Biodegradation of benzyl butyl phthalate and dibutyl phthalate by *Arthrobacter* sp. via micellar solubilization in a surfactant-aided system

Moumita Nandi, Tanushree Paul, Dipak Kumar Kanaujiya, Divya Baskaran, Kannan Pakshirajan and G. Pugazhenthi

**ABSTRACT**

Endocrine-disrupting chemicals (EDCs) like phthalates, mostly discharged in industrial effluents, cause detrimental effects to different life forms, and hence their removal from constituent wastewater is necessary. This study investigated the kinetics of biomass growth and biodegradation of benzyl butyl phthalate (BBP) and dibutyl phthalate (DBP) by *Arthrobacter* sp. in a surfactant-aided batch system. The effect of different surfactants on aqueous solubility of BBP and DBP was initially examined, which showed that Tween 80 resulted in maximum bioavailability and biodegradation efficiency of the phthalates by the bacterium and without inhibiting the biomass growth. Compared with BBP, DBP was found to be efficiently degraded and supported the bacterial growth within a short period of time over the entire concentration tested in the range 100–1,000 mg L\(^{-1}\). A maximum biomass concentration of 1.819 g L\(^{-1}\) was obtained at 120 h for a DBP concentration of 600 mg L\(^{-1}\) in the presence of Tween 80, which is 5.66-fold increase in biomass concentration as compared with only DBP as the sole substrate. For evaluating the biokinetic parameters involved in DBP biodegradation, the experimental data on DBP utilization were fitted to various kinetic models as reported in the literature.

**Key words** | *Arthrobacter* sp., bioavailability, biodegradation, kinetic modelling, phthalates, surfactant-aided system

**HIGHLIGHTS**

- Biokinetics of surfactant-aided biodegradation of phthalates is reported.
- Tween 80 enhanced phthalate biodegradation by improving its bioavailability.
- *Arthrobacter* sp. showed direct uptake of micellar solubilized phthalates.
- Efficient biodegradation is achieved overcoming growth inhibition.

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INTRODUCTION

Endocrine-disrupting chemicals (EDCs), in particular phthalates, are environmental pollutants which pose a serious threat to human beings, wildlife and the ecosystem due to their toxic, mutagenic and teratogenic nature (Lauretta et al. 2019; Margina et al. 2019; Godfray et al. 2019; La Merrill et al. 2019). Hence, their removal from contaminated systems is of recent interest. Among the different methods to treat wastewater containing EDCs, biodegradation using microorganisms is proving to be the most promising, economic and environmentally sound method (Eltoukhy et al. 2020; Gadupudi et al. 2020; Roccuzzo et al. 2020). However, success of this biological method is limited owing to the highly hydrophobic nature and very low bioavailability of phthalates and other EDCs to degrading microorganisms (Grinbaum et al. 2019; Gani & Kazmi 2020). For instance, the solubility of phthalates in water is 0.71–2.69 mg L⁻¹ for benzyl butyl phthalate (BBP) and 11.2 mg L⁻¹ for dibutyl phthalate (DBP) at 25 °C and hence bioavailability of these compounds is very poor. Moreover, high octanol–water partition coefficient values of these compounds indicate their high hydrophobicity which causes their accumulation in soils, sediments and even organisms (Sacan et al. 2005; Net et al. 2015; Gao & Wen 2016; Shi et al. 2019; Li et al. 2019). Hence, different strategies to improve the bioavailability and therefore biodegradation of these compounds have been proposed, which include application of surfactants (Franzetti et al. 2008; Paria 2008), decrease in temperature (Thomsen et al. 2001), solid-dispersion, particle size reduction, nanonization, cosolvency and inclusion complexation (Kumar & Singh 2016).

Among the afore-mentioned strategies, surfactants are conventionally applied to increase the aqueous solubility of these phthalates for enhancing their bioavailability and biodegradation (Aryal & Liakopoulou-Kyriakides 2013). Surfactants distinctively enhance the bioavailability of hydrophobic organic pollutants either by decreasing interfacial tension between aqueous and non-aqueous phases (Cuny et al. 1999) or by solubilization of hydrophobic organic compounds in the core of the micelles formed by surface-active agents (Sartoros et al. 2005; Hadibarata & Tachibana 2010). In the case of poorly water-soluble compounds, it has been reported that bacteria adhere to the liquid–liquid interface for lowering the interfacial tension and causing direct uptake of these pollutants, thereby enhancing their biodegradation rates (Volkering et al. 1998).

Among the different microorganisms reported to degrade highly recalcitrant compounds, such as phenolics, polycyclic hydrocarbons, bacteria belonging to the genus Arthrobacter have been shown to be highly effective (Abatenh et al. 2017; Guo et al. 2019). For instance, Arthrobacter chlorophenolicus A6 is reported to effectively degrade a mixture of phenolic compounds, including 4-chlorophenol, 4-nitrophenol and phenol (Unell et al. 2008). In addition, polycyclic aromatic hydrocarbons like phenanthrene and naphthalene were shown to be efficiently degraded by halotolerant bacteria belonging to the genus...
Arthrobacter (Plotnikova et al. 2011). Interestingly, some studies have also reported the potential of Arthrobacter sp. strain YC-RL1 for utilizing bisphenol A (BPA) as the sole source of carbon for its growth and metabolism (Ren et al. 2016). The organism Arthrobacter sp. DAT1 was isolated and characterized to efficiently degrade atrazine (Wang & Xie 2015). From Table 1, biodegradation of DBP and BBP by various microorganisms is well reported in the literature (Fang et al. 2010; Wu et al. 2010; Kumar et al. 2017; Feng et al. 2018; Zhang et al. 2018). However, except for a study carried out by Chatterjee & Dutta (2008) on Arthrobacter spp. for BBP degradation, its potential to degrade other phthalates is largely unknown. Moreover, there has been no study carried out to establish the potential of Arthrobacter sp. to degrade EDCs in a surfactant-aided system. Besides, details of kinetics of biodegradation and biomass growth by the organism using phthalates as the carbon source are lacking for establishing its application potential. Hence, this study was aimed at biodegradation of phthalates by Arthrobacter sp. using BBP and DBP as the carbon source. In order to enhance the bioavailability of these compounds, four chemical surfactants, viz. Tween 20, Tween 80, Triton X-100 and sodium dodecyl sulfate (SDS), were assessed as to their capability to solubilize BBP and DBP in aqueous media.

The results of biomass growth and degradation of BBP and DBP by Arthrobacter sp. obtained in the batch experiments conducted at different initial concentrations of the compounds and in the presence of a surfactant were compared with that obtained in experiments without any added surfactant. In order to gain further insight into the biokinetics of DBP biodegradation and biomass growth by Arthrobacter sp. in a surfactant-aided system the experimental data were fitted to different kinetic models reported in the literature and the biokinetic parameters involved were evaluated in the study. To our knowledge, this is the first study to show some new evidence for the biodegradation of both BBP and DBP using Arthrobacter sp. along with a detailed study on the biodegradation of these compounds.

**MATERIALS AND METHODS**

**Chemicals and reagents**

Benzyl butyl phthalate (>97% pure) and dibutyl phthalate (>97% pure) used in the study were obtained from TCI Chemicals Pvt. Ltd (Chennai, India). Analytical grade chemicals for preparing the bacterial growth media were obtained from Merck (Mumbai, India). Solvents (HPLC grade), including methanol, acetonitrile, and water were procured from Finar Limited (Ahmedabad, India). The surfactants Tween 20, Tween 80, Triton X-100 and SDS were purchased from either Hi-Media, Merck or Sigma-Aldrich.

**Table 1** Microorganisms reported in the literature on biodegradation of BBP and DBP

| Bacterial strain | Compound | Concentration | Degradation (%) | Incubation time | References |
|------------------|----------|---------------|-----------------|-----------------|------------|
| Ochrobactrum sp. JDC-41 | DBP | 500 mg L⁻¹ | 87 | 48 hr | Wu et al. (2010) |
| Rhodococcus sp. HS-D2 | BBP | 500 mg L⁻¹ | 100 | 96 hr | Zhang et al. (2018) |
| Pleurotus ostreatus | DBP | 500 mg L⁻¹ | 99.6 | 312 hr | Ahuactzin-Pérez et al. (2018b) |
| | | 1,000 mg L⁻¹ | 94 | 504 hr | |
| Fusarium culmorum | DBP | 500 mg L⁻¹ | 99 | 168 hr | Ahuactzin-Pérez et al. (2018a) |
| | | 1,000 mg L⁻¹ | 99 | 228 hr | |
| Acinetobacter sp. HS-B1 | DBP | 300 mg L⁻¹ | 100 | 3 days | Wen et al. (2014) |
| Arthrobacter sp. C21 | | | | | |
| Pseudomonas sp. V21b | DBP | 1,000–2,000 mg L⁻¹ | 57 | 192 hr | Kumar et al. (2017) |
| Comamonas sp. 51F | | | 46 | 192 hr | |
| Bacillus megaterium YJB3 | DBP | 100 mg L⁻¹ | 82.5 | 5 days | Feng et al. (2018) |
| Arthrobacter sp. WY | BBP | 1 g L⁻¹ | 95 | 44 days | Chatterjee & Dutta (2008) |
| Acinetobacter sp. 33F | DBP | 2,000 mg L⁻¹ | 82.45 | 192 hr | Vinay & Maitra (2016) |
Microorganism and culture conditions

The bacterial isolate *Arthrobacter sp.* capable of phthalate biodegradation was kindly donated by Department of Microbiology, Bose Institute, Kolkata, India. For routine growth and maintenance of the culture, Luria Bertani (LB) broth containing (g L⁻¹) peptone (10), yeast extract (5), and NaCl (5), was used. For phthalate biodegradation, minimal salt medium (MSM) containing (g L⁻¹) MgSO₄·7H₂O (0.409), CaCl₂ (0.0200), KH₂PO₄ (1), NH₄NO₃ (1), Na₂HPO₄·12H₂O (6), and FeCl₃·6H₂O (0.0833) and 1% (v/v) of each trace metal solution, i.e., 50 μL trace elements (g L⁻¹): FeCl₃·6H₂O (28.33), CaCl₂·2H₂O (0.8), ZnSO₄·7H₂O (0.356), CuSO₄·5H₂O (0.1712), MnSO₄·H₂O (0.224), CoCl₂·6H₂O (1.465), H₃BO₃ (0.1) and Na₂MoO₄·2H₂O (0.3), pH 7, was used (Paul et al. 2019). The bacterium was cultured at 28°C temperature and 180 rpm agitation conditions (Chatterjee & Dutta 2013).

Aqueous solubility of phthalates with surfactants

Based on the chemical nature, i.e., ionic or non-ionic, four different surfactants, namely Triton X-100, Tween 80, Tween 20 and sodium n-dodecyl sulfate (SDS), were evaluated for enhancing the aqueous solubility of BBP and DBP (Mahanty et al. 2007). To determine the extent of phthalate aqueous solubility in the presence of these surfactants, six different standards for each of the four surfactants were prepared from their respective stock solutions containing 5–30 times the critical micellar concentration (CMC) of the surfactants. To each of the standards, 100 mg L⁻¹ of the phthalates were added in separate flasks followed by agitating the flasks overnight on an orbital shaker maintained at 180 rpm (Scigenics Biotech, Chennai, India). The resulting mixture was filtered using a 0.45-micron nylon membrane filter (Axiva Sichem Biotech, Delhi, India) to separate any insoluble compound. Soluble phthalate concentration in surfactant solutions was subsequently analyzed by high performance liquid chromatography (Shimadzu SPD-20A, Japan) as detailed later in Analytical methods.

Effect of surfactants on bacterial growth

The effect of different non-ionic surfactants (Tween 20, Tween 80, Triton X-100) and the anionic surfactant SDS on *Arthrobacter* sp. biomass growth was investigated at five different concentrations above their respective CMC values, i.e., 10–30 times of CMC of these surfactants. In these experiments, the individual surfactants served as the sole carbon and energy source. *Arthrobacter* sp. growth in the presence of surfactants was determined by measuring optical density of the culture at 600 nm using a UV-Vis spectrophotometer (Antech, California, USA). A flask without any added surfactant served as the control in this study, whereas the other flasks were incubated with different surfactants at concentrations above their respective CMC values as mentioned earlier.

Surfactant-aided biodegradation of phthalates

Based on the results of the previous experiments, biodegradation of phthalates by *Arthrobacter* sp. was evaluated by using Tween 80 as the surfactant, which yielded maximum solubility of BBP and DBP and also showed the least toxic effect on the bacterium growth as compared with the other surfactants. All experiments in this study were performed in duplicate using Erlenmeyer flasks (250 mL) with 100 mL working volume, and each of the flasks contained MSM, inoculum (10% (v/v)), phthalate (BBP or DBP) and the surfactant (Tween 80). The flasks were added with different initial BBP/DBP concentrations of 100, 200, 400, 600, 800, 1,000 mg L⁻¹ in MSM (pH 7), and 10% (v/v) freshly grown bacterial cultures from the mid-log phase were used as the inoculum for this batch study. The flasks were then incubated for 192 h at 28°C and continuous shaking of 180 rpm in an orbital incubator shaker. Control experiment was carried out using a flask containing either BBP or DBP but without any added surfactant ( Tween 80) and keeping all the other parameters the same. Samples were collected at regular time intervals of 12 h and analyzed for biomass and BBP/DBP concentrations.

Biomass dry weight was expressed as cell dry weight (CDW, mg L⁻¹), and the biomass specific growth rate (μ)
was calculated as per the following Equation (1):

$$\mu = \frac{1}{X} \frac{dX}{dt}$$  \hspace{1cm} (1)

where $\mu$ is the biomass specific growth rate (h$^{-1}$), $X$ is the biomass concentration (g L$^{-1}$) and $t$ is time (h).

The biokinetics of DBP utilization by *Arthrobacter* sp. were analyzed by fitting the experimental data to first order, logarithmic and Logistic kinetic models (Equations (2)–(4)).

First order

$$S = S_0 e^{(-K_0 t)}$$  \hspace{1cm} (2)

Logarithmic

$$S = S_0 + X_0 [1 - e^{(\mu_{\text{max}} t)}]$$  \hspace{1cm} (3)

Logistic

$$S = \frac{S_0 + X_0}{1 + \frac{X_0}{S_0} e^{\left(\frac{\mu_{\text{max}} K_s}{S_0} (S_0 + X_0) t\right)}}$$  \hspace{1cm} (4)

where $S$ and $S_0$ are the instantaneous and initial DBP concentrations (g L$^{-1}$), respectively. $X_0$ is the initial biomass concentration (g L$^{-1}$), $K_0$ is the first-order rate constant (h$^{-1}$), $\mu_{\text{max}}$ is the maximum DBP specific utilization rate (h$^{-1}$), and $K_s$ is the half-saturation coefficient (mg L$^{-1}$). In addition, the following modified form of the Gompertz model was used to explain DBP utilization by *Arthrobacter* sp.

$$S_0 - S = S_{\text{max}} e^{-\lambda} \left[1 - \left(\frac{r_{\text{max}} S_{\text{max}}}{S_{\text{max}}}\right)^{\frac{1}{\lambda-1}}\right]$$  \hspace{1cm} (5)

where $r_{\text{max}}$ is the maximum rate of DBP utilization (mg L$^{-1}$ h$^{-1}$), $S_{\text{max}}$ is the maximum utilizable DBP concentration (mg L$^{-1}$) and $\lambda$ is lag time for DBP utilization (h).

The effect of initial DBP concentration on its specific utilization rate ($q$, h$^{-1}$) by the bacterium was calculated by using the following Equation (6), and the experimental data fitted to different bio-kinetic models reported in the literature (Equations (7)–(13)).

$$q = -\frac{1}{X} \frac{dS}{dt}$$  \hspace{1cm} (6)

Monod

$$q = \frac{q_{\text{max}} S}{K_s + S}$$  \hspace{1cm} (7)

Haldane

$$q = \frac{q_{\text{max}} S}{K_s + S + \frac{S^2}{K_i}}$$  \hspace{1cm} (8)

Han–Levenspiel

$$q = \frac{q_{\text{max}} S}{K_s + S + \left(\frac{S^2}{S_m}\right)^n}$$  \hspace{1cm} (9)

Edward

$$q = \frac{\frac{q_{\text{max}} S}{K_s + S + \left(\frac{S^2}{S_m}\right)^n}}{1 + \left(\frac{S^2}{K_s + S + \left(\frac{S^2}{S_m}\right)^n}\right)}$$  \hspace{1cm} (10)

Moser

$$q = \frac{q_{\text{max}} S^n}{K_s + S^n}$$  \hspace{1cm} (11)

Yano and Koga

$$q = \frac{q_{\text{max}} S}{K_s + S + \left(\frac{S^2}{S_m}\right)^n}$$  \hspace{1cm} (12)

Luong

$$q = \frac{q_{\text{max}} S}{K_s + S \left[1 - \left(\frac{S}{S_m}\right)^n\right]}$$  \hspace{1cm} (13)

where $q$ is the specific DBP utilization rate (h$^{-1}$), $q_{\text{max}}$ is the maximum specific DBP utilization rate (h$^{-1}$), $S$ is the DBP concentration (mg L$^{-1}$), $S_m$ is the DBP concentration above which net growth ceases (mg L$^{-1}$), $K_s$ is the
half-saturation constant (mg L\(^{-1}\)), \(K_i\) is the inhibition constant (mg L\(^{-1}\)), \(K_1\) is a positive constant and \(n\) and \(m\) are empirical constants.

In addition to the above, the following two bio-kinetic models (Equations (14) and (15)) were further applied to simulate the biomass growth.

Gompertz

\[
X - X_0 = X_{\text{max}} e^{-e^{-\left(\frac{r_{\text{max}} \times 2.71828}{X_{\text{max}}} \right)^{\frac{t}{n+1}}}}
\]  

Logistic

\[
X - X_0 = \frac{X_{\text{max}}}{1 + e^{-\left(\frac{4r_{\text{max}} X_{\text{max}}}{X_{\text{max}}} \right)^{(t-t_0)^2}}}
\]  

where \(X_0\) is the initial biomass concentration, \(X\) is the biomass concentration at time \(t\), \(X_{\text{max}}\) is the maximum biomass concentration (g L\(^{-1}\)) and \(r_{\text{max}}\) is the maximum rate of biomass growth (g L\(^{-1}\) h\(^{-1}\)).

**Analytical methods**

Residual concentrations of BBP and DBP in the samples were quantified using a high pressure liquid chromatography (HPLC) (Shimadzu SPD-20A, Japan) fitted with C-18 column (Shim-pack GIST-HP C18) of dimensions 250 mm \(\times\) 4.6 mm and a UV detector set at 220 and 254 nm (dual mode on) at an oven temperature of 30 \(^\circ\)C. Acetonitrile and deionized water in the ratio 70:30 was used as the mobile phase at a flow rate of 0.5 mL min\(^{-1}\) and a run time of 25 min for this analysis.

The biomass growth of *Arthrobacter* sp. in this study was estimated by analyzing optical density (OD\(_{600}\)) of the culture, and for which absorbance (optical density) of the bacterial culture was measured at 600 nm using a UV-Vis spectrophotometer. Biomass determination in this study was verified based on its cell dry weight (CDW) by lyophilizing the biomass obtained after centrifugation followed by washing the cell pellet with sterile 0.8% w/v NaCl saline and weighing of dry biomass.

**RESULTS AND DISCUSSION**

Screening of surfactants based on enhancement of phthalate aqueous solubility and effect on *Arthrobacter* sp. biomass growth

Figure S1 (supplementary information) shows the effect of different surfactants on enhancing the aqueous solubility of BBP and DBP, which revealed the following order for both the compounds: SDS > Tween 80 > Triton X-100 > Tween 20. Hence, Tween 80 and SDS proved effective as compared with the other chemical surfactants. In the literature, Aryal & Liakopoulou-Kyriakides (2013) reported increased solubilization in presence of surfactant and successive biodegradation of phenanthrene and pyrene by *Arthrobacter* strain using Tween 20 and Tween 80 as the surfactants. The authors also observed that the biodegradation of both the polycyclic aromatic hydrocarbons in the presence of the surfactants followed the first-order kinetics model. Additionally, molar solubilization ratio (MSR) of pyrene using different surfactants was studied by Mahanty et al. (2007), which showed that Tween 80 has a higher MSR value compared to other surfactants for enhancing the aqueous solubility of the polycyclic aromatic hydrocarbons (PAH).

The effect of non-ionic (Tween 20, Tween 80, and Triton X-100) and anionic (SDS) surfactants on the biomass growth of *Arthrobacter* sp. after 48 h of incubation was further analyzed, and the results revealed that the bacterial growth enhanced with a rise in Tween 80 (Figure S2.a) and Tween 20 (Figure S2.b) concentrations compared to that without any added surfactant in the medium. However, in the case of Triton X-100 and SDS, the bacterial growth was either reduced or totally inhibited with an increase in their concentration in the medium. However, in the case of Triton X-100 and SDS, the bacterial growth was either reduced or totally inhibited with an increase in their concentration in the media (Figure S2.c and d). These results showed that *Arthrobacter* sp. utilized Tween 80 and Tween 20 as the sole source of carbon for its metabolism and growth. In the literature, Chatterjee & Dutta (2008) reported that biodegradation of BBP with Tween 80 caused a two-fold increase in degradation rate as compared with that in the absence of any added surfactant. SDS and Triton X-100 are known to inhibit biomass growth of *Streptococcus faecalis* ATCC 9790 by inducing lysis of cells in the exponential growth phase (Cornett & Shockman 1978).
Besides, Mahanty et al. (2007) reported that surfactants with a high MSR value, e.g., Tween 80 exhibits a low toxicity on microorganisms capable of degrading PAHs. However, Aryal & Liakopoulou-Kyriakides (2013) observed an increase in cell number of *Arthrobacter* strain Sphe3 with increasing Tween 80 and Tween 20 concentrations. Hence, based on the results obtained in this study, Tween 80 was further chosen for performing the batch biodegradation experiments with *Arthrobacter* sp.

**Effect of Tween 80 on biomass growth by *Arthrobacter* sp. at different phthalate concentrations**

Figure 1 compares the time profile of *Arthrobacter* sp. biomass growth at different initial concentrations of BBP with or without Tween 80, which reveals that the bacterial biomass (CDW) steadily increased with an increase in BBP concentration even at 1,000 mg L\(^{-1}\) in the presence of the surfactant. From Figure 1(a) and 1(b), it is observed that in the presence of Tween 80 a biomass concentration of 1.744 g L\(^{-1}\) is achieved at 120 h for 600 mg L\(^{-1}\) initial BBP concentration, which is 4.59 fold increase in biomass concentration value obtained without the surfactant addition. Similarly, for an initial DBP concentration of 600 mg L\(^{-1}\) in the presence of Tween 80, a biomass concentration (maximum) of 1.819 g L\(^{-1}\) is obtained at the end of 120 h, which is a 5.66 fold increase in biomass concentration without the surfactant (Figure 2(a) and 2(b)). These results clearly reveal that the biomass growth rate is relatively lower in the absence of surfactant, probably due to poor solubility and therefore very low bioavailability of these compounds in the media for an efficient utilization by the bacterium. All these results clearly reveal that in the presence of surfactant, the bacterium is very capable of utilizing phthalates as the sole carbon source. However, in the presence of Tween 80, *Arthrobacter* sp. showed a lag period in its growth due to BBP, which was not observed with DBP. The lag phase in growth of the bacterium due to BBP can be attributed to its greater recalcitrance compared to DBP. Hence, in order to further confirm the uptake of BBP and DBP by the bacterium for its growth, their biodegradation in the presence of Tween 80 was analyzed.

**Effect of Tween 80 on phthalates biodegradation by *Arthrobacter* sp. at different initial concentrations**

Time profile of BBP and DBP biodegradation at different initial concentrations in the absence or presence of Tween 80 are depicted in Figures 3 and 4, respectively. From Figures 3(a) and 4(a), it could be seen that both the compounds were almost completely degraded (98%) for an initial concentration of BBP and DBP up to 600 mg L\(^{-1}\). At a concentration above 600 mg L\(^{-1}\) and in the absence of Tween 80, the degradation efficiency decreased due to limited solubility of the compounds in the liquid medium; the values were 30.1% and 29.2% for 1,000 mg L\(^{-1}\) of
Figure 2 | Time profile of Arthrobacter sp. biomass growth at different initial concentrations of DBP in (a) absence and (b) presence of Tween 80.

Figure 3 | Time profile of BBP biodegradation at different initial concentrations in (a) absence and (b) presence of Tween 80.

Figure 4 | Time profile of DBP biodegradation at different initial concentrations in (a) absence and (b) presence of Tween 80.
BBP and DBP, respectively. Conversely, in the presence of Tween 80, BBP and DBP degradation rapidly increased after 24 and 12 h of culture, respectively (Figures 3(b) and 4(b)). These results further demonstrate that DBP was almost completely degraded (99.1%) even up to 1,000 mg L$^{-1}$ concentration. In the case of BBP, complete degradation was achieved only up to 800 mg L$^{-1}$ concentration, and a degradation efficiency of 58% was obtained for 1,000 mg L$^{-1}$ initial concentration. Hence, compared with BBP, DBP is more effectively degraded by *Arthrobacter* sp. due to its less recalcitrant structure. It is reported that owing to the presence of a single benzene ring, DBP is more easily degradable than the other phthalates (Wu *et al.* 2013; Vinay & Maitra 2016).

Surfactants are known to stimulate the uptake of micellar pollutants, resulting in their efficient biodegradation and an increased growth rate of microorganisms (Kaczorek & Olszanowski 2021). This phenomenon occurs mainly due to either phthalate and surfactant being utilized co-metabolically or direct uptake of phthalate containing surfactant micelles (Volkering *et al.* 1998). Hence, corroborating the results of biodegradation of BBP and DBP by *Arthrobacter* sp. with that of the previous experiment on biomass growth of the bacterium, it could be surmised that the latter is the mechanism of action by Tween 80 in this study.

It has been reported that DBP degradation by *Bacillus megaterium* YJB3 involves intermediates such as mono-butyl phthalate (MBP), phthalic acid (PA), dihydroxyphthalate, and protocatechuate (PCA) (Feng *et al.* 2018). Besides these compounds, mono-butyl phthalate and PA are the two transient metabolites formed during the degradation of DBP by *Enterobacter* sp. T5 (Wu *et al.* 2010). The biodegradation pathway of these two phthalates (BBP and DBP) used in this study by *Arthrobacter* sp. follows a similar pattern as already reported in various literature mentioned before. The pathway of BBP and DBP degradation was investigated by analyzing transient metabolites/intermediates at every stage of degradation using LC-MS, and the results revealed that both BBP and DBP followed the same degradation pathway (PA pathway) (Figure 5). The first degradation product of both BBP and DBP was mono-butyl phthalate, which was further broken down by

![Figure 5](http://iwaponline.com/ws/article-pdf/21/5/2084/920242/ws021052084.pdf)
phthalate 3,4-dioxygenase enzyme to form PA. During the degradation process, other pathway metabolites formed were 3,4-dihydroxyphtalate, protocatechuic acid, β-carboxy-muconate, γ-carboxy-muconolactone, followed by its successive degradation to form succinyl coenzyme A (CoA) which then enters Krebs (tricarboxylic acid) cycle via β-oxidation.

In the literature, *Pleurotus ostreatus* is reported to degrade 500 and 1,000 mg L\(^{-1}\) of DBP with efficiencies 99.6 and 94% at the end of 312 and 504 h, respectively (Ahuactzin-Pérez et al. 2018a). In another study, *Fusarium culmorum* was observed to degrade DBP at the same initial concentrations with 99% efficiency after an incubation period of 168 and 228 h, respectively (Ahuactzin-Pérez et al. 2018b). Compared with these literature reports, *Arthrobacter* sp. is found to be highly efficient in quickly degrading DBP even up to 1,000 mg L\(^{-1}\). In order to gain further insight into DBP degradation by the bacterium, kinetics of the biodegradation process were further analyzed.

**Kinetics of DBP utilization and biomass growth by *Arthrobacter* sp.**

Experimental and predicted DBP utilization by *Arthrobacter* sp. obtained using different biokinetic models are shown in Figure 6, and estimated values of biokinetic parameters from these models are presented in Table 2. Among the three different models tested – First order, Logarithmic, and Logistic kinetic models, the Logistic model accurately fitted the experimental data for all the initial concentrations of DBP with a coefficient of determination (R\(^2\)) value greater than 0.98 (Table 2 and Figure 6(c)). Hence, it could be appropriately stated that the utilization of DBP by *Arthrobacter* sp. is growth-associated. The estimated maximum DBP utilization rate value of 0.0012–1.8309 h\(^{-1}\) from this growth-associated model further confirmed that *Arthrobacter* sp. is highly efficient in utilizing DBP in the presence of Tween 80. Wen et al. (2014) reported complete degradation of DBP as the sole substrate for an initial concentration of 300 mg L\(^{-1}\) within 3 days of culture, and kinetic analysis of the results revealed that DBP biodegradation followed first-order kinetics but only for an initial concentration less than 100 mg L\(^{-1}\).

In order to further understand the growth-associated kinetics of DBP utilization by the bacterium, the values of specific DBP utilization rate obtained for all initial concentrations of the compound were fitted to various kinetic models as described in the literature. Kinetic variables involved were evaluated by fitting the experimental data to Luong, Han–Levenspiel, Yano and Koga, Moser, Monod, Haldane and Edward kinetic models values of the kinetic parameters are presented in Table 3. The results show that the Moser model well described the experimental data with R\(^2\) value of 0.9998 followed by the other models. However, the Yano–Koga and Han–Levenspiel models yielded low R\(^2\) values of less than 0.8. The values of maximum specific DBP utilization rate (q\(_{\max}\)) and half-saturation coefficient (K\(_s\)) using the best-fitting Moser model were calculated to be 9.5561 h\(^{-1}\) and 535.03 mg L\(^{-1}\), respectively. Figure 7 further confirms that the Yano–Koga and Han–Levenspiel models did not accurately fit the experimental values. Moreover, the substrate inhibition constant (K\(_i\)) value of 15,236.4 estimated from the Haldane model proved that *Arthrobacter* sp. is highly capable of utilizing a very high concentration of DBP in presence of Tween 80 without causing inhibition on its growth (Sinharoy et al. 2019).

The effect of different initial DBP concentration on *Arthrobacter* sp. biomass growth was further modelled using modified Gompertz and Logistic models. Figure 8 presents the experimental and the predicted *Arthrobacter* sp. biomass growth at different initial DBP concentrations; the biokinetic parameters assessed from the two models are presented in Table 4. It can be clearly observed that the modified Gompertz model is better than the Logistics model in explaining the experimental results (R\(^2\) > 0.97). The predicted maximum specific growth rate of *Arthrobacter* sp. increased from 0.0161 to 0.0352 h\(^{-1}\), with an increase in the initial DBP concentrations from 100 to 1,000 mg L\(^{-1}\), and the predicted values of maximum biomass concentration varied in the range from 0.4011–2.769 g L\(^{-1}\) for 100–1,000 mg L\(^{-1}\) initial concentrations of DBP. The experimental values of maximum biomass concentration obtained in the range 0.540–2.829 g L\(^{-1}\) were also close to the values estimated by the modified Gompertz model. Moreover, these results confirm that an increase in concentration of DBP did not inhibit the biomass growth of *Arthrobacter* sp. which agrees with the findings reported by Dou et al. (2009).
Figure 6 | Experimental and predicted values of DBP utilization due to (a) First order, (b) Logarithmic and (c) Logistic kinetic models.
Hence, the results of kinetic analysis of biomass growth and DBP degradation by *Arthrobacter* sp. are useful for further upscaling of the present simple batch degradation system to a continuous pilot bioreactor system. Furthermore, proper monitoring and control of biokinetic parameters involved in the biodegradation process can help maintain high degradation efficiency of phthalates by the bacterium at a large scale. However, owing to their very low CMC, bio-surfactants instead of the chemical surfactant Tween 80 evaluated in this study can be evaluated for enhancing bioavailability and biodegradation of EDCs by potent organisms. Moreover, identification of metabolites formed during

### Table 2 | Estimated values of bio-kinetic model parameters on DBP utilization in this study

| Model        | Initial DBP concentration (mg L\(^{-1}\)) |
|--------------|------------------------------------------|
|              | 100  | 200  | 400  | 600  | 800  | 1,000 |
| First order  |      |      |      |      |      |       |
| \(S_0\) (mg L\(^{-1}\)) | 106.283 | 225.071 | 470.897 | 718.358 | 971.707 | 1,209.646 |
| \(K_1\) (h\(^{-1}\)) | 0.0398 | 0.0281 | 0.0237 | 0.0200 | 0.0170 | 0.0140 |
| \(R^2\)     | 0.989 | 0.890 | 0.849 | 0.797 | 0.770 | 0.7610 |
| Logarithmic  |      |      |      |      |      |       |
| \(S_0\) (mg L\(^{-1}\)) | 54.073 | 135.186 | 297.540 | 505.005 | 755.028 | 1,000.926 |
| \(X_0\) (g L\(^{-1}\)) | 3,578.791 | 9,743.567 | 20,232.296 | 45,480.901 | 48,270.741 | 32,082.66 |
| \(U_{max}\) (h\(^{-1}\)) | 0.0001 | 0.0001 | 9.83986E-05 | 7.43076E-05 | 0.0001 | 0.0001 |
| \(R^2\)     | 0.548 | 0.658 | 0.666 | 0.7607 | 0.845 | 0.9156 |
| Logistic     |      |      |      |      |      |       |
| \(S_0\) (mg L\(^{-1}\)) | 98.883 | 192.720 | 398.155 | 588.892 | 791.764 | 992.143 |
| \(X_0\) (g L\(^{-1}\)) | 11.347 | 4.264 | 7.632 | 7.333 | 13.524 | 24.621 |
| \(U_{max}\) (h\(^{-1}\)) | 0.223 | 0.0973 | 0.641 | 0.090 | 0.001 | 1.830 |
| \(K_S\) (mg L\(^{-1}\)) | 241.854 | 185.362 | 2,913.940 | 667.982 | 15.877 | 37.098.27 |
| \(R^2\)     | 0.998 | 0.999 | 0.999 | 0.987 | 0.987 | 0.980 |

### Table 3 | Estimated values of biokinetic model parameters on specific DBP utilization rate by *Arthrobacter* sp.

| Model             | \(q_{max}\) (h\(^{-1}\)) | \(K_s\) (mg L\(^{-1}\)) | \(K_i\) (mg L\(^{-1}\)) | \(S_m\) (mg L\(^{-1}\)) | \(n\) | \(m\) | \(K_p\) | \(R^2\) |
|-------------------|---------------------------|-------------------------|--------------------------|--------------------------|------|------|--------|--------|
| Luong             | 9.838                     | 477.34                  | –                        | 3,846.5                  | 1,497.1 | –     | –      | 0.997  |
| Han-Levenspiel    | 11.501                    | 596.08                  | –                        | 999.6                    | 3.492  | 3.971 | –      | 0.778  |
| Yano–Koga         | 5.932                     | 108.92                  | –                        | –                        | –0.1705 | –     | 999.9  | 0.600  |
| Moser             | 9.556                     | 535.03                  | –                        | –                        | 1.029  | –     | –      | 0.999  |
| Monod             | 9.838                     | 477.34                  | –                        | –                        | –      | –     | –      | 0.999  |
| Haldane           | 10.577                    | 529.49                  | 15,236.4                 | –                        | –      | –     | –      | 0.999  |
| Edward            | 10.718                    | 541.71                  | –                        | 34,898.2                 | –      | –     | –      | 0.997  |

**Figure 7** | Experimental and predicted values of specific DBP utilization rate at different initial DBP concentrations.
phthalates biodegradation and details of the pathway followed by *Arthrobacter* sp. would throw more light on controlling the biodegradation process. In addition, biodegradation of phthalates in the presence of co-pollutants might be studied to establish the robustness of the process in a realistic situation.

**CONCLUSIONS**

Among the different surfactants evaluated in this study, Tween 80 was found to be highly effective for enhancing the bioavailability of both BBP and DBP and their biodegradation by *Arthrobacter* sp. Phthalate biodegradation and biomass growth by the bacterium in the presence of Tween 80 indicated that the phthalate uptake mechanism involved direct uptake of surfactant micelles containing the pollutant. Kinetics of DBP utilization, *Arthrobacter* sp. biomass growth and specific DBP utilization rate revealed that *Arthrobacter* sp. was highly capable and effective in degrading DBP even at very high concentrations in the presence of the surfactant without any inhibitory effect on its biomass growth. This study demonstrated an excellent potential of *Arthrobacter* sp. for the successful degradation of phthalates using a surfactant-aided system.

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**DATA AVAILABILITY STATEMENT**

All relevant data are included in the paper or its Supplementary Information.

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