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An efficient approach to bioconversion kinetic model generation based on automated microscale experimentation integrated with model driven experimental design

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ABSTRACT

Reliable models of enzyme kinetics are required for the effective design of bioconversion processes. Kinetic expressions of the enzyme-catalysed reaction rate however, are frequently complex and establishing accurate values of kinetic parameters normally requires a large number of experiments. These can be both time consuming and expensive when working with the types of non-natural chiral intermediates important in pharmaceutical syntheses. This paper presents an automated microscale approach to the rapid and cost effective generation of reliable kinetic models useful for bioconversion process design. It incorporates a model driven approach to the experimental design that minimises the number of experiments to be performed, while still generating accurate values of kinetic parameters. The approach has been illustrated with the transketolase mediated asymmetric synthesis of L-erythrulose. Experiments were performed using automated microwell studies at the 150 or 800 μL scale. The derived kinetic parameters were then verified in a second round of experiments where model predictions showed excellent agreement with experimental data obtained under conditions not included in the original experimental design. In comparison with conventional methodology, the modelling approach enabled a nearly 4-fold decrease in the number of experiments while the microwell experimentation enabled a 45-fold decrease in material requirements and a significant increase in experimental throughput. The approach is generic and could be applied to a wide range of enzyme catalysed bioconversions.

1. Introduction

Obtaining sufficient data for the establishment of detailed kinetic models for bioconversion processes is time consuming and generally requires significant investment in experimental resources. This is especially true when dealing with the types of non-natural reactants typically used in pharmaceutical syntheses which often feature significant substrate and/or product inhibition (Pollard and Woodley, 2007; Yazbeck et al., 2004; Rozzell, 1999). In these cases kinetic expressions can become complex and require a large number of kinetic measurements to be performed in order to obtain accurate values of model kinetic parameters. At the early stages of pharmaceutical development these novel chiral substrates can also be expensive and in short supply (Pollard and Woodley, 2007) limiting the number of experiments that can be performed. Consequently, approaches to minimise the number of experiments (Blackmond, 2005) and to reduce the scale at which they are performed are now receiving significant attention.

Based on analysis of bioconversion kinetic characteristics and expressions, we recently developed a model driven method for improved kinetic parameter estimation (Chen et al., 2008). Using this method, the number of experiments for biocatalytic reaction kinetic parameter identification can be considerably decreased while still guaranteeing model accuracy. Ourselves and others have also established microscale bioprocessing techniques, based on shaken microwell bioreactors, to reduce the scale of experiments and increase experimental throughput (Duetz et al., 2000; John and Heinzle, 2001; Lye et al., 2003). The application of laboratory automation in particular and the capacity to carry out experiments in parallel enables a wider range of process variables to be explored within a reasonable time frame (Nealon et al., 2005; Micheletti and Lye, 2006). Such new approaches are considered essential during the initial stages of pharmaceutical product development if bioconversion is to be considered alongside chemical syntheses and when complex chiral intermediates are expensive and in short supply (Lye et al., 2002; Pollard and Woodley, 2007).

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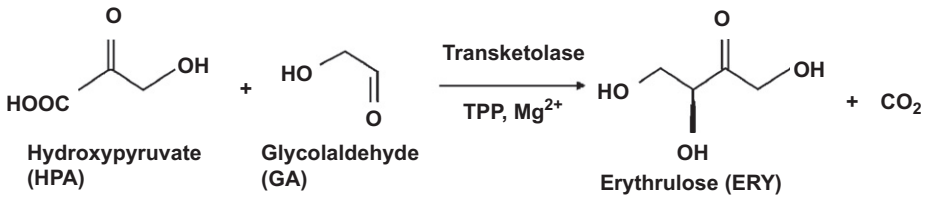

Fig. 1. Reaction scheme for the wild-type transketolase mediated synthesis of L-erythrulose (ERY) from β-hydroxypyruvate (HPA) and glycolaldehyde (GA).

In this work, we present an efficient approach to kinetic model generation based on automated microscale experimentation. The approach is considered to be generic but is illustrated for the known transketolase-mediated L-erythrulose (ERY) synthesis from β-hydroxypyruvate (HPA) and glycolaldehyde (GA) as shown in Fig. 1. Comparison of the techniques reported here with conventional experimental approaches is made and the potential benefits of the new approach in terms of increased speed to model generation and reduced experimental costs are also discussed.

2. Model driven experimental design

In our previous work (Chen et al., 2008), a model driven method for kinetic parameter identification was developed and validated using the synthesis of 1,3-dihydroxypentan-2-one from propionaldehyde and β-hydroxypyruvate by a mutant transketolase (D469T) as an example. Here the approach is briefly summarised using a second system, the synthesis of ERY from HPA and GA by wild-type transketolase. In this case we study a bioconversion with known reaction mechanism and published kinetic parameter values in order to quantitatively compare the values generated here using automated microscale experimentation. The reaction kinetics of the TK-mediated ERY synthesis from HPA and GA follow the ping-pong bi-bi mechanism with competitive substrate inhibition (Gyamerah and Willetts, 1997). The kinetic expression can be written as:

$$\frac{d[Q]}{dt} = \frac{k_{cat}E_i[A][B]}{K_b[A]\left(1+\frac{[A]}{K_{ia}}\right)+K_a[B]\left(1+\frac{[B]}{K_{ib}}\right)+[A][B]+\frac{K_a}{K_{iq}}[B][Q]+\frac{K_aK_{ib}}{K_{iq}}[Q]} \quad (1)$$

where $[A]$, $[B]$ and $[Q]$ represent the concentrations of HPA, GA and ERY, respectively. k_{cat} is the reaction rate constant, K_a and K_b are the Michaelis constants of A and B and K_{ia}, K_{ib} and K_{iq} are the inhibition constants of A, B and Q, respectively. E_i is the enzyme concentration in the reaction (mmol L^{-1}). Now, at low initial substrate concentrations, Expression (1) for the initial rate of reaction can be simplified to:

$$\frac{d[Q]}{dt} = \frac{k_{cat}E_i[A][B]}{K_b[A]+K_a[B]+[A][B]} \quad (2)$$

The step-by-step procedure for subsequent kinetic parameter identification is illustrated in Fig. 2. In this figure 'full kinetic model' and 'simplified model' refer to Expressions (1) and (2), respectively.

The experiments required to then generate the rate data, from which the kinetic parameters are determined, can be summarised in the following steps:

(1) Measure the initial reaction rate at different enzyme concentrations under (pseudo) constant substrate concentration to find the linear region for reaction rate variation with enzyme concentration.
(2) Quantify the initial reaction rates at different initial substrate concentrations to determine the region of substrate concentration in which inhibition effects can be considered negligible.

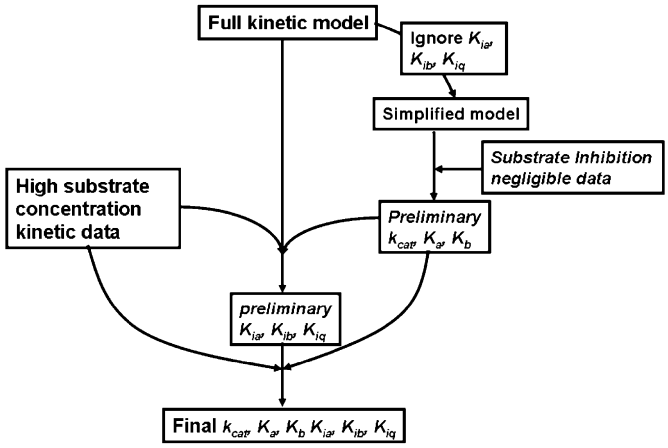

Fig. 2. Schematic representation of the integrated experimental and model driven approach for rapid kinetic parameter identification. Kinetic data obtained from automated microwell experiments as described in Section 3.2 (Micheletti et al., 2006).

(3) Produce progress curves, i.e., concentrations of substrate and product as a function of time, at high substrate concentrations and relatively high enzyme concentrations to obtain data for estimation of inhibition constants. This final set of data is also re-used in obtaining the final values of the full kinetic model parameters.

3. Materials and methods

3.1. Microbial fermentation and bioconversion reaction kinetics

3.1.1. Fermentation medium and lysate preparation

The strain used for the transketolase bioconversion was *Escherichia coli* BL21 grown on a modified version of Luria–Bertani (LB) medium containing glycerol (10 g L^{-1}). This contained plasmid pQR412 for the over-expression of wild-type *E. coli* transketolase, with molecular weight 72 KDa (Hobbs, 1994). The overnight culture was prepared as previously reported by Ferreira-Torres and coworkers (2005) and was used to inoculate 100 mL of medium in 1 L shake flasks at 10% of the final working volume. Flasks were incubated at 300 rpm, 37 °C for 8–12 h.

Cells were harvested by centrifugation (4000 rpm, 10 min) and pellets suspended in sodium phosphate buffer (5 mM, pH 7.0) to give a final concentration of 0.1 g wet cell paste mL^{-1}. After cell disruption by sonication, cell debris was removed by centrifugation and the lysate stored at −20 °C.

3.1.2. Microscale TK bioconversion kinetics

Bioconversions using a TK containing lysate from *E. coli* (prepared as described in Section 3.1.1) were carried out in 96-deep square well (96-DSW) and 96-standard round well (96-SRW) microplate formats using HPA and GA as substrates at varying initial concentrations. In *E. coli* BL21, TK expression is constitutive and therefore no induction was required. The bioconversion experiments were carried out essentially as described by Hobbs (1994) with HPA and ERY concentrations quantified as described in Section 3.3. All microwell experiments were carried out in triplicate.

3.2. Automated microscale bioconversion processes

Automated microscale process sequences involving bioconversion set-up and operation, sample acquisition and processing were established for each of the experiments outlined in Section 2. Automation was achieved using a Genesis Workstation (Tecan, Berkshire, UK) equipped with a liquid handling arm and a RoMa arm for plate manipulation. Disposable tips were used for reagent addition. The accuracy and precision of pipetting were determined for the low viscosity liquids and the dispense volumes used in this work and the error was less than 5% in all cases (Micheletti et al., 2006). All the experiments were carried out at room temperature (24 °C) in a Class II robotic containment cabinet.

Initially, the experiments described in Section 2, step 1 were carried out in 96-DSW plates using a reaction volume of 500 μL. The purpose of these experiments was to define the linear range of the relationship between initial reaction rate and enzyme concentration. At a fixed equimolar substrate concentration (30 mM), samples were taken at different reaction times, between 1 and 20 min, to correctly identify the linear range of the initial rate. Once this range had been identified, the linear relationship between initial rate and enzyme concentration was also confirmed.

Based on the information obtained in step 1, additional automated experiments were designed in order to determine the substrate concentration range in which any inhibition effects could be neglected i.e., Section 2, step 2. Only two bioconversion samples were needed in order to obtain estimates of the initial reaction rate and therefore a smaller reaction volume of 150 μL in a 96-SRW plate format was used. Two sets of experimental protocols were established: firstly, GA was kept constant at 50 mM and the initial rate variation with HPA concentrations was studied; secondly, HPA concentration was maintained at 30 mM and GA concentration was varied between 5 and 500 mM (the enzyme concentration used in each set of experiments was also determined to ensure compatibility).

The final set of automated experiments involved obtaining reaction progress curves that could be used for kinetic parameter identification and refinement; Section 2, step 3. For these experiments the reaction volume was 800 μL in 96-DSW plates and 12 samples for HPLC analysis, each of 20 μL, were withdrawn over time for each reaction condition, i.e., for each set of substrate and enzyme concentration described in Table 2.

3.3. Analytical techniques

A Dionex (Camberley, Surrey, UK) HPLC was used to quantify HPA and ERY concentrations throughout the TK bioconversion as described by Miller and coworkers (2007). The HPLC assay, based on an Aminex 87H column and UV detection, is not able to quantify GA depletion, which was therefore obtained by mass balance based on the measured HPA and ERY concentrations. Control experiments, in which GA concentration was monitored by HPLC with electrochemical detection, showed no GA degradation and confirmed the validity of the mass balance approach. The concentration of TK used in each experiment was determined by densitometry measurements of SDS-PAGE gels using a calibration curve based on densitometry measurements of pure TK samples of known concentration (Hibbert et al., 2007).

4. Results and discussion

4.1. Reaction rate variation with enzyme concentration

As outlined in Section 2, the first step in the model driven experimental design was to establish the linear region for reaction rate variation with enzyme concentration. It is quite common that increasing the enzyme concentration in the reaction does not always increase the overall rate in a linear manner due to mass transfer limitations (Law et al., 2006). Thus it is necessary to establish the range of enzyme concentrations over which there is a proportional increase in reaction rate with increasing enzyme concentration. All enzyme concentrations used in subsequent experiments to determine kinetic parameters then need to be within this defined linear region.

Fig. 3 shows the experimental results in the case of the TK mediated ERY synthesis at low initial concentrations of the two substrates HPA and GA (present at an equimolar ratio). The concentration of TK in the lysate preparations used for these experiments was determined by protein densitometry as described in Section 3.3. The results indicate that a linear relationship between enzyme concentration and initial reaction rate can be maintained up to enzyme concentrations of $5\,g\,L^{-1}$. The actual TK concentrations used in all subsequent kinetic experiments were much lower than this due to the high activity of the enzyme used in the synthesis of L-erythrulose from HPA and GA.

4.2. Substrate inhibition characterisation

The second set of experiments required in the experimental design (Section 2, step 2 and Fig. 2) explores the extent of substrate inhibition of the enzyme in order to determine the 'inhibition negligible' region of the reaction rate. This can be established from a plot of initial reaction rate against substrate concentration. For the TK reaction studied here it is well known that there is substrate inhibition of enzyme activity due to both HPA and GA (Gyamerah and Willetts, 1997; Vasic-Racki et al., 2003). For experiments

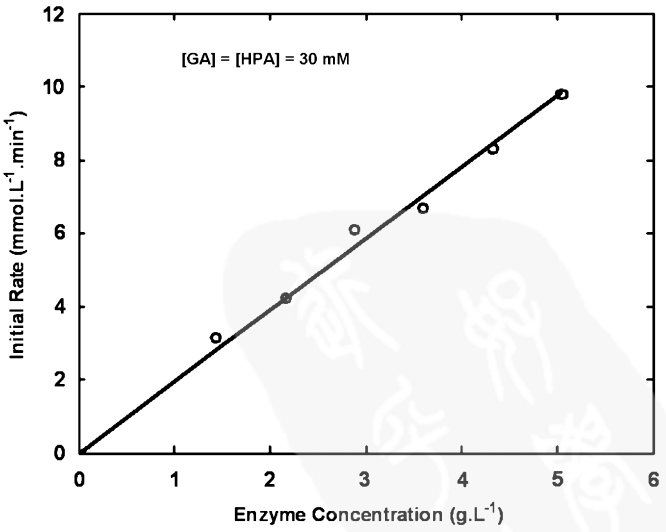

Fig. 3. Variation of initial reaction rate with transketolase concentration. A linear relationship is seen to hold up to $5\,g\,L^{-1}$ enzyme concentration. Experiments performed as described in Section 3.2. Solid line fitted by linear regression ($R^2 = 0.985$).

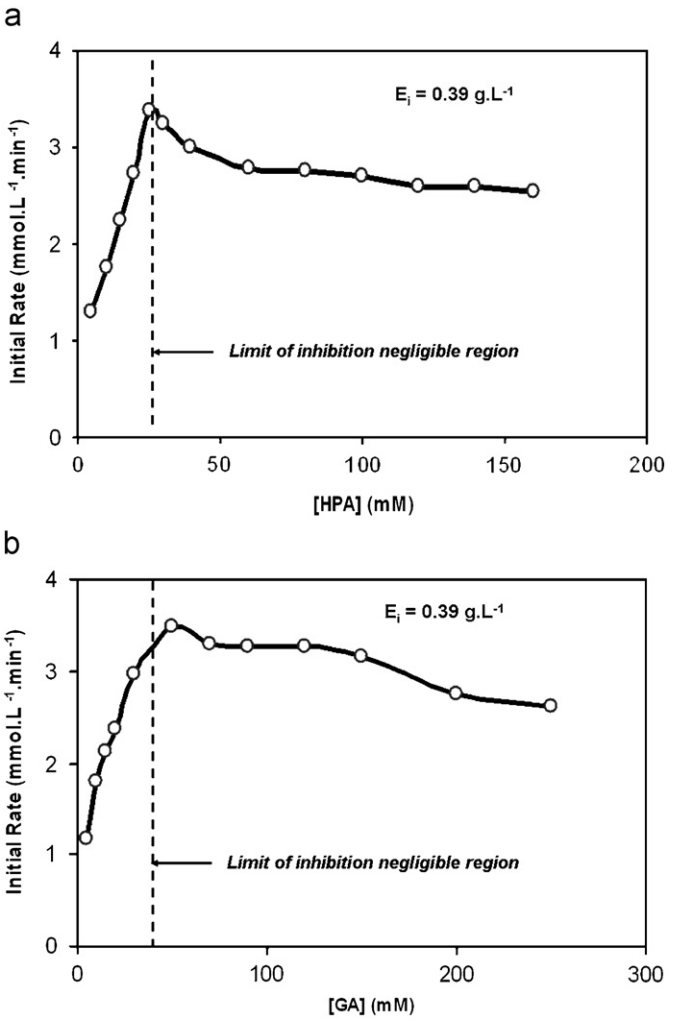

Fig. 4. Variation of initial reaction rate with substrate concentration for determination of inhibition negligible regions: (a) HPA plot indicates inhibition becomes significant at [HPA] > 25 mM; (b) GA plot indicates inhibition becomes significant at [GA] > 30 mM. Experiments performed as described in Section 3.2.

involving new enzyme mutants (Hibbert et al., 2007) or previously unstudied substrates this step of the experimental procedure also allows potential inhibition problems to be identified early.

At this point in the experimental design very accurate initial rate values are not necessary for the parameter identification based approach described in Section 2. This is because the initial parameters obtained using these data will be reconciled later when full progress curves are measured over a range of initial substrate concentrations (Fig. 2). Instead, values of the product concentration change with time as close as possible to the beginning of the reaction are all that is required in order to estimate the initial rate. In this work, experimental samples were taken for analysis just 1 min after initiation of the reaction. This helps to increase experimental throughput and reduces the analytical load (Lye et al., 2003).

Fig. 4(a) shows a plot of the measured initial reaction rate as a function of varying concentrations of substrate HPA when the concentration of the second substrate GA is kept constant at 50 mM. Fig. 4(b) shows a similar plot for variation in GA concentration while the HPA concentration is kept constant at 30 mM. The regions in which substrate inhibition is negligible are seen to be 0–25 mM and 0–30 mM for HPA and GA, respectively. The enzyme concentration in these experiments was kept constant at 0.39 g L^{-1}. Therefore, the

Table 1
Experimental initial rate data in the inhibition negligible region used for preliminary k_{cat}, K_a, and K_b estimation.

[GA] (mM)	[HPA] (mM)	Initial rate (mmol L^{-1} min^{-1})
50	5	1.29
50	10	1.76
50	15	2.23
50	20	2.72
5	30	1.16
10	30	1.79
15	30	2.11
20	30	2.37

Experiments performed at the microwell scale as described in Section 3.2.

Table 2
Experimental conditions used for obtaining progress curves at high substrate concentrations for refinement of initial kinetic parameters.

[HPA] (mM)	150	150	120	200	200	200	300	300	320
[GA] (mM)	120	150	140	220	250	180	350	285	360
E_i (g L^{-1})		0.52			0.78			1.04	

Experiments performed at the microwell scale as described in Section 3.2.

first four values of initial rates in each set of experiments (summarised in Table 1) were used to determine the preliminary values of rate and Michaelis parameters. These can be established using either linear plotting or nonlinear regression based on the simplified kinetic model (Expression (2)). The calculated initial values of k_{cat}, K_a and K_b based on the data in Table 1 were 2200 min^{-1}, 19 and 17 mM, respectively, as determined by nonlinear curve fitting in this case.

4.3. Kinetic parameter identification

The next stage in the experimental design involves obtaining complete reaction progress curves over a range of higher substrate concentrations where any substrate inhibition effects on the reaction kinetics will be evident (Section 2, step 3 and Fig. 2). The sets of substrate and enzyme concentrations used for obtaining progress curves at these high substrate concentrations in the case of TK are summarised in Table 2. Substrate concentrations chosen should represent those under which the final bioconversion process might be performed and should certainly be beyond the substrate inhibition negligible regions identified in Fig. 4. For the TK experiments performed here each progress curve had 12 sampling points for determination of substrate and product concentration with time to ensure the accuracy of the model fit to the experimental data. Putting the values obtained earlier for k_{cat}, K_a and K_b of 2200 min^{-1}, 19 and 17 mM respectively into the full kinetic model (Expression (1)), K_{ia}, K_{ib} and K_{iq} can be estimated by nonlinear regression based on the goodness of the model fit to all the experimentally determined progress curves. The preliminary values of K_{ia}, K_{ib} and K_{iq} (Fig. 2) were found to be 50, 800 and 533 mM using the 'pattern search' algorithm available in the 'Genetic Algorithm and Direct Search Toolbox' in Matlab® (MathWorks, Natick, MA, USA).

The values of K_{ia}, K_{ib} and K_{iq} obtained up to this point are based on the initial estimated values of k_{cat}, K_a and K_b found using the simplified kinetic model (Expression (2)) as described in Section 2. Any bias when determining k_{cat}, K_a and K_b using the simplified kinetic model will obviously be propagated to the values of K_{ia}, K_{ib} and K_{iq}. Thus the values of the kinetic parameters need to be reconciled by using the full kinetic model combined with nonlinear regression. In this final step (Fig. 2), all the parameter values obtained up to this point are used as initial values for nonlinear regression. For the model TK reaction described here the initial values of k_{cat},

Table 3
Final kinetic parameters determined for the TK mediated synthesis of L-erythrulose from HPA and GA.

Kinetic parameters	This work	Gyamerah and Willetts (1997)
Michaelis constant for HPA: K_a (mM)	18	13.2
Michaelis constant for GA: K_b (mM)	16	16.1
Inhibition constant for HPA: K_{ia} (mM)	40	42.2
Inhibition constant for GA: K_{ib} (mM)	570	597.6
Inhibition constant for ERY: K_{iq} (mM)	536	565.8
Rate constant: K_{cat} (min^{-1})	2442	5076

Kinetic parameters established from the model driven approach described in Fig. 2. Also shown are the kinetic parameter values determined by Gyamerah and Willetts (1997).

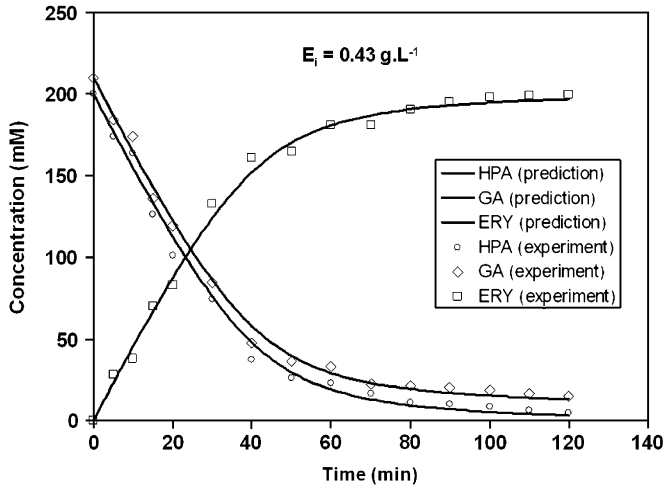

Fig. 5. Verification of model predictions with an experimental data set not included in the original experimental design. Experimental data obtained at microwell scale as described in Section 3.2. Solid lines represent kinetic model predictions based on Expression (1) and the parameters listed in Table 3.

K_a, K_b, K_{ia}, K_{ib} and K_{iq} were set at 2200 min^{-1}, and 19, 17, 50, 800 and 533 mM, respectively. The final 'reconciled' values of each kinetic parameter, obtained by the 'pattern search' algorithm based on all the experimental progress curves and the full kinetic model expression (Expression (1)) are shown in Table 3. The final parameter values are somewhat different to the results obtained in the previous stage (used as initial values in this step), especially for K_a and K_{ib} with 25% and 40% relative difference, respectively. Such changes to the final values of the kinetic parameters at this point are not unexpected (Chen et al., 2008).

4.4. Evaluation of model fit and kinetic parameter validation

In order to illustrate the capacity of the final kinetic model to accurately describe the reaction progress curves, experimental and predicted concentration–time profiles were compared. The agreement between modelled and experimental values was excellent for all initial substrate concentrations used in Table 2 to establish the model parameters. To test the predictive power of the full kinetic model Fig. 5 shows the comparison of model predicted progress curves and a further set of experimental data obtained under conditions not previously used for parameter estimation. Again there is excellent agreement between model predictions and actual values. This serves to verify both the model driven approach outlined in Fig. 2 and the kinetic model and constants described by Expression (1) and in Table 3, respectively.

4.5. Comparison of the model driven approach with conventional methods

The experimental system for the TK mediated synthesis of L-erythrulose from HPA and GA was specifically chosen since comparative data from a detailed kinetic investigation by Gyamerah and Willetts (1997) were available in the literature. These authors used exactly the same reactants and enzyme as used here and applied a linear plotting method to obtain the six kinetic parameters described in Expression (1). The kinetic parameters obtained by Gyamerah and Willetts are compared to those determined here in Table 3. Good quantitative agreement is seen between K_a, K_b, K_{ia}, K_{ib} and K_{iq}. The largest difference is seen for the value of k_{cat}. This depends strongly on the enzyme concentration used since k_{cat} is given by V_{max}/[TK]. In the work of Gyamerah and Willetts the value for [TK] is only approximated based on the assumption that the TK enzyme accounts for 40% w/w of the total cell protein. In contrast the TK concentration in each experiment described here was experimentally determined (as described in Section 3.3) and so we are confident that the k_{cat} value of 2442 min^{-1} is closest to the true value.

Table 4 summarises the range and number of experiments performed by Gyamerah and Willetts (1997). In their work the full combination of concentrations for each substrate pair was used, requiring a total of 130 experiments. In contrast using the model driven approach exemplified here, the number of experiments for obtaining the same kinetic parameters can be significantly reduced to just 34 (Table 4). It should be noted that the number of experiments necessary for the preliminary determination of the linear range of enzyme concentration (as shown in Fig. 3) are not included in either total as these represent standard experiments that would be performed when screening any bioconversion prior to establishment of kinetic parameters. Consequently the approach exemplified here represents a 4-fold reduction in the number of experiments to be performed.

Not only is it important to reduce the number of required experiments, but it is also desirable to be able to automate those experiments that are necessary in order to reduce the amount of often expensive substrate required (Pollard and Woodley, 2007). The ability to perform all the required experiments at the microwell scale and in an automated fashion meets these additional requirements for rapid bioconversion process development (Lye et al., 2003; Micheletti and Lye, 2006). The potential savings in material requirements between the conventional laboratory approach and the experimental studies described here are also illustrated in Table 4. These are based on the amount of HPA required to perform the necessary experiments, as this is the most expensive of the reagents used, by a factor of 8 on a molar basis, or 15 on a mass basis. The experiments performed by Gyamerah and Willetts (1997) were carried out in small stirred reactors with a working volume of 10 mL. In contrast, the microwell experiments performed here had total working volumes in the range of 150–800 μL. Based on these, and the concentrations of HPA used, Table 4 shows that there is a 45-fold decrease in material requirements. The use of the microwell format also allows for at least an 18-fold increase in experimental throughput since six kinetic experiments could be performed in parallel (each in triplicate) while those of Gyamerah and Willetts (1997) had to be performed sequentially.

5. Conclusions

Compared to conventional methods the combination of automated microscale experimentation with model driven experimental design offers a rapid, efficient and cost-effective approach to kinetic model generation (Table 4). For a reaction with known kinetics and mechanism, i.e., the TK mediated synthesis of ERY from HPA and

Table 4
Detailed comparison of the number of experiments and the amount of HPA required (the most expensive reactant) for obtaining TK kinetic parameters based on the work of Gyamerah and Willetts (1997) and the model driven microwell approach described here.

Experiments	Gyamerah and Willetts (1997)			This work	
	Conditions	Number of experiments	Required HPA (mg)	Number of experiments	Required HPA (mg)
For k_{cat}, K_a, K_b	[GA] = 30, 50, 100 mM [HPA] = 10, 20, 30, 40, 50 mM	15	495	25 (Fig. 4)	12.8
For K_{ia}	[GA] = 10, 20, 30, 40, 50 mM [HPA] = 200, 300, 400 mM	15	4950	9 (Table 2)	170.7
For K_{ib}	[GA] = 200, 300, 400, 500 mM [HPA] = 10, 20, 30, 40, 50 mM	20	660		
For K_{iq}	[ERY] = 0, 20, 40, 60, 80 mM [GA] = 20, 30, 50, 50 mM [HPA] = 10, 20, 30, 40 mM	80	2200		
Total		130	8305	34	183.5

Experiments performed by Gyamerah and Willetts (1997) at the 10 mL scale while those performed here were either at 150 μL (for k_{cat}, K_a, K_b) or 800 μL (for K_{ia}, K_{ib}, K_{iq}) scales. Preliminary screening experiments have not been included in this analysis.

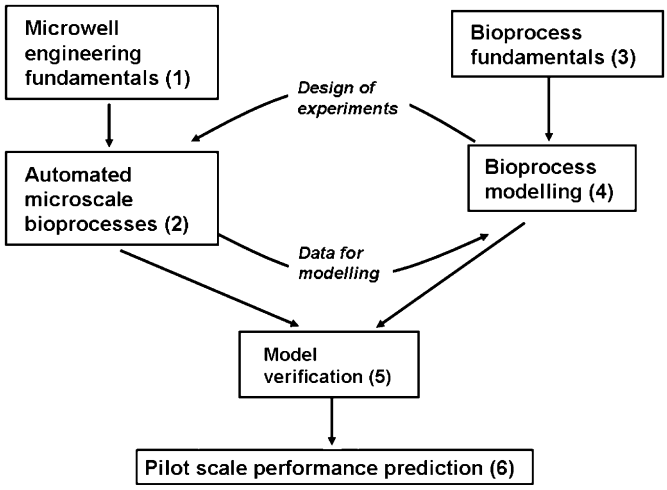

Fig. 6. Representation of the integrated microwell and modelling approach to rapid kinetic model generation and larger scale bioconversion performance prediction. References illustrating the component elements: (1) Doig et al. (2005), Zhang et al. (2008), (2) Lye et al. (2003), Ferreira-Torres et al. (2005), (3) Blanch and Clark (1997), (4) Chen et al. (2007), Islam et al. (2007), (5) this work, (6) Micheletti et al. (2006), Islam et al. (2008).

GA, this new approach enabled an 4-fold reduction in the number of experiments required, a 45-fold decrease in material requirements and an 18-fold increase in experimental throughput. For alternative reactions different kinetic expressions may be required. In these cases the selection of suitable kinetic expressions would clearly be informed by knowledge of the enzyme class and any previously published kinetic data. The microwell experimental approach can be readily implemented in virtually any laboratory equipped with standard microplate shakers and plate readers. Even in the absence of specialist laboratory robots for process automation most of the throughput and cost-saving benefits can be obtained by the straightforward adoption of parallel microlitre scale experimentation.

At this stage the microscale experimentation is demonstrated for a homogeneous reaction system and further work is necessary to adapt the experimental methods for use with the poorly water soluble and multiphase reaction systems often employed industrially. Related research on the engineering characterisation of shaken microwell systems (e.g., Weiss et al., 2002; Hermann et al., 2003; Doig et al., 2005; Zhang et al., 2008) has established suitable bases for scale-up of microwell experimental results (Fig. 6). In this way it can be ensured that kinetic expressions established at the microwell scale can be predictive of both laboratory (Ferreira-Torres et al., 2005; Micheletti et al., 2006) and pilot scale (Islam et al., 2008) performance.

Notation

[A]HPA concentration, mM
[B]GA concentration, mM
E_i enzyme concentration in reaction solution
ERY Erythrulose
GA glycolaldehyde
HPA β-hydroxypyruvate
k_{cat} reaction rate constant, min^{-1}
K_a, K_b Michealis constant of A and B, mM
K_{ia}, K_{ib}, K_{iq} inhibition constants of A, B and Q, mM
[Q]ERY concentration, mM
t time, min

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