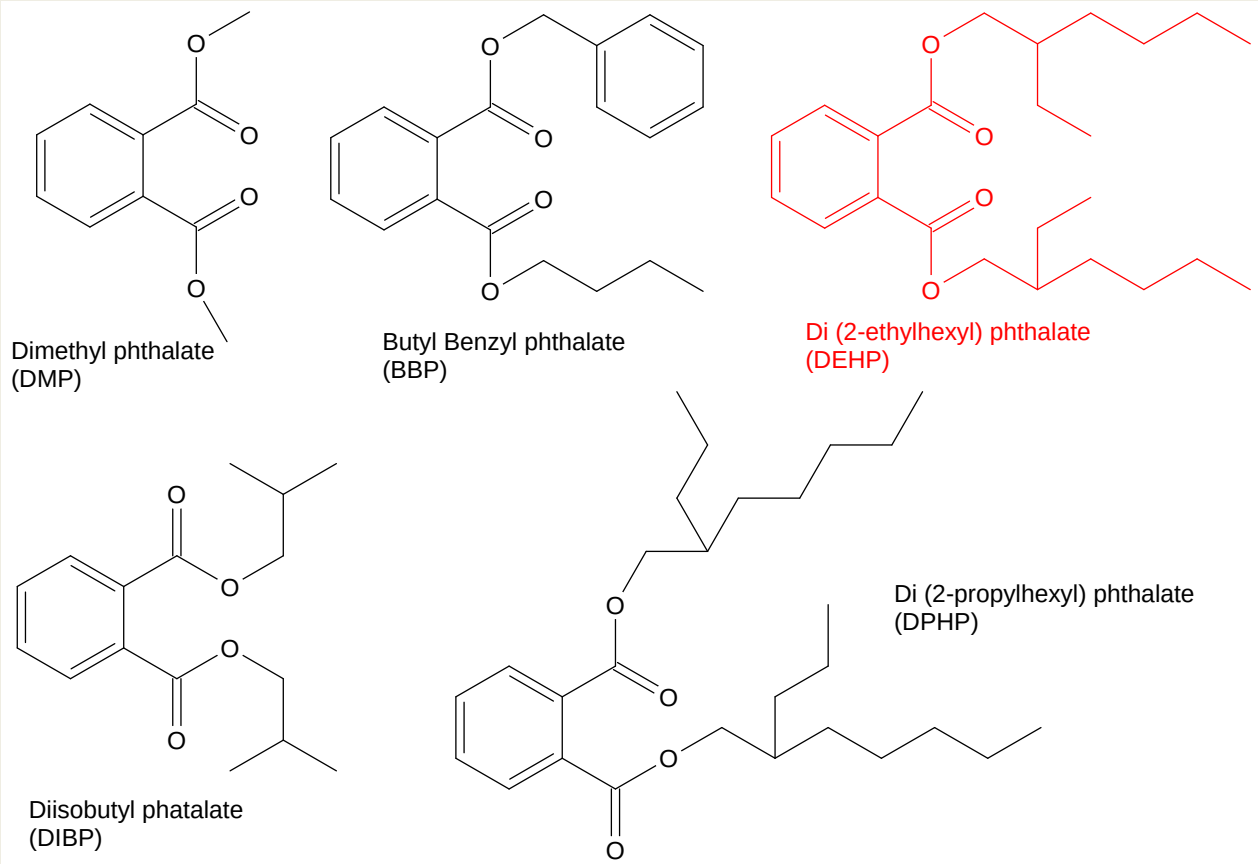


BACKGROUND

Phthalate esters (PAEs)

- ✓ Used as **plasticizers**
- ✓ Endocrine disrupting chemicals (EDCs)
- ✓ Physically (rather than chemically) bonded to the plastic matrix



BACKGROUND

Di(2-ethylhexyl) phthalate (DEHP)

- ✓ One of the most cost effective and widely available general purpose plasticizers
- ✓ PVC, toys, and medical devices
- ✓ Dysfunction of endocrine, reproductive, and nervous systems
- ✓ Possible human **carcinogen** since 1987 (USEPA)

DEHP concentration in (waste)water in China (Wang et al., 2011)

Contaminant	industrial wastewater (μg/L)	well water (μg/L)	pond water (μg/L)	Standard drinking water (μg/L)
DEHP	**42.4**	**14.2**	**135.7**	**8**

Wang et al., 2011. Environ. Sci. Pollut. Res. 18, 987-996

BACKGROUND

Physicochemical processes

✓ Adsorption, Membrane filtration, Advanced oxidation processes (AOPs)

No mineralization
Generation of by-products
Not cost effective

Biological process

✓ Anaerobic and aerobic conditions

Environmentally friendly
Cost effective
Contaminant mineralization

No research about the effects of microelements (inhibitory/stimulatory) on PAEs biodegradation

OBJECTIVES

- ✓ To remove DEHP from artificially contaminated water using indigenous bacterial isolate, *Acinetobacter* sp.
- ✓ To optimize the DEHP biodegradation process
- ✓ To evaluate growth kinetics and biodegradation pathway for DEHP by the isolate
- ✓ To evaluate the effects of microelements (Fe^{3+} and Mn^{2+}) on DEHP biodegradation

MATERIALS AND METHODS

Microbial isolation

✓ Activated sludge samples from wastewater treatment plant (Macau SAR, China)

Enrichment

✓ Enriched in nutrient broth (3 g l^{-1} beef extract + 5 g l^{-1} peptone)

✓ Cultured in a basal salt medium (BSM) with increasing DEHP concentration (from 10 to 500 mg l^{-1}) as sole carbon source

Microbial identification

✓ 16S rRNA gene sequence analysis

✓ Sequences deposited in NCBI GenBank under the accession number KX_670538

MATERIALS AND METHODS

Experimental setup

Temperature, pH, and microelements

Inoculum (5 ml)
BSM solution (45 ml) spiked with 100 mg l^{-1} DEHP

Temperatures (25°C, 30°C, 35°C)
pHs (3, 5, 7, 8, 9)
Microelements Fe^{3+} and Mn^{2+} (100, 500, 1,000 µg l^{-1})

Kinetic studies:

Initial DEHP concentrations (10-500 mg l^{-1}) at pH 7.0±0.2 and 30°C.

- ✓ Treatments incubated in the dark at 150 rpm and 30ºC, in replicates
- ✓ DEHP concentrations and OD_{600} were determined in every 24 h for 5 days
- ✓ One-way analysis of variance (ANOVA) at the 95% confidence interval

MATERIALS AND METHODS

Analytical methods

DEHP concentration: HPLC/DAD (Thermo Fisher Scientific, USA)

Column: AcclaimTM C18 (5 μm, 4.6 x 150 mm), temperature 45℃

Mobile phase: acetonitrile:deionized water (9:1), flow rate 0.5 ml min^{-1}

- Linearity:10 to 500 mg l^{-1} (n=3), r^2 0.9977
- Precision (100 mg l^{-1}):

  Repeatability (n=6), 0.51%
  Intermediate precision (d=6), 1.35%

- Accuracy (100 mg l^{-1}): 100.4±1.67% spike-recovery

Biodegradation pathway: HPLC-MS (Thermo Fisher Scientific, USA)

Electrospray ionization (ESI) source

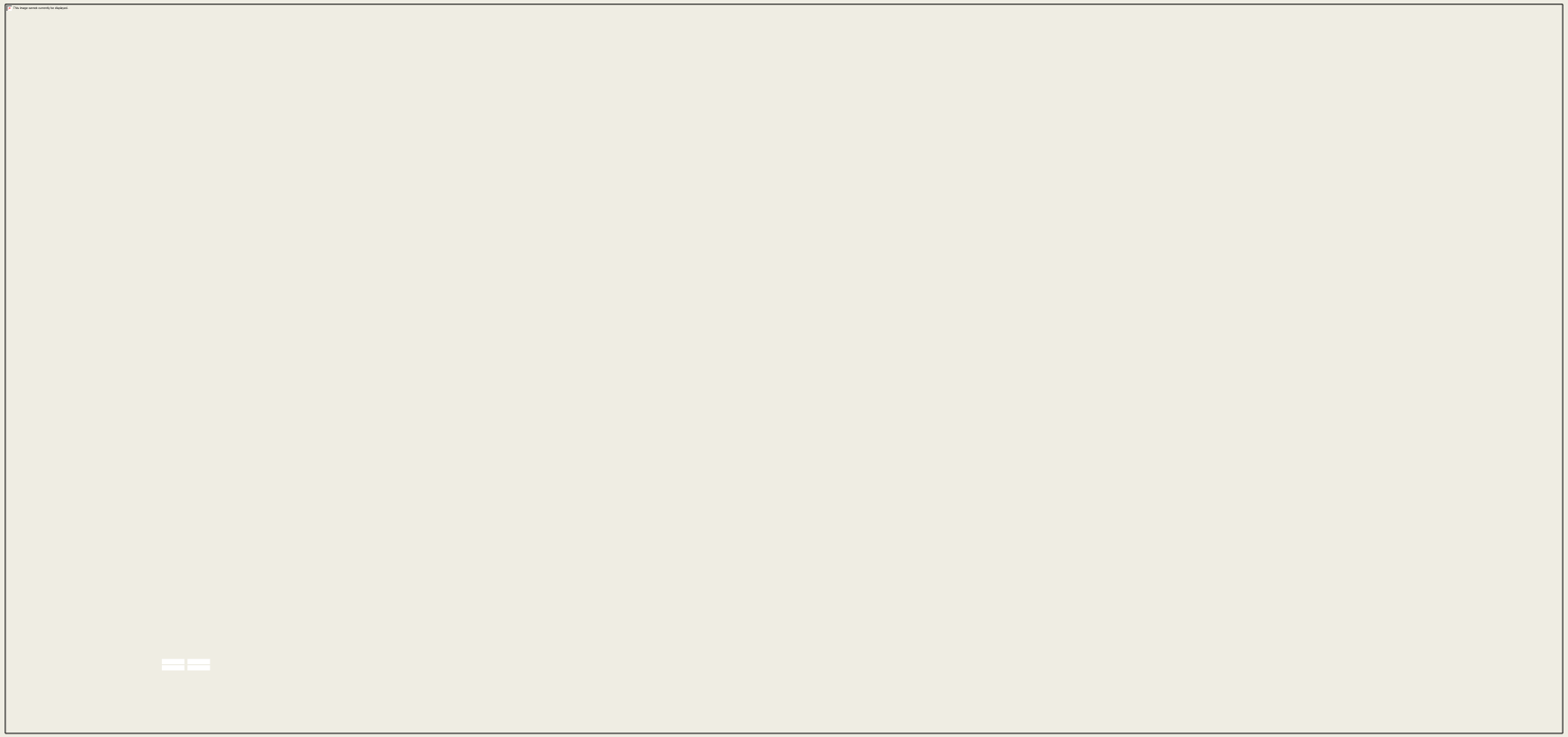
Probe temperature: 300℃

Polarity: positive

Optical density: UV mini-1240 spectrophotometer (Shimadzu, Japan)

RESULTS

Microorganism identification



Phylogenetic tree for the isolate *Acinetobacter* sp.

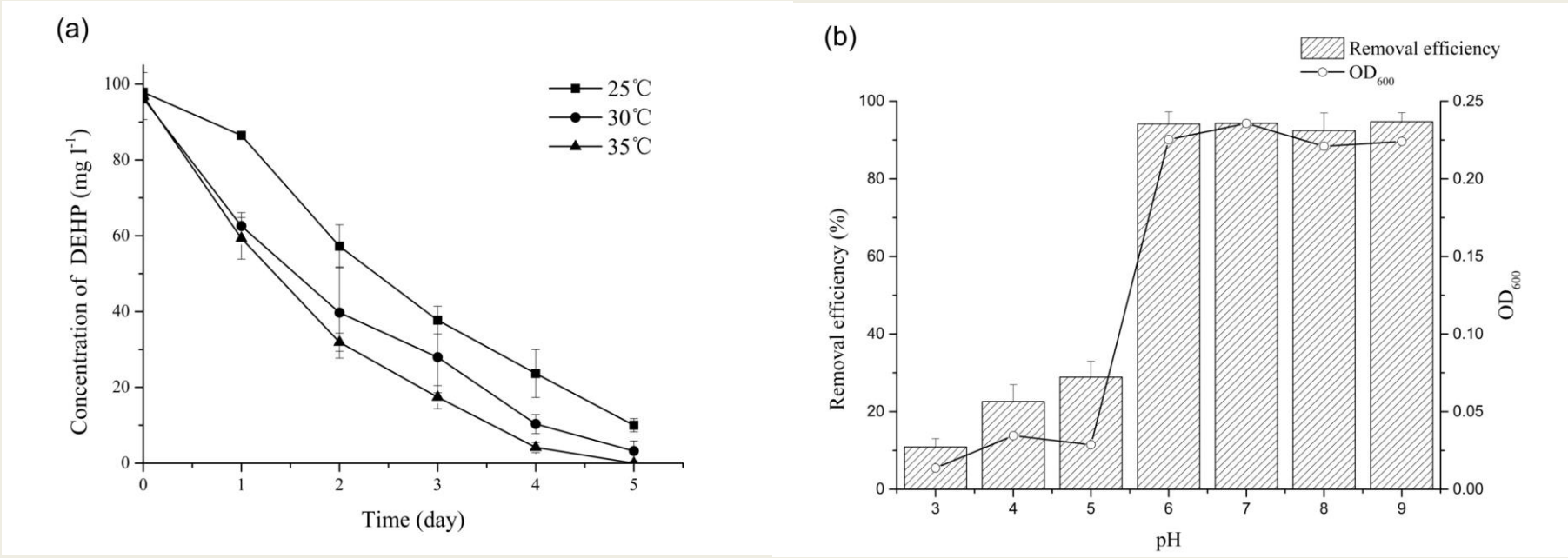
RESULTS

Effects of temperature and pH

There was no significant difference in DEHP biodegradation:

35°C and 30°C (p=0.22)
pH 6-9 (p=0.87)

Neutral pH is also considered optimal for the growth of other *Acinetobacter* spp.



Effects of temperature (a) and pH (b) on DEHP biodegradation

RESULTS

Biodegradation kinetics

Substrate inhibition kinetics:

$$D = D_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}}$$

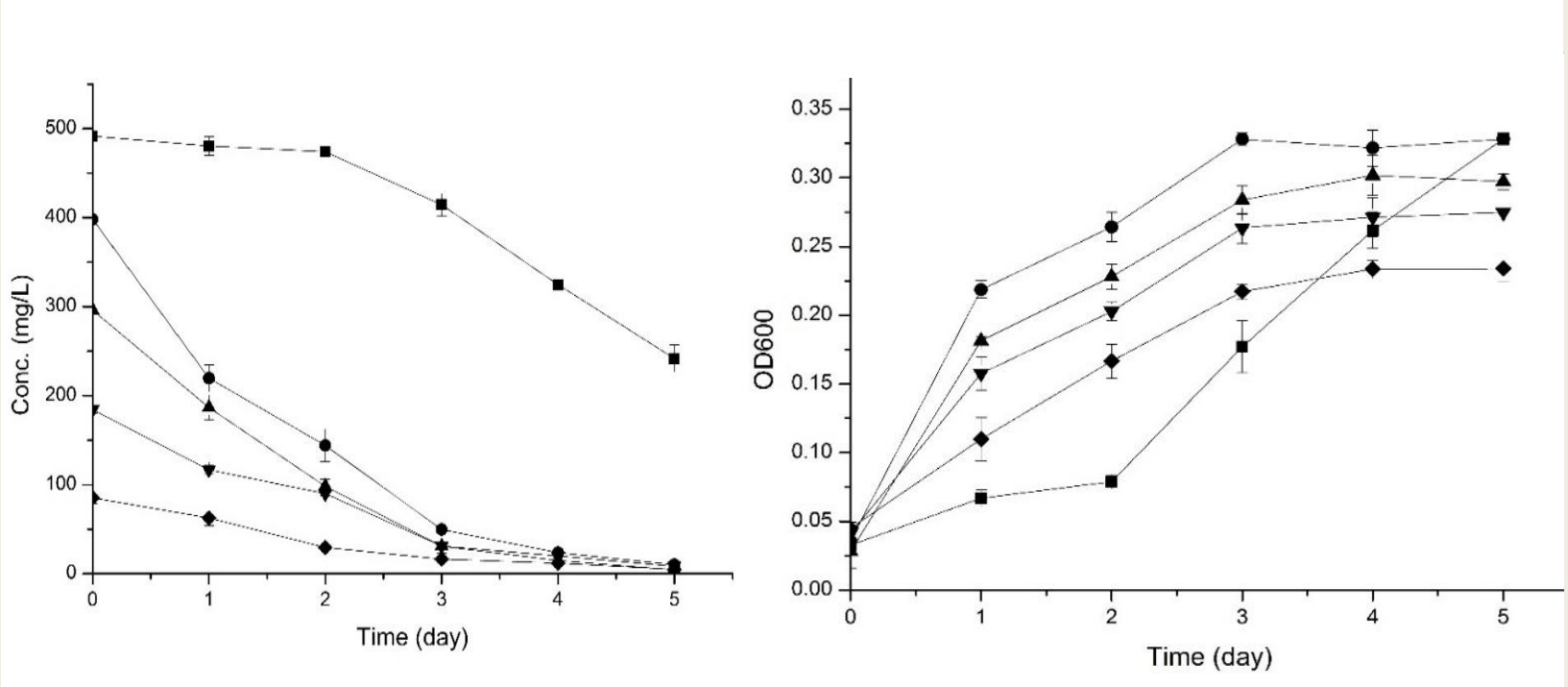
$$\mu = \mu_{max} \frac{S}{K_s + S + \frac{S^2}{K_i}}$$

Biodegradation kinetics parameters for the isolate *Acinetobacter* sp. grown on DEHP

Parameter	Degradation rate	Specific growth rate
D_{max} (mg/L•day)	124.8	-
μ_{max} (day^{-1})	-	0.1192
K_S (mg/L)	272.3	137.6
K_i (mg/L)	720.5	850.3

RESULTS

DEHP biodegradation and cell growth

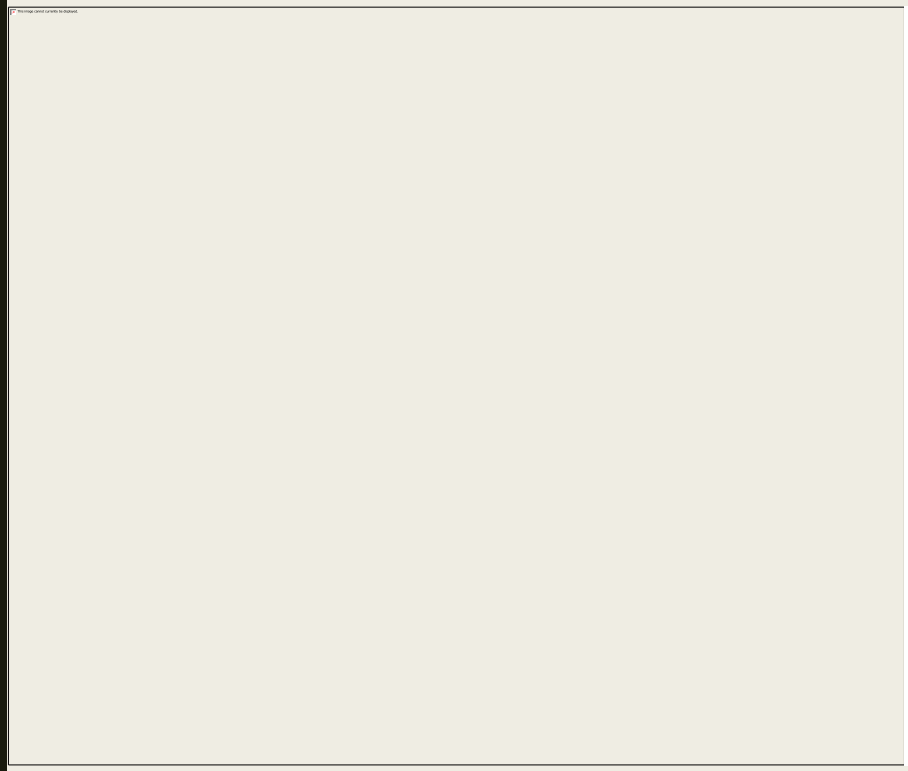


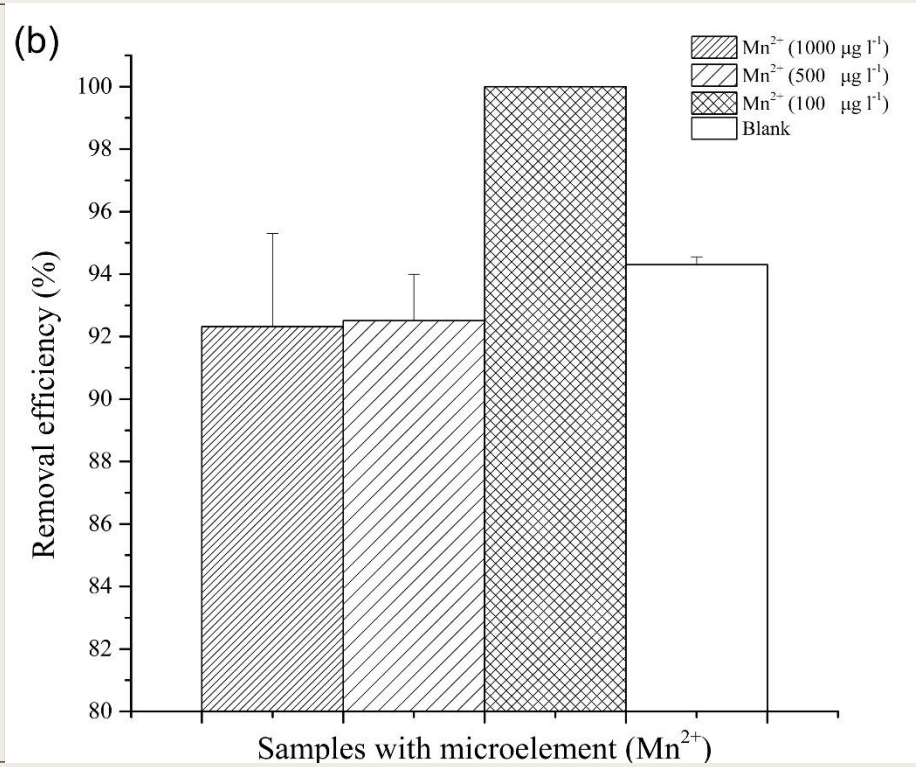
(A) **(B)**

DEHP biodegradation (A) and cell growth (B) at different initial concentrations: (-■-) 500; (-●-) 400; (-▲-) 300; (-▼-) 200; and (-◆-) 100 mg/L

RESULTS

Effects of microelements

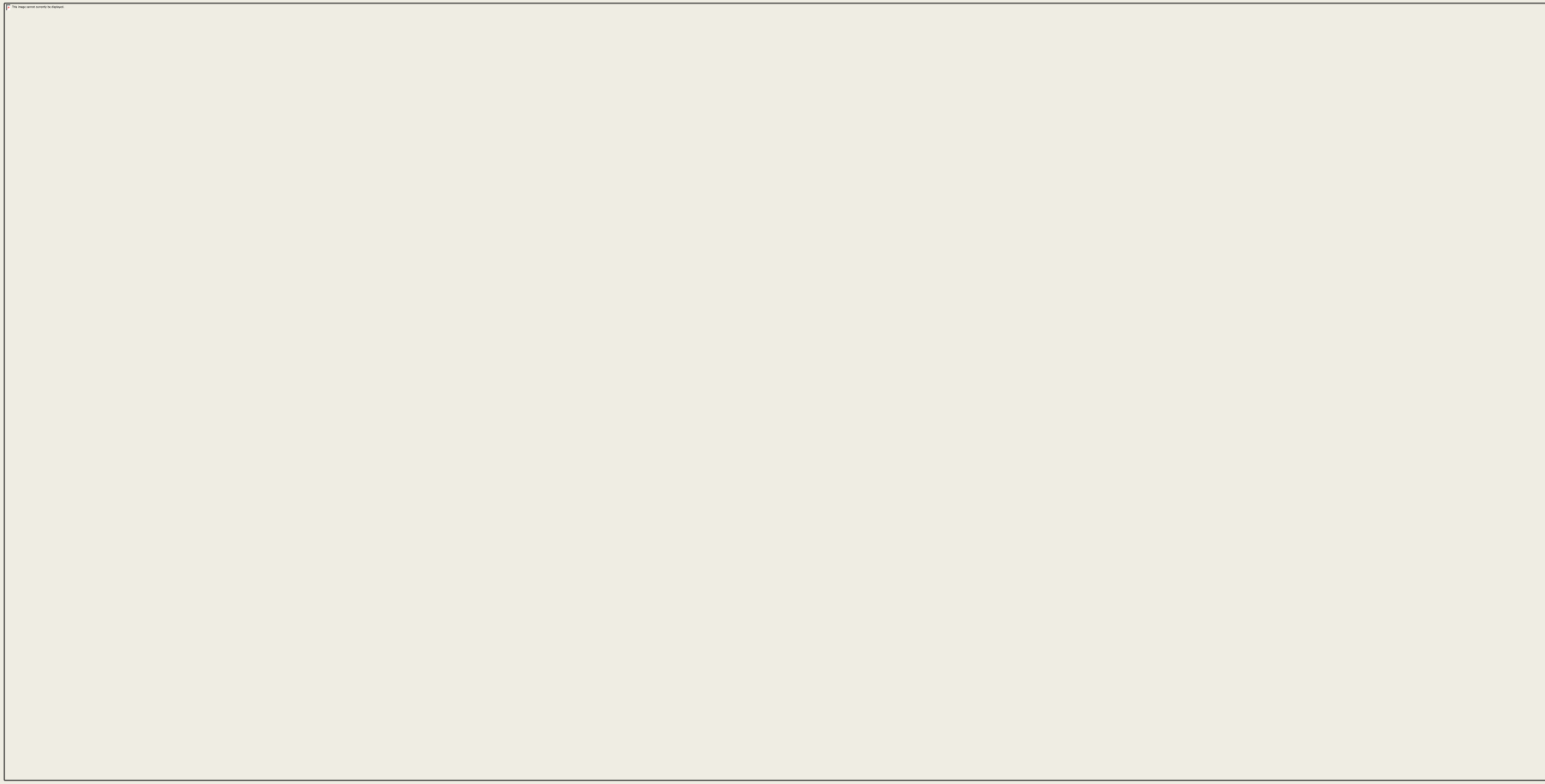




DEHP removal efficiencies after the addition of (a) Fe^{3+} and (b) Mn^{2+} at different concentrations

RESULTS

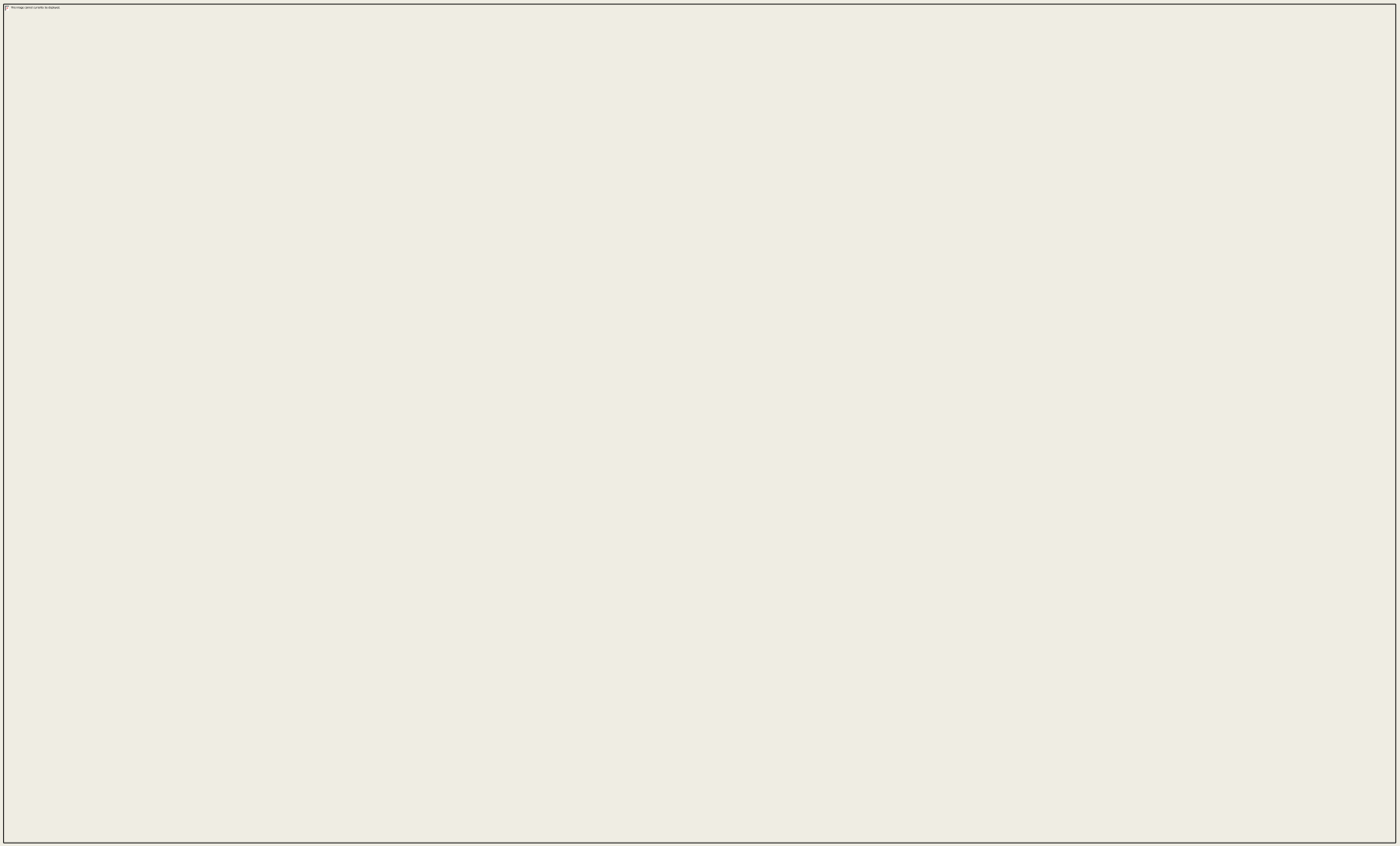
Intermediates identification by HPLC-MS



Mass spectra of DEHP (a), MEHP (b), β-carboxy-*cis*,*cis*-muconic acid (c), 3-katoadipate (d), and di-ethyl hexanoic acid (e)

RESULTS

DEHP biodegradation pathway



Proposed DEHP biodegradation pathway for the isolate

CONCLUSIONS

- ✓ The optimal temperature for the biodegradation is considered 30ºC and the neutral and alkaline conditions are shown favourable for DEHP degradation by *Acinetobacter* sp. SN13.

- ✓ High concentrations of DEHP (500 mg/L) were inhibitory to both biodegradation and cell growth.

- ✓ Ferric ion at 100-1,000 μg l^{-1} showed the stimulatory effect on the DEHP biodegradation, while Mn^{2+} was stimulatory at the lower concentration (100 μg l^{-1}) but inhibitory at higher concentrations (500-1,000 μg l^{-1}).

- ✓ The biodegradation pathway for DEHP by the isolate is proposed with some metabolic products identified.

- ✓ The biological process could be further scaled up and applied to treat different types of wastewater, especially the ones containing high concentration levels of DEHP and other PAEs generated from the plastics industries.

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