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Quantitative Polymerase Chain Reaction for Microbial Growth Kinetics of Mixed Culture System

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Microbial growth kinetics is often used to optimize environmental processes owing to its relation to the breakdown of substrate (contaminants). However, the quantification of bacterial populations in the environment is difficult owing to the challenges of monitoring a specific bacterial population within a diverse microbial community. Conventional methods are unable to detect and quantify the growth of individual strains separately in the mixed culture reactor. This work describes a novel quantitative PCR (qPCR)-based genomic approach to quantify each species in mixed culture and interpret its growth kinetics in the mixed system. Batch experiments were performed for both single and dual cultures of *Pseudomonas putida* and *Escherichia coli* K12 to obtain Monod kinetic parameters (μ_{max} and K_s). The growth curves and kinetics obtained by conventional methods (*i.e.*, dry weight measurement and absorbance reading) were compared with that obtained by qPCR assay. We anticipate that the adoption of this qPCR-based genomic assay can contribute significantly to traditional microbial kinetics, modeling practice, and the operation of bioreactors, where handling of complex mixed cultures is required.

Keywords: Microbial growth, Monod, quantitative PCR (qPCR), *Pseudomonas putida*, *Escherichia coli* K12

Introduction

Microbial growth kinetics is an indispensable tool in the field of microbiology. It addresses the knowledge gap between the physiology, genetics, and ecology of microorganisms to enable further development of biotechnology [5, 10, 17]. Bioprocesses, including biodegradation or wastewater treatment, are optimized *via* the study of the microbial growth kinetics [15, 19]. Despite more than half a century of research, many fundamental questions about the validity and application of growth kinetics are still unanswered [9].

Currently, there are several mathematical expressions that describe the rate of biotransformation. One of the most prominent approach is the Monod expression [2, 13]. This relatively simple empirical model proposed by Jacques Monod half a century ago continues to dominate the field of microbial growth kinetics [10]. The extraction of the Monod parameters involves the measurement of the limiting substrate (S) as well as the biomass growth (m) [10]. Biomass can be quantified in three ways: total suspended solids (TSS) as grams of dry or wet weight per liter of sample; number count of viable/dead cells per milliliter; or optical density measurement of the sample. In the last method, the absorbance of the sample measured with a spectrometer is correlated to either the dry weight or the number of cells per volume [20]. Nevertheless, these methods are inappropriate for mixed culture samples because they cannot quantify the growth of individual cultures present in mixed samples.

Quantification of bacterial populations in the environment is difficult owing to the challenges of isolating and identifying a specific bacterial population within a diverse microbial community. Most studies neglected the fact that microorganisms in nature grow mostly with a mixture of substrates, that growth may not be controlled by only a single nutrient, and that kinetic properties of a cell might change as a result of adaptation [10]. Another challenge lies

in the interactions of microorganisms in the population. Competitive or synergistic growth between microorganisms in the microbial ecology makes it difficult to predict the individual culture growth in mixed populations based on single culture kinetics.

Advancements in molecular techniques have lately been used to address issues and challenges that were encountered in past research. Techniques such as the polymerase chain reaction (PCR) provide a way to detect specific genes of various individual bacterial strains at very low concentrations. More recently, quantitative PCR (qPCR) has been used to provide quantitative measurement of bacteria by targeting genomic information. This gene quantification technique can potentially be used as an alternative to prior methodologies of biomass quantification. More importantly, it provides quantitative information pertaining to specific genes (or bacteria) present in mixed culture or environmental samples in the absence of isolation or culturing steps.

Pseudomonas species are ubiquitous microorganisms found in all major natural environments and in intimate association with plants and animals [24]. A prominent attribute of some species or strains is their metabolic versatility, making them attractive candidates for use in bioremediation [8, 14, 22, 23]. Studies have shown that *Pseudomonas putida* can degrade aromatic hydrocarbons that range in size from single ring to polycyclic aromatics [23], with a substrate affinity constant K_s = 4–9 mg/l [15, 24]. Another very well researched bacteria with similar K_s is *Escherichia coli (E. coli)*. Because of its wide use as a model organism in microbial genetics and physiology, and its use in industrial applications, *E. coli* K12 (K_s = 7 mg/l) is one of the most extensively studied microorganisms. We have used these two species above as model bacteria to simulate a mixed dual culture for qPCR application to microbial growth kinetics [18].

In this work, qPCR assay was used for biomass quantification to address microbial growth kinetics. Microbial kinetic parameters were calculated and compared between conventional approaches and qPCR assay. Pure culture was used for validation and mixed culture was used to highlight the capability of qPCR in monitoring biomass of individual species in a mixed system. This work presents a new genomic approach to quantify cell density of a specific strain in complicated environmental samples, to utilize it to the microbial growth kinetics.

Materials and Methods

Bacterial Culture Preparation for Kinetic Experiments

The *Pseudomonas putida* strain (DSM 8368) was purchased from German Collection of Microorganisms and Cell Collection (DSMZ, Braunschweig, Germany) and cultured in trypticase soy broth (TSB; Bacto, Sparks, MD, USA) at 28°C. The *Escherichia coli* K12 strain (ATCC 27325) was purchased from American Type Culture Collection (ATCC, Manassas, VA, USA) and cultured in Luria-Bertani (LB) medium (Difco, Sparks, MD, USA) at 37°C. Both cultures revived in 1 ml of medium was sequentially transferred to bigger volumes once it reached to a stationary phase based on the absorbance reading at 550 nm by a Nanodrop ND 1000 spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Prior to kinetic experiments, each culture (*Pseudomonas putida* and *E. coli*) was transferred to M9 medium (Amresco, Solon, OH, USA) with 20% concentration of glucose as a sole carbon source [9]. M9 medium is a minimal medium containing PO_4^{2-} (pH buffer), NH_4^+ (nitrogen source), NaCl (isotonicity), and Mg^{2+}/Ca^{2+} (supplements for growth), and it is known to support the growth of *E. coli* [16] and *Pseudomonas putida* [9]. Each pure culture was washed to remove all nutrients, particularly carbon source, from the previous medium. Briefly, 30 ml of culture was centrifuged for 10 min at 5,000 rpm. The supernatant was discarded and the pellet was resuspended in deionized water to a volume of 10 ml. After the samples were further centrifuged to separate all media, and the biomass pellet was subsequently transferred to the kinetic reactors and homogenized manually in 30 ml of M9 medium. The batch experiment for each single culture was performed in a 500 ml Erlenmeyer flask with 300 ml of M9 medium and 30 ml of biomass, with a range of glucose (substrate) concentration (175–1,000 mg/l). The following batch experiment for mixed culture growth was performed in the same manner above, except using 15 ml of each culture biomass and the initial glucose concentration of 175 mg/l.

Kinetic Experiments and Glucose Substrate Monitoring

Each batch experiment was implemented as a function of time (hours). Two main variables, (i) the consumption of growth-controlling substrate (D-glucose) and (ii) the increase in biomass concentration, were measured. The biomass was monitored by three quantification methods: (a) total suspended solids (TSS), (b) optical density (OD), and (c) quantitative PCR assay. Following sampling, 2 ml of each sample was immediately stored at −80°C for further genomic DNA extraction.

Glucose concentration in the medium was measured using the Substrate D-glucose enzymatic kit (R-Biofarm AG, Darmstadt, Germany) according to the manufacturer's procedure. In summary, through a series of enzymatic reactions, the amount of NADPH formed in this reaction is stoichiometric to the amount of D-glucose. The increase in NADPH is measured by means of its light absorbance at 340 nm using a SpectraMax M2 spectrophotometer (Molecular Devices, Sunnyvale, CA, USA) with a 100 µl sample in a 1 cm light path cuvette (Plastibrand; Sigma-Aldrich, St. Louis, MO, USA).

Biomass Monitoring by TSS Measurement and UV-Vis Spectrophotometry

TSS measurement. TSS (mg/l) were obtained using a

conventional dry cell weight method. Cells were separated from the medium by filtration using Whatman Qualitative Filter Papers (Grade 1), in duplicates, with a sample volume of 10 ml. A vacuum pump was applied to pull the liquid through the filter. The cell paste was subsequently dried in an oven at 105°C for 24 h. The dry weight was then recorded in order to calculate the difference in weight for biomass calculations.

UV-Vis spectrophotometry. Samples (2 ml) were taken every hour for a period of 10 h and the absorbance was immediately measured at 550 nm using a SpectraMax M2 spectrophotometer in a Nunc 96-well plate (Roskill, Denmark). Triplicates were measured with a sample volume of 100 µl.

Genomic DNA (gDNA) Extraction

The gDNA was extracted from each sample (5 ml, single or mixed culture sample) of kinetic experiments by a FastDNA SPIN kit for soil (MP Biomedicals, Solon, OH, USA). The DNA concentration and purity were determined by UV absorption at 260 nm and 280 nm using a ND1000 spectrophotometer. To confirm the presence and size of gDNA extracted from the samples, gel electrophoresis was conducted on a 1% agarose gel. A molecular ruler 50–2,000 bp ladder was used to determine the correct size of the band (BioRad, Hercules, CA, USA).

Polymerase Chain Reaction

PCRs targeting the 16S rRNA specific to *Pseudomonas putida* and *E. coli* K12 were performed to generate the standard gene templates for the following qPCR assay. All PCR amplifications were conducted in an Applied Biosystems 2720 Thermal Cycler (Life Technologies, Foster City, CA, USA).

The primers for *Pseudomonas* specific 16S rRNA (990 bp) were Ps-for, 5′-GGTCTGAGAGGATGATCAGT-3′ and Ps-rev, 5′-TTAGCTCCACCTCGCGGC-3′ [22]. The PCR mixture was prepared using 1× Takara PCR buffer without $MgCl_2$, 1.5 mmol/l $MgCl_2$, 0.13 mmol/l dNTPs, 0.8 mmol/l forward and reverse primer, 0.75 U of Takara *Taq* polymerase, 5 µl of gDNA, and UltraPure DNase/RNase-free distilled water (Life Technologies-Invitrogen, Grand Island, NY, USA) to bring the final reaction volume to 25 µl. The thermal cycling protocol was as follows: initial denaturation of 95°C for 5 min, followed by 30 cycles of 45 sec at 94°C; 1 min at 66°C; 1 min at 74°C; and a final extension for 10 min at 74°C [6, 22].

The primers for *E. coli* K12 specific 16S rRNA (101 bp) were forward 5′-GCTACAATGGCGCATACAAA-3′ and reverse 5′-TTCATGGAGTCGAGTTGTTGCAG-3′ [12]. Each reaction mixture was prepared using 2× Takara PCR buffer without $MgCl_2$, 3 mmol/l $MgCl_2$, 0.4 mmol/l dNTPs, 1 µmol/l forward and reverse primer, 2 U Takara *Taq* polymerase, 2 µl gDNA, and *Taq* nanopure water to bring the final reaction volume to 25 µl. The thermal cycling protocol was as follows: initial denaturation of 94°C for 5 min, followed by 30 cycles of 30 sec at 94°C; 30 sec at 55°C; 30 sec at 72°C; and a final extension at 72°C for 7 min.

Following PCR, gel electrophoresis for both PCR products was performed in a 1.5–2% agarose gel to verify the product size. The PCR products were subsequently purified using a Zymo DNA Clean and Concentrator 5 kit (Zymo, Irvine, CA, USA) following the manufacturer's protocol. The purified product was imaged again to validate the purification. The concentration of the product was measured using the ND 1000 spectrophotometer and the copy number was calculated accordingly [11, 21].

Biomass Monitoring by qPCR

Ten-fold dilution from PCR-purified products (*i.e.*, 5.8×10^2 to 5.8×10^{10} genes/reaction for *Pseudomonas putida*; 8.4 to 8.4×10^{12} genes/reaction for *E. coli* K12) was used to construct the qPCR calibration curve (R^2 more than 0.95 for both targets).

$$\text{Gene copy numbers} = \frac{6.02 \times 10^{23}\ (\text{number/mol}) \cdot \text{DNA amount (g)}}{\text{DNA length (bp)} \cdot 660\ (\text{g/mol/bp})} \quad (1)$$

All qPCRs were conducted in the Applied Biosystems Step One Real-Time PCR system (Life Technologies, Grand Island, NY, USA). All runs for *Pseudomonas putida* were performed in triplicates. The reaction mixture included 10 µl of 2× Fast SYBR Green Master Mix (Life Technologies), 700 nmol/l of *Pseudomonas* specific 16S rRNA primers, 5.0 µl of standard or sample DNA, and UltraPure DNase/RNase-free distilled water (Life Technologies) to bring the final reaction volume to 20 µl. The thermal cycling protocol was as follows: initial denaturation for 15 min at 95°C followed by 40 cycles of 95°C for 10 sec, at 65°C for 15 sec, and at 72°C for 20 sec, followed by a dissociation cycle of 95°C for 30 sec, 65°C for 1 min, and 95°C for 30 sec [1].

For E. coli K12, all runs were performed in duplicates, and each reaction mixture included 4.0 µl of 2× Fast SYBR Green Master Mix, 500 nmol/l of *E. coli* K12 specific 16S rRNA primers, 2.0 µl of standard or sample gDNA, and filter-sterilized DNase/RNase free water to bring the final reaction volume to 20 µl. The thermal cycling protocol was as follows: initial denaturation for 10 min at 95°C followed by 35 cycles of 95°C for 5 sec, 60°C for 5 sec, and 72°C for 5 sec. The fluorescence signal was measured at the end of each extension step at 72°C [12]. For the sample gDNA extracted from both single and mixed cultures, dilutions of 1, 2, and 10 for template gDNA were applied prior to qPCR. For the mixed culture of *Pseudomonas putida* and *E. coli* K12, two qPCR assays for each gene target were performed using the same template gDNA.

Microbial Kinetic Models and Fitting

Using the measured variables (X and S), the Monod model and its kinetic parameters were calculated for each batch reactor.

For the fitting of the Monod's model S (mg/l) was obtained by glucose measurement from each sample. The specific growth rate constant (µ, 1/h) was calculated from X (mg/l) based on Eq. (2).

$$\mu = \frac{\ln X - \ln X_0}{t} \quad (2)$$

The kinetic parameters, μ_{max} and K_s, were extracted from the data using a nonlinear curve fit regression in Excel software. This method involves manual data entry and graphing of data, followed by curve fitting and displaying the resulted curve fit [3]. The fitting lines were drawn using the m_{max} and K_s obtained from this fitting method. In comparison, the series of S and μ (obtained from X based on Eq. (2)) are used to plot the kinetic graphs as experimental data (points). It is important to note that the linear regression or Lineweaver-Burk method has been found to give a deceptively good fit, even with unreliable data points. Nonlinear regression analysis is reported to yield better parameter estimations in laboratory experiments [7].

To obtain kinetic parameters from the qPCR assay, the gene copy number per qPCR was converted to mg biomass per liter of bacteria culture by multiplying a conversion factor that is a ratio between the mass of bacteria and the mass of purified PCR products. The mass of purified PCR products was obtained by the absorbance reading for typical DNA quantification (OD_{260}). The mass of bacterial culture at stationary phase was also obtained by absorbance reading at OD_{550} and the calibration curve between concentration and OD_{550}. The calibration curve was obtained from the experiment of comparing OD_{550} and TSS for the pure culture. By extracting from it, an OD_{550} of 0.1 was equivalent to 3,000 mg/l bacterial cells of *Pseudomonas putida*. In the same manner, an OD_{550} of 0.05 was equivalent to 3,000 mg/l of *E. coli* K12.

Results and Discussion

Biomass Quantification

Biomass (X) that represents microbial growth in batch experiments was quantified and compared, based on the three methods. They include two conventional methods: dry weight measurement (depicted as TSS afterwards) and absorbance reading by UV-vis spectrophotometry. The third method is the qPCR-based genomic assay technique. The result is shown in Fig. 1. For 48 h duration, the growth curves for *Pseudomonas putida* measured by absorbance and qPCR showed a typical microbial growth pattern. In other words, the growth went through three phases of microbial growth: lag, exponential, and stationary phases. On the contrary, the data observed from TSS data did not present a similar growth pattern when compared with the other two methods. The result indicated that TSS, which is often employed for biomass measurement in a number of engineering practices, may not be a good indicator to represent biomass. Further experiment for microbial kinetics was performed using absorbance and qPCR assay, based on the finding in Fig. 1.

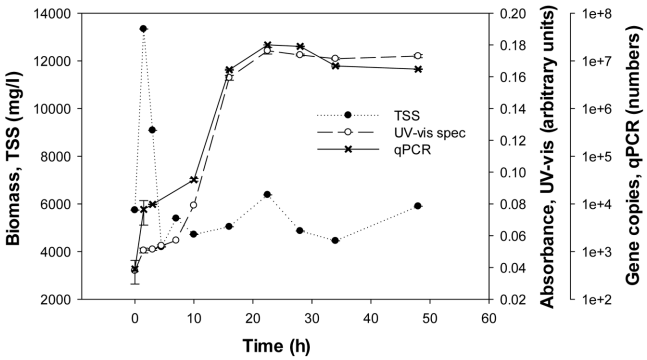

Fig. 1. Biomass (X) measured by TSS (total suspended solids), UV-Vis spectrophotometry, and qPCR for microbial growth of single *Pseudomonas putida* culture.

TSS is expressed by biomass (mg/l, closed circles) on the left y-axis. Both spectrophotometry and qPCR are depicted by absorbance (arbitrary units, open circles) and gene copies (numbers, crosses) on the right y-axis, respectively.

Microbial Growth and Substrate Depletion in Single Culture System

The kinetic experiment was conducted for both *Pseudomonas putida* and *E. coli* K12 as a single culture to verify the growth and substrate depletion over time (12 h duration). The initial substrate (D-glucose) concentration for each culture was 175 mg/l for both *Pseudomonas putida* and *E. coli* K12. The initial concentration of substrate was selected such that substrate depletion became apparent over the range of glucose concentration: 175–1,000 mg/l (each data not shown). Figs. 2A and 2B show the substrate depletion (open circles) and the growth (closed circles) for *Pseudomonas putida* and *E. coli* K12, respectively. The graphs showed the typical pattern for substrate depletion and bacterial growth. The growth curve reached the stationary phase when the glucose was completely depleted in the reactor (Fig. 2A). Note that the growth in Figs. 2A and 2B was monitored by absorbance reading of UV-vis spectrophotometry.

Monod Kinetic Parameters for Single Cultures

Kinetic parameters, μ_{max} and K_s, were determined from the experimental data by nonlinear regression and are presented in Fig. 2 and Table 1. The curve of glucose (S) and the specific growth rate (μ) for the two single cultures are depicted in Figs. 2C and 2D. In the graphs, specific growth rate as a function of glucose substrate was determined by absorbance experiment (points) and the fitted line was obtained by nonlinear regression based on the Monod equation. The μ_{max} and K_s for *Pseudomonas putida* and *E. coli* K12 were 0.044 h^{-1} and 39.050 mg/l, and 0.099 h^{-1} and 10.003 mg/l, respectively (Table 1). As shown in Table 1, we have compared our experimental values

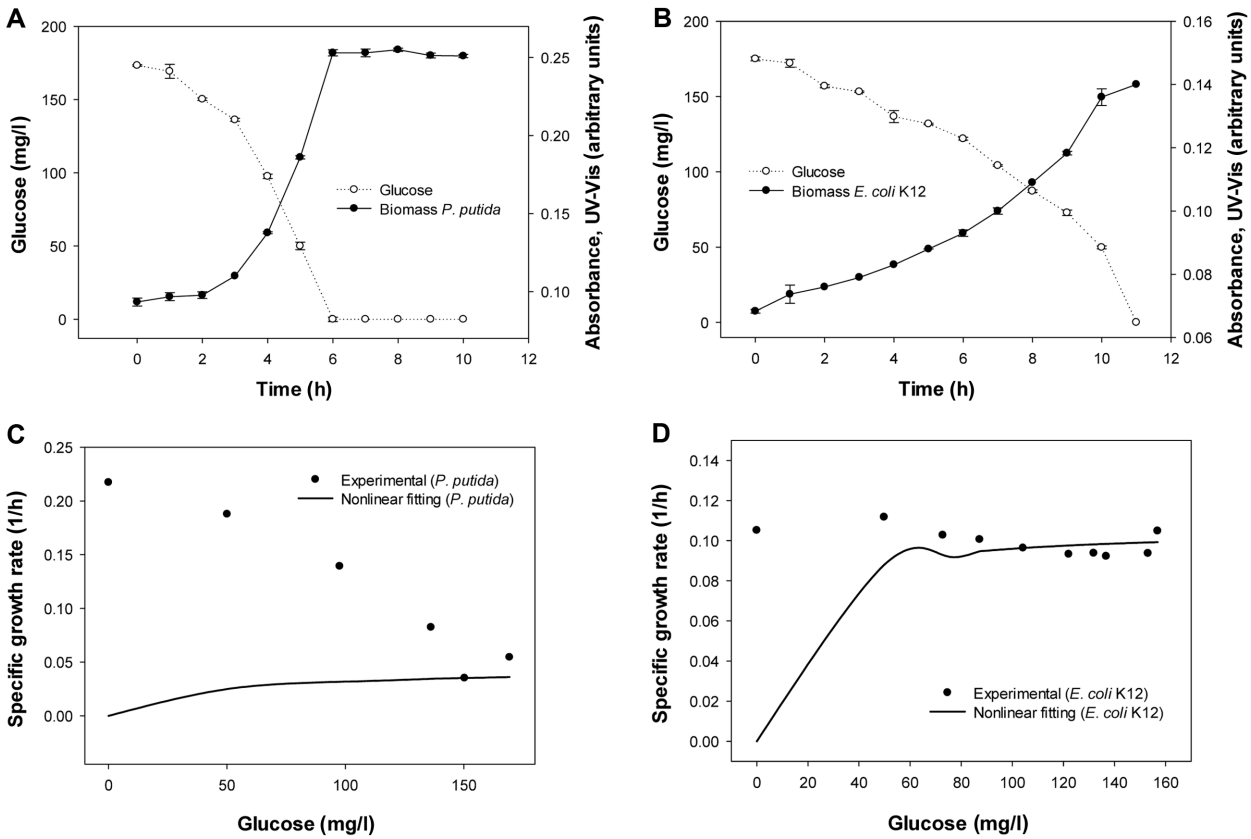

Fig. 2. Kinetics of single culture.
Microbial growth (biomass, closed circles) and substrate (glucose, open circles) depletion curves for (**A**) *Pseudomonas putida* and (**B**) *E. coli* K12. Microbial growth kinetics of single culture were determined by the absorbance experiments (points), and the fitting by nonlinear regression (line) was based on the Monod growth kinetics.

with previously published values in the references for both culture. Both *E. coli* K12 and *Pseudomonas putida* have the similar growth conditions as the previous works [4, 15], which show less than one order of magnitude difference. Reference values of μ_{max} and K_s for *Pseudomonas putida* and *E. coli* K12 were 0.014–0.200 h^{-1} and 4.860–9.300 mg/l, and 0.760 h^{-1} and 7.160 mg/l, respectively.

Comparison between UV-Vis and qPCR Methods

Figs. 3A and 3B show the comparison of biomass increase

Table 1. Monod kinetic parameters for single culture and mixed dual culture.

Name of strain	Description	Kinetic parameters		Reference
		μ_{max} (h^{-1})	K_s (mg/l)	
Pseudomonas putida	Single culture	0.044	39.050	This study
	Reference value	0.014–0.200	4.860–9.300	Oliveira *et al.* [15]
	In dual culture (qPCR)	1.217	17.847	This study
Escherichia coli K12	Single culture	0.099	10.003	This study
	Reference value	0.760	7.160	Dykhuizen [4]
	In dual culture (qPCR)	0.403	89.120	This study
Mixed dual culture	-	0.107	57.279	This study
	Additive model[a]	0.143	49.053	This study

[a]Additive model refers to the average of each single culture based on Eq. (2).

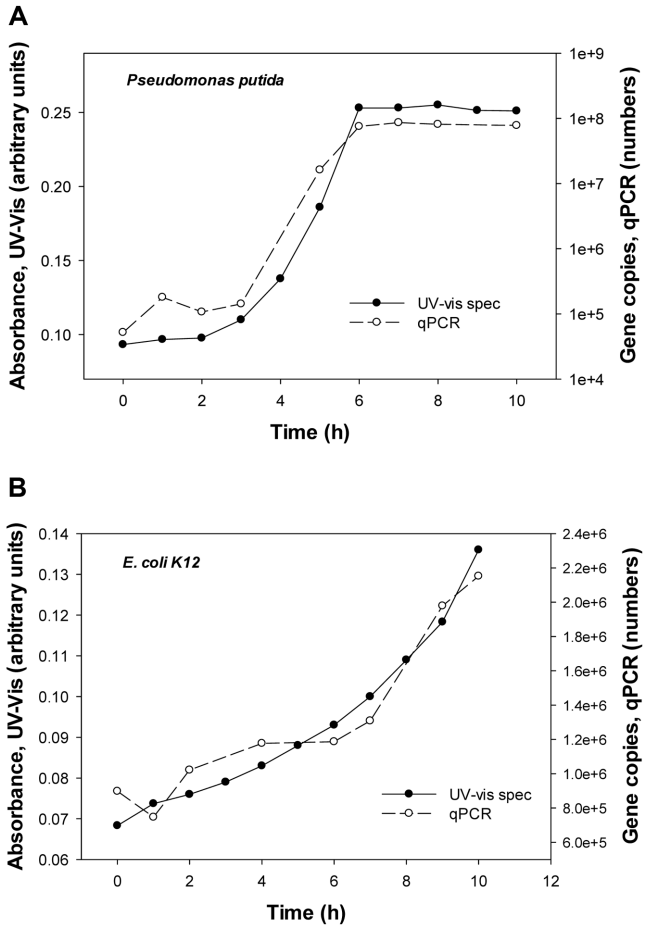

Fig. 3. Comparison of biomass (X) monitoring by both UV-vis spectrophotometry (absorbance, closed circles) and qPCR assay (gene copy numbers, open circles) for (**A**) *Pseudomonas putida* and (**B**) *E. coli* K12 as a single culture.

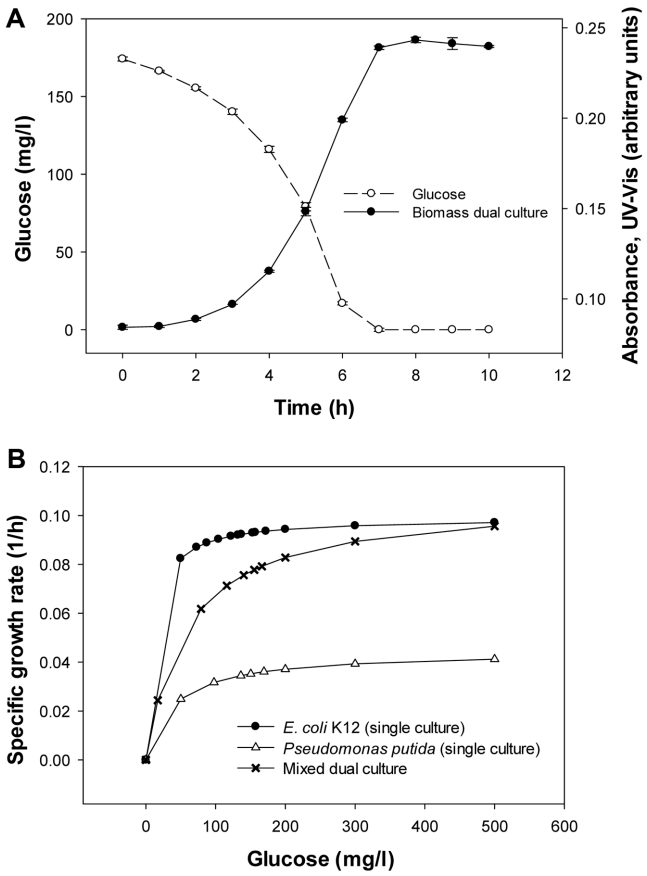

Fig. 4. Kinetics of mixed dual culture.
(**A**) Microbial growth (biomass, closed circles) and substrate (glucose, open circles) depletion curves for mixed culture of *Pseudomonas putida* and *E. coli* K12. (**B**) Microbial growth kinetics of two single cultures and mixed dual culture as determined by nonlinear regression fitting.

measured by absorbance and genomic assay for *P. putida* and *E. coli* K12, respectively. The absorbance reading had a similar pattern as the qPCR reading in both single cultures. The absorbance measures the turbidity of microbial cells, whereas qPCR measures the gene copies of 16S rRNA for each culture. This indicates that qPCR can be a good alternative for biomass monitoring, where absorbance reading is not adequate for the mixed culture system or contaminated samples.

Microbial Growth in Mixed Dual Culture System

The mixed dual culture was prepared from each *Pseudomonas putida* and *E. coli* K12 culture at the stationary phase to simulate the mixed culture system for further analysis of microbial kinetics. The growth by absorbance reading and substrate depletion curve is presented in Fig. 4A at initial glucose concentration of 175 mg/l. The bacterial growth and glucose consumption curves of mixed culture also followed the typical trend in the same way as the single culture system.

Similar to that in the single culture system, the kinetic parameters in the dual culture system were also determined based on Monod kinetics. The results are presented in Table 1 and Fig. 4B. The values for K_s and μ_{max} were 57. 279 mg/l and 0.107 h^{-1}, respectively. The plot of glucose (S) and the specific growth rate (μ) for the mixed dual culture (crosses) is presented along with the single cultures (closed circles and open triangles) in Fig. 4B. The three plots exhibited similar trends at three significantly different specific growth rates. The mixed culture plot showed a specific growth rate in between *E. coli* K12 and *Pseudomonas putida*, although it was closer the *E. coli* K12 growth rate.

Recent research has demonstrated that, in the event no

substrate interactions are identified (*i.e.*, single substrate system), simple Monod terms can be added in sum kinetics [9]. Based on the experimental design, a proposed additive model using the single culture systems to predict mixed culture Monod parameters was calculated.

$$\mu_{max}\ (\text{mixed}) = \mu_{max}\ (E.\ coli) + \mu_{max}\ (P.\ putida)$$
$$K_s\ (\text{mixed}) = K_s\ (E.\ coli) + K_s\ (P.\ putida) \quad (4)$$

The above additive model was employed to verify that kinetic parameters of a dual culture system could be derived from the averaged kinetic parameters of single culture systems. This will in turn allow us to investigate the possibility of extrapolating parameters from a simple (*i.e.*, pure) culture system to derive parameters for a complicated culture system. In the proposed additive model (Eq. (4)) for our system, we were able to demonstrate this possibility. The additive model for the mixed culture reactor gave K_s = 49.053 mg/l and μ_{max} = 0.143 h^{-1}. As compared with the experimental value (K_s and μ_{max}, 57. 279 mg/l and 0.107 h^{-1}, respectively), the additive model was reasonably similar.

Application of qPCR Assay in Mixed Culture Kinetics

The growth trend of individual species, *E. coli* K12 (crosses) and *Pseudomonas putida* (open circles), present in a mixed dual culture was determined by qPCR assay and is presented in Fig. 5A. For comparison purpose, the absorbance reading (closed circles) for the mixed dual culture is also depicted in Fig. 5A. *Pseudomonas putida* in mixed culture (open circles in Fig. 5A) followed a trend similar to that of the single (Fig. 2A) or mixed culture (Fig. 4A) obtained so far. However, it was observed that the growth curve of *E. coli* K12 (crosses in Fig. 5A) differed significantly in the mixed dual culture. The exponential growth shown in the single culture reactor for *E. coli* K12 only (Fig. 2B) was not observed in the *E. coli* K12 in dual culture (crosses in Fig. 5A) that was obtained by qPCR assay.

The kinetics of both bacteria in dual culture were also determined using qPCR assay results and are presented in Fig. 5B and Table 1. The μ_{max} and K_s for *Pseudomonas putida* and *E. coli* K12 were 1.217 h^{-1} and 17.847 mg/l, and 0.403 h^{-1} and 89.120 mg/l, respectively (Table 1). Interestingly, *Pseudomonas putida* had a faster growth rate than *E. coli* when they co-existed in one reactor, as shown in Fig. 5B. Even though the specific interaction between the two species in the reactor is fully understood, it is important to note that the kinetics obtained from each single culture was different from that obtained from dual culture. This indicated that the interaction between species greatly affects the kinetics of individual species, and it can be

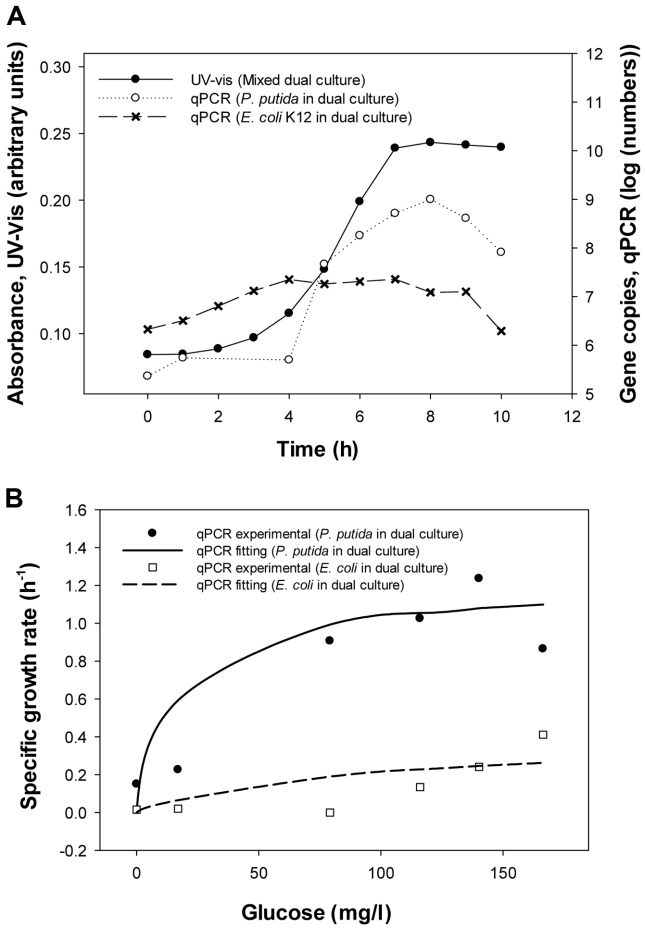

Fig. 5. Kinetics of individual cultures present in mixed culture. (**A**) Biomass (X) measured by spectrophotometry (absorbance), and qPCR (gene copies) for microbial growth of mixed dual culture of *Pseudomonas putida* and *E. coli* K12. Absorbance (closed circle) was measured for the mixed culture only, and qPCR (open circle and cross) was performed to target each culture present in the dual culture. (**B**) Microbial growth kinetics of individual bacteria in the mixed dual culture as determined by the qPCR experiments (points) and the fitting by nonlinear regression (line).

monitored by targeting specific species in mixed culture samples by techniques such as qPCR. Even though this study is preliminary and requires more elucidation on the interactions between species, it demonstrated the potential of qPCR as a critical tool for the study of individual species kinetics in complicated mixed samples.

As such, this approach can be useful in a variety of environmental and microbiological applications, such as wastewater treatment and bioremediation. It has potential to detect one species' efficacy based on specific kinetics of target species in environmental samples. In addition, the method described in this study can shift the paradigm of

current numerical modeling approaches for microbial kinetics, as our findings have shown that the commonly accepted model – in which growth patterns observed in single culture reactors represent growth patterns in mixed culture consortiums – is not true at all times.

Acknowledgments

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