Process Analysis of the Conversion of Styrene to Biomass and Medium Chain Length Polyhydroxyalkanoate in a Two-Phase Bioreactor

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ABSTRACT: The improvement and modeling of a process for the supply of the volatile aromatic hydrocarbon, styrene, to a fermentor for increased biomass production of the medium chain length polyhydroxyalkanoate (mcl-PHA) accumulating bacterium Pseudomonas putida CA-3 was investigated. Fed-batch experiments were undertaken using different methods to provide the styrene. Initial experiments where styrene was supplied as a liquid to the bioreactor had detrimental effects on cell growth and inhibited PHA polymer accumulation. By changing the feed of gaseous styrene to liquid styrene through the air sparger a 5.4-fold increase in cell dry-weight was achieved (total of 10.56 g L⁻¹) which corresponds to a fourfold improvement in PHA production (3.36 g L⁻¹) compared to previous studies performed in our laboratory (0.82 g L⁻¹). In addition this final improved feeding strategy reduced the release of styrene from the fermentor 50-fold compared to initial experiments (0.12 mL total styrene released per 48 h run). An unstructured kinetic model was developed to describe cell growth along with substrate and oxygen utilization. The formation of dispersed gas (air) and liquid (styrene) phases in the medium and the transfer of styrene between the aqueous and dispersed liquid droplet phases was also modeled. The model provided a detailed description of these phase transitions and helped explain how the feeding strategy led to improved process performance in terms of final biomass levels. It also highlighted the key factors to be considered during further process improvement.


KEYWORDS: styrene; unstructured kinetic model; two phase system; polyhydroxyalkanoate; Pseudomonas putida CA-3

Introduction

Pseudomonas putida strains are known for being able to metabolize a broad range of substrates (Wackett, 2003) and to accumulate the biodegradable polymer, medium chain length polyhydroxyalkanoate (mcl-PHA) (Chen, 2009; Hartmann et al., 2005; Witholt and Kessler, 1999). Mcl-PHA is a partially crystalline elastomer with a thermal degradation temperature close to 300°C and desirable properties such as biodegradability and biocompatibility which allow for a wide range of applications from packaging to medical (Furrer et al., 2008; Keshavarz and Roy, 2010; Valappil et al., 2006).

Recent legislation on waste diversion from landfill (2008/98/EC), has driven the search for technologies to recycle wastes such as plastic (Aguardo et al., 2008; Panda et al., 2010). We have previously developed a two-step chemobiotechnological conversion of polystyrene, a major post-consumer waste, to mcl-PHA whereby polystyrene is converted to styrene by pyrolysis and the styrene monomer is fermented by bacteria that accumulate mcl-PHA intracellularly (Goff et al., 2007; O’Connor et al., 1995; Ward et al., 2006). Early experiments in stirred bioreactors with styrene as the sole carbon source yielded cell dry weight concentrations of approximately 1 g L⁻¹ (Ward et al., 2006). The process was improved through the controlled feeding of nitrogen to the growth medium which resulted in a twofold increase in biomass and a 1.4-fold increase in mcl-PHA accumulation (Goff et al., 2007). To date we have reported on the growth and mcl-PHA accumulation by P. putida CA-3 from gaseous styrene (Goff et al., 2007; Ward et al., 2006). The supply of styrene through a liquid feed should increase its rate of supply to the bioreactor, thus providing the potential for increased biomass production. Furthermore, liquid feeding of styrene is more practical on a larger scale.
air pump was used for the air supply and the air flow was adjusted manually to 5 L min⁻¹. The pH of the reactor liquid was maintained at 6.9 ± 0.2 using 1 M HCl and 1 M NaOH. The pumps and pH were controlled by Electrolab FerMac 360 controller, which in turn was controlled by PC using the Electrolab FerMac Management Software. The software was used for data acquisition of the online measurements (DO, pH, temperature, acid/base dosage). The pH and dissolved oxygen (DO) concentration as a percentage of air saturation, was monitored using Mettler Toledo electrodes.

The growth medium used was MSM containing per liter: 9 g Na₂HPO₄·12H₂O, 1.5 g KH₂PO₄, 0.2 g MgSO₄·7H₂O, 0.002 g CaCl₂, and 1 mL of trace element solution (Schlegel et al., 1961). The nitrogen source (NH₄Cl; Sigma–Aldrich, Dublin, Ireland) was supplied as described in Table I. The sole carbon and energy source, styrene (Sigma–Aldrich) was either (A) pumped as liquid through the feed port and droplets fell into the liquid medium or (B) as a liquid pumped into the air sparger tube (Fig. 1). When the air sparger was used fine droplets of styrene liquid entered the liquid medium at the bottom of the fermentor vessel using a peristaltic pump (Electrolab, low flow rate pump).

A series of different fermentation conditions were used in this study. These conditions were divided into experiments (A–E) with the conditions in each experiment run in duplicate. For runs within experiment A (Table I), an incremental increase in the rate of liquid styrene supply through the feed port was applied i.e. first 2 h of fermentation styrene was supplied at a rate of 94 µL h⁻¹, followed by 189 µL h⁻¹ for 2 h and further 4 h at 360 µL h⁻¹. The styrene supply rate was increased for the following 8 h to 432 µL h⁻¹, followed by 10 h at 468 µL h⁻¹ and further 10 h at 512 µL h⁻¹ and further 8 h at 730 µL h⁻¹. To ensure all styrene was completely utilized before harvesting of cells from the fermentor the rate of styrene supply at 44 h was decreased to 360 µL h⁻¹ for 2 h, followed by 2 h at 94 µL h⁻¹.

For the runs in experiments B–D, styrene was supplied through the air sparger (Fig. 1), using various feeding profiles. For runs in experiment B the feeding rate profile

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**Materials and Methods**

**Bacterial Strain, Media and Culture Conditions**

*Pseudomonas putida* CA-3 (NCIMB 41162) was previously isolated from a bioreactor containing styrene (O’Connor et al., 1995). *Pseudomonas putida* CA-3 cultures were maintained on minimal mineral salt (MSM) agar plates supplied with styrene vapor as carbon source, as previously described (Nikodinovic et al., 2008). The inoculum (100 mL) was grown in the MSM medium with styrene as a sole carbon source in the 500 mL conical flask at 30°C, for 28 h shaking at 200 rpm. Fermentation cultures were grown in a 5 L (working volume) glass stirred tank reactor (Electrolab, Tewkesbury, UK) with temperature controlled by electrical heating pad and maintained at 30°C. The reactor was equipped with two standard six-blade turbines. A separate

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**Table I.** List of fermentation conditions used in this study. The conditions have been sorted into experiments A–E in accordance with varying nutrient supply conditions and were run in duplicate. The styrene supply rates for experiments B–D are provided in Supporting Information Figure 1 and runs in experiment E are shown in Figure 3.

<table>
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<th>Styrene supply</th>
<th>Nitrogen supply</th>
<th>Impeller speed</th>
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<td>A</td>
<td>Stepwise liquid droplets to medium</td>
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<tr>
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<td>Larger less frequent alterations to volume of styrene supplied over time: Liquid supplied through air sparger</td>
<td>Batch; 0.5 g L⁻¹</td>
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<td>C</td>
<td>Smaller more frequent alterations to volume of styrene supplied over time: Liquid supplied through air sparger</td>
<td>Fed batch; 0.5 g L⁻¹ at T0. Feed 125 mg h⁻¹ (T17–T27). Increase feed to 200 mg h⁻¹ (T27–T47)</td>
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<tr>
<td>D</td>
<td>Smaller more frequent alterations to volume of styrene supplied over time: Liquid supplied through air sparger</td>
<td>Fed batch; 0.5 g L⁻¹ at T0. Feed 125 mg h⁻¹ (T22–T34). Increase feed to 300 mg h⁻¹ (T34–T47)</td>
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<td>E</td>
<td>Smaller more frequent alterations to volume of styrene supplied over time: Liquid supplied through air sparger</td>
<td>Fed batch; 0.5 g L⁻¹ at T0. Feed 125 mg h⁻¹ (T12–T24). Decrease feed to 2 mg h⁻¹ (T24–T46)</td>
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Finding a way to increase styrene supply without inhibiting bacterial growth was a key challenge of the present study. The aim was to investigate options for improved transfer of styrene to the fermentation medium in a stirred tank bioreactor in order to maximize the biomass production without affecting polymer production and secondly to develop a mechanistic understanding of the process by means of a mathematical model.

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was as follows: 94 μL h⁻¹ for 4 h; 188 μL h⁻¹ for 4 h; 360 μL h⁻¹ for 5–7 h; 855 μL h⁻¹ for 11 h; 1185 μL h⁻¹ for 7 h; and 1440 μL h⁻¹ for 18 h. During runs within experiments C and D styrene supply rates were increased more often in smaller increments in comparison to experiment B runs (Supporting Information Fig. 1). The styrene feeding profile for the runs in experiment E is provided in Figure 3.

Estimation of Oxygen Mass Transfer Coefficient (kLaO₂)

The mass transfer coefficient of oxygen (kLaO₂) from the dispersed gas phase to the continuous aqueous phase was measured experimentally via the dynamic gassing out method (Vantriet, 1979). A number of impeller speeds and aqueous phase compositions (medium without cells) were tested, with the results used in the mathematical model to predict the supply of oxygen to the aqueous phase.

Analytical Methods

Biomass

Cell growth was monitored spectrophotometrically (OD₅₄₀; Unicam Helios δ UV/VIS spectrophotometer, Thermo Scientific, Dublin, Ireland) and cell dry weight was obtained via a calibration curve of optical density (OD₅₄₀) versus dry weight where cell suspensions with optical densities ranging from 0.1 to 0.8 were filtered through previously weighed glass fiber membranes (Whatman) and dried at 90°C until a constant weight was achieved. Aliquots of fermentation broth (5 mL) were centrifuged at 5,000 g for 10 min at 4°C (Eppendorf 5804 R bench top centrifuge, Hamburg, Germany) and cell pellets washed in potassium phosphate buffer (10 mM, pH 7.4) two times prior to application of the cells to the membranes.

Styrene

Styrene concentration in the air stream leaving the fermentor was determined similarly to a method previously described (Prado et al., 2006) using a solid-phase extraction cartridges Oasis HLB (Waters, Milford) that were fitted at the exhaust line. Cartridges were changed at 1–2 h intervals. Retained styrene was eluted using 2 mL of diethyl ether (4°C) at approximately 0.5 mL min⁻¹. Gas chromatography (GC) was used for detection of styrene eluted from the filters. A Fison 8000 series GC equipped with an Agilent HP-1 capillary column (30 m, 0.25 mm inner diameter, 0.25 μm film thickness; J&W Scientific, Agilent Technologies, Santa Clara) was used with splitless injection and hydrogen as the carrier gas. A temperature gradient of 50–180°C was implemented with an increment of 10°C min⁻¹. Detection was done using a flame ionization detector (FID). Styrene was identified by comparison of retention times with commercially available standard.

Ammonium

The nitrogen concentration in the growth medium was determined by the indophenol method as previously described (Scheiner, 1976).

mcl-PHA

Cells collected at different time points of fermentation were harvested by centrifugation (5,000 g for 10 min at 4°C) and washed twice with an equal volume of phosphate buffer (50 mM, pH 7). Cells were centrifuged again (5,000 g for 10 min at 4°C) and freeze dried. The polymer content was determined by subjecting 5–10 mg of lyophilized whole cells to acidic methanolysis according to previously described protocols (Brandl et al., 1988; Lageveen et al., 1988). This method degrades the intracellular PHA to methyl esters of its constituent 3-hydroxyalkanoic acids. The 3-hydroxyalkanoic acid methyl esters were assayed by GC using a Hewlett-Packard HP6890 chromatograph equipped with a BP-20 capillary column (30 m by 0.25 mm, 0.25-μm film thickness; J&W Scientific) and a FID. A temperature program 60°C for 3 min; temperature ramp of 5°C min⁻¹; 200°C for 1 min was used. For the peak identification, methyl esters of 3-hydroxyalkanoic acid were prepared in a similar manner and PHA standards from P. putida CA-3 were used (Ward et al., 2005).
Mathematical Model Development

The fermentation of styrene is a complex combination of biological and physical transport processes. In order to dissect and understand these processes, a simple kinetic model was constructed to account for biomass growth and substrate and oxygen utilization, while the physical processes of substrate and oxygen supply and mass transfer were also modeled.

Model Type

An unstructured, non-segregated model was applied to this system. This type of model considers neither internal structure of cells (unstructured) nor any diversity in the cell population (non-segregated) (Nielsen and Villadsen, 1992). These characteristics are often seen in plant and animal cell cultures but only in certain microbial populations. Thus, it is satisfactory to assume for this system, that the microbial composition is nearly constant along the culture cycle (Bailey and Ollis, 1986). Another key assumption of this model type is that the time constants for the biological kinetic processes are much larger than the mixing and mass transfer time constants (Roels, 1983).

Rate of Cell Growth

The specific exponential growth rate of the cells was expressed as a function of substrate concentration using the Monod equation:

$$\mu = \mu_{\text{max}} \frac{S}{K_s + S}$$

where, $S$ is the concentration of the growth limiting substrate, $K_s$ is the saturation constant for this substrate, and $\mu_{\text{max}}$ is the maximum growth rate of the cell under substrate sufficient conditions.

Rate of Substrate and Oxygen Utilization

The specific rate at which substrate, oxygen and nitrogen were consumed was related to the specific growth rate predicted by the Monod equation by the means of a yield coefficient (Enfors and Häggström, 2000):

$$q_s = \frac{\mu}{Y_{x/s}}$$

$$q_o = \frac{\mu}{Y_{x/o}}$$

where, $q_s$ and $q_o$ are the specific rates of consumption of substrate (S) and oxygen (O), respectively, and $Y_{x/s}$ and $Y_{x/o}$ are the yield coefficients of biomass on substrate and oxygen, respectively.

Mass Balances

Mass balances were constructed to track changes in concentration over time (Enfors and Häggström, 2000):

$$\frac{dX}{dt} = \mu X$$

$$\frac{dS}{dt} = -q_s X$$

$$\frac{dO}{dt} = -q_o X$$

where, $C_{d}$ and $C_{c}$ are the styrene concentration in the dispersed liquid droplets and aqueous phase, respectively, $k_{L}a_{\text{styrene}}$ is the liquid–liquid styrene mass transfer coefficient and $m$ is a phase partition coefficient (equilibrium mole fraction ratio of styrene in the respective phases).

Styrene and Oxygen Mass Transfer

Styrene was fed through the gas sparger in the form of fine liquid droplets and due to its low solubility in water (350 mg L$^{-1}$ at 30°C) (Verschueren, 1996), formed dispersed liquid droplets in the continuous aqueous phase. The rate of mass transfer of styrene from the dispersed liquid droplets into the aqueous phase ($N_{A,\text{styrene}}$) was described as follows (Doran, 1995):

$$N_{A,\text{styrene}} = k_{L}a_{\text{styrene}} \left( \frac{C_{d}}{m} - C_{c} \right)$$

$$m = \frac{Z_{d}}{Z_{o}}$$

where, $C_{d}$ and $C_{c}$ are the styrene concentration in the dispersed liquid droplets and aqueous phase, respectively, $k_{L}a_{\text{styrene}}$ is the liquid–liquid styrene mass transfer coefficient and $m$ is a phase partition coefficient (equilibrium mole fraction ratio of styrene in the respective phases).

Oxygen was fed in the form of air which formed a dispersed gas phase in the reactor medium. The rate of mass transfer of oxygen ($N_{A,O_2}$) from this dispersed gas phase to the continuous aqueous phase was described as follows (Doran, 1995):

$$N_{A,O_2} = k_{L}a_{O_2} \left( C_{L} - C_{c} \right)$$

where, $C_{L}$ is the saturation concentration of oxygen in the medium, $C_{L}$ is the instantaneous oxygen concentration in the aqueous phase and $k_{L}a_{O_2}$ is oxygen gas–liquid mass transfer coefficient.
Model Assumptions

A number of assumptions were made during the model construction:

1. Only the data for the runs in experiment E was modeled, as it was the optimized configuration for styrene feeding. Only the first 20 h of this data was modeled as the focus of the present work was on the maximization of biomass. PHA accumulation did not occur during the first 20 h of operation and as such biomass composition remained constant over this period.

2. It was assumed that the only growth limiting factor (over the 10–20 h period) was $k_l a_{styrene}$ and it was on this basis that $k_l a_{styrene}$ was fitted to the experimental data over this time period. This assumption is explained further in the Results Section.

3. The solubility of styrene was based on water and not medium containing cells.

4. It was assumed that during the first 10 h of the runs in experiment E that the cells were growing at their maximum specific growth rate. This was based on an examination of the transfer of styrene to the aqueous phase during the initial hours of the run (using the fitted value of $k_l a_{styrene}$) which showed an accumulation of dissolved styrene in the aqueous phase. This lead to an excess supply of styrene to the cells and it was assumed that this excess allowed the cells to grow at their maximum specific growth rate. Accordingly, the maximum specific growth rate applied to the model was the experimentally observed specific growth rate over the 0–10 h period.

5. The inhibition of growth due to styrene toxicity has been neglected. Some inhibition of growth may have taken place, but there is no evidence of any major inhibition in the experimental data. It was also seen for the degradation of benzene by a number of different Pseudomonas strains, that the fundamental, qualitative result did not vary through the use of either inhibitory or non-inhibitory growth kinetics, despite inhibition by high concentrations of substrate being witnessed (Kim et al., 2005).

Model Parameters

$Y_{x/a}$ and $Y_{s/o}$ were estimated from the experimental data and later refined by fitting. The saturation constant ($K_s$) of $P. putida$ SN1 grown on gaseous styrene and reported as 2.5 $\mu$M (Park et al., 2005), was used initially and refined by fitting. $k_l a_{styrene}$ was assumed constant and also fitted to the experimental data. The final model parameters applied are shown in Table II.

Model Implementation

The model was constructed and implemented using the DynoChem (Scale-up Systems Ltd, Dublin, Ireland) computer modeling package. This package was chosen due to its solver power, built in tools and suitability as a cross disciplinary tool. Once constructed, the ordinary differential equations were solved using the built-in Rosenbrock optimization routine.

$Y_{x/a}, Y_{s/o}, K_s$, and $k_l a_{styrene}$ were fitted to the experimental data using the Dynochem built-in fitting routine. This routine uses the Levenberg-Marquardt algorithm to minimize the sum of squares between the model predictions and experimental data.

Results and Discussion

*Pseudomonas putida* CA-3 can metabolize styrene to yield carbon and energy for growth (O’Connor et al., 1995). When challenged with an essential nutrient limitation (such as nitrogen), this bacterium accumulates carbon in the form of a polymer mcl-PHA (Ward et al., 2005).

From a single 48 h fermentation Goff et al. obtained 9.6 g (1.92 g L$^{-1}$) of cell dry weight (of which 5.5 g was PHA-free biomass and 4.1 g mcl-PHA) using styrene vapor as sole source of carbon and energy and feeding the nitrogen source at 1.5 mg L$^{-1}$ h$^{-1}$ (Goff et al., 2007). During that fermentation approximately 15 mL of styrene was used (Goff et al., 2007). In the first set of experiments in the present study (experiment A; Table I) styrene was directly added to the liquid medium as droplets. Within 48 h of fermentation a total cell dry weight of between 5 and 8 g was achieved (1–1.6 g L$^{-1}$) (Table III). However, the dried cell pellet appeared greasy and contained no mcl-PHA. During these runs about 24 mL of styrene was supplied to the bioreactor. Styrene (25–30%) supplied was collected on the filters. Furthermore, during these runs droplets of styrene could be observed on the interface of the medium and the headspace. The runs in experiment B involved the supply of styrene through the air sparger (Fig. 1). This approach was proposed to increase the dispersion of styrene in the liquid medium. A threefold increase in cell mass in comparison to the conditions in the experiment A runs (3.5–4.4 g L$^{-1}$) was obtained after 48 h. However a significant fraction (about 30%) of the supplied styrene was detected in the air exhaust as determined by filter measurements (Table III).

Alterations to the styrene feed profile were undertaken in experiments C and D to reduce the adverse effect of styrene supply on oxygen transfer, biomass productivity and reduce...
the amount of styrene in the fermentor exhaust (Table I). In addition, a switch in the nitrogen supply strategy from batch to fed batch was attempted to further increase biomass. In the runs in experiments C and D the final biomass concentration ($7 \, \text{g\,L}^{-1}$) was improved 5.4-fold compared to fermentation condition A (Table III). In addition, styrene collected on the filters was reduced 10-fold to 2–3% of total styrene supplied.

The styrene feeding rate during experiment E runs was based on a strategy of matching styrene supply to demand. Accordingly, biomass concentration was measured hourly and the feed pump adjusted to supply at a rate that matched the specific styrene utilization rate multiplied by the biomass concentration prevailing at that time point. Additionally, nitrogen feeding rate was reduced at hour 24 from 200 to 2 mg·h$^{-1}$, to induce mcl-PHA accumulation by cells (Fig. 2). The amount of polymer accumulated by hour 48 was 32% of total cell dry weight which is 1.3-fold lower PHA levels (42% CDW) obtained in previous studies (Goff et al., 2007). However, the final cell dry weight achieved at 48 h in experiment E was 10.3 g·L$^{-1}$ corresponding to a 5.4-fold improvement in biomass which corresponds to a fourfold improvement in PHA production compared to previous studies (Goff et al., 2007). Furthermore, styrene released to the exhaust was further reduced to 0.2% of total supplied.

An analysis of the experimental data for experiment E (Fig. 2) showed a sudden drop in specific growth rate from 0.63 between 0–10 h to 0.29 h$^{-1}$ between hours 10 and 20. However, at the lower growth rate, the DO level within the reactor fell at an increased rate in comparison to during the higher growth rate period. This increased fall in DO, was not caused by a change in air flow rate or impeller speed and was most likely not due to an increased rate of consumption by the cells due to the observed fall in growth rate. Therefore, it is proposed that it must have been caused a decrease in $k_L a_O$. A similar fall was observed during the experimental measurement of $k_L a_O$ following addition of styrene to the aqueous phase (data not shown). Thus, the most likely cause of the fall in DO was the accumulation of styrene as dispersed droplets in the medium. It was on this basis that $k_L a_{styrene}$ was fitted to the experimental data (as per assumption 2).

<table>
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<tr>
<th>Experiment</th>
<th>Styrene total volume supplied (mL) to 5 L medium</th>
<th>Styrene total volume collected (mL) on exhaust</th>
<th>Final biomass (g) from 5 L fermentation</th>
</tr>
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<tr>
<td>A</td>
<td>22.0</td>
<td>6.00</td>
<td>6.75</td>
</tr>
<tr>
<td>B</td>
<td>46.7</td>
<td>15.23</td>
<td>19.55</td>
</tr>
<tr>
<td>C</td>
<td>51.2</td>
<td>1.23</td>
<td>35.10</td>
</tr>
<tr>
<td>D</td>
<td>46.6</td>
<td>0.93</td>
<td>33.6</td>
</tr>
<tr>
<td>E</td>
<td>106.6</td>
<td>0.12</td>
<td>52.8</td>
</tr>
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*Average of two runs. Standard error ranged from 0.5% to 2.5%.
Model Predictions

The model was implemented for the experimental conditions used in the experiment E runs. The styrene feed profile and its predicted dissolution in the aqueous phase and dispersion as droplets in the medium are shown in Figure 3. A plot of model prediction versus experimental data for biomass and DO is shown in Figure 4.

Styrene Supply and Phase Transition

At the start of the experiment E runs, the total styrene requirement of the cells was lower than the rate of styrene supply, thus enabling the cells to grow at their maximum specific growth rate. This also allowed for the accumulation of dissolved styrene in the aqueous phase and as dispersed droplets in the reactor medium (Fig. 3). This continued until hour 7, by which time the total styrene requirement of the cells was higher than its rate of transfer between phases.

From hours 7 to 10, the cells continued growing at their maximum rate, but there was no further accumulation of dissolved styrene in the aqueous phase. However, styrene continued to accumulate as dispersed droplets in the medium. By hour 10, most of the styrene in solution in the aqueous phase (that had been accumulated over the first 7 h) was consumed. There was also significant accumulation of styrene as droplets in the medium.

At hour 10, the specific growth rate fell from 0.63 to 0.29 h\(^{-1}\) as styrene in solution in the aqueous phase was no longer in excess. From hour 10 to 20, the growth rate was dictated by the rate of styrene transfer into solution in the aqueous phase. This is evident as the concentration of dissolved styrene in the aqueous phase was very low (~0.1% saturation) and any styrene transferred to the aqueous phase was almost instantly consumed.

From hour 10 to 12, some of the styrene accumulated as droplets in the medium was transferred to the aqueous phase and consumed. However, after this time, styrene continued to accumulate as droplets in the medium (albeit at a lower rate) even though the driving force for transfer of
styrene was near its maximum. This confirmed that $k_L a_{\text{styrene}}$ was the growth-limiting factor over this period. It has also been seen that substrate mass transfer was the slowest step in the biodegradation of benzene and toluene by P. putida F1 for substrate concentrations $>1$ mM (Reardon et al., 2000).

As the styrene concentration dissolved in the aqueous phase could not be monitored accurately due to its low solubility, it was confirmed indirectly through the DO data as follows. The predicted oxygen consumption rate of the cells over the first 10 h was 180 nmol min$^{-1}$ mg cells$^{-1}$. This compares very favorably to the range of 150–250 nmol min$^{-1}$ mg cells$^{-1}$ measured experimentally for P. putida CA-3 cells extracted from the same fermentor (Electrolab) which were supplied with gaseous styrene (Ward et al., 2006). This agreement confirmed the fitted value of $Y_{x/o}$ and showed that the model could accurately predict the oxygen consumption of the cells.

Using this value of $Y_{x/o}$, the DO level was predicted using a constant $k_L a_{O_2}$ value (as measured experimentally for pure medium). The model prediction was significantly higher over hours 12–20 than that witnessed experimentally (Fig. 5). The oxygen consumption of the cells was shown to be accurate; meaning the constant $k_L a_{O_2}$ value applied was too high over this time period.

As observed experimentally, the addition of styrene to the medium reduced $k_L a_{O_2}$ significantly. Thus, the value of $k_L a_{O_2}$ applied to the model was adjusted based on the predicted accumulation of styrene over the course of the runs (Fig. 3). This led to very good agreement between the experimental data and model prediction (Fig. 5). This confirmed the predicted accumulation of dispersed styrene droplets in the medium over the course of the runs, the value of $k_L a_{\text{styrene}}$ fitted to the data and the dissolved styrene concentration in the medium.

There is good agreement between the experimental data and model predictions for both biomass and DO (Fig. 4). There was some deviation from the data in the DO prediction over the 8–12 h period (where the specific growth rate fell from 0.63 to 0.29 h$^{-1}$). This deviation is characteristic of the Monod kinetics applied and is likely caused by the unsteady state conditions over this short time period corresponding to a transition to a lower specific growth rate.

### Improvement in Total Biomass Yields

The implementation of the model has helped explain how the modifications to styrene feeding method improved the biomass yield. Feeding large liquid droplets of styrene directly to the reactor (as per experiment A) resulted in poor dispersion throughout the reactor and poor mass transfer of styrene into the aqueous medium. Consequently, styrene was not available to the cells in sufficiently high quantities in order to achieve high yields. The feeding of styrene through the gas sparger was likely to have produced very fine liquid droplets that were well dispersed throughout the reactor. This resulted in a higher styrene liquid–liquid mass transfer coefficient, allowing a much greater supply of styrene to the cells in order to achieve the increase in yields witnessed. However, even with this improved feeding regime, the mass transfer rate of styrene continued to be growth limiting. Methods to improve the rate of styrene mass transfer to the medium and its solubility in the medium need to be examined in order to improve yields further. For example, the use of different impeller configurations and agitation strategies should improve both styrene and oxygen mass transfer. Also, small quantities of additives such as silicone oil (Dumont et al., 2006) could be used to increase styrene mass transfer and hence its availability to the cells. However, further studies are required regarding the possible toxicity of these compounds to microbial growth. The model could be used to examine the effect of these changes on mass transfer rate and to design feeding profiles to increase the biomass yield even further.

### Nomenclature

- $C_o$: continuous medium phase styrene concentration (M)
- $C_d$: dispersed styrene phase concentration (M)
- $C_e$: equilibrium medium phase DO concentration (M)
- $C_L$: medium DO concentration (M)
- $k_L a_{O_2}$: oxygen gas–liquid volumetric mass transfer coefficient (s$^{-1}$)
- $k_L a_{\text{styrene}}$: styrene liquid–liquid volumetric mass transfer coefficient (s$^{-1}$)
- $K_s$: saturation constant for growth limiting substrate (M)
- $m$: styrene phase partition coefficient (–)
- $N_a a_{O_2}$: rate of transfer of oxygen between gas and medium phases (M s$^{-1}$)
- $N_a a_{\text{styrene}}$: rate of transfer of styrene between dispersed and medium phases (M s$^{-1}$)

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**Figure 5.** Examination of DO level based on different $k_L a_{O_2}$ values. Experimental DO (●) plotted against model predictions based on constant $k_L a_{O_2}$ (---) and varying $k_L a_{O_2}$ based on styrene accumulation as dispersed droplets in medium (—-).
\( \dot{q}_o \) specific oxygen uptake rate (g·g\(^{-1}\)·h\(^{-1}\))

\( \dot{q}_s \) specific substrate uptake rate (g·g\(^{-1}\)·h\(^{-1}\))

\( S \) concentration of growth limiting substrate (M)

\( X \) total biomass (mol)

\( Y_{w/o} \) yield of biomass on oxygen (g·g\(^{-1}\))

\( Y_{w/s} \) yield of biomass on substrate (g·g\(^{-1}\))

\( Z_o \) equilibrium mole fraction of styrene in dispersed phase (mol·mol\(^{-1}\))

\( Z_s \) equilibrium mole fraction of styrene in continuous phase (mol·mol\(^{-1}\))

\( \mu \) specific growth rate (h\(^{-1}\))

\( \mu_{\text{max}} \) maximum specific growth rate (h\(^{-1}\))

References


