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Aerobic Bioremoval of BTEX, cis-DCE, and TCE Mixtures from the Contaminated Environment

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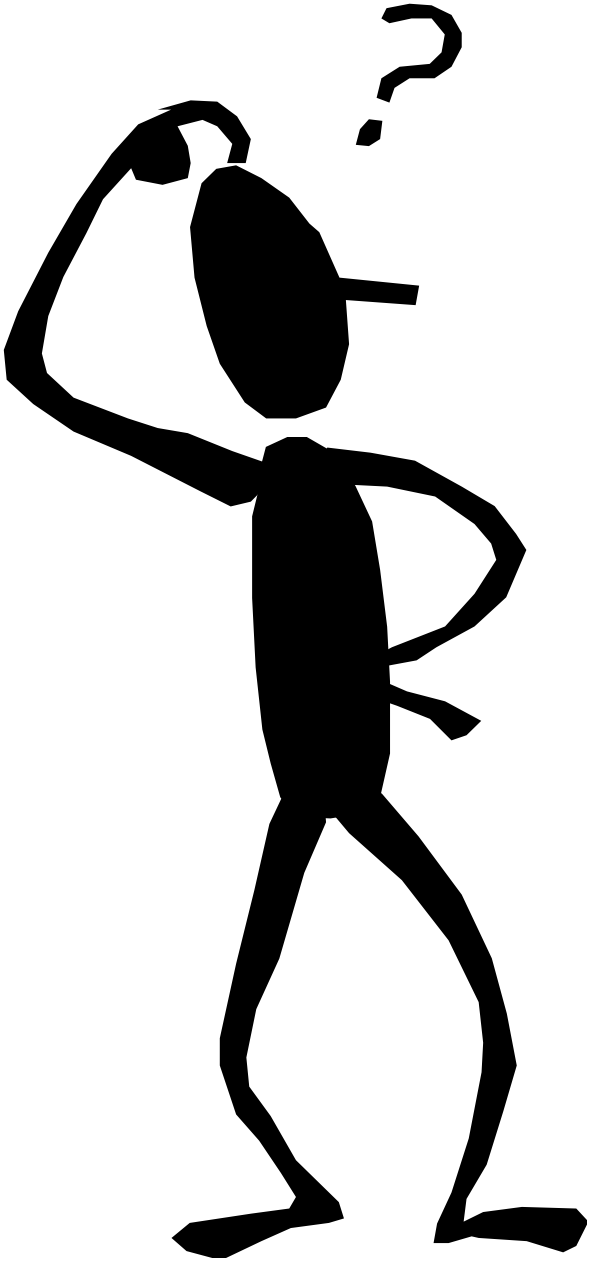
Terms

- Benzene, toluene, ethylbenzene, & three isomers (*ortho-, meta-, & para-*) of xylene, collectively known as BTEX, major aromatic components in unleaded gasoline;

- Chlorinated aliphatic hydrocarbons (CAHs): PCE, TCE, (1,1-/*cis*-1,2-/*trans*-1,2-)DCE, VC;

- Volatile organic compounds (VOCs).



WHY BTEX & CAHs?

Introduction

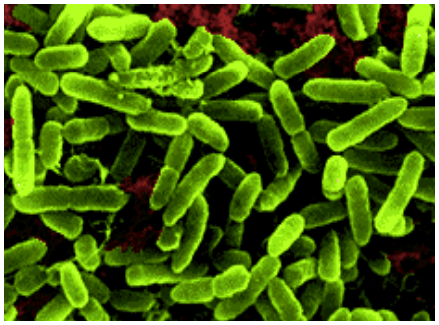
- **BTEX & CAHs are toxic (volatile and known carcinogenic) and very commonly found contaminants in subsurface (soil and groundwater)**

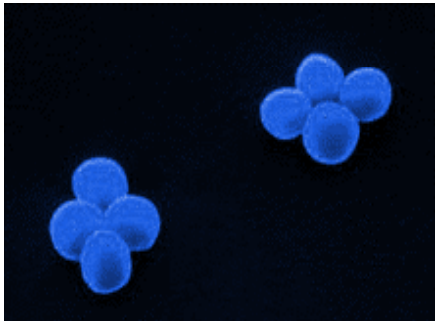
- **BTEX even at low concentrations can cause damage to liver and kidney and paralyze central nervous system**

- **CAHs used extensively as degreasing and dry cleaning solvents; especially TCE, suspected carcinogen and considered toxic to humans, and among US EPA's (2005) priority pollutant list**

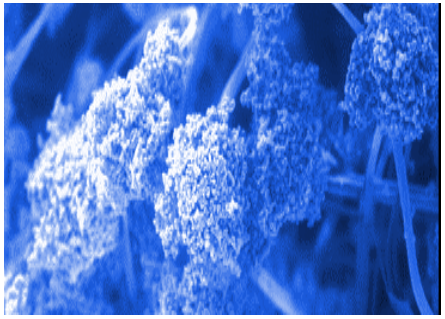
Introduction

- Biological remediation considered not only cost-effective but also more environmentally sound approach as indicated by reduced ecotoxicity
- Bacteria, most important





- Fungi also play important roles



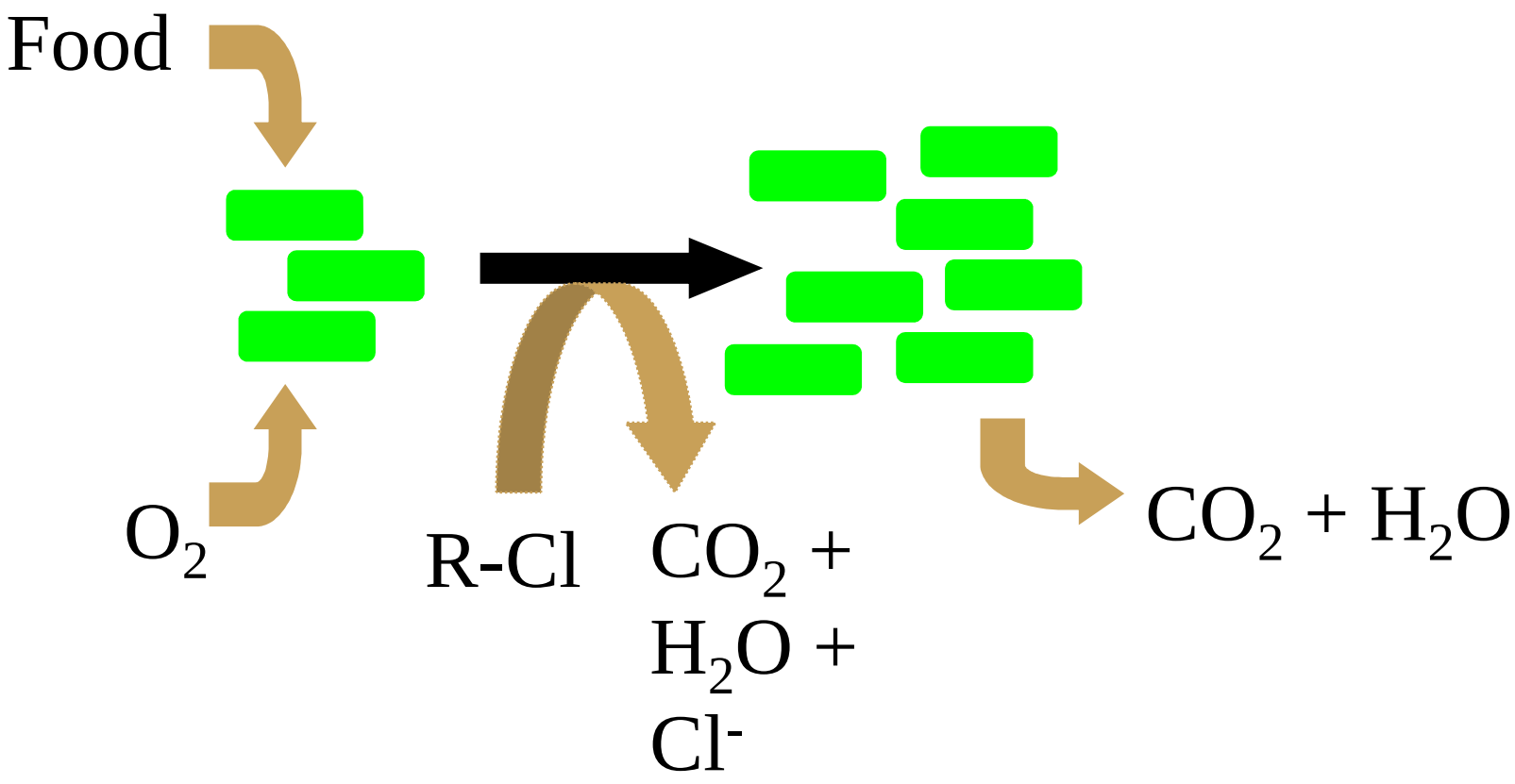
Introduction

- No microbial isolate yet to grow on TCE as sole carbon and/or energy source

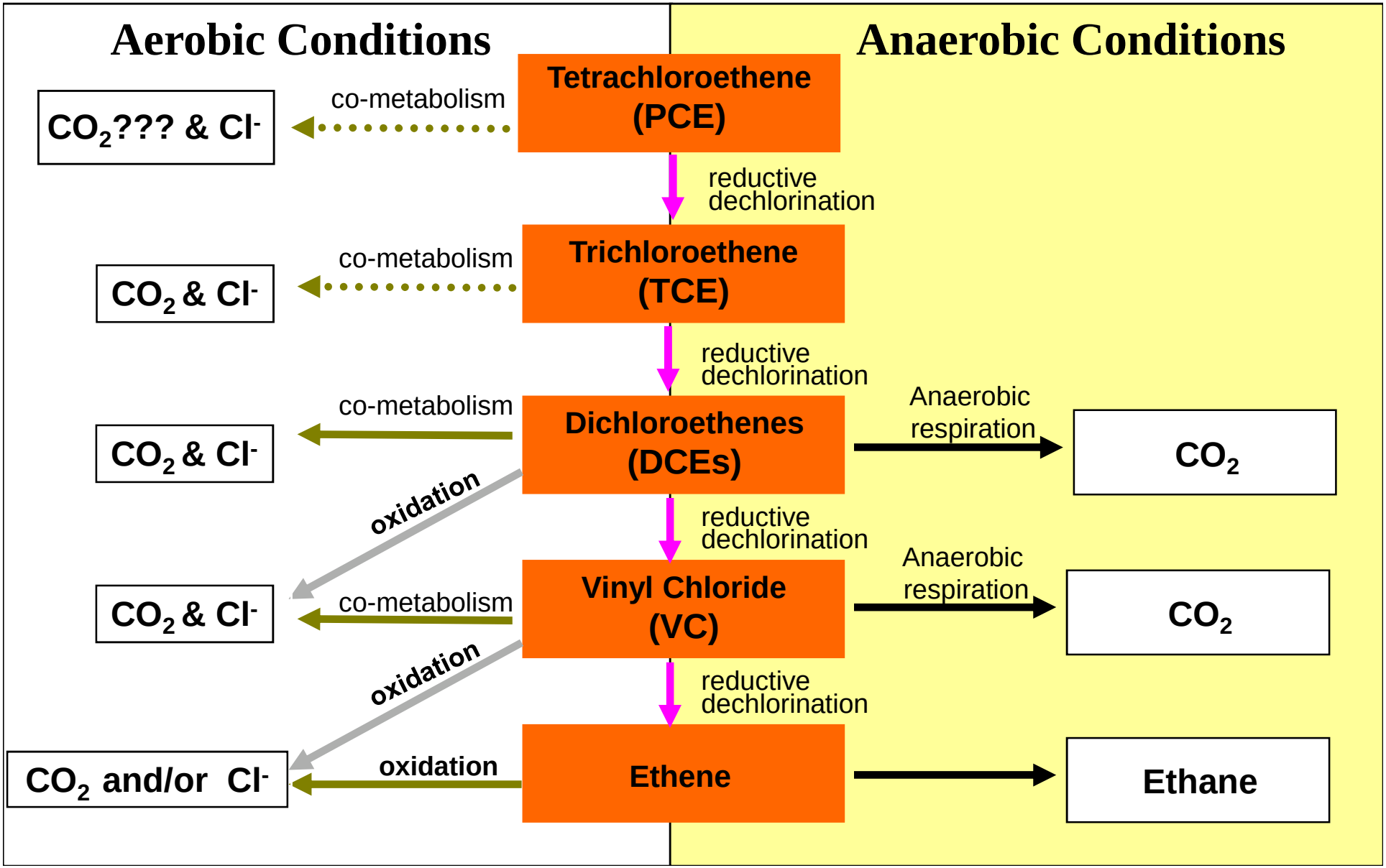
- TCE transformed/co-metabolized through catalysis of microbial enzymes (e.g., TOM, TOD, ToMO, MMO, AMO) with presence of substrates (e.g., T, *o*-X, methanol, ammonia)

- When BTEX & CAHs co-exist, interactions (stimulatory/inhibitory) among them toward degradation/co-metabolism, results complicated and contradictory so far, but understanding these interactions will be beneficial to the applications of bioremediation

Aerobic Co-metabolism



PCE/TCE Degradation Pathways



Introduction

- **This anaerobic process for PCE & TCE is often incomplete when it does occur, resulting primarily in the formation of *cis*-DCE & VC. *cis*-DCE along with TCE chosen as representative CAHs;**

- **Co-contaminated environments generally considered difficult to remediate due to the mixed nature of contaminants; when contaminated together (e.g., airport), more beneficial (cf. co-metabolism of CAHs with gasoline/petroleum hydrocarbons as substrates);**

- **This study focuses on the optimization of aerobic bioremoval of BTEX and CAHs in soil**

Research methodology: Isolation of Microorganisms

Table 1 Indigenous microbial isolate

Isolate	Location of the collected sample	Medium
1	A construction site nearby a gas station in Fai Chi Kei, Macau SAR	Soil
2	A construction site nearby a gas station in Fai Chi Kei, Macau SAR	Soil
3	A heavily oil-contaminated site in Xiamen	Soil
4	Macau airport wastewater treatment plant	Sludge
5	Macau airport wastewater treatment plant	Sludge
6	A canal near the New Century Hotel & Casino in Taipa, Macau SAR	Sediment
7	A canal near the New Century Hotel & Casino in Taipa, Macau SAR	Sediment
8	A canal near the New Century Hotel & Casino in Taipa, Macau SAR	Sediment

Methodology: Isolation and cultivation

Pure Culture

Methodology: analysis

- VOCs concentrations measured by GC system (Agilent, 6890N) equipped with FID

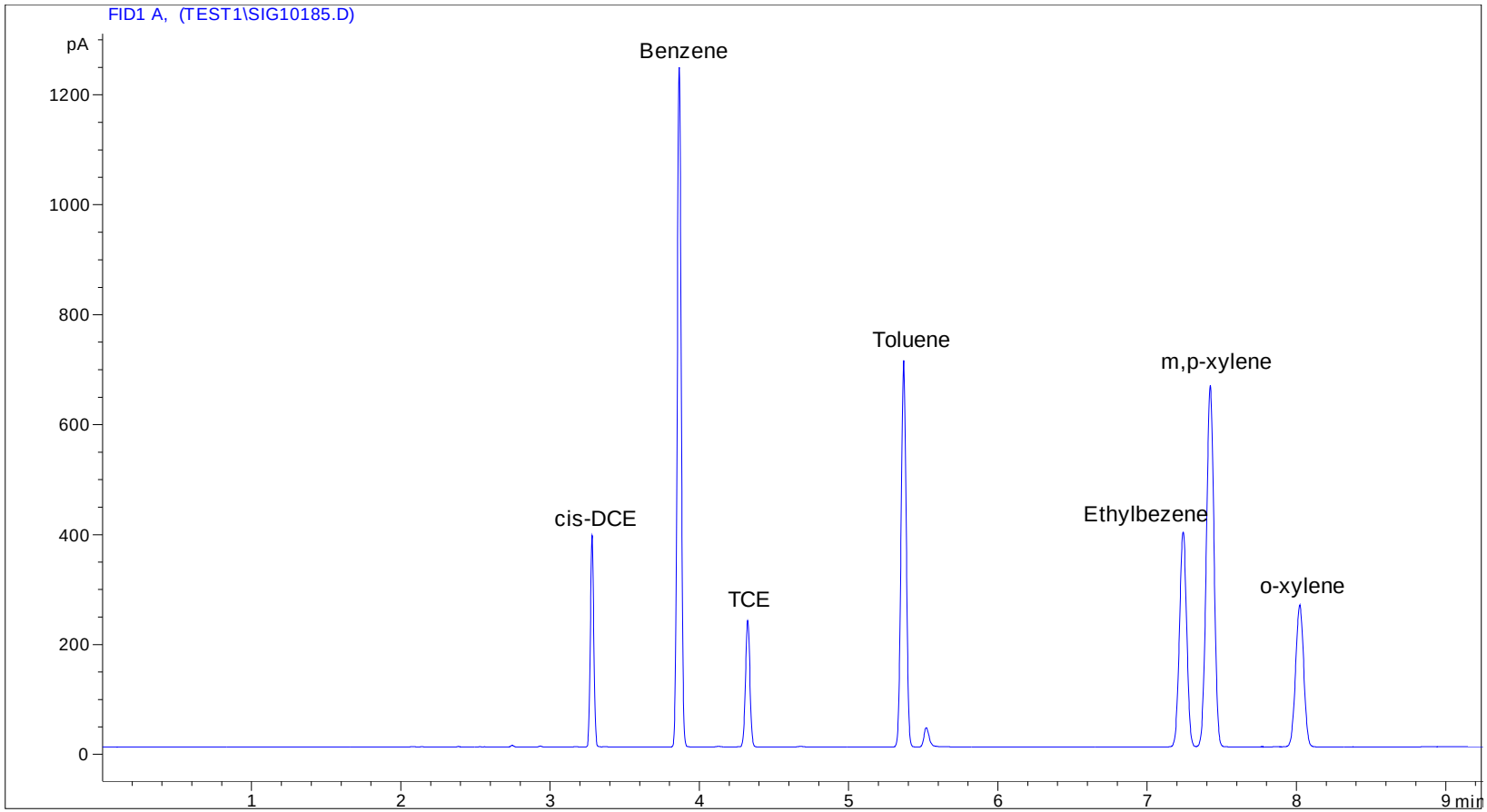


Fig.1 Gas chromatograms for VOCs (each, 100 ppm)

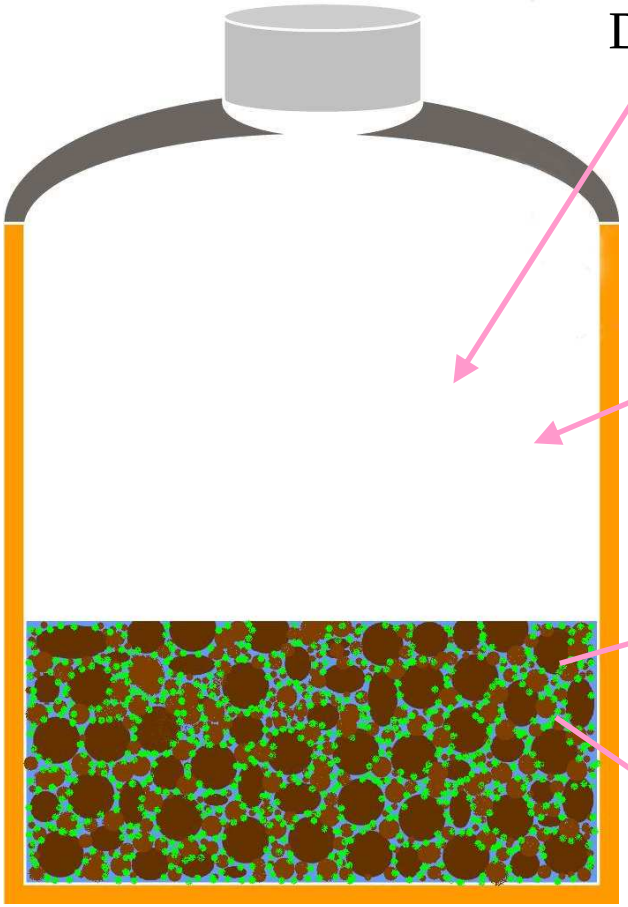
Location of the soil sample

Fig.2 Location of the soil sample used in the experiment

Table 2 Basic soil properties

Parameter	Result	Method
Organic carbon	2.94 %	Wet oxidation
Total nitrogen	1,775.3 mg/kg	Kjeldahl method
Bioavailable nitrogen	41.7 mg/kg	Extracted using 2N KCl solution
Ammonia-nitrogen	36.6 mg/kg	Extracted using 2N KCl solution
Total phosphorus	175.3 mg/kg	Spectrophotometry
Olsen phosphorus	1.42 mg/kg	Extracted using 0.5M $NaHCO_3$ solution
Total potassium	40,042.1 mg/kg	Flame photometry
pH	4.96	1:2.5 w/v soil to distilled water ratio
Moisture	1.58%	Gravimetrical method
WHC	50.14%	Gravimetrical method
Soil texture	clay	Pipette method
sand: 0.05-2 (mm)	36.32%	
silt: 0.002-0.05 (mm)	21.27%	
clay: <0.002 (mm)	42.41%	

Experimental Design



160 mL serum bottles

Desired concentration of BTEX, *cis*-DCE and TCE

Desired level of water and nutrient

20 g of soil (DW)

5 mL early stationary phase inocula

Pretreatment of soil sample

- Prior to use, 20 g of soil (dry weight) was added into the serum bottles, covered with aluminum foil, and autoclaved for 1 h (121℃, 103.5 KPa) on three consecutive days to eliminate viable microorganisms;
- The soil pH was adjusted by adding NaOH (2 N) solution. Five milliliters of early stationary phase inocula (with the incubation period of 72 h at 25℃ and 150 rpm) with OD (600 nm) of 0.437±0.018 was transferred as microorganism source;
- All the treatments were carried out in triplicate, and each bottle was monitored twice.

Results & Discussion

A four-factor central composite design (CCD) was employed to study the effect of pH, temperature, moisture and concentration of CAHs on the removal efficiency of VOCs.

Table 3 Experimental ranges and levels of independent variables for experiments

	Range and levels				
Factors	-2	-1	0	+1	+2
pH	6	7	8	9	10
Temperature (℃)	15	20	25	30	35
Moisture (% of water holding capacity) [a]	70	90	110	130	150
Concentration of CAH (ppm) [b]	0	2.5	5	7.5	10

[a]50.14%, [b]The growth substrate concentration is 400 ppm.

Table 4 Central composite design in experimental runs & biodegradation efficiencies.

Run	X_1	X_2	X_3	X_4	benzene	toluene	ethylbenzene	*cis*-DCE	TCE	*m,p*-xylene	*o*-xylene
1	-1(7)	-1(20)	-1(90)	-1(2.5)	*37.6*	44.3	39.8	11.9	21.3	19.2	14.3
2	1(9)	-1(20)	-1(90)	-1(2.5)	93.5	87.0	72.3	24.7	28.3	25.4	12.3
3	-1(7)	1(30)	-1(90)	-1(2.5)	20.2	33.1	36.0	18.1	*13.6*	29.4	26.4
4	1(9)	1(30)	-1(90)	-1(2.5)	*77.6*	87.1	70.4	14.4	*18.6*	12.4	6.2
5	-1(7)	-1(20)	1(130)	-1(2.5)	96.9	95.7	84.2	21.7	29.3	34.8	27.2
6	1(9)	-1(20)	1(130)	-1(2.5)	97.4	94.0	77.2	19.7	20.0	10.5	7.3
7	-1(7)	1(30)	1(130)	-1(2.5)	88.3	85.9	70.4	15.3	16.4	22.9	14.9
8	1(9)	1(30)	1(130)	-1(2.5)	85.7	84.0	64.9	7.8	5.1	0.2	0
9	-1(7)	-1(20)	-1(90)	1(7.5)	68.2	72.5	72.9	15.1	22.5	47.8	44.7
10	1(9)	-1(20)	-1(90)	1(7.5)	92.2	*86.4*	*77.5*	20.0	25.5	43.7	37.3
11	-1(7)	1(30)	-1(90)	1(7.5)	0.6	5.4	9.9	*1.0*	3.0	3.8	1.7
12	1(9)	1(30)	-1(90)	1(7.5)	22.1	42.2	37.7	3.5	8.0	7.4	4.2
13	-1(7)	-1(20)	1(130)	1(7.5)	96.7	96.3	85.1	*5.4*	22.1	15.5	7.9
14	1(9)	-1(20)	1(130)	1(7.5)	97.9	95.3	79.2	15.1	12.5	12.8	3.5
15	-1(7)	1(30)	1(130)	1(7.5)	83.4	86.4	77.9	9.9	15.3	43.3	38.7
16	1(9)	1(30)	1(130)	1(7.5)	86.4	87.8	69.2	7.1	3.6	7.4	3.4
17	-2(6)	0(25)	0(110)	0(5)	13.7	27.4	35.9	10.9	20.3	33.4	31.3
18	2(10)	0(25)	0(110)	0(5)	88.1	89.8	71.8	9.7	11.5	12.1	5.8
19	0(8)	-2(1.5)	0(110)	0(5)	98.7	98.6	94.2	27.0	37.1	52.2	46.5
20	0(8)	2(35)	0(110)	0(5)	9.1	19.2	21.6	*5.6*	9.8	17.6	14.9
21	0(8)	0(25)	-2(70)	0(5)	85.8	88.5	74.2	10.3	10.8	23.4	18.3
22	0(8)	0(25)	2(150)	0(5)	*97.0*	*95.2*	*86.2*	18.6	21.3	43.5	35.5
23	0(8)	0(25)	0(110)	-2(0)	97.4	95.9	90.3	-	-	48.6	36.1
24	0(8)	0(25)	0(110)	2(10)	94.7	94.4	82.4	15.9	14.0	19.1	11.6
25	0(8)	0(25)	0(110)	0(5)	97.4	96.3	90.3	14.1	12.5	39.4	24.9
26	0(8)	0(25)	0(110)	0(5)	97.4	96.3	90.3	14.1	12.5	39.4	24.9
27	0(8)	0(25)	0(110)	0(5)	97.4	96.3	90.3	14.1	12.5	39.4	24.9
28	0(8)	0(25)	0(110)	0(5)	97.4	96.3	90.3	14.1	12.5	39.4	24.9
29	0(8)	0(25)	0(110)	0(5)	97.4	96.3	90.3	14.1	12.5	39.4	24.9
30	0(8)	0(25)	0(110)	0(5)	97.4	96.3	90.3	14.1	12.5	39.4	24.9

The values in italic were ignored toward the model analysis owing to the comparatively high differences between the predicted and actual values;

-which means the missing data due to the original concentrations of both *cis*-DCE and TCE in Run 23 were 0;

Statistical analysis

- **Multiple regression coefficients were determined to quadratic polynomial models for the response variables studied. The experimental results of response surface methodology (RSM) were fitted via the response surface regression procedure, using the following second order polynomial equation:**

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_{ij} \beta_{ij} X_i X_j + \sum_{ii} \beta_{ii} X_i^2$$

Generalized polynomial model:

$$Y_i = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{33} X_3^2 + \beta_{44} X_4^2 + \beta_{12} X_1 X_2 + \beta_{13} X_1 X_3 + \beta_{14} X_1 X_4 + \beta_{23} X_2 X_3 + \beta_{24} X_2 X_4 + \beta_{34} X_3 X_4$$

Reduced response surface models

- The data were subjected analysis of variance (ANOVA). The significances of the model equation and the equation parameters for each response variable were assessed by F-ratio at a probability (p) of 0.05 and 0.10, respectively.

Table 5 One-way ANOVA for RSM parameters fitted to polynomial equation

Source of variation	benzene	toluene	ethylbenzene	*cis*-DCE	TCE	*m,p*-xylene	*o*-xylene
F-value	18.76	17.98	12.38	11.77	33.43	5.03	3.87
Probability > F	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0070	0.0141
R^2 *	0.8988	0.8999	0.8320	0.7370	0.9093	0.3673	0.3821
CV (%)	16.17	13.71	15.76	21.88	17.41	46.51	57.73
Adi-R^2	0.8509	0.8498	0.7649	0.6744	0.8821	0.2943	0.2833
Pre-R^2	0.6857	0.6745	0.5121	0.5601	0.8341	0.0745	0.0437

* multiple correlation coefficient

Table 6 Reduced response surface model equations

Response variables	Polynomial equations
benzene	$98.01292 + 12.90686X_1 - 16.45294X_2 + 17.25672X_3 - 2.29757X_4 - 9.79368X_1X_3 + 7.85651X_2X_3 - 6.80293X_2X_4 - 12.69636X_1^2 - 11.94269X_2^2$
toluene	$97.10361 + 12.51437X_1 - 14.56037X_2 + 12.67881X_3 - 0.45032X_4 - 11.3554X_1X_3 + 7.27520X_2X_3 - 8.06196X_2X_4 - 10.13153X_1^2 - 10.04923X_2^2$
ethylbenzene	$86.78920 + 5.99899X_1 - 12.38052X_2 + 10.84520X_3 - 0.89973X_4 - 7.88298X_1X_3 - 5.51466X_2X_4 - 10.05898X_1^2 - 9.04572X_2^2$
***cis*-DCE**	$15.28669 - 0.30882X_1 - 4.09630X_2 - 1.82766X_4 - 2.36165X_1X_2 - 1.14219X_1^2$
TCE	$14.19812 - 1.67208X_1 - 6.92365X_2 + 0.75117X_3 - 1.34401X_4 - 4.71566X_1X_3 + 2.34899X_2^2$

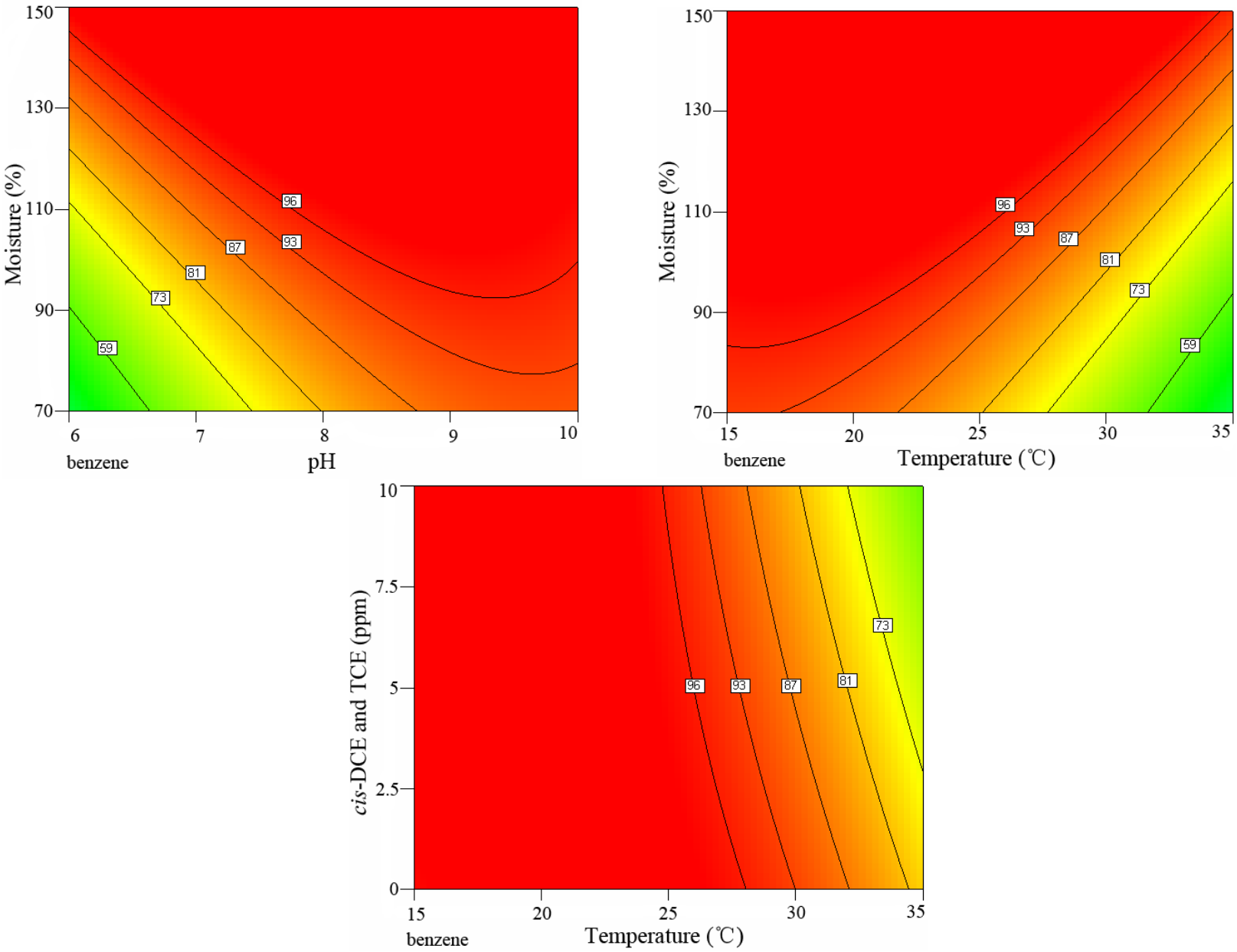


Fig.3 Contour surface plot for bioremoval of benzene

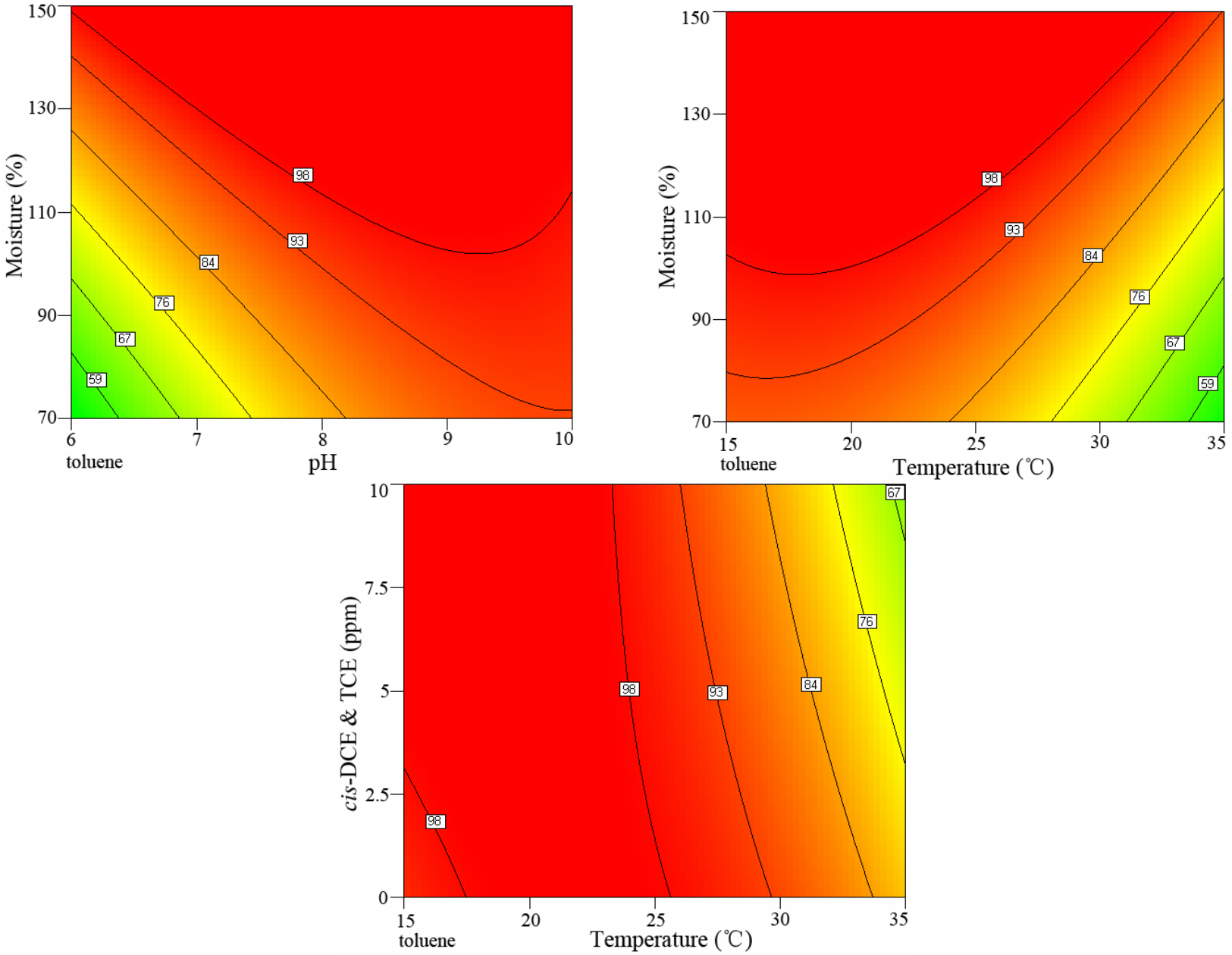


Fig.4 Contour surface plot for bioremoval of toluene

Contour surface plots

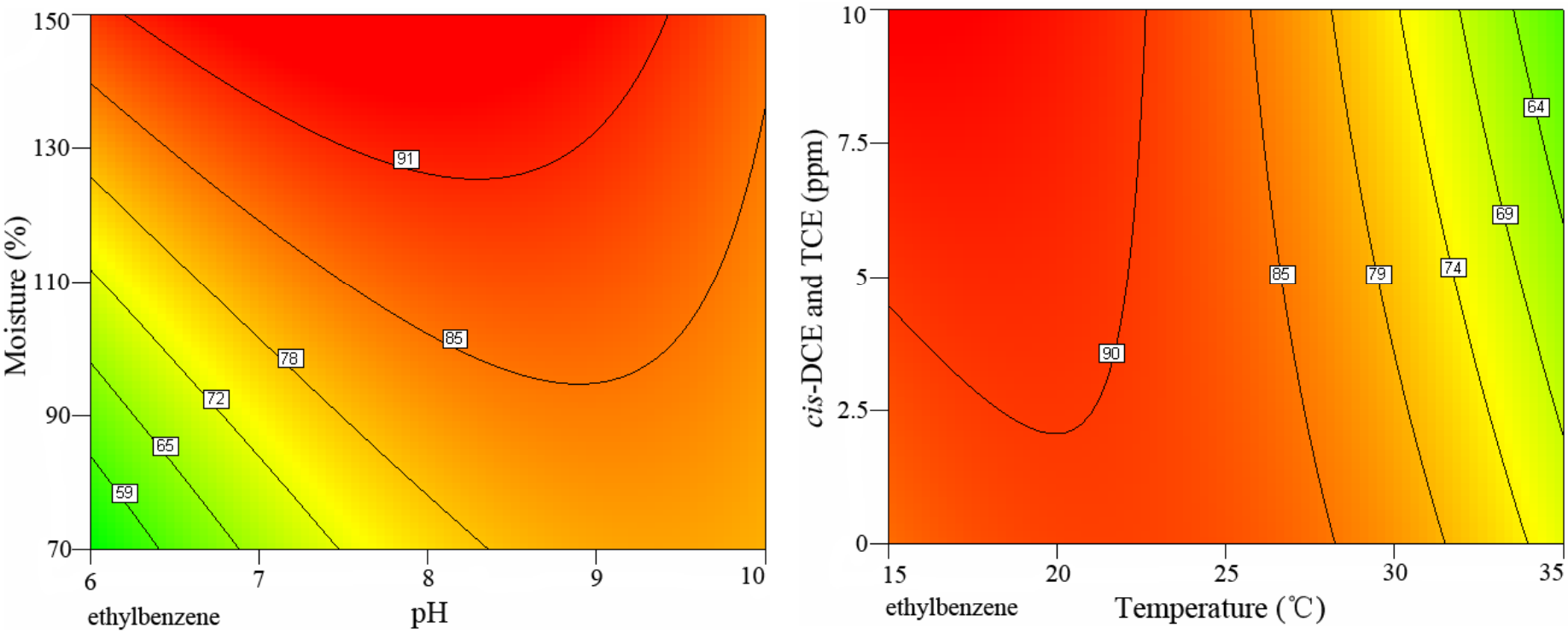


Fig.5 Contour surface plot for bioremoval of ethylbenzene

Contour surface plots

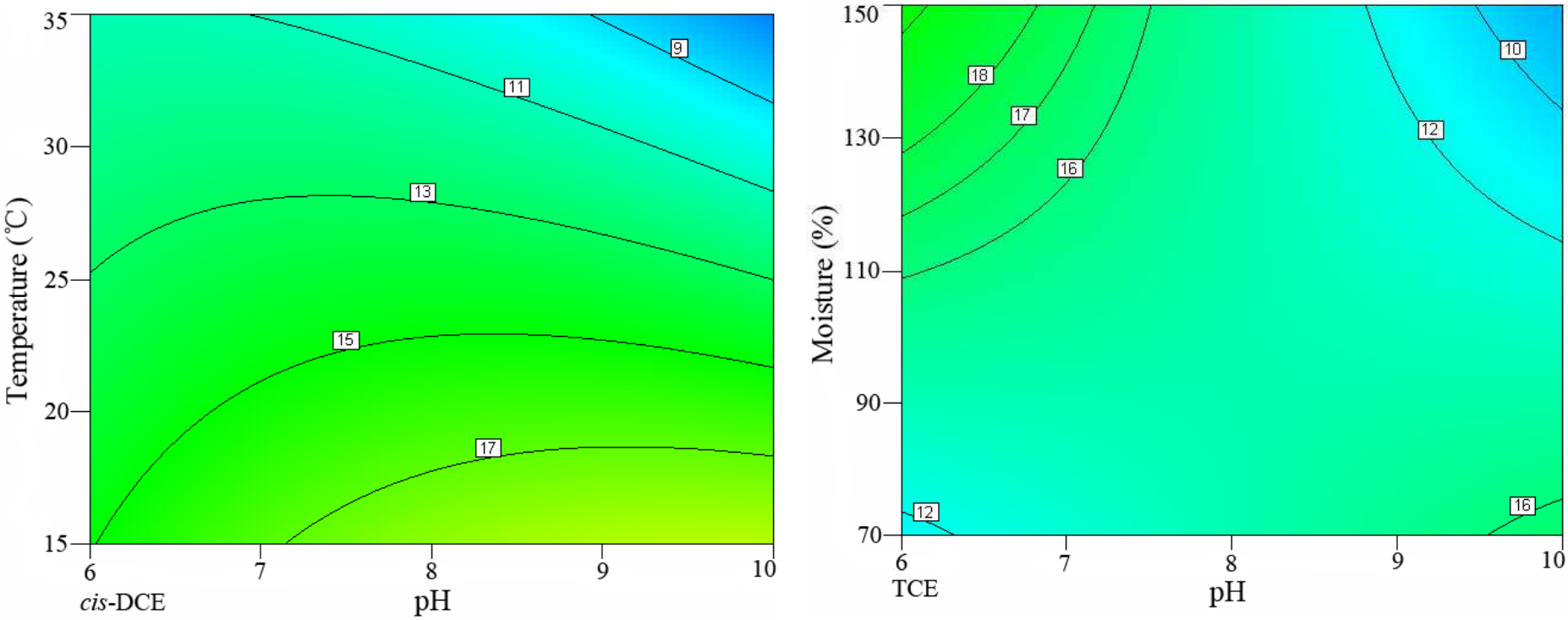


Fig.6 Contour surface plot for bioremoval of *cis*-DCE & TCE

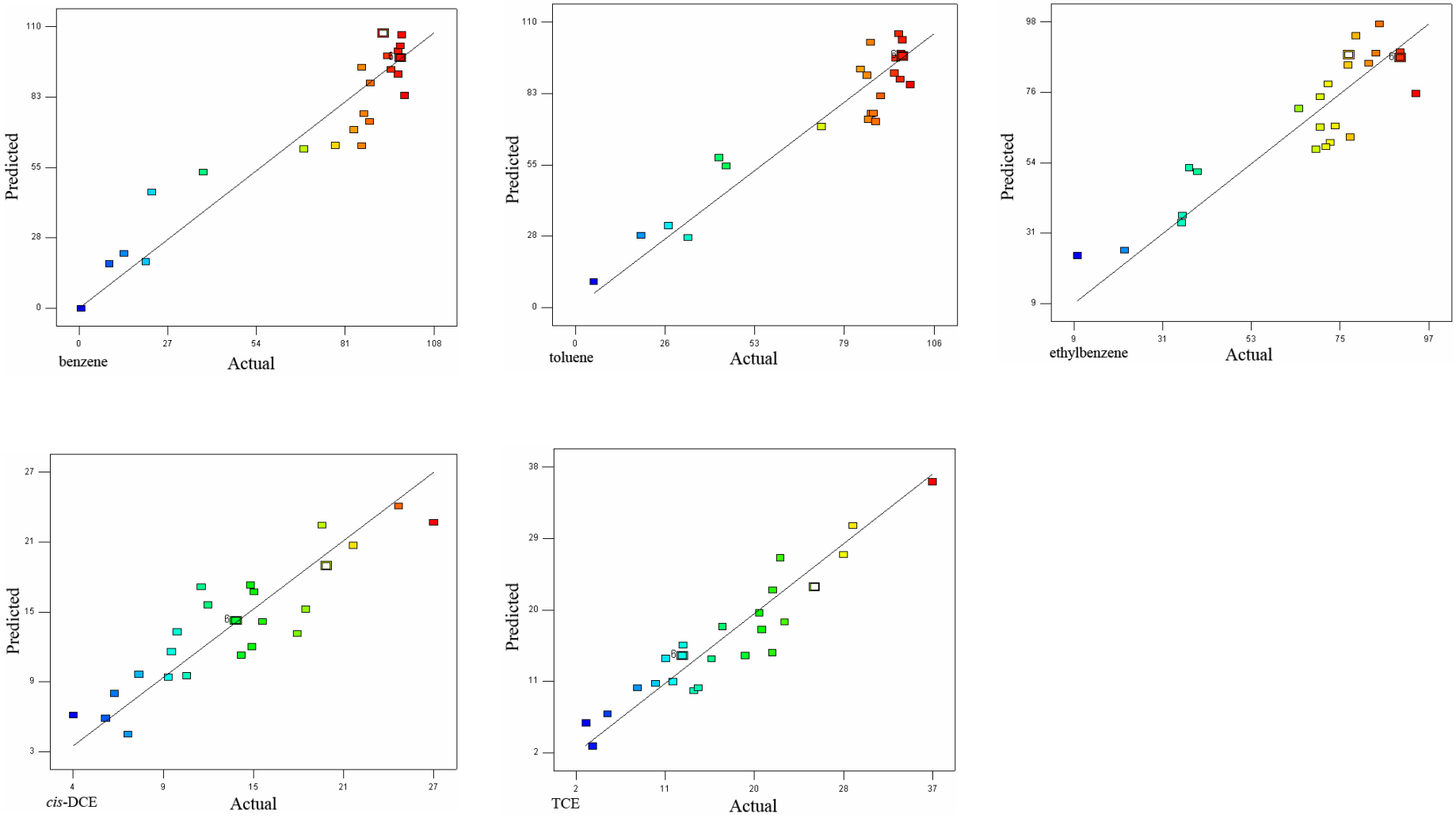


Fig.7 Diagnostics plot of predicted versus actual for VOCs bioremoval

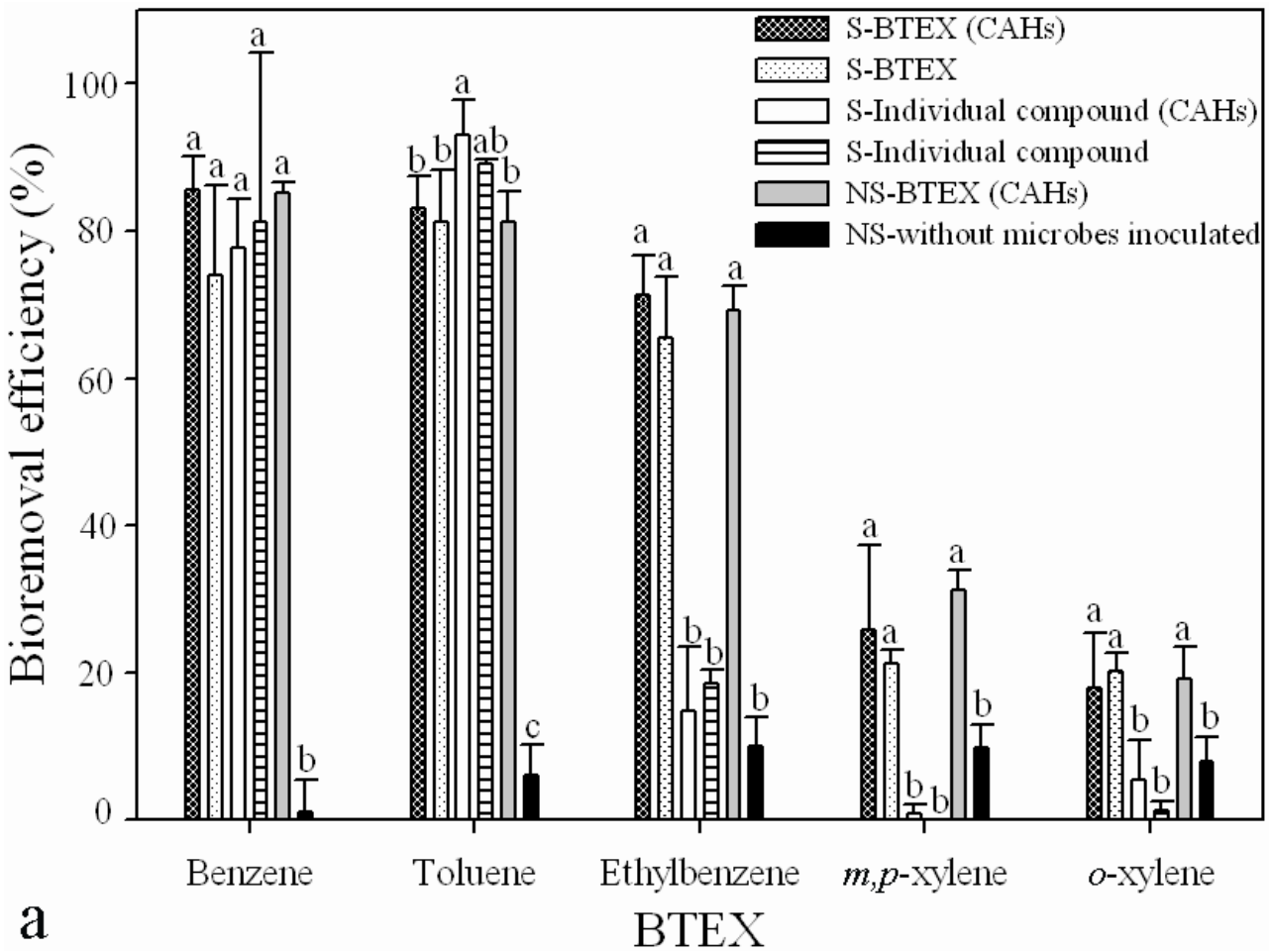


Fig.8a Biodegradation non-sterile/sterile soil spiked simultaneously & individually with BTEX with or without the presence of *cis*-DCE and TCE

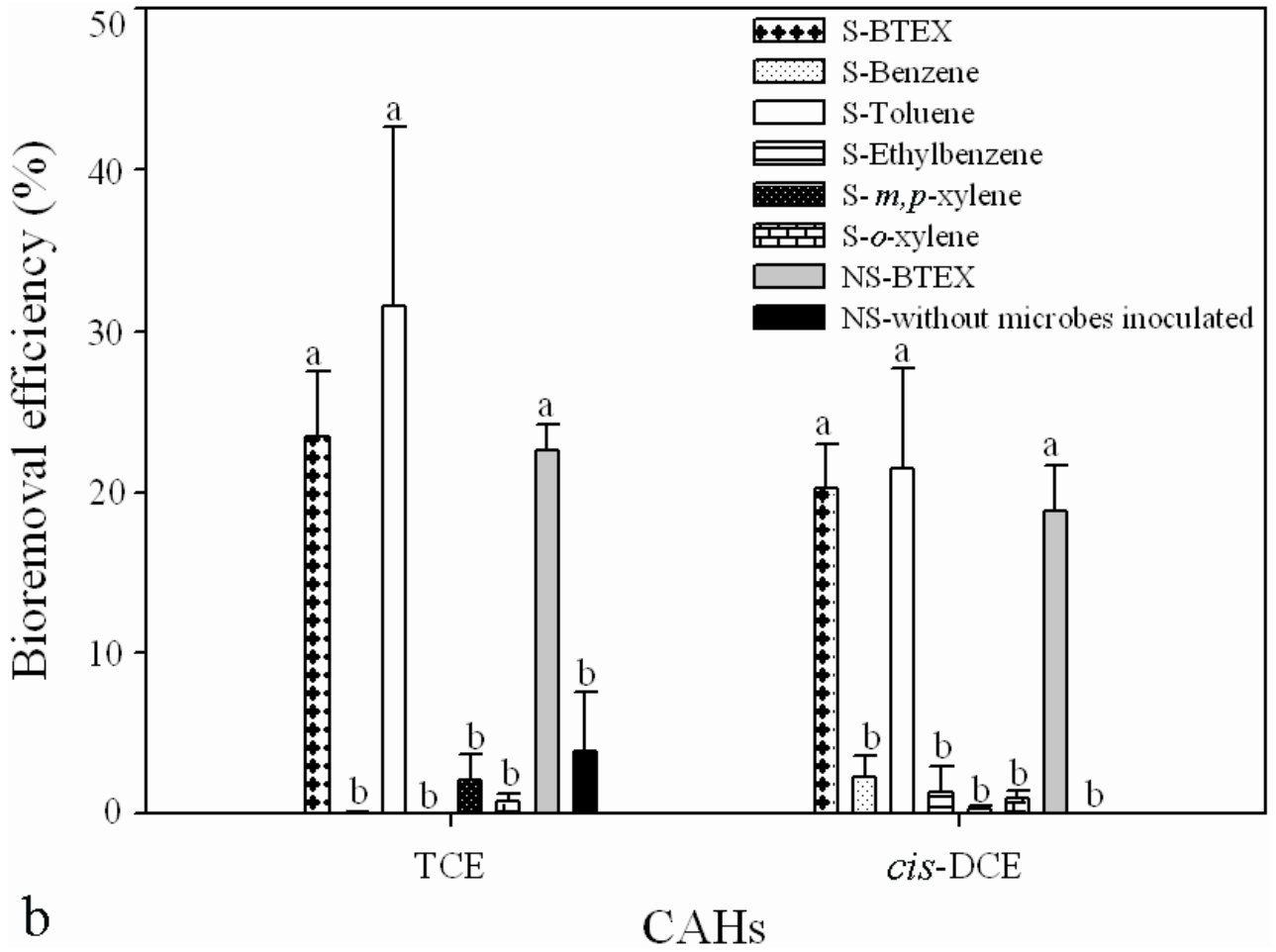


Fig.8b Biodegradation non-sterile/sterile soil spiked simultaneously & individually with BTEX with or without the presence of *cis*-DCE and TCE

Conclusions

- **The overall optimal condition for the biodegradation predicted to be at the combined levels of pH 8-10, temperature 15-20℃, soil moisture 110-150% of WHC, and concentrations of *cis*-DCE and TCE 10 ppm each.**

- **The RSM found useful to study the operating variables and their interactions with BTEX, *cis*-DCE, and TCE biodegradation in a statistically significant manner.**

- **The presence of toluene enhanced the bioremoval of other compounds.**

Acknowledgements

- University of Macau Research Committee

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Thank you

Q & A