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A comparative cytotoxicity study of isomeric alkylphthalates to metabolically variant bacteria

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ABSTRACT

This work investigated the toxicity of two isomeric alkylphthalates, *i.e.*, di-*n*-octyl phthalate (DOP) and di-2-ethylhexyl phthalate (DEHP) to two model bacteria, *Escherichia coli* (*E. coli*) and *Bacillus subtilis* (*B. subtilis*), which have been previously used to study the toxicity of environmental pollutants. Microcalorimetry was used as the key analytical tool alongside scanning electron microscopy (SEM) and traditional microbiology techniques. The thermokinetic parameters from microcalorimetry showed that the phthalates had a biphasic effect on the metabolic activities of the bacteria; serving as energy sources for the bacteria thereby stimulating their growth at low dosages ($\leq$150 μg/mL), but displaying inhibitory effects at higher dosages ($\geq$300 μg/mL), indicated by a sharp decrease in growth rate constants at 450 μg/mL. The SEM revealed that the bacterial cells were morphological deformed, with shrunk cells and elongated strands at 600 μg/mL of both phthalates. The elongated strands inferred that the phthalates inhibited the reproductive processes of the bacteria by possibly impeding some stages of cell division. The half inhibitory concentrations of the phthalates showed that DEHP was more toxic than DOP. Additionally, *E. coli*, a facultative anaerobe, was more susceptible to the toxic effects of phthalates than *B. subtilis*, an obligate aerobe capable of forming endospores crucial for tolerating extreme environmental conditions.

1. Introduction

In recent years, phthalates have attracted growing attention due to their widespread use, environmental ubiquity, and public health concerns especially with regards their endocrine-disrupting activities [1,2]. They are synthetic compounds used as plasticizers to improve the mechanical properties of plastic materials [3] used in building, home furnishing, textile, transportation and in relatively lesser quantities in food and medical packaging materials [4]. They are also used as additives in lubricants, insecticides, dielectric fluids [5] and a wide range of consumer products including cosmetics. Global phthalate production has increased over the past half century to a current annual production of about 3.5 million metric tons, with di-2-ethylhexyl phthalate (DEHP) alone accounting for approximately one third of the production [6].

Phthalates are released into the environment through diverse ways including discharge of wastewater from polymer and related industries. A study in China suggests the use of agricultural polyvinyl chloride films on soils as potential phthalate pollution source and identifies DEHP as the most widespread [7]. As plasticizers, phthalates are merely embedded into the parent polymer material but not linked via any covalent interaction and can therefore easily leach out or volatilize from plastic materials during usage or after disposal [8,9]. Their low water solubility and strong adsorption to solid particles make them easily precipitate from waste water and accumulate in sludge and sediment [10]. Phthalates released into the air could be deposited on dust particles, soil or in surface water and could be taken-up by crops intended for human or livestock consumption, thus entering the food chain [11]. They could enter food or infant formulas from plastic packaging materials [12] and the highest concentrations are detected in fatty foods.

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Humans are directly exposed to phthalate contamination during medical procedures like blood transfusion or dialysis from equipments containing phthalates [13]. Even at very low concentrations, phthalates are suspected to interfere with the reproductive systems in humans and wildlife through the disturbance of the endocrine system [14]. The acute toxicity of a phthalate ester strongly depends on the carbon chain length of its alcohol moiety [15]. The two phthalates studied in this work, di-*n*-octyl phthalate (DOP) and DEHP have been indicted as human carcinogens, causing damage to the liver and kidney, influencing the malfunction of reproductive organs and interfering with human development by acting as mimics of the sex hormone estrogen [16]. As a result, several regulatory bodies including the US Environmental Protection Agency, the European Union Environmental Agency, and China National Environmental Monitoring Center have classified phthalates as the top priority pollutants for risk assessment and have also mandated their control and reduction [17].

The two bacteria used in this work, *Bacillus* (*B.*) *subtilis* and *Escherichia* (*E.*) *coli*, are model laboratory organisms that have been previously used for evaluating the toxicity of organic compounds [18,19]. They possess striking differences in both their morphology and metabolic characteristics which could be used for the purpose of comparing the toxicity of various phthalates and in assessing microbial stress response to this class of pollutants. *B. subtilis* could be described as an obligate aerobe and forms protective endospores helpful in tolerating extreme environmental conditions whereas *E. coli* is a facultative anaerobe and non-sporulating [20].

The interactions of phthalates, like other organic pollutants, with microorganisms are biological in nature and accompanied by heat changes [21]. Thus, the biological response to toxicants could be manifested by a change in metabolic heat production rate of cultured cells. This phenomenon serves as the basis for using microcalorimetry in this study, which is sensitive and continuously monitors microbial metabolic activity without disturbing the system by sampling procedures [22]. Moreover, microcalorimetry provides more quantitative information that is relevant to toxicity assessment than most other analytical techniques. The power–time curves obtained from microcalorimetry provide several toxicity indices, which estimate the susceptibility of microorganisms at doses considered. In addition, other methods like optical density (OD) and scanning electron microscopy (SEM) are used to validate the microcalorimetric results. OD monitors the influence of toxicants on the biomass growth while SEM records the morphological changes that result from the toxic effect of phthalates to the bacteria.

In the past, most researches on phthalates have targeted the extent of contamination and possible degradation options [23,24]. Although microbial breakdown is the major route known for phthalates degradation in the environment, no investigations on the toxicity of phthalates to microorganisms using microcalorimetry is to our knowledge. In this research, the toxicities of two isomeric phthalate (DOP and DEHP) are studied and compared with the aim of establishing a relationship between dose and effect on the growth, metabolic activity and morphology of the two bacteria. The results will deduce the relative toxicity of the two phthalates and the relative susceptibility/tolerance of the two bacteria to phthalate pollution. Overall, the investigation should throw light on the cytotoxicity of phthalates to microorganisms that are bio-indicators of environmental pollution.

2. Experimental

2.1. Materials

B. subtilis (G^+ 010, CCAM020074) and *E. coli* (DH5α) strains were kindly provided by the State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University (Wuhan, PR China). DOP and DEHP with 99% purity were obtained from Acros Organics (Geel, Belgium). The stock solutions were prepared by dissolving phthalates in ethanol (5000 μg/mL) and diluted to give the desired concentrations (0, 150, 300, 450, and 600 μg/mL) in the growth medium.

The peptone culture medium used to incubate both bacteria was prepared by dissolving 10.0 g peptone, 5.0 g beef extract (Beijing Shuangxuan Microbe Culture Medium Products Factory, Beijing, PR China) and 5.0 g NaCl (Sinopharm Group Chemical Reagent Co., Ltd., Beijing, PR China) in 1.0 L of deionized water at pH 7.2. Solid growth medium was prepared by adding 1% w/v agar to the peptone medium. The resulting medium was sterilized in an autoclave at 120 °C for 30 min. Both bacteria were aerobically grown at 37 °C between 12 and 16 h with constant shaking at 200 rpm. All reagents of analytical grade or above were used as received from supplier.

2.2. Growth measurement

The toxic effects of phthalates on the growth of the bacteria were monitored by OD measurement and viable (bacteria) count method in triplicates. These experiments were carried out simultaneously with appropriate controls to eliminate any possible effect of the solvent. The first control contained the growth medium and the solvent (ethanol) without bacteria; the second contained the growth medium, solvent and bacteria; and the third contained the growth medium and the phthalate solution (of the same dose as the main experiment) without bacteria. The bacteria cells were inoculated into 150 mL growth media containing various doses of phthalates in 250-mL Erlenmeyer flasks and incubated at 37 °C with continuous shaking at 150 rpm. The bacteria growth was monitored by removing small amounts of the growth medium for viable count and OD measurement at 600 nm and 2-h intervals over a 24-h period. The growth medium went through a three-serial dilution with ultra-pure water. After vigorous shaking, the sample was then transferred into a 2.0-mL quartz cuvette in a Spectrumlab752 s UV–visible absorption photometer (Lingguang Co., Ltd., Shanghai, PR China). The OD of the bacteria growth medium was determined by comparing the absorptions of the sample and the third control containing various concentrations of phthalates without microbe. For the viable count, 100 μL of various serial dilutions of the growth medium was spread on LB agar plates and incubated overnight at 37 °C. The number of colonies formed was counted and expressed as colony forming units per mL (CFU/mL).

2.3. Microcalorimetric method

A TAM III multi-channel thermal activity monitor (Thermometric, Järfälla, Sweden) was used to record the heat flow rate of the microbial growth activity of each bacterium in growth media containing various concentrations of phthalates. A microcalorimeter comprising a reference and five sampling channels was used. Each channel has a calorimetric unit at the bottom and one at the top, separated by a small 'primary' heat sink that is in thermal contact with a surrounding steel tube [21]. The channels are inserted into a precise liquid thermostat which serves as the main heat sink for all the calorimetric units. The equipment is calibrated by the release of electrical energy in a resistor and the thermal effects of the sample ampoules are adjusted to an electrical calibration [25]. The microcalorimeter has precise control over the isothermal conditions in the thermostatic bath and the detection of the thermal events in the system [26]. 4.0 mL stainless steel ampoules were hermetically sealed with Teflon discs to prevent evaporation and inserted into the top units of the

channels. The thermal effects were obtained at a constant temperature of 37 °C. All experiments were performed in clean and sterilized ampoules containing 2.0 mL medium, different doses (0–600 μg/mL) of phthalate (DOP or DEHP) and 0.10 mL bacterial suspension containing 10^6 cells/mL of *B. subtilis* or *E. coli* (microscopically counted by a bacteria chamber). The thermal effects associated with the metabolic activity of each bacterium under the influence of the phthalates were recorded as a function of time on a computer using the TAM III software. The associated metabolic parameters were then calculated from the power–time curves [27,28]. OriginLab 8.0 software (OriginLab Corporation, Northampton, MA, USA) was used for data processing and statistical analysis.

2.4. Scanning electron microscopy

The effect of phthalate on the morphology of the bacteria was monitored on a JEOL JSM-6510 LV SEM (Tokyo, Japan). For

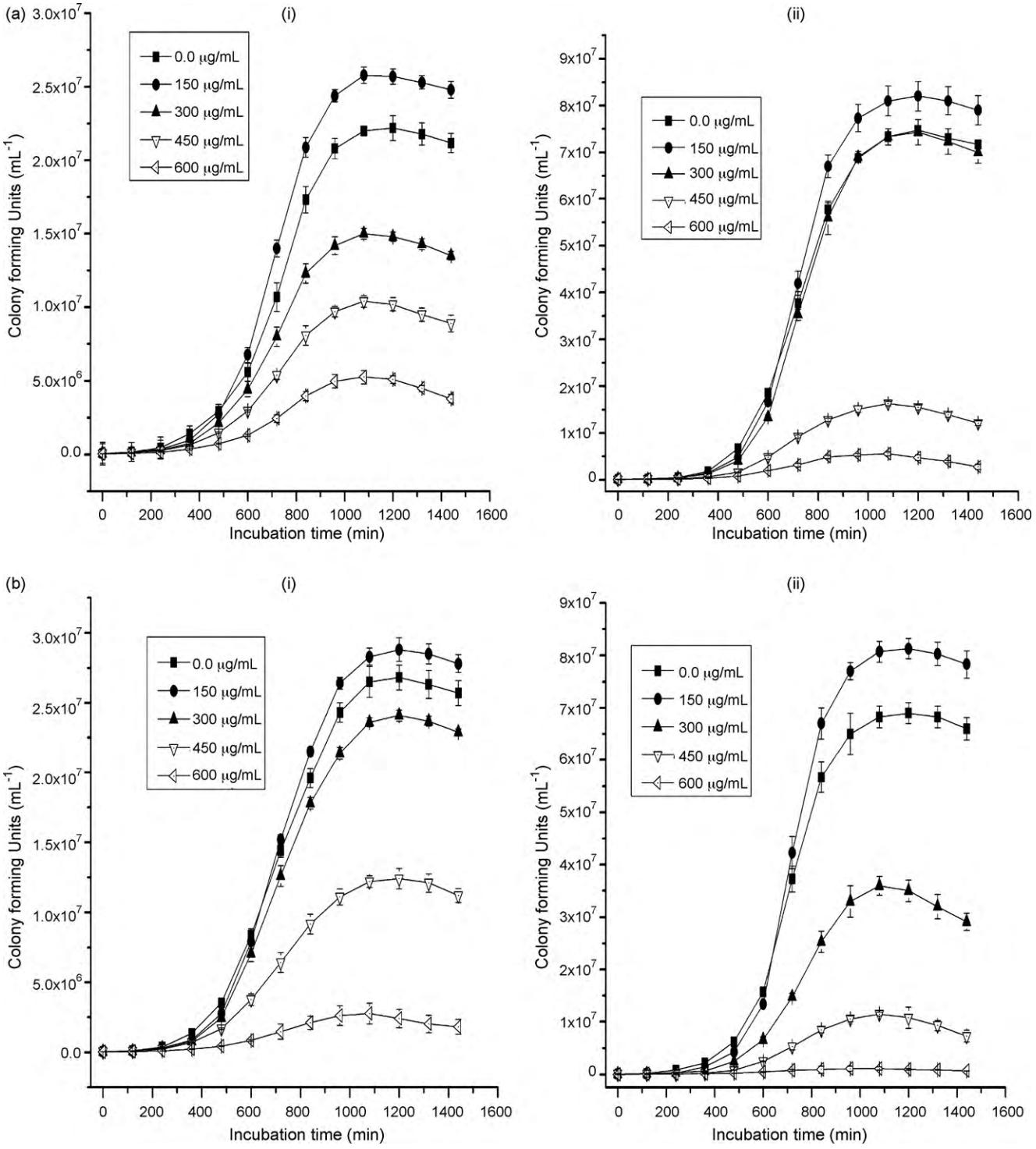

Fig. 1. Trends in colony forming units by the viable count method showing the effects of various doses of (a) DOP on the growth of (i) *B. subtilis* and (ii) *E. coli*, and (b) DEHP on the growth of (i) *B. subtilis* and (ii) *E. coli*.

Table 1
Growth rate constants of *B. subtilis* and *E. coli* under various doses of DOP and DEHP obtained from optical density and viable count methods.

	Growth rate constant k (min^{-1}) × 10^{-3} obtained			
	From optical density measurement		From viable count method	
Dose (μg/mL)	DOP	DEHP	DOP	DEHP
B. subtilis				
0.0	9.28 ± 0.05	9.49 ± 0.04	7.83 ± 0.03	8.01 ± 0.01
150	13.51 ± 0.09	15.47 ± 0.10	11.47 ± 0.09	12.95 ± 0.06
300	6.92 ± 0.14	4.43 ± 0.03	5.39 ± 0.02	1.79 ± 0.11
450	0.95 ± 0.06	0.39 ± 0.07	0.64 ± 0.08	0.12 ± 0.07
600	0.29 ± 0.03	0.11 ± 0.02	0.18 ± 0.06	0.07 ± 0.02
E. coli				
0.0	10.55 ± 0.13	9.83 ± 0.07	8.27 ± 0.09	8.35 ± 0.06
150	15.29 ± 0.04	16.23 ± 0.02	12.93 ± 0.07	13.74 ± 0.10
300	8.47 ± 0.09	2.44 ± 0.11	7.95 ± 0.02	0.67 ± 0.08
450	0.35 ± 0.07	0.09 ± 0.02	0.10 ± 0.05	0.06 ± 0.03
600	0.05 ± 0.03	N	N	N

Data are represented as mean ± S.D. ($n = 3$ and $P < 0.0001$).
DOP: Di-*n*-octyl phthalate; DEHP: Di-2-ethylhexyl phthalate; N: negligible.

comparison purposes, three concentrations of phthalates (0, 300 and 600 μg/mL) were impregnated in solid agar growth medium. Freshly activated cells of each bacterium were separately inoculated on the surface of the impregnated solid medium and allowed to grow for 12 h. It was at about the mid-exponential phase of bacterial growth, estimated from the viable count. Colonies of the bacteria cells were collected, transferred into a fixative glutaraldehyde solution and kept for 5 h or overnight. The cells were then washed three times with ultra-pure water and dehydrated serially by 30%, 50% and 70% alcohol solutions. In each case, the bacteria suspensions were centrifuged and the supernatant liquid decanted. Afterwards, the cells were freeze-dried for 6 h and viewed under the SEM.

3. Results and discussion

3.1. Optical density and viable count

Fig. 1 displays the effects of (a) DOP and (b) DEHP on the growth process of (i) *B. subtilis* and (ii) *E. coli* monitored by viable count method respectively with trends essentially identical to those obtained from OD measurement. Table 1 gives the calculated growth constants obtained from these determinations. The growth rate constants from the OD measurement were slightly higher than those of the viable count method. However, both data showed similar trends depicting enhanced growth at the lowest concentration (150 μg/mL), which subsequently decline at higher concentrations (≥300 μg/mL) of phthalates. It was clearly manifested from the growth curves that the exponential growth phases of the bacteria were impeded as a function of the phthalate concentration. In the absence of phthalates, the lag phases of the growth curves for both bacteria were shorter than those at higher doses. This can be accounted for by the fact that the bacteria required some time to adapt to the presence of phthalates in the growth medium which posed a challenge to their metabolic activities at the initial stage. However, after surmounting the growth barrier posed by the phthalates, the metabolic activities of the bacteria were subsequently stimulated at the lowest concentration (150 μg/mL). But a general inhibitory effect was noted at higher concentrations (≥300 μg/mL) as evidenced by the trends in growth constant shown in Table 1. The growth of both bacteria at the lowest concentration of phthalates (150 μg/mL) was higher than that of the control but was suppressed at higher concentrations. The decrease in growth of *E. coli* was relatively more drastic than *B. subtilis* at doses ≥300 μg/mL. The metabolic activities of the bacteria at doses >150 μg/mL were inhibited as a function of phthalate concentration. However, the growth curves obtained from the viable count method showed that the metabolic activity of *E. coli* was relatively stimulated at the second highest dose of DOP (300 μg/mL) compared to *B. subtilis*. This would infer that *E. coli* being a facultative anaerobe can better metabolize phthalates at lower concentrations. In addition, the growth rate constants of the same bacteria were higher for DOP than DEHP (300–600 μg/mL), inferring that DEHP poses a higher inhibition effect on the bacteria than DOP. This fact is also supported by the trend of growth shown in Figs. 2–5.

3.2. Scanning electron microscopy

The SEM images reveal crucial evidences about the nature and extent of toxicity of phthalates to the microorganisms as displayed in Figs. 2 and 3. The selected incubation time of 10 h was estimated from the preliminary experiments. By then, the bacterial cells would have been at about mid-way through their exponential growth phase and actively involved in reproductive processes. It is observed from Figs. 2a and 3a that the bacteria cells grew and carried out their life processes normally in terms of shape or size in the absence of phthalates. Some of the cells existed as single cells or with obvious signs of being at the peak of cell division. By contrast, in the presence of phthalates especially at the highest concentrations (Figs. 2c and e and 3c and e), structural deformities of most cells were observed with irregular shapes either shrunk or elongated. It can be envisaged that the extent of cell deformity is a function of phthalate concentration even just by observing the physical shapes of the cells. The results also manifest that the cell division processes of the bacteria were interrupted resulting in elongated strands of cells that were unable to divide into single cells. Phthalate esters as possible mutagenic compounds could have acted as exogenous agents causing DNA damage in bacterial cells. This could have prompted a global response by the cells in attempts to repair such damages via various mechanisms (post-replication DNA repair response system, non-homologous end joining, etc.), which are mostly reflected by cell cycle arrest and inhibition of cell division [29]. Finally, it was observed that DEHP exerted more adverse toxic effect on the bacteria than DOP. At the highest dose, both bacteria were more deformed and elongated in the DEHP medium (Figs. 2e and 3e) as compared to DOP (Figs. 2c and 3c).

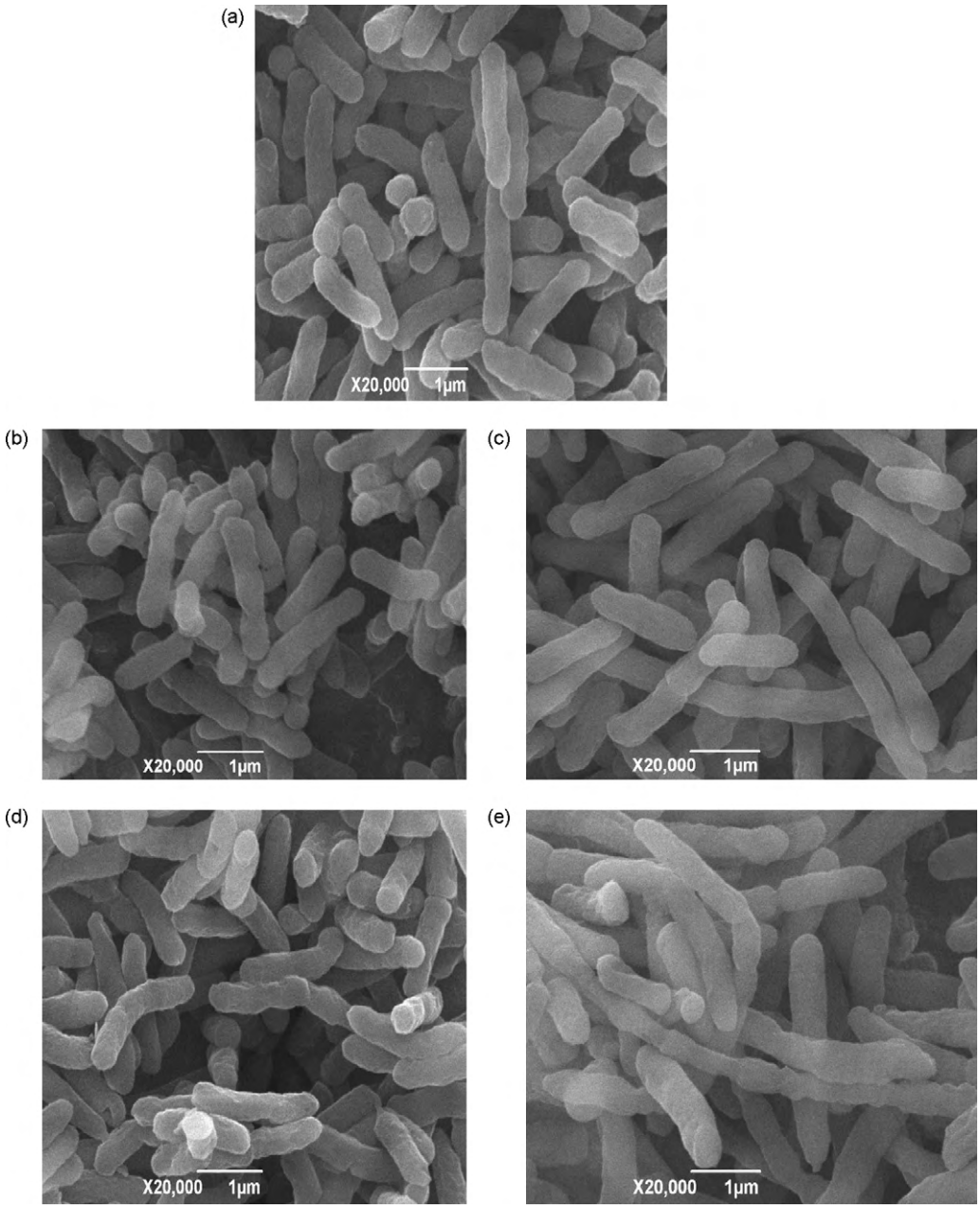

Fig. 2. SEM images of *B. subtilis* under various concentrations of phthalates: (a) 0.00, (b) 300, (c) 600 μg/mL DOP, (d) 300, and (e) 600 μg/mL DEHP.

3.3. Microcalorimetric study

The metabolic activities of the bacteria at different concentrations of phthalates are shown in Fig. 4a–d. Single peaks were observed for each concentration and the curves show distinct lag, exponential, stationary and decline phases. The total heat produced (Q_T), maximum power (P_{max}) and corresponding time (T_{max}), the microbial growth rate constant (k), generation time (T_G), inhibitory ratio (I) and IC_{50} are summarized in Table 2.

The growth rate constant, k, is a good indicator of the chemical stress posed by the phthalates to the bacterial cells [30]. It is calculated as the slope of the plot of time against the natural logarithm of the heat response (power) from the power–time curves (Fig. 4) for each dose of phthalate:

$$\ln P_t = \ln P_0 + kt \quad (1)$$

where P_t and P_0 are the powers at time t and $t = 0$, respectively.

The inhibitory ratio, I, which gives a quantitative estimation of the relative inhibitory effect of each dose of phthalate on the metabolic activity of the bacteria, was calculated using the following equation:

$$I = \frac{k_0 - k_C}{k_0} \times 100\% \quad (2)$$

where k_0 is the rate constant of the control, and k_C is the rate constant for microbial activity inhibited by an inhibitor with concentration C. When I is 50%, the corresponding half inhibitory concentration of the inhibitor is represented as IC_{50}. The generation times, T_G, of the bacteria at various doses were obtained as $(\ln 2)/k$. The total heat produced Q_T, which relates to the use and degradation of the carbon source, was calculated by integrating each power–time curve from the beginning to the end. P_{max} and T_{max} are the maximum heat output power and time at the peak of the power–time curve, respectively. The parameters Q_T, k and

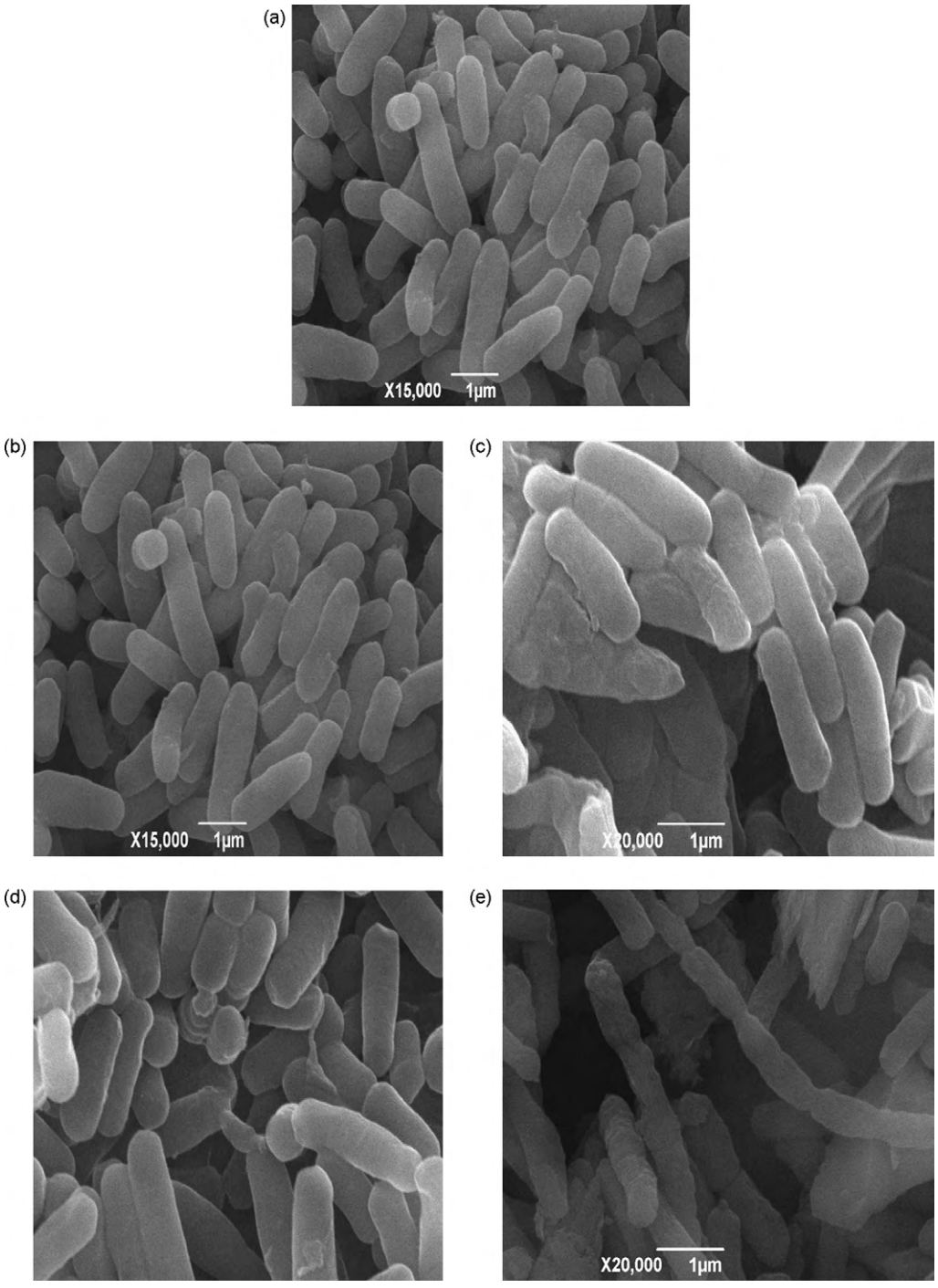

Fig. 3. SEM images of *E. coli* under various concentrations of phthalates: (a) 0.00, (b) 300, (c) 600 μg/mL DOP, (d) 300, and (e) 600 μg/mL DEHP.

P_{max}, which estimate the extent of metabolic activity of the bacteria, are highest for both phthalates at the lowest dosage (150 μg/mL). However, at doses above 150 μg/mL, these values decreased and remarkably so for concentrations ≥450 μg/mL. This observation suggests that at lower doses (≤150 μg/mL), the bacteria utilized phthalates as energy sources, thus stimulating their growth and metabolic activities. On the other hand, they posed threat to the growth and metabolic activities of the bacteria at higher doses (>150 μg/mL). This observation suggests that phthalates have a biphasic effect on the growth and metabolism of microorganism, *i.e.*, serving as energy sources and thus stimulating microbial metabolic growth at very low doses, but having inhibitory effects at higher doses.

The inhibitory ratio, *I*, could be negative indicating a stimulating effect on the metabolic activity of bacteria or positive indicating an inhibition effect relative to the control. Negative *I* values were obtained for the least dose (150 μg/mL) of both phthalates for both bacteria and also the second highest dose (300 μg/mL) of DOP for *E. coli*. However, the *I* values were positive and increased dramatically for higher doses, suggesting the decrease in metabolic activities relative to the control. T_G which indicates the time required for the cell population to double in number shows a similar trend to

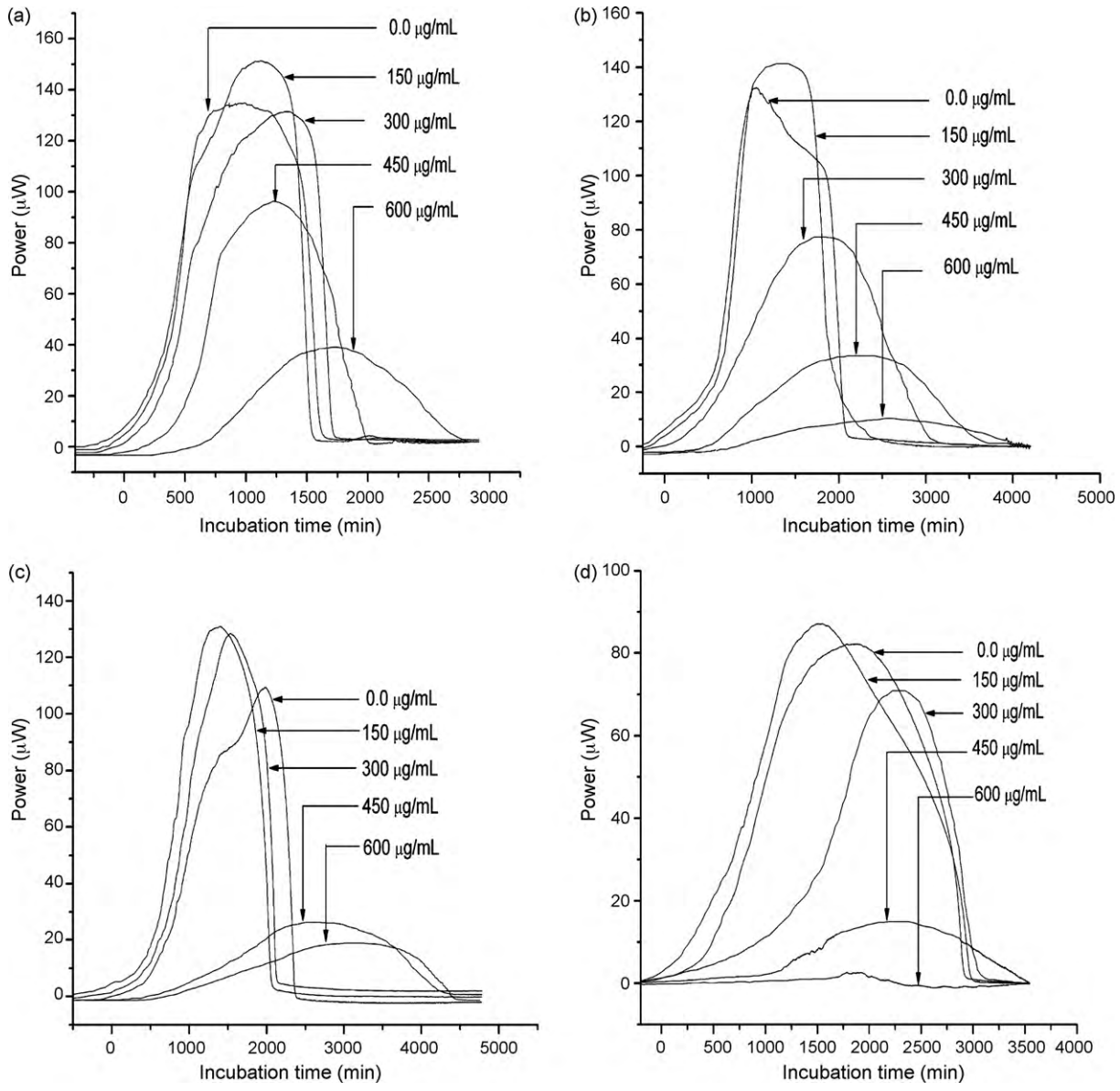

Fig. 4. Power–time curves of the metabolic activities of (a) *B. subtilis* at different doses of DOP. (b) *B. subtilis* at different doses of DEHP. (c) *E. coli* at different doses of DOP. (d) *E. coli* at different doses of DEHP.

I. The lowest values of T_G for the least doses of phthalates indicate favorable conditions for cell growth and metabolism. The increase in T_G at higher doses manifests an impediment to the microbial activities of bacteria. These facts underscore previous observations noted from viable count results.

The IC_{50} obtained by plotting *I* against concentration of phthalates at $I = 50\%$ is depicted in Fig. 5. This parameter has an inverse relationship with the toxicity of the compounds. Thus, it can be deduced that DEHP with lower IC_{50} for both bacteria is relatively more toxic than DOP. Also, the IC_{50} of each phthalate with respect to *E. coli* is smaller than *B. subtilis*, inferring that *E. coli* is generally more susceptible to the toxicity of phthalates.

The T_{max} is the time required to attain maximum heat peak during the metabolic process of the microorganisms. It gives an indication of the extent of barrier encountered by the cells in the course of their metabolic activity. As shown in Table 2, T_{max} increased with increase in phthalate dose. Higher T_{max} suggests that the bacteria cells encountered some form of growth barrier that prevented the cells from attaining their fullest growth potential. The power–time curves (Fig. 4) also show that the exponential phases were delayed as a function of increasing phthalate concentration. These observations established the fact that the mere presence of phthalates in bacteria growth media poses some stress to bacterial cell growth giving rise to the need for extra time and energy for adaptation. This assertion is also supported by the T_G data.

By comparing the *k* and T_G of the two bacteria at the lowest doses of both phthalates, it can be envisaged that the metabolic activity of *E. coli* was relatively higher than *B. subtilis*. However, at the highest concentration of both phthalates, the growth inhibition was considerably higher for *E. coli* than *B. subtilis*. This highlights the fact that *B. subtilis* could be more resistant to the toxic effects of phthalates due to its adaptive features. It forms endospores which render it much more resistant to the toxic effects of phthalates than *E. coli*. In addition, although both phthalates are toxic to the bacteria at higher doses, DEHP is significantly more toxic than DOP. This is possibly attributed to the structural variability of the two phthalates. DEHP with a branch alcohol moiety is more volatile and soluble in organic medium and thus can migrate more readily across the cell membranes of microorganisms. This observation corroborates pre-

Table 2
Effect of various concentrations of phthalates on microbial activities of *B. subtilis* and *E. coli*.

	Q_T (J g^{-1})	$k \times 10^{-3}$ (min^{-1})	P_{max} (μW)	T_{max} (min)	T_G (min)	I (%)	IC_{50} (μg/mL)
B. subtilis							
Concentration of DOP (μg/mL)							
0.0	8.90	15.4 ± 0.04	135	988	44.97	–	
150	9.12	16.3 ± 0.05	151	1120	42.52	−5.74	
300	8.53	11.8 ± 0.06	132	1340	58.88	23.6	
450	6.07	3.13 ± 0.07	96.4	1225	221.6	79.7	
600	2.89	0.59 ± 0.06	39.0	1714	1180	96.2	415
Concentration of DEHP (μg/mL)							
0.0	9.13	15.6 ± 0.11	132	1045	43.49	–	
150	9.84	18.2 ± 0.07	141	1283	38.05	−14.3	
300	7.69	8.64 ± 0.05	77.4	1715	80.27	45.8	
450	3.97	0.79 ± 0.09	33.7	2097	875.2	95.0	
600	1.14	0.22 ± 0.05	10.5	2548	3214	98.7	314
E. coli							
Concentration of DOP (μg/mL)							
0.0	7.39	16.8 ± 0.04	109	1983	41.39	–	
150	8.82	19.1 ± 0.07	131	1405	36.22	−5.32	
300	8.56	17.4 ± 0.09	128	1537	39.90	19.8	
450	3.44	2.06 ± 0.11	26.2	2529	337.0	94.6	
600	2.49	0.14 ± 0.12	18.9	3016	5040	97.5	386
Concentration of DEHP (μg/mL)							
0.0	8.55	14.7 ± 0.08	82.2	1890	47.03	–	
150	9.01	17.8 ± 0.10	87.1	1502	38.90	−14.3	
300	5.59	5.00 ± 0.15	71.0	2225	138.6	−15.7	
450	1.52	0.43 ± 0.09	15.0	2240	1606	87.7	
600	0.0512	N	2.64	1787	N	99.2	269

k are represented as mean ± S.D. ($n = 3$ and $P < 0.0001$).
Q_T: Total metabolic thermal effect; k: Metabolic rate constant; P_{max}: Maximum metabolic heat.
T_{max}: Time at P_{max}; T_G: Generation time; I: Inhibitory ratio; IC_{50}: Half inhibitory ratio.
DOP: Di-*n*-octyl phthalate; DEHP: Di-2-ethylhexyl phthalate.
N: negligible.

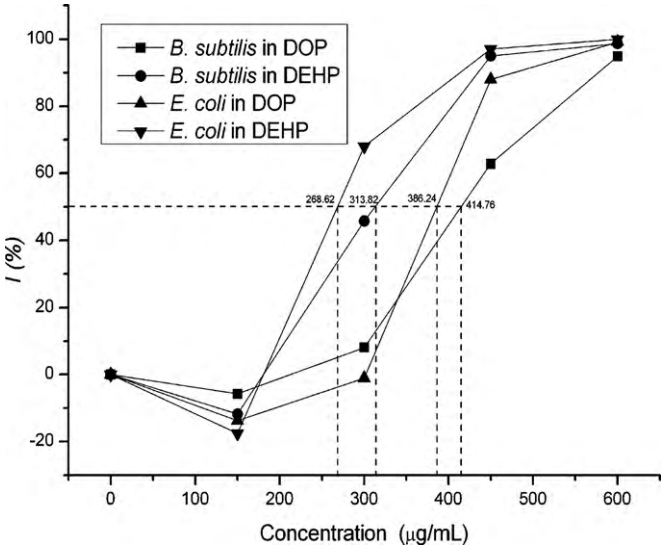

Fig. 5. Relationship between the inhibitory ratio I of bacteria and the concentration of phthalates.

vious research that DEHP is one of the most recalcitrant and toxic phthalates in the environment [20].

4. Conclusion

It can be asserted that phthalates like other organic compounds have biphasic effects on the growth and metabolic activity of bacteria. On one hand, they could stimulate the growth and metabolic processes of the bacteria at low doses (≤150 μg/mL) but on the other, could be seriously toxic, thereby impeding the growth and metabolic processes of microorganisms at higher doses. DEHP with a branched alcohol moiety attached to the parent phthalate acid structure is comparatively a better growth and metabolic stimulant at lower doses but more toxic to both bacteria at higher doses than its isomeric congenial DOP. This is attributed to its higher solubility (higher octane/water ratio) in organic medium, inferring its faster mobility through cell membranes. The SEM images show that these compounds interfere with the reproductive processes of the bacteria by possibly inhibiting the microbial cell division at some stages. The inhibitory ratio manifests that *E. coli*, a facultative anaerobe better metabolizes the phthalates at lower doses than *B. subtilis*, an obligate aerobe. However, the half inhibitory concentrations (IC_{50}) of both phthalates with respect to the bacteria prove that *E. coli* is generally more susceptible to the toxic effects of phthalates especially at higher doses. *B. subtilis* with the ability of forming endospores, a characteristic uncommon to *E. coli*, is more resistant to the toxicity of phthalates.

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