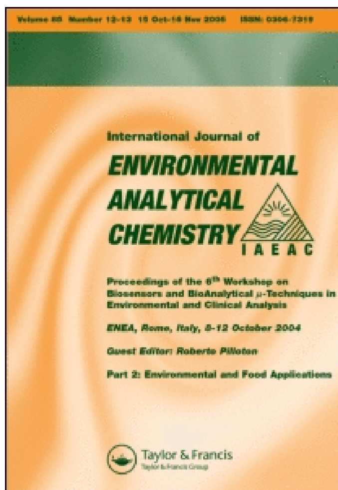
This article was downloaded by:
On: *17 January 2011*
Access details: *Access Details: Free Access*
Publisher *Taylor & Francis*
Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



International Journal of Environmental Analytical Chemistry
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713640455

Spectrophotometric verification of biodegradation of phenol in a flow dynamic biocers-based bioreactor system

Martin Mkandawire[a]; Jochen Förster[a]; Dirk Fiedler[b]; Horst Böttcher[b]; Wolfgang Pompe[a]
[a] Institute of Materials Science, Dresden University of Technology, Dresden, Germany [b] Department of Functional Coatings, GMBU e.V., Dresden, Germany

To cite this Article Mkandawire, Martin , Förster, Jochen , Fiedler, Dirk , Böttcher, Horst and Pompe, Wolfgang(2009) 'Spectrophotometric verification of biodegradation of phenol in a flow dynamic biocers-based bioreactor system', International Journal of Environmental Analytical Chemistry, 89: 7, 529 — 541
To link to this Article: DOI: 10.1080/03067310802714839
URL: http://dx.doi.org/10.1080/03067310802714839

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: http://www.informaworld.com/terms-and-conditions-of-access.pdf

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Intern. J. Environ. Anal. Chem.
Vol. 89, No. 7, 15 June 2009, 529–541

Taylor & Francis
Taylor & Francis Group

Spectrophotometric verification of biodegradation of phenol in a flow dynamic biocers-based bioreactor system

Martin Mkandawire[a]*, Jochen Förster[a], Dirk Fiedler[b], Horst Böttcher[b] and Wolfgang Pompe[a]

[a]*Institute of Materials Science, Dresden University of Technology, Dresden, Germany;* [b]*Department of Functional Coatings, GMBU e.V., Dresden, Germany*

(*Received 3 September 2008; final version received 29 December 2008*)

A quick fingerprint spectrophotometric procedure based on the absorbance in the ultraviolet spectrum region was developed to verify biodegradation of phenol in flowing contaminated water. The procedure was employed to test the functional feasibility of a biocers-packed bioreactor to filter phenol on a bench-scale fluidic dynamic system, which should simulate a macroscopic microorganism-based remediation system. The biocers contained *Rhodococcus* sp. P1, a phenol degrading bacteria. An influent with initial phenol concentration of $500\,mg\,L^{-1}$ was fed into the bioreactor at different flow rates, and the effluent was continuously monitored for residual phenol concentration by coupling the fluid dynamic system to a UV-vis spectrophotometer. Within 3 days, the effluent from bioreactor reached a minimum residual concentration of phenol of $<40\,mg\,L^{-1}$. Therefore, the aim of the current paper is to report results of the procedure for on-line spectrophotometric detection of phenol, and the feasibility of a biocers-packed bioreactor for degradation of phenol.

Keywords: sol–gel matrix; UV-vis spectrophotometer; bioremediation; *Rhodococcus* sp., phenol biodegradation

Downloaded At: 13:45 17 January 2011

1. Introduction

Most discharges of contaminants into the environment are either low concentrated but long-term, or spontaneously high but short-term. In some instance, most discharges do not originate from a point source. Such type of discharges may not warrant establishment of high technology wastewater treatment plants because of the economic implications. Instead, passive remediation technologies using biological systems are preferred to conventional wastewater treatment technology based on sophisticated and high technology chemical and civil engineering approach [1,2]. For instance, use of biological process to filter phenols from contaminated water have become promising because a few micro-organisms species have shown high capacity of biodegrading phenol [3,4]. One of species with high phenol biodegradation capacity is a bacterial strain of *Rhodococcus* sp. [5,6].

Many microbes like *Rhodococcus* sp. have phenol degradation potential. However, their introduction into the natural environment may turn invasive and cause more ecological harm than benefits. Therefore, immobilisation of microbial cells on inert

*Corresponding author. Email: martin.mkandawire@nano.tu-dresden.de

ISSN 0306–7319 print/ISSN 1029–0397 online
© 2009 Taylor & Francis
DOI: 10.1080/03067310802714839
http://www.informaworld.com

materials helps to retain the microbes within a reactor and to prevent suspended bacterial biomass in the effluent [7–10]. Further advantages of immobilisation of microbial cells include protection of cells against phenol toxicity, high degradation efficiency, and good operational stability [11]. Thus, there have been intense efforts to develop methods for immobilising bacteria in different materials. One of the promising procedures is embedding environmental beneficial microbes in sol–gel matrices called biocers [12], which can be used in passive water treatment for both inorganic and organic pollutants [13–15]. In view of the developments, the current study tested performance of a column biofilter (i.e., bioreactor) packed with biocers containing *Rhodococcus* sp. P1 for removal of phenol from contaminated water. Phenol biodegradation by suspended cells of *Rhodococcus* sp. in continuous culture systems [8,9,16] and by *Rhodococcus* sp. immobilised in calcium alginate beads or on granular activated carbon [17] has been reported to function well under laboratory conditions [18,19]. The advantage for preference of *Rhodococcus* sp. immobilised in biocer to the earlier methods is that the silica matrix of the biocer is inert that it does not influence the chemical processes, while at the same time it can prolong cells viability [12,20]. Further, the porous structure of the biocers allows easy flow of fluids to supply necessary nutrients to the microorganism.

In order to evaluate the efficiency of such an approach, it is important to monitor the biotransformation process on-line. UV-vis spectrophotometry has high potential for this on-line fingerprint verification of phenol biodegradation due to its easiness to operate and its low operational costs. Determination of phenol in water using 4-aminoantipyrine and potassium ferricyanide reaction and measuring at 500 nm wavelength has been in use for some years [6]. Unfortunately, the procedure is limited for on-line application in flow dynamic systems due to high dependence on the phenol-amino-antipyrine reaction kinetics and the stoichiometry, which may be difficult to determine under continuous flow conditions. Consequently, we developed a quick and cheap fingerprint spectrophotometric determination procedure based on phenol absorbance in the ultraviolet wavelength region. Therefore, we report in this paper 2-fold results *vis-a-vis* capacity and limitation of the spectrophotometric procedure, which we developed; and the feasibility of a biocers-packed bioreactors for degradation of phenol.

Downloaded At: 13:45 17 January 2011

2. Experimental

2.1 *Chemicals*

Phenol and all other chemicals were purchased from Merck (Darmstadt, Germany). All reactants and standard solutions were prepared in deionised water in concentrations of 1.0 mol L^{-1} from chemicals of analytical grade.

2.2 *Sol–gel matrix and biocers material*

The biocers impregnated with *Rhodococcus* sp. P1 (2.3 wt%) as well as the pure sol–gel matrix samples (used as control material) were kindly provided by the project partner, GMBU e.V. (Rossendorf, Dresden). The preparation process of the sol–gel samples and the impregnation with bacteria have been described in detail in Raff *et al.* [14], Soltmann *et al.* [13], and Matys [21]. The biocers were delivered as cylinders of 12 mm in diameter by 10 mm high and ~2 mm thick with estimated nominal porosity of ~30%.

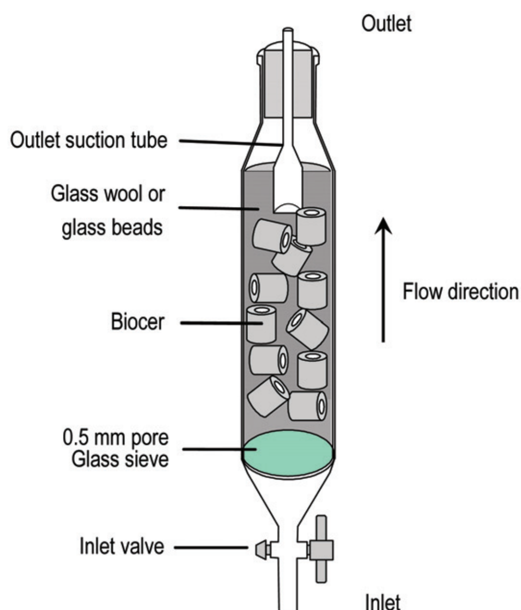


Figure 1. Structure of a bioreactor filled with biocer. Four of these bioreactors were used in the fluid dynamics system for laboratory bench experiments of biodegradation of phenol.

Downloaded At: 13:45 17 January 2011

2.3 *Bioreactor and fluid dynamic system*

The bioreactor was made from glass columns of 30 cm length and 2.5 cm internal diameter with a sinter-glass filter at the bottom, and packed with 10 biocer samples to sum up to an average of 20 g (Figure 1). The samples were stabilised by either glass beads or glass wool. Control bioreactors were filled with only sol–gel matrix samples. At full operation, each bioreactor contained about 100 mL of either phenol-containing or only pure mineral medium. The fluid dynamics system was constructed by connecting the bioreactors to the medium source (i.e., container) from which the medium was pumped using peristaltic pump with flow-rate regulation ability. An intermediate flask was placed between the bioreactor and the media source where the medium was bubbled with an air stream before supplied into the bioreactor (Figure 2). This was done because direct aeration to the bioreactor would cause cell desorption and presence of suspended cells in the effluent of the aerated column reactor. Outlet was connected to a modified flow cell of a UV–vis spectrophotometer (Varian Cary 100, Australia) where degradation of phenol was continuously monitored. Between the bioreactor and the UV–vis spectrophotometer, another intermediate bottle was placed, where stirring facilitated release of gasses from the bioremediation processes. Microbial growth was assessed routinely and directly through turbidity measurement as absorbance at 600 nm in the UV–vis spectrophotometer (Figure 2). The complete fluid dynamics system had four bioreactors.

2.4 *Phenol biodegradation assay*

Phenol biodegradation was assayed in continuous flow mode in biocers-packed bioreactors using synthetic groundwater based on mineral medium of Schlegel *et al.* [22] and modified with 500 mg L^{-1} phenol. The media composition is presented in Table 1.

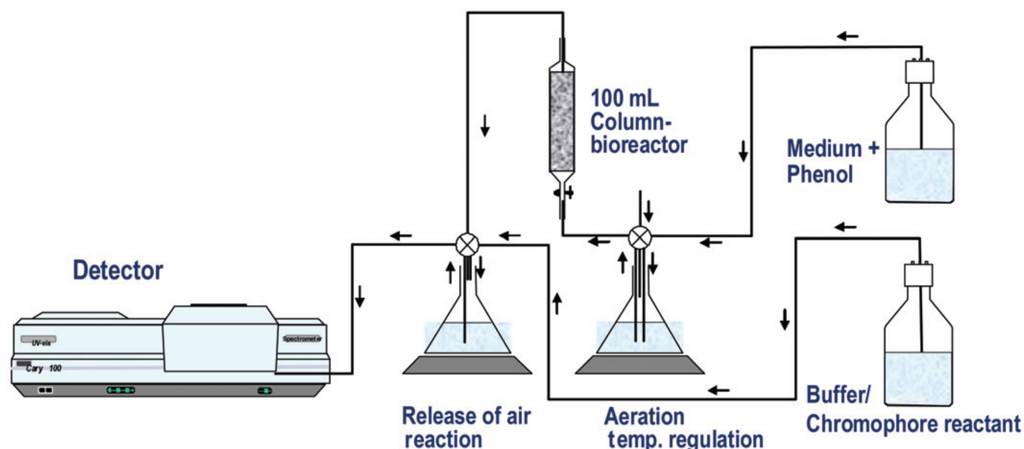


Figure 2. Scheme of the biocer column reactors of the fluid dynamics system coupled to UV-vis-spectrophotometer set-up for long-term continuous phenol biodegradation mode. The bottle labelled 'Buffer/chromophore reactant' and the subsequent connections were used only in the colorimetric detection of phenol using 4-aminoantipyrine and potassium ferricyanide procedure.

Downloaded At: 13:45 17 January 2011

Table 1. Composition of Schlegel medium for bacteria culture. The synthetic ground water was based on this mineral composition with phenol as sole source of carbon or in combination with $10\,g\,L^{-1}$ sucrose.

	Components	Concentration
Macroelement	$Na_2HPO_4{\cdot}12H_2O$	$4.5\,g\,L^{-1}$
	KH_2PO_4	$0.75\,g\,L^{-1}$
	NH_4Cl	$1.0\,g\,L^{-1}$
	$MgSO_4{\cdot}7H_2O$	$0.2\,g\,L^{-1}$
	$CaCl_2{\cdot}2H_2O$	$0.0265\,g\,L^{-1}$
Microelement	$FeSO_4{\cdot}7H_2O$	$0.01\,g\,L^{-1}$
	H_3BO_4	$50.0\,mg\,L^{-1}$
	$MnSO_4{\cdot}4H_2O$	$40.0\,mg\,L^{-1}$
	$ZnSO_4{\cdot}7H_2O$	$40.0\,mg\,L^{-1}$
	$NaMnO_4{\cdot}2H_2O$	$24.0\,mg\,L^{-1}$
	KI	$10.0\,mg\,L^{-1}$
	$CuSO_4{\cdot}5H_2O$	$10.0\,mg\,L^{-1}$

The reactor temperature was maintained at 30°C. Three hydraulic retention times and loading rates were controlled by the volumetric flow-rates of $0.01\,ml\,s^{-1}$; $0.03\,ml\,s^{-1}$; and $0.07\,ml\,s^{-1}$. The inlet pH value was 7.2. The system operated for 6 h to allow the bacteria transformation from senescence before monitoring of the biodegradation of phenol commenced. The measured parameter was mainly the phenol concentration outlet. Phenol degradation in the medium was monitored in the bioreactor effluent through a direct coupling to UV-vis spectrophotometer flow using (a) colorimetric method of 4-aminoantipyrine and potassium ferricyanide [6], and (b) a self developed procedure (detailed below). A control reactor filled with sol–gel samples (i.e. without the biocomponent) was operated in parallel and monitored under the same conditions. The whole biodegradation assay ran for 5 days and each experiment was replicated four times. To ascertain whether the phenol depletion in the bioreactors of the fluid

dynamics system was biologically initiated, a few selected samples of spent media were analysed with IR spectroscopy for existence of known transient compounds of the phenol biodegradation pathways.

2.5 *Analysis*

2.5.1 *Scanning electron microscopy investigation*

After the 4 days in phenol biodegradation assay the spent biocer likewise sol–gel matrix samples were observed on scanning electron microscopy (SEM) (Carl Zeiss, Jena Germany) to determine the extent and pattern of bacterial growth in the biocer, as well as, whether the bacterial growth affects the stability and structure of the biocer. The spent biocer samples were selected randomly from each bioreactor of the fluid dynamics system, then rinsed twice with deionised water and dehydrated in an ethanol gradient (30–50–70–90–100% v/v) for 1 h at 4°C. Subsequently, the specimens were dried at 30°C in oven (Binder, Tuttlingen Germany) until a constant weight was reached. Then, samples were carbon coated for 30 s, followed by palladium-gold coating for another 30 s before inspection on scanning electron microscope at acceleration voltage between 2.0 and 5.0 kV.

Downloaded At: 13:45 17 January 2011

2.5.2 *UV-vis spectrophotometric analysis*

Phenol biodegradation in the bioreactor was determined through measuring the residual phenol concentration in the effluent using colorimetric methods. Consequently, there was some modification in coupling the fluid dynamics system to the UV-vis spectrophotometer as explained in the caption of Figure 2. First, the phenol was determined using 4-aminoantipyrine and potassium ferricyanide procedure [6]. 16.7 g L^{-1} 4-aminoantipyrine ($C_{11}H_{13}N_3O$), 10 g L^{-1} $K_3[Fe(CN)_6]$ and buffer of 0.07 M (i.e. 23.6 g L^{-1}) $Na_2HPO_4 \cdot 12H_2O$ were pumped to mix with sample (see Figure 2) and incubated for 15 min before measuring on the UV-vis spectrophotometer at 500 nm. The volume ratios were 1 : 8 : 16 : 20 of $C_{11}H_{13}N_3O$ to $K_3[Fe(CN)_6]$ to $Na_2HPO_4 \cdot 12H_2O$ to the sample, respectively. The measurements were accurate within the concentration range of 0–10 g L^{-1} phenol. Furthermore, a second and independent colorimetric measurement using an own developed method was applied. In our modified procedure, the effluent was pumped directly into the flow cell of the UV-vis spectrophotometer and measured in the UV spectrum region, and calibrated at 270 nm wavelength.

In both colorimetric methods, a flow cell was used in the UV-vis spectrophotometer for continuous sampling and determination of phenol in the effluent. The sampling was set to start automatically after 6 h of experimental operation. All samples were measured against a reagent blank. The results were verified by analysing selected samples with gas chromatography and IR. The product compounds found were compared with known compounds in the phenol biodegradation pathway.

3. Results and discussion

3.1 *Bacterial growth in the biocers*

Analysis and observation of the surface and internal structure of the biocer on the SEM revealed that bacteria could grow on the surface of the biocer, and could also penetrate

into the internal pores (Figure 3). The biocer has macro- and micro-pores (Figure 3a and b), which allow good supply of the medium and gaseous exchange that facilitate bacteria growth and the biodegradation of phenol. The microporous structure might also have acted like sponge, which store up the growth medium for a long time.

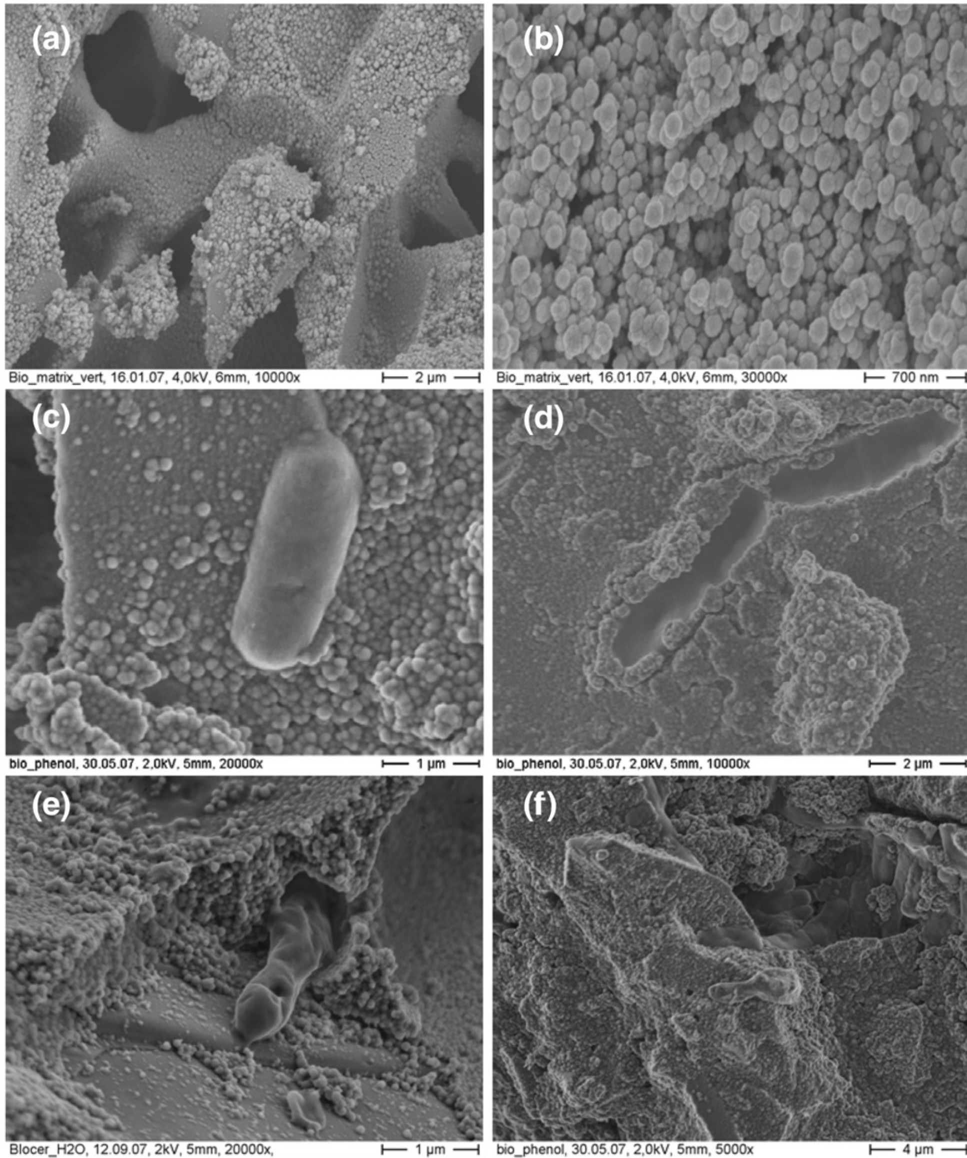


Figure 3. SEM micrographs of sections of the biocer revealing the following: (a) internal macropore structure which support microbial growth and anchoring; (b) the matrix grain structure of the same biocer at higher magnification revealing micropores; (c) a full vegetative cell of *Rhodococcus* sp. P1 attached on matrix surface; (d) the cell growth space for bacterial imbedded in the interior of the matrix; (e) single cells partially attached (using filament-like structure) on the surface; and finally, (f) cells colonising macropores of the biocer.

Downloaded At: 13:45 17 January 2011

The micrographs also show that the cells attached themselves on the surface of the biocer (Figure 3c) where they grew within a pocket-like growth space (Figure 3d). The bacteria transformation from senescence and quiescence to vegetative form was successful in both media containing phenol as the sole source of carbon and sucrose as supplement. No bacterial growth was observed in the media that contained neither phenol nor sucrose (data not shown). The first vegetative bacteria were observed between 4 and 6 h after start of the experiments. For most spores-producing bacteria, they may start germinating and transforming to vegetative form between 15 and 90 min after inoculation when incubated at 30°C [15,23–25]. Logically, the *Rhodococcus* sp. P1 should quickly transform from the senescence and quiescence. However, we observed a prolonged transformation time in the current study, which might have been due to adaptation to phenol as a sole source of carbon. Therefore, determination of the time dependence of bioactivity of *Rhodococcus* sp. like measuring reliable O_2 exchange rate would be important to quantify the actual effect of phenol on the bacterial growth. Nevertheless, the SEM study already showed that the biocer supported the growth of *Rhodococcus* sp. P1 supplied with media where phenol is sole source of carbon.

Downloaded At: 13:45 17 January 2011

3.2 *Confirmation of biodegradation of phenol*

Few samples obtained at random for analysis with IR spectroscopy found no transient products of phenol degradation but only traces of residual phenol ($46.3 \pm 12.7\,mg\,L^{-1}$) in the effluent. The current study was conducted under full aerobic conditions. Figure 4 shows possible biodegradation pathways of phenol by difference bacteria. Under aerobic conditions, phenol biodegrades to carbon dioxide [26–28] while under anaerobic conditions, it degrades to carbon dioxide or methane [5,28–31]. Therefore, the result in the current study confirms that aerobic biodegradation of phenol took place, where the end-products were probably CO_2 and H_2O. The absence of transient products in the effluent can be explained in two ways. The sample of the effluent represents probably

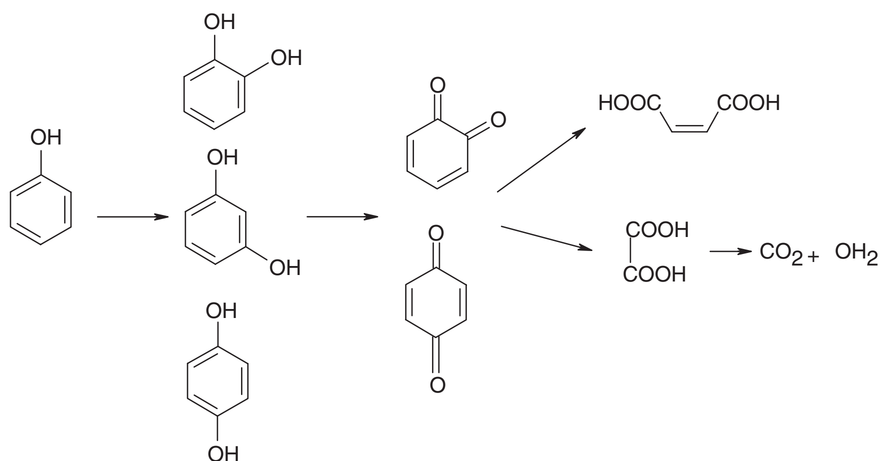


Figure 4. Possible phenol biodegradation pathways. The aerobic degradation of phenolic compounds is metabolised by different strains through either the ortho- or the meta-cleavage pathway. The ideal pathway should end with CO_2 and water.

the end of the biodegradation process, or alternatively the transient products continued to break down in the period between sampling and analysis – the transient products are unstable. Under aerobic condition, oxygen is used as electron acceptor for the transfer of electrons from phenol the electron-donor, which is catalysed by special enzymes of the microorganisms and defines the metabolic pathway [32]. The reaction in which phenol degrades provides the bacteria with the required energy for growth and reproduction.

3.3 *Performance of the spectrophotometric procedure*

In the pre-experiment runs, depletion of phenol in the bioreactor effluent corresponded to decrease in absorbance intensity on UV-vis spectroscopy both measured with (a) the standard 4-aminoantipyrine determination procedure, and (b) in the ultraviolet absorption region without any addition of chromophore (Figure 5). Under standard procedure of using 4-aminoantipyrine determination for phenol, there is a band at around 500 nm wavelength at which the phenol concentration is calibrated against the corresponding absorbance intensity. In the UV region absorbance, phenol has two broad peak bands between 200 and 235 nm, and again two peaks on a band at around 270–280 nm. These values correspond broadly with literature values for phenol absorbance at around 235 and 275 nm [33–35]. As the phenol concentration in the water decreased, the absorbance peaks in the former band shifts to even lower wavelengths (Figure 5), which can be attributed probably to the decrease of the peak height at the higher wavelength. At lower wavelength in the ultraviolet region, the absorbance band is relatively very wide at high initial phenol concentrations, for instance 500 mg L^{-1} (Figure 5). In such instances, it is difficult to attribute the band to phenol absorbance only because the probabilities of presence of congeners are high because the phenol

Downloaded At: 13:45 17 January 2011

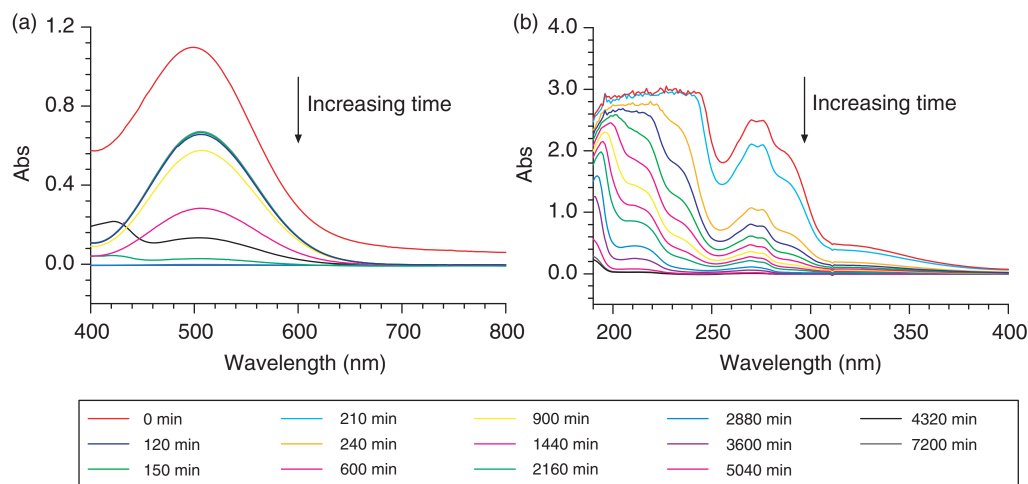


Figure 5. Changes in the peaks of the UV spectrum for phenol measured in the effluent of the bioreactor, which verify reduction of phenol in the media during a 5-day experiment: (a) spectrograms of phenol in the visible light region after the 4-aminoantipyrine reaction procedure; and (b) absorption spectrograms of phenol in the ultraviolet region. The values are means of four replications.

biodegradation pathway (Figure 4) might have been incomplete. The presence of congeners can lead to spectral interferences, which result in widely overlapped absorption bands. Therefore, we used the absorbance at 270 nm to calibrate phenol (Figure 6).

Comparison of the calibration using the absorbance at 270 nm with those obtained using the 4-aminoantipyrene procedure shows very slight insignificant difference (Figure 7). Consequently, there were negligible differences in prediction of phenol concentrations between the two procedures. When the predicted concentration was correlated with the actual phenol concentration added (Figure 7), the 4-aminoantpyrene procedure had higher standard deviation in low concentrations of phenol, which can be attributed to a number of factors like the procedure reaching lower detection limit, and possible effects of non-reacted reagents (i.e. $C_{11}H_{13}N_3O$ and $K_3[Fe(CN)_6]$). For the approach, when phenol concentration was predicted using the '270 nm' procedure, higher standard deviation was observed in higher concentration. This was probably

Downloaded At: 13:45 17 January 2011

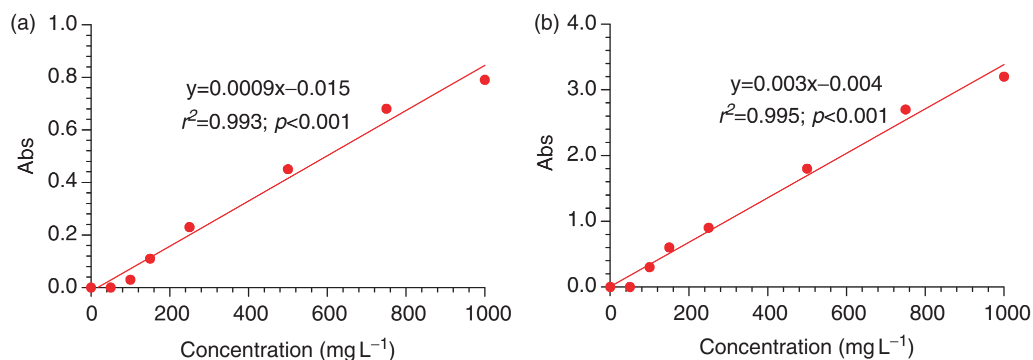


Figure 6. Calibration curve for phenol at (a) 500 nm with standard 4-aminoantipyrine reaction procedure, and (b) 270 nm wavelength in the UV absorption region. Standard phenol concentration (Merck, Darmstadt Germany) was added related to the corresponding absorption on UV-vis spectrophotometer and the regression was tested within 95% confidence interval.

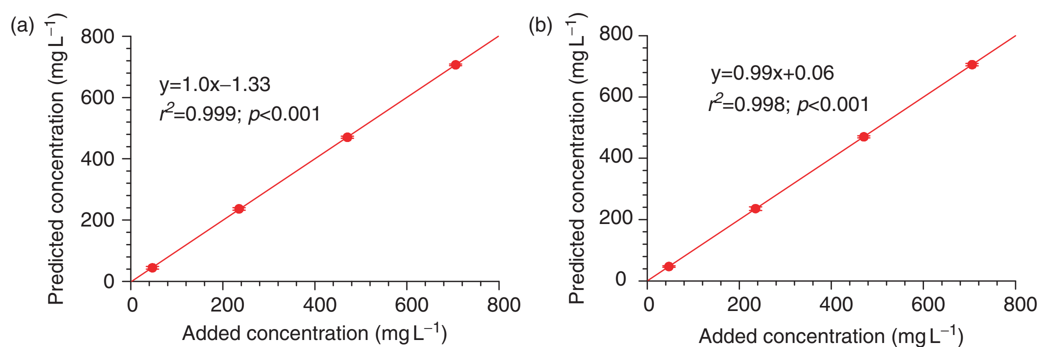


Figure 7. The relationship between the predicted concentration of phenol and the concentration actually added: (a) the relationship obtained with the 4-aminoantipyrine reaction procedure measured at 500 nm in the visible light region; and (b) the relationship when measured at 270 nm wavelength in the ultraviolet region. The values are means of five replications.

Table 2. Estimation of error between the actual added and the UV-vis spectrophotometric predictions of concentration.

	Phenol concentration ($mg\,L^{-1}$)				
Sample	Added	Predicted at 270 nm absorbance	Error (%)	Predicted with 4-aminoantipyrine procedure	Error (%)
1	50.0	49.9	−0.3	50.0	−0.1
2	250.0	250.4	0.2	251.3	0.6
3	500.0	499.7	−0.1	499.9	0.0
4	750.0	750.0	0.0	751.0	0.1

because the procedure might have been approaching the upper detection limit, which may indicate that the procedure functions optimally in low concentration (Figure 5). However, the prediction errors were tolerable (Table 2). This showed that the prediction of phenol using absorbance at 270 nm could be used effectively as a fingerprint verification of phenol biodegradation in a fluidic dynamics system likewise in monitoring biodegradation of phenol under natural environment.

The determination of phenol concentration in the lower ultraviolet region of spectrum can be improved when proper multivariate calibration methods involving mathematical models are applied, which will improve the spectrophotometric resolution of the components with partial overlap of spectra absorbance. With appropriate mathematical models applied to the analytical data, the model parameters can be obtained to estimate the concentration in the samples. Among those, linear regression, partial least squares regression and principal components regression have been used with success in complex determinations [34,36–39].

Downloaded At: 13:45 17 January 2011

3.4 *Monitoring biodegradation of phenol*

The depletion of phenol in bioreactor was observed through measuring the absorbance at 270 nm wavelength in the ultraviolet region range in the UV-vis spectrophotometer. The result shows that there was significant degradation of phenol in the bioreactor, depending on the dilution rate and residence time when phenol is a sole source of carbon (Figure 8a). Non-inoculated control flasks run in parallel showed that sol–gel matrix neither adsorbs nor degrades phenol (data not shown). The effect of the dilution rate on the performance of bioreactor was studied by varying the influent flow-rate. The calculated maximum rates of phenol biodegradation in the first 3.5 days were 10.2, 13.1, and $3.6\,g\,L^{-1}$ per day for flow-rates of 0.01, 0.03, and $0.07\,mL\,s^{-1}$, respectively. After 2.5 days, the biodegradation rate for the slowest flow-rate ($0.01\,mL\,s^{-1}$) suddenly decreases significantly to about $1.3\,g\,L^{-1}$ per day, while degradation in highest flow rate is constantly low. This is attributed to inadequate source of carbon to sustain the cell growth due to an insufficient amount of carbon during the long residence time of the samples in the lowest flow-rate, which eventually leads to cell death. Contrary, when the flow-rate is high, the cells do not have enough time to degrade the phenol. Therefore, small stepwise increases in phenol concentration at a fixed low dilution rate or alternatively changes of the dilution rate should be directly related to the cells

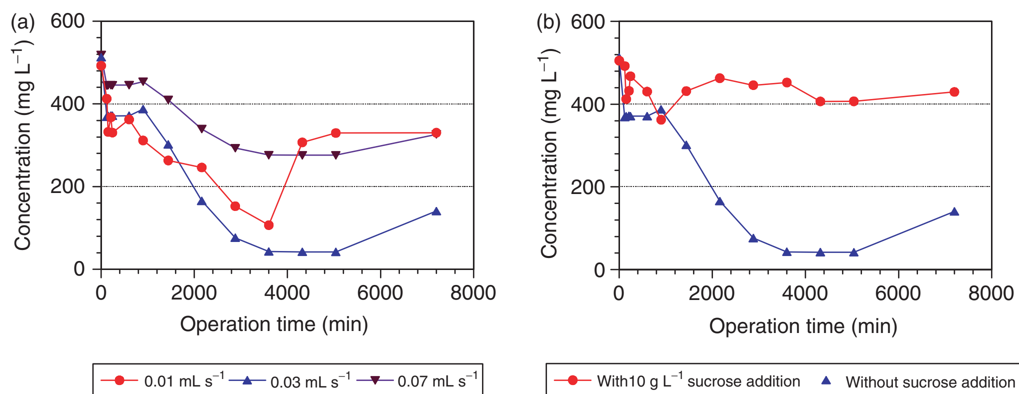


Figure 8. Biodegradation of phenol in the fluid dynamics system during a 5-day operation: (a) biodegradation under different influent–effluent flow rates of the media in which phenol is the sole source of carbon; and (b) comparison of biodegradation of phenol in media where either phenol is the sole source of carbon or sucrose is added. All values are mean of four replications.

Downloaded At: 13:45 17 January 2011

growth rate. However, the rates of phenol biodegradation reported here are higher than most values reported by other authors for *Rhodococcus* sp. in free cell cultures [40–43].

The capacity of the bioreactor to degrade phenol was significantly reduced when 10 g L^{-1} of sucrose was added to the media as a supplement source of carbon (Figure 8b). This suggests that *Rhodoccocus* sp. P1 degrades phenol only when phenol is the sole source of carbon. This may pose an inherent problem for application of the bioreactor where cultures degrade specific compounds in a well-defined environment, but are not effective to degrade the same compound in a real environment due to the presence of other pollutants, carbon sources or bacterial populations. Therefore, more investigations are necessary to develop *Rhodococcus* sp. P1 culture that can function in natural environment too. Furthermore, the physiological status of *Rhodococcus* sp. P1 should be investigated for a possible regeneration of the biocer after a first phenol biodegradation process.

4. Conclusion

The rapid biodegradation of phenol from 500 mg L^{-1} to 40 mg L^{-1} within 2 days in the bench scale fluid dynamic system signifies that biocer-based bioreactor technology is feasible. Thus, it can be up-scaled easily to real life phenol remediation in continuous groundwater flow. However, there should be seriously considerations of flow rates and possible alternative carbon sources when developing the bioreactor for natural ground. Secondly, the colorimetric procedure in the UV spectrum region is applicable to verify biodegradation of phenol in fluid dynamic system. The procedure provides instant information on the performance of the bioreactor system with the continuous water flow. However, more investigations are necessary particularly in application of our current UV-vis spectrophotometer procedure where transient species interfere strongly with phenol spectrum. Hence, we recommend a multivariate calibration technique (e.g. Partial Least-Squares Regression, PLSR) for our procedure. Thus, the current study laid foundation for further investigation and improvement of the procedures.

Acknowledgements

This work was supported under BMBF Project grant No. 02WR0694. The biocer samples used in the experiments were kindly supplied by our project partner, GMBU e.V. Department of Functional Coatings at the Rossendorf Technology Centre (ROTECH) in Dresden. Professional advice from Dr Ulrich Soltmann and Dr Sabine Matys is highly acknowledged.

References

[1] M. Mkandawire and E.G. Dudel, in *VII International Congress of Ecology: New Tasks for Ecologists after Rio 1992*, edited by A. Farina, J. Kennedy, and V. Bossu (INTECOL, Florence, 1998), p. 296.

[2] E.G. Dudel, C. Brackhage, H. Dienemann, M. Mkandawire, J. Rotsche, A. Weiske, in: *8th International Conference Radioactive Waste Management and Environmental Remediation*, Bruges, Belgium, A. Taboas, R. Vanbrabant, G. Benda (Eds.), American Society of Mechanical Engineers and US Department of Energy 2001, p. 8.

[3] S.R. Verma, S. Rani, A.K. Tyagi, and R.C. Dalela, Water Air Soil Pollut. **14**, 95 (1980).

[4] J. Seo, J. Jeon, S.-D. Kim, S. Kang, J. Han, and H.-G. Hur, Wat. Sci. Technol. **55**, 163 (2007).

[5] P. Olguìn-Lora and E. Razo-Flores, J. Chem. Technol. Biotechnol. **79**, 554 (2004).

[6] A.E. Greenberg, L.S. Clescerl, A.D. Eaton (Eds.), *Standard methods for the analysis of water and wastewater*, Series (American Public Health Association, Washington, D.C. 1995).

[7] M. Prieto, A. Hidalgo, C. Rodríguez-Fernández, J. Serra, and M. Llama, Appl. Microbiol. Biotechnol. **58**, 853 (2002).

[8] M.B. Prieto, A. Hidalgo, J.L. Serra, and M.J. Llama, J. Biotechnol. **97**, 1 (2002).

[9] M. Veselý, M. Pátek, J. Nešvera, A. Čejková, J. Masák, and V. Jirků, Appl. Microbiol. Biotechnol. **61**, 523 (2003).

[10] Y.-H. Kim and K.-H. Engesser, Appl. Environ. Microbiol. **70**, 4398 (2004).

[11] K. Vidya Shetty, R. Ramanjaneyulu, and G. Srinikethan, J. Haz. Mat. **149**, 452 (2007).

[12] H. Böttcher, U. Soltmann, M. Mertig, and W. Pompe, J. Mat. Chem. **14**, 2176 (2004).

[13] U. Soltmann, J. Raff, S. Selenska-Pobell, S. Matys, W. Pompe, and H. Böttcher, J. Sol-Gel Sci. Technol. **26**, 1209 (2003).

[14] J. Raff, U. Soltmann, S. Matys, S. Selenska-Pobell, H. Böttcher, and W. Pompe, Chem. Mater. **15**, 240 (2003).

[15] S. Matys, J. Raff, U. Soltmann, S. Selenska-Pobell, H. Böttcher, and W. Pompe, Chem. Mater. **16**, 5549 (2004).

[16] R. Margesin, P.-A. Fonteyne, and B. Redl, Res. Microbiol. **156**, 68 (2005).

[17] S.-L. Pai, Y.-L. Hsu, N.-M. Chong, C.-S. Sheu, and C.-H. Chen, Biores. Technol. **51**, 37 (1995).

[18] M. Tirrell, E. Kokkoli, and M. Biesalski, Surf. Sci. **500**, 61 (2002).

[19] H.K. Baca, C. Ashley, E. Carnes, D. Lopez, J. Flemming, D. Dunphy, S. Singh, Z. Chen, N. Liu, H. Fan, G.P. Lopez, S.M. Brozik, M. Werner-Washburne, and C.J. Brinker, Sci. **313**, 337 (2006).

[20] D. Fiedler, A. Thron, U. Soltmann, and H. Bottcher, Chem. Mater. **16**, 3040 (2004).

[21] S. Matys, in: *Fakultät Maschinenwesen*, Technischen Universität Dresden, Dresden, p. 154. (2005).

[22] H.G. Schlegel, H. Kaltwasser, and G. Gottschalk, Arch. Mikrobiol. **38**, 209 (1961).

[23] R. Leblanc and G. Lefebvre, Bull. Math. Biol. **46**, 447 (1984).

[24] R.G.K. Leuschner and P.J. Lillford, Lett. Appl. Microbiol. **29**, 228 (1999).

[25] S.C. Stringer, M.D. Webb, S.M. George, C. Pin, and M.W. Peck, Appl. Environ. Microbiol. **71**, 4998 (2005).

[26] K. Kryst and D.G. Karamanev, Indust. Engin. Chem. Res. **40**, 5436 (2001).

[27] H.-q. Chang, X. Yang, Y.-y. Fang, P.-m. Pu, Z.-k. Li, and Z. Rengel, J. Zhejiang B **7**, 521 (2006).

Downloaded At: 13:45 17 January 2011

[28] P.M. van Schie and L.Y. Young, Biorem. J. **4**, 1 (2000).
[29] R. Satsangee and P. Ghosh, Worl. J. Microbiol. Biotechnol. **12**, 409 (1996).
[30] P.b.M. Tuah, in: *Faculty of Science*, Universiti Teknologi Malaysia 2006.
[31] M. Eiroa, A. Vilar, L. Amor, C. Kennes, and M.C. Veiga, Wat. Res. **39**, 449 (2005).
[32] D.H. Pieper and W. Reineke, Curr. Opin. Biotechnol. **11**, 262 (2000).
[33] A.C. Oomens and F.G. Schuurhuis, Int. Arch. Occup. Environ. Health **52**, 43 (1983).
[34] K.V.D. Souza and P. Peralta-Zamora, An. Acad. Bras. Ciênc. **73**, 519 (2001).
[35] I. Nukatsuka, S. Nakamura, K. Watanabe, and K. Ohzeki, Anal. Sci. **16**, 269 (2000).
[36] M.S. Arayne, N. Sultana, and S.S. Bahadur, Pak J. Pharm. Sci. **20**, 163 (2007).
[37] M.S. Collado, V.E. Mantovani, H.C. Goicoechea, and A.C. Olivieri, Tal. **52**, 909 (2000).
[38] P. Peralta-Zamora, A. Kunz, N. Nagata, and R.J. Poppi, Tal. **47**, 77 (1998).
[39] J.A. Arancibia, G.M. Delfa, C.E. Boschetti, G.M. Escandar, and A.C. Olivieri, Anal. Chim. Acta **553**, 141 (2005).
[40] A.M. Gerrard, J.P. Júnior, A. Kostečková, J. Páca, M. Stiborová, and C.R. Soccol, Braz. Arch. Biol. Technol. **49**, 669 (2006).
[41] S. Vijayaraghavan, T. Srinivasaraghavan, S. Musti, S. Kar, T. Swaminathan, and A. Baradarajan, Bioprocess Biosyst. Eng. **12**, 227 (1995).
[42] P. Bergauer, P.-A. Fonteyne, N. Nolard, F. Schinner, and R. Margesin, Chem. **59**, 909 (2005).
[43] B. Marrot, A. Barrios-Martinez, P. Moulin, and N. Roche, Biochem. Eng. J. **30**, 174 (2006).

Downloaded At: 13:45 17 January 2011