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Degradation kinetics of resorcinol by *Enterobacter cloacae* isolate

Marissa R. Jablonski[1], Shraddha Shaligram[2], Asifa Qureshi[2]*, Hemant Purohit[2] and John R. Reisel

[1]Department of Civil Engineering and Mechanics, University of Wisconsin, Milwaukee, 3200 N. Cramer St., Milwaukee, WI 53211, USA.
[2]Environmental Genomics Division (EGD), National Environmental Engineering Research Institute (NEERI), Nagpur India, Nehru Marg, Nagpur, 440020, India.
[3]Department of Mechanical Engineering, University of Wisconsin, Milwaukee, 3200 N. Cramer St., Milwaukee, WI 53211, USA.

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Resorcinol was utilized as the sole carbon and energy source by *Enterobacter cloacae* (identification by 16S rDNA nucleotide sequencing Genbank Accession Number JN093148). The different concentration of resorcinol utilized by the bacterial isolate ranged between 55 and 220 mg l^{-1} at 30°C and pH of 7.0. It was observed that the batch experimental results were best fitted for Michaelis-Menten and Monod models (for 220 mg l^{-1} resorcinol) with time under defined conditions. The kinetics constants for the Michaelis-Menten equation (enzyme kinetics) were K_m = 11.00 mM and V_{max} = 0.03 mM min^{-1} and for the Monod equation (growth kinetics) was μ_{max} = 0.0371 h^{-1} in the inhibitory region and K_S = 22.09 mg l^{-1}. It was assumed that enzyme reactions limit biomass production (Monod kinetics) during resorcinol degradation by *E. cloacae*. The enzyme kinetic model (Michaelis-Menten) used was fit to the resorcinol degradation profiles with a set of model parameters such as using pre-induced *E. cloacae* cells on 220 mg l^{-1} resorcinol.

Key words: Resorcinol, Michaelis-Menten, Monod, aerobic, *Enterobacter cloacae*.

INTRODUCTION

Release of wastewater from textile production is a major pollutant of watersheds around the world. Dye wastewater is not only toxic and mutagenic but has the ability to deconstruct ecosystems by blocking sunlight from reaching plant life upon release of the color into the environment. Studies of industrial areas where azo dyes are in use have connected azo dye presence in soil, surface water, and groundwater to the direct deterioration of these areas (Pandey et al., 2007). Azo dyes have a wide appeal throughout textile, leather and paper Industries, as they are the largest class of synthetic dyes and contain the broadest color variations (Balan et al., 2001; Bafana et al., 2007; Pandey et al., 2007). Specifically, their production was estimated in 1994 to comprise 50% of the then annual global production of one million tons of commercial dyes (Pandey et al., 2007). The textile Industry effluent requires decolorization due to the high percentage of dye in the wastewater effluent (10 to 15%) (Bafana et al., 2007). This can be a major problem for that industry considering that some dyes are not decolorized at concentrations as low as 1 mg l^{-1} (Pandey et al., 2007).

*Corresponding author. E-mail: a_qureshi@neeri.res.in. Tel: +91-712-2249885. Fax: +91-712-2249900.

Resorcinol has been selected as a model compound for this study because it is a precursor to azo dyes and is dihydroxy benzene or hydroxy aromatic compound, containing two hydroxyl groups in the *meta* position of the phenol ring (Ferreira Guedes et al., 2010; PubChem NCBI). Resorcinol is commonly used as an industrial solvent and raw material in the leather, textile, paper, pharmaceutical, steel, cosmetics, and petrochemical Industries. Resorcinol is distinctly used in the cosmetics industry as a disinfectant and exfoliating agent for many acne treatments which may cause thyroid defects with daily use (Ferreira Guedes et al., 2010). Resorcinol's solubility in water is 1100 g/L at 20°C. It is flammable when unprotected against high temperatures or oxidants, is more toxic than phenol, and causes severe body impairment if ingested or if it comes in contact with skin, eyes, or mucous membranes (Subramanyam and Mishra, 2007; Ferreira Guedes et al., 2010). A variety of kinetic substrate utilization and inhibition models have been used to describe the dynamics of aerobic microbial growth on organic contaminants including phenol, benzene, toluene, nitrophenol, catechol and even orcinol, but not on resorcinol (Chapman and Ribbons, 1976; Zeyer and Kearney, 1984; Meulenberg et al., 1996; Reardon et al., 2000; Kim et al., 2005; Kumar et al., 2005; Yao et al., 2006; Agarry and Solomon, 2008; Shumkova et al., 2009). Resorcinol studies tend to focus more on degradation under different levels of stress (Ferreira Guedes et al., 2010). The present study focuses on kinetics of resorcinol in order to predict its degradation in both aerobically growing cells and resting cells using different initial substrate concentrations.

In this study, Monod and Michaelis-Menten mathematical models were used to anticipate and assess the biodegradation rates of resorcinol and illustrate the dynamics of microbial growth in two experiments: growth/decay and resting cell. To ensure the successful kinetic evaluation of the bacterial degradation of resorcinol, indigenous bacteria from the common effluent treatment plant, CETP (an industrial wastewater treatment facility) in Gujarat, India was used. This is a similar approach to that used in the study by Shinozaki et al. (2002), which used indigenous bacteria to degrade *p*-nitrophenol. Effective kinetics research investigates the degradation potential of bacteria. In order to be successful, the experiment requires a bacterium able to consume and utilize the resorcinol as its main carbon and energy source (Kim et al., 2005). In this study, enzyme kinetics were applied to resorcinol degradation using *Enterobacter cloacae*.

The objective of this study was to investigate the degradation potential of an indigenous *E. cloacae* isolated from a wastewater treatment plant in Gujarat, India and the effect that initial resorcinol concentration has on its degradation ability. Few reports are available on the aerobic degradation kinetics of resorcinol. This study on a pure indigenous isolated facultative anaerobic bacterium, *E. cloacae*, from a local industrial wastewater treatment facility appears to be new. While most degradation studies occurred under anaerobic conditions, *an E. cloaca is* shown here to work under microaerophilic and aerobic conditions. Since the strain used in this study was isolated from CETP effluent, chances of obtaining *E. cloacae* with hazardous characteristics is very rare. Despite the fact that *E. cloacae* is reported as a Class I (or Class II in Canada) biohazard, the strain isolated in this study is lacking hazardous status as determined by 16S rDNA BLAST analysis. In future studies, the kinetics of *E. cloacae* towards biodegradation of resorcinol in aerobic reactors could be applicable if bioaugmented.

MATERIALS AND METHODS

Resorcinol substrate

Resorcinol has been selected as a model compound for this study because it is a precursor to azo dyes, which are heavily produced and cause extensive environmental damage if left untreated prior to disposal. Resorcinol is a dihydroxy benzene (Ferreira Guedes et al., 2010; PubChem NCBI). The chemical was purchased from Sigma-Aldrich and its chemical formula is C_6H_4-1,3-$(OH)_2$ and has a molecular weight of 110.11. Spectrophotometer scanning determined resorcinol's absorption maxima (λ_{max}) to be 274 nm (240 with 0.1X NaOH dilution) (Perkin Elmer UV Lambda 900 WinLab).

Isolation of microorganisms and culture conditions for growth and decay experiments

Activated sludge, procured from the CETP in Gujarat State, India, was enriched with 110 mg l^{-1} resorcinol. The samples were covered in aluminum foil, to prevent photo-oxidation, and placed in Erlenmeyer flasks, sealed with cotton and paper, and stored in an orbital shaker for 4 - 5 days at 30°C and 120 rpm. After 4 - 5 days, 100 µl of growth cultures were spread onto 1X new minimal media (NMM) plates with 110 mg l^{-1} resorcinol substrate. A 100 ml of 1X NMM plate solution consists of 0.2 ml 10% $MgSO_4$, 0.25 ml 10% NH_4Cl, 0.1 ml 100mM $CaCl_2$, 2 ml Hutners Solution (Selvakumaran et al., 2011), 0.224 ml 1 M Phosphate Buffer with pH 7, 2% agar for solidification, with the remaining volume filled with deionized water. The new minimal media was amended with resorcinol (110 mg l^{-1}) as the carbon substrate. The bacteria that grew were considered to be using resorcinol as a carbon and energy source and in turn degrading the substrate. The serial dilutions resulted in twelve individual bacteria isolates from which the *NR-1* isolate (later identified as *E. cloacae*) was chosen due to its strong degradation potential based on the color change seen in the samples. One colony of the isolate was transferred from the plate to 5 - 10 ml of 1X NMM broth with 55 mg l^{-1} resorcinol to cultivate biomass growth. All glassware was autoclaved at 121°C for 15 min.

Biodegradation batch experiments

The effect that the initial resorcinol concentration has on the *E. cloacae* growth kinetics was examined using the analysis of cell growth and resorcinol decay in batch experiments using spectrophotometric curve analysis. Different concentrations (220, 550, 880 and 1100 mg l^{-1}) of resorcinol were prepared in 100 ml of 0.1X NMM in 200-250 ml Erlenmeyer flasks leaving headspace for oxygen levels sufficient for cell growth, sealed with cotton and pa-

per, and stored in an orbital shaker at 120 RPM for 95 h at 30°C. Using a sterile pipette, samples were taken every 5 -12 h and either analyzed immediately or stored at -20°C. Samples were also drawn from un-inoculated control flasks that were also incubated and shaken equal to the others. Since resorcinol compound is photo oxidizable, the presence of residual resorcinol during degradation experiments interferes with OD_{600} estimation of cell growth. To prevent the interference of photochemically oxidized resorcinol, samples were centrifuged to remove the brown colored oxidized resorcinol supernatant at 4°C after which the cells were resuspended in 1 ml of distilled water for OD_{600} estimation. The UV-visible spectrophotometer absorbance of the bacteria cells at 600 nm determined the bacterial growth, and the absorbance of the supernatant at 274 nm determined the resorcinol degradation. All experiments were repeated three times at separate times to ensure accuracy and mean values are shown.

Growth kinetics

The Monod equation was used for growth kinetics. The Monod equation analyzes the utilization of substrate during concurrent bacterial growth or decay using specific growth rate and specific substrate consumption rate (Kim et al., 2005; Ostendorf et al., 2007; Agarry and Solomon, 2008). During the use of Monod kinetics, assumption that enzyme reactions limit biomass production was allowed. Using a single bacterial isolate and a single substrate in minimal media allowed for little to no effect on growth kinetics. The non-inhibitory Monod equation was used for this case looking for an increase in growth rate corresponding to an increased substrate concentration that reaches a maximum growth rate asymptotically (Kim et al., 2005).

Monod kinetics is an empirical model that can fit the substrate degradation of the resorcinol by *E. cloacae* to the equation (Kovarova-Kovar and Egli, 1998; Ferreira Guedes et al., 2010):

$$\mu = \frac{1}{X}\frac{dX}{dt} = \frac{\mu_{max} S}{K_S + S} \quad (1)$$

where μ is the specific growth rate per hour, μ_{max} is the maximum biomass growth rate per hour taken as the maximum biomass on the growth curve, K_S is the half-saturation constant in mg l^{-1}, S is the initial substrate concentration in mg l^{-1}, and X is the microbial cell concentration in mg l^{-1} found with:

$$X = X_0 e^{\mu t} \quad (2)$$

μ can be solved using

$$m = \frac{\ln\left(\frac{m_1}{m_2}\right)}{t_2 - t_1} \quad (3)$$

where m is biomass concentration in mg l^{-1}.

To solve for K_S and μ_{max}, the units of substrate were in mg l^{-1} and biomass OD_{600} was also in mg l^{-1}. The mM conversion was done as follows: 2 mM or 2000 mole per liter of resorcinol is multiplied by 0.110 mg per mole (110 g is the molecular weight of resorcinol) amounting to 220 mg per liter as substrate (S) used initially. For biomass, the cells taken were measured in terms of dry weight as 330 mg l^{-1}. μ is found by using the first and last points from the exponential curve as m_1 and m_2, and t_1 and t_2.

Biomass cultivation for resting cell experiment

To cultivate the biomass of the *E. cloacae* for the resting cell experiment, test tubes of 1X Luria broth (10% tryptone, 5% yeast extract, 5 g NaCl per liter distilled water with a pH of 7) with 55 mg l^{-1} resorcinol were inoculated with *E. cloacae* and placed in the orbital shaker at 30°C at 120 RPM. Once the biomass exceeded an optical density (OD) of 1, the cells were transferred to 0.1X NMM.

Respirometric analysis of resting cell experiment (cellular respiration)

The objective of the resting cell experiment was to analyze all the enzymes of the degradation pathway of resorcinol in the *E. cloacae* isolate. It was assumed that in the resting cell experiment, cells pre-grown on the resorcinol substrate will not multiply, but the induced enzymes in bacterial cells will remain activated and react in the presence of the substrate; so degradation can be modeled using the Michaelis-Menten equation. The aerobic bacteria were reported to undergo oxidative reactions yielding central intermediates (Reineke, 2001), from which the oxygen uptake experiments were designed. Cells pre-grown on resorcinol were considered as induced cells and were harvested to obtain resting cells. Based on the growth curve results showing best growth peaks for *E. cloacae* at 1.5 h, cells were harvested at 1.5 h and then used for oxygen uptake analysis. Resting cell experiments require 1.0 OD_{600} of pre-grown cells per 1 ml of media equivalent to 330 mg l^{-1} with a concentration of substrate experimentally predetermined. A digital oxygen system (model 10, Rank Brothers, Bottisham UK) was used to track the oxygen uptake changes over a total of 15 min at 30°C. The machine uses 3 ml of solution, constant stirring to allow for complete mixing. The 50 mM phosphate buffer is added into the machine followed by 3 OD of *E. cloacae*. The initial 4 mins measure endogenous activity presenting the absence of substrate. After 180 s, varying molarities of the substrate are added to the mixture and the readings are recorded for the next 11 min. The oxygen uptake experiment is based on the resting cells where the cells have grown using resorcinol as their carbon source and are then starved. All the enzymes of the resorcinol degradation pathway are assumed to be induced while applying the oxygraph test. Also, the Michaelis-Menten enzyme kinetic model used was fit to the resorcinol degradation only with the use of pre-induced *E.* cloacae cells as a set of model parameters. The oxygen uptake reading in µmol min^{-1} per mg cell is determined by the difference in slope of degradation before and after the substrate is added to the mixture followed by a division by the dissolved oxygen in water (8 ppm) and multiplied by the mg of cells (0.33 g). The oxygen uptake rates were expressed as nanomoles of oxygen per minute per mg of cells. Weight of cells was determined by drying cell pellets at 70°C until a constant weight was obtained.

Enzyme kinetics

Michaelis-Menten kinetics, also known as enzymatic kinetics, is related to Monod kinetics by using a similar equation but at steady state conditions; it is also known as Monod no-growth kinetics (Blum et al., 2009). The steady state condition is created when the enzyme binds to the substrate faster than the product formation (Costa et al., 2010). Use of the Michaelis-Menten equation tests all the enzymes of the pathway assuming that all of the enzymes act at the same time in the presence of one substrate and one product (Costa et al., 2010). They must be tested during a steady state of zero growth and are therefore analyzed after resting cell experiments with varying substrate concentrations using oxygen uptake which was performed. Varying substrate concentrations determine spanning from non-saturated to saturated, in order to derive the

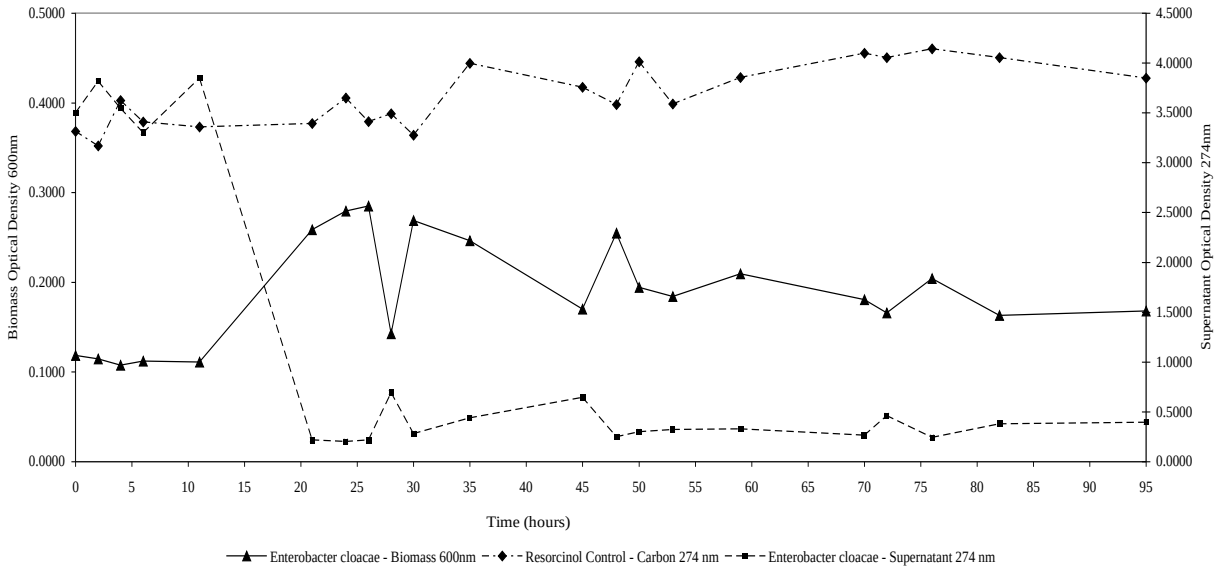

Figure 1. Growth/decay curve of *E. cloacae* over 95 h time span showing biomass exponential growth (triangle) as well as substrate presence decay (square) contrasting the substrate constant (diamond) taken at the same interval to reassess the photo-oxidation in resorcinol substrate. All data were analyzed spectrophotometrically using UV L900 WinLab.

Lineweaver-Burke plot from which the kinetic parameters V_{max} and K_m can be derived (Valsaraj, 2009; Bueno et al., 2010; Chauhan et al., 2010). The Michaelis-Menton equation is shown in Equation 4, where r is the substrate degradation in mM per minute, V_{max} is the enzyme's maximum rate of reaction once it is saturated with the substrate, S is the initial substrate concentration and K_m is the Michaelis-Menten growth rate constant:

$$r = \frac{V_{\max}[S]}{K_m + [S]} \quad (4)$$

The inverse of Equation 4 is used in the Lineweaver-Burke plot:

$$\frac{1}{r} = \frac{K_m}{V_{\max}}\frac{1}{[S]} + \frac{1}{V_{\max}} \quad (5)$$

Analytical methods: Degradation analysis using UV-visible spectrophotometer

Samples of 1 ml size were drawn and centrifuged to separate the bacterial cells from the supernatant for analysis. Spectrophotometric analysis determined the absorbance of the bacterial cells resuspended with 1 ml of deionized water at 600 nm and of the supernatant at 274 nm (Perkin Elmer, Model Lambda 900).

RESULTS AND DISCUSSION

Growth curve-degradation of resorcinol

A resorcinol degrading bacteria, *E. cloacae*, was tested in batch experiments using a single limiting substance to record its resorcinol biodegrading potential. Growth curve experiments were conducted for initial substrate concentration of 220 mg l^{-1} of resorcinol. Concentrations more than 220 mg l^{-1} such as 550, 880 and 1100 mg l^{-1} of resorcinol were also used to demonstrate the effect of the initial substrate concentration on *E. cloacae* cell growth over a 95-h time span using intermittent sampling. Higher concentrations were inhibitory for cells and also, due to self-photo oxidation characteristic of resorcinol (dark brown coloration) the spectrophotometric analysis of cell growth and residual resorcinol, caused interference for estimations (data not shown). The model concentration of resorcinol selected was 220 mg l^{-1} due to the success of the degradation coinciding with biomass growth. Figure 1 shows the result where the 220 mg l^{-1} initial concentration of resorcinol in 0.1X NMM inoculated with *E. cloacae* significantly degraded resorcinol. Table 1 lists the parameter values. Following Reardon et al. (2000), death rates were set to zero to obtain best-fit results. *E. cloacae* cells with an initial OD_{600} of approximately 0.1 were in a lag phase for the first 10 h, while resorcinol had an initial OD_{274} of approximately 4.0 for the same period of time. Figure 1 represents typical biomass growth since the degradation proceeds with cell growth. This was followed by the exponential phase when cells at OD_{600} increased to almost 3.0 between 10 to 25 h, while the OD_{274} of the supernatant decreased to approximately 0.25 during the same period of time. Biomass accumulation ended at approximately the same time that the consumption of the resorcinol ceased. The final phase of the growth/decay was a stationary phase where both cells and supernatant

Table 1. Monod kinetics parameter results for *Enterobacter cloacae* isolate.

Bacteria	K_S (mg/l)	μ (h^{-1})	μ_{max} (h^{-1})	S (mg/l)	t_1 (h)	t_2 (h)	t_{max} (h)	m_1 (mg/l)	m_2 (mg/l)	m_{max} (mg/l)
Enterobacter cloacae isolate	22.09	0.0338	0.0371	220	0	25	21	39.105	94.05	85.305

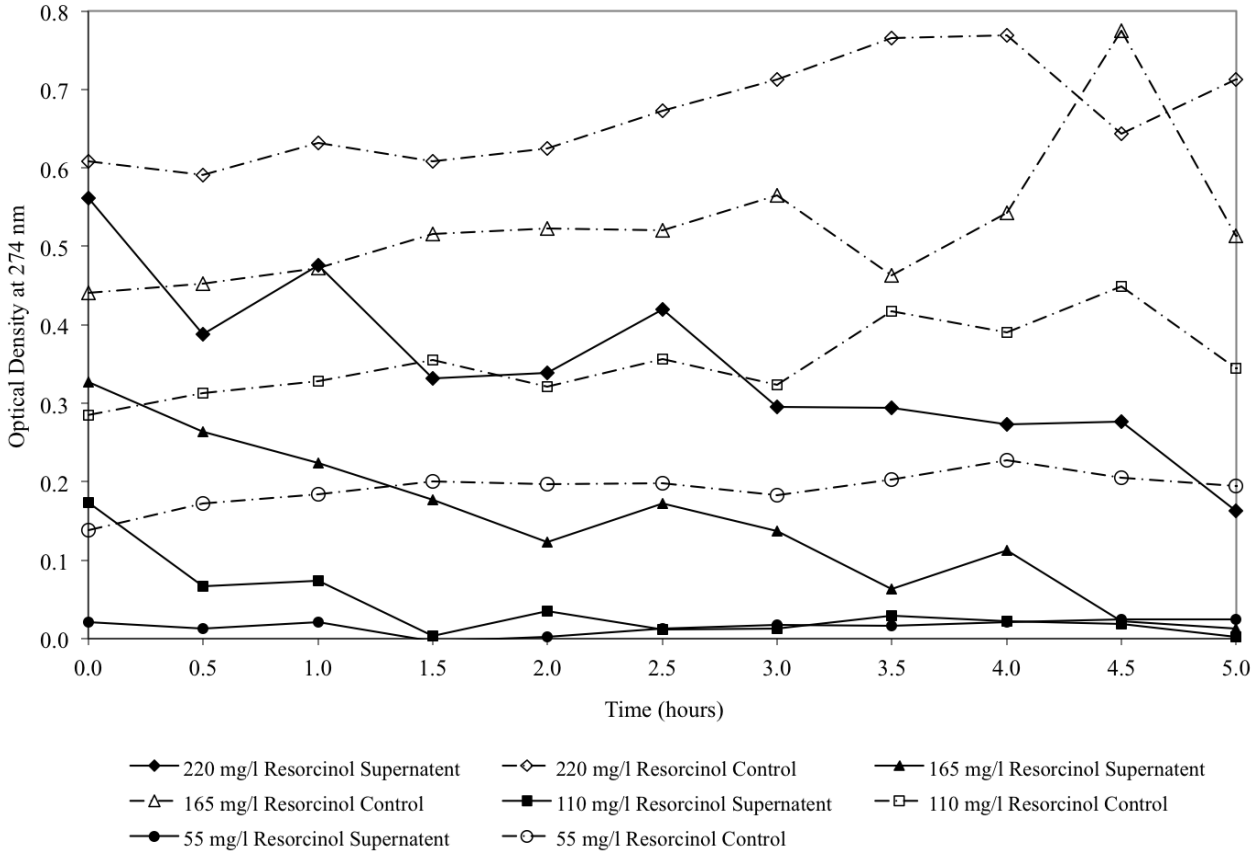

Figure 2. Resorcinol resting cell data for *E. cloacae* isolate analyzed over a 5 h time span showing samples of different substrate concentrations ranging from 55 to 220 mg l^{-1}: 55 mg l^{-1} (circle), 110 mg l^{-1} (square), 165 mg l^{-1} (triangle), 220 mg l^{-1} (diamond). The graph depicts control samples in dashed lines and empty marker shapes, and supernatant samples in solid lines and blackened marker shapes. All data were analyzed spectrophotometrically using UV L900 WinLab at a wavelength of 274 nm.

OD leveled off to a steady OD_{600} of 0.2 and OD_{274} of 0.4. Since resorcinol was utilized by *E. cloacae* (biomass increase), it can be assumed that *E. cloacae* can use resorcinol as its source of carbon.

Monod kinetics

Based on the growth curve (Figure 1) of *E. cloacae*, the decay of resorcinol was modeled by Monod kinetics (Equation 1). Monod kinetics required S and biomass in mg l^{-1}. μ is found in Equation 3. State 1 is the initial conditions and state 2 is chosen to be at the peak of the exponential phase of the growth curve. μ_{max} is found by using the same equation as for μ, but looking at the highest value μ rather than the peak of the exponential phase of the curve. At t_0, m_0 is 39.1050 mg l^{-1}, at t_{26}, m_{26} is 94.0500 mg l^{-1} resulting in μ being 0.0338 h^{-1}. At t_{21}, m_{21} is 85.3050 mg l^{-1} providing a μ_{max} of 0.0371 h^{-1}. Using this data, K_S is 22.09 mg l^{-1}. Degradation kinetics for resorcinol by *E. cloacae* is being reported for the first time in this paper, whereas different strains of *Pseudomonas putida* have been reported to degrade resorcinol and use it as its sole carbon and energy source (Chapman and Ribbons, 1976). Kinetic parameters of resorcinol degradation using Monod during growth and Michaelis-Menten during resting cell experiments for *E. cloacae*, when compared with Ferreira Guedes et al. (2010) Monod-Michaelis Menten kinetic parameters of resorcinol degradation using *Penicillium chrysogenum* CLONA2 are compared

Table 2. Kinetic parameters of resorcinol degradation using Monod during growth and Michaelis-Menten during resting cell experiments for *E. cloacae* when compared with Ferreira et al. (2010) Monod-Michaelis Menten kinetic parameters of resorcinol degradation using *P. chrysogenum* CLONA2.

Culture	S_0 (mg/l)	K (mM) (Reference)
Enterobacter cloacae isolate (Michaelis-Menten)	220	11.00 (This study)
Penicillium chrysogenum CLONA2	50-300	11.27 (Ferreira Guedes et al., 2010)

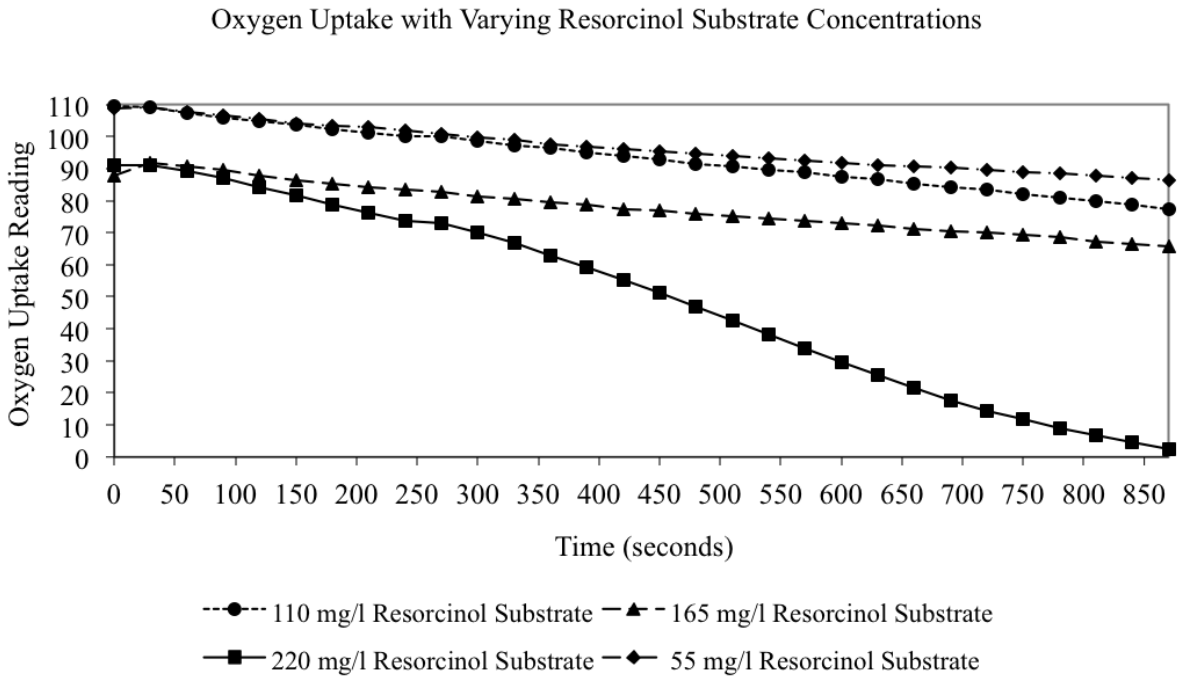

Figure 3. Representative graphs of oxygen uptake studies of *E. cloacae* cells (3 OD_{600nm} cells per reaction) with resorcinol substrate concentrations used during analysis in respirometer. Resorcinol substrate 55 mg l^{-1} (diamond), 110 mg l^{-1} (circle), 165 mg l^{-1} (triangle), 220 mg l^{-1} (square) was added after 240 s (4 m) in each reaction. *E. cloacae* cells used were pre-grown in 165 mg l^{-1}resorcinol substrate for 1.5 h and harvested for analysis.

in Table 2.

Resting cell-degradation of resorcinol (respirometric assays)

Enzyme kinetics was studied when enzymes were activated but cell growth is at rest. A common model for such enzymatic kinetics is the Michaelis-Menten equation (Equation 4). The resting cell experiment involved growing 1 optical density (OD) of cells per 1 ml of media. Different concentrations of resorcinol substrates below 220 mg l^{-1} concentration were compared (55, 110, 165 and 220 mg l^{-1}). The study did not allow for the isolation of a single enzyme, but considered them as a single enzyme in crude lysate form. Figure 2 presents the spectrophotometric analysis at absorbance 274 nm of the supernatant of the resting cells over a 5-h span. Solid lines represent supernatant measurements and dashed lines, the control. Despite keeping samples covered in aluminum foil to prevent photo-oxidation, color change did occur. In Figure 2, diamonds represent 220 mg l^{-1} substrate sample starting at almost OD_{290} of 0.6 and decreasing to 0.2; triangles represent 165 mg l^{-1} substrate sample starting at OD_{290} of 0.35 and decreasing to 0; squares represent 110 mg l^{-1} substrate sample starting at OD_{290} of 0.2 and decreasing to 0; and circles represent 55 mg l^{-1} substrate sample starting at OD_{290} of 0.03 and decreasing to 0.

Figure 3 shows the respirometric analysis of the consumption of oxygen by *E. cloacae* during the enzymatic consumption of substrate. In Figure 3, diamonds represent 55 mg l^{-1} substrate sample starting at 100 and decreasing to 87; circles represent 110 mg l^{-1} substrate sample starting at 110 and decreasing to 77; triangles represent 165 mg l^{-1} substrate sample starting at 88 and decreasing to 66; and squares represent 220 mg l^{-1} substrate sample starting at 91 and decreasing to 2.5.

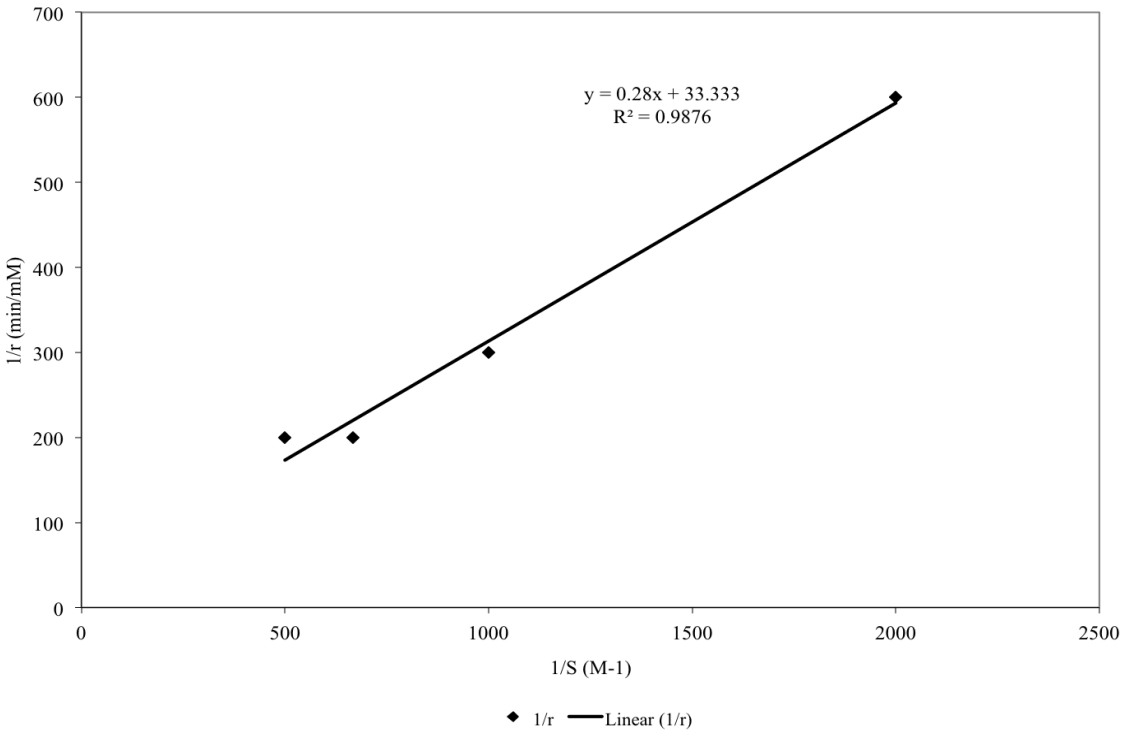

Figure 4. Lineweaver-Burke plot of 1/r vs 1/S for obtaining a straight-line equation that ease the calculation of V_{max} and in turn K_m.

Table 3. Experimental data for enzymatic kinetics including averages to be used in the calculation for K_m in the Michaelis-Menten equation.

Resorcinol concentration [S] (mol/l)	Rates [r] (mM/min)	1/[S]	1/[r]
0.00200	0.00500	500	200
0.00150	0.00500	666.67	200
0.00100	0.00333	1000	300
0.00050	0.00167	2000	600
Averages: 0.00125	0.00375	1041.67	325

It is apparent that the 220 mg l^{-1} resorcinol substrate is the optimal tested concentration of resorcinol for *E. cloacae* to be consumed as its sole source of carbon and energy. The enzymes used in the degradation pathway are able to consume 220 mg l^{-1} concentration completely, whereas a lesser concentration does not provide enough carbon and energy source to sustain the *E. cloacae* demands within the observation time.

Michaelis-Menten kinetics

The Michaelis-Menten equation was chosen as the enzymatic kinetics modeling equation. Curve-fitting regression analysis was allowed for solution for initial rate (Jonh et al., 2009) while the classic method of solving for kinetic parameters was to linearize the graphs using reciprocals (Agarry and Solomon, 2008). To solve the Michaelis-Menten equation (Equation 4), it was best to plot the data according to 1/r vs. 1/S (data in Table 3) and obtain a straight-line equation (Figure 4). The equation was solved for the y-intercept, and its reciprocal is V_{max}. The Lineweaver-Burke equation (Equation 5) was used to ease solving of the model, as previously done by Kovarova and Egli (1998) and Chauhan et al. (2010). Experimental data and this simple calculation generated all the variables in the Michaelis-Menten equation except for K_m, which was found. V_{max} was 0.03 mM/min and K_m was equal to 0.011 moles, empirical averages for r of 0.00375 mM of O_2 for substrate utilization/min/mg dry weight of cells and for S of 0.00125 moles. The Michaelis - Menten equation finds K_m to be the theoretical amount of substrate present when velocity was at half of its maximum (V_{max}).

DISCUSSION

The present study reports the biodegradation growth kinetics and steady state enzyme kinetics for resorcinol by *E. cloacae*. Pure culture degradation kinetics of organic contaminants has been reported in the past by many researchers (Bafana et al., 2007). Although a consortium was often recognized as beneficial due to the favorable synergistic interactions that often result, but in this study indigenous *E. cloacae* was found to degrade resorcinol. Resorcinol degradation differs from other phenolic compounds due to the position of the hydroxyls. Resorcinol degradation required the addition of a hydroxyl transfer whereas catechol is easily converted to benzoquinone and phenol to catechol (Yao et al., 2006; Ferreira Guedes et al., 2010). It is also reported that resorcinol toxicity, despite its dependence on the surrounding environment affects the ability of *P. putida* DSM6414 to utilize it easily (Ferreira Guedes et al., 2010). It is very rare to find literature describing an aerobic bacterium that is able to use resorcinol as its sole source of carbon and energy; however, *Penicillium* fungus has been proven to use resorcinol (Ferreira Guedes et al., 2010), and *Rhodococcus opacus* has anaerobically degraded resorcinol in the gut of *Macrotermes michaelseni* (Ngugi et al., 2005).

The results in Table 2 display the Monod, Michaelis-Menten and Monod-Michaelis Menten kinetic analysis of different bacterial strains on resorcinol (up to 220 mg l^{-1}). Since Michaelis-Menton is based on the Monod equation, this comparison is valid. The half-saturation constant was solved when the biomass was kept at a steady no-growth constant (Ferreira Guedes et al., 2010). The higher half-saturation constant was detected for substrates that are more difficult to degrade (Ferreira Guedes et al., 2010). The data reported were highly comparable with that (11.27 mM) reported by Ferreira Guedes et al. (2010). Agarry and Solomon (2008) suggested that the most reliable data reporting better physiology of the bacterial strain is the rate of substrate degradation. Despite the higher Monod constant found for *E. cloacae* listed in Table 2, *E. cloacae* degraded 220 mg l^{-1} resorcinol almost completely in the first 25 h of growth, whereas *Penicillium chrysogenum* CLONA2 took over 80 h to degrade approximately the same concentration of resorcinol substrate (Ferreira Guedes et al., 2010). Kim et al. (2005) stated that the highest specific growth per substrate concentration was another good indicator of competitive advantages when comparing bacterial strains. This was depicted as μ_{max} divided by K_S. The Monod equation for resorcinol degradation using *E. cloacae* resulted in a low μ_{max} of 0.0371. It has been reported in the literature that μ_{max} for benzene degradation using *Pseudomonas aeruginosa*, *P. fluorescens*, and *P. putida* ranges from 0.30 to 0.62, resulting in a μ_{max}/K_S ratio in the range of 0.004 to 0.376 (Abuhamed et al., 2004; Kim et al., 2005). In comparison, resorcinol degradation by *E. cloacae* specific growth per substrate concentration outcome was as low as 0.00168, explaining that it is a difficult substrate to be used by bacteria as a sole source of carbon and energy under aerobic conditions. The outcome offered a possible reason why resorcinol is rarely used in organic compound degradation studies.

Conclusions

The performance of the indigenous *E. cloacae* in the diluted defined minimal media using 220 mg l^{-1} resorcinol substrate concentration showed aerobic degradation of the single limiting substrate. The K_m value observed was 11.00 mM and μ_{max} value was 0.037 h^{-1}. Concentrations of resorcinol substrate lower than 220 mg l^{-1} proved to be insufficient in carbon and energy to allow growth, while resorcinol concentrations greater than 220 mg l^{-1} (550, 880, 1100 mg l^{-1}) proved inhibitory for cell growth. The present study is believed to be the first publication describing aerobic degradation kinetics for resorcinol (a photo-oxidizable compound) using an isolated bacterium *E. cloacae*, from an activated sludge of industrial waste water treatment facility.

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