In Situ Measurement of Bioluminescence and Fluorescence in an Integrated Microbioreactor

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Abstract: Reporter strains of bacteria that emit light or a fluorescent marker in response to specific conditions in their environment are having a significant impact in many areas of biology, including toxicity assays for environmental pollutants, chemical detection, and gene expression profiling. We have demonstrated methods for in situ measurements of bioluminescence and fluorescence from bacterial cultures grown in 50 μL instrumented microbioreactors. Results from microbioreactors were compared to results obtained from conventional 500 mL batch bioreactors and shake flasks. Experiments were conducted with reporter strains of *Escherichia coli* in which *luxCDABE* or *gfp* was fused to a promoter that was either expressed constitutively, or that responded to oxygen limitation. With these reporter strains, we have demonstrated the ability to obtain information on growth conditions within the microbioreactor. We have also shown that the large aspect ratio of the microbioreactor provides a unique advantage over measurements in larger bioreactors by reducing the inner filter effect in on-line measurements and eliminating the need for error-prone off-line dilutions. In addition, continuous on-line monitoring of genes in real-time, when expanded to include entire reporter libraries, could potentially provide a true dynamic picture of cellular gene expression from which the kinetics of gene expression can be untangled and elucidated.

Keywords: in situ measurements; bioluminescence; fluorescence; instrumented microbioreactors; *Escherichia coli*; *luxCDABE*; *gfp*

INTRODUCTION

Light emission from luminescent and fluorescent bacteria (and more recently, yeast) created to act as reporters for various environmental conditions is finding application in several areas of biology, including toxicity assays for environmental pollutants, chemical detection, and gene expression profiling (Andreescu and Sadik, 2004; Baeumner, 2003; March et al., 2003; Jansson, 2003; Naylor, 1999; Rhodius et al., 2002).

The importance of bioluminescence as a marker for gene expression was first recognized by Engebrecht (1985), who used a DNA fragment from the marine bacteria *Vibrio fischeri* to construct recombinant *Escherichia coli* strains that produced light in response to transcriptional activation of a specific gene. Concurrent with this practical application was the development of an understanding of bioluminescence biochemistry (Hastings et al., 1985; Meighen, 1988, 1991). The use of green fluorescent protein (GFP) as a gene expression marker was first described by Chalfie et al. (1994), the properties of GFP having been described by Shimomura et al. (1962) and later by Morin and Hastings (1971) and Morise et al. (1974).

There are three major areas in which luminescent and fluorescent reporters are being used. The first is for non-specific environmental reporting. For these applications, the *lux* operon (Bechor et al., 2002; Rozen et al., 2001; Rupani et al., 1996; Van Dyk et al., 1994) or *gfp* gene (Cha et al., 1999; Funabashi et al., 2002; Lu et al., 2003; Seo et al., 2003) is fused to a stress response promoter that responds to a number of environmental and chemical stresses. For instance, the heat shock response is activated whenever environmental conditions cause changes in protein structure, and the SOS regulatory circuit is activated in response to DNA damage. A second area is that of monitoring for specific substances in the environment. Examples include reporters for metals (Riether et al., 2001; Selifonova et al., 1993) and organic compounds (Belkin et al., 1996; King et al., 1990; Rozen et al., 1999). Finally, libraries of strains have been developed in which *lux* and *gfp* fusions representing large portions of the bacterial genome can be used as an alternative to microarray technology (Van Dyk et al., 2001b) and for

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clarifying metabolic pathways (Kalir et al., 2001; Lu et al., 2004; Sabina et al., 2003; Van Dyk et al., 1998). Similar examples of these applications exist with yeast as the model organism (Dimster-Denk et al., 1999; Gupta et al., 2003; Li et al., 2002).

The choice between the use of luminescence and fluorescence is application-specific. The advantages of the *lux* system are a faster response time (Hakkila et al., 2002; Van Dyk et al., 1994) and a lower detection limit due to the lack of interference that cellular autofluorescence causes when *gfp* is used (Hakkila et al., 2002). The advantages of *gfp* include the fact that *gfp* has not been found to be a self-regulator like *lux* (Forsberg et al., 1994), and that the response of autofluorescent *gfp* is independent of substrate concentrations in the medium, as is the case with *lux*.

For all of the applications discussed, it is clearly desirable to have the ability to carry out multiple fermentations in parallel. Currently, high-throughput experiments with fluorescent and luminescent bacteria are generally carried out on agar plates (Van Dyk et al., 2001a), in microtiter plates (Lu et al., 2004), or shake flasks. These approaches yield limited data since many growth parameters cannot be measured online in such systems. Alternatively, bioreactors are used when growth data is needed (DeLisa et al., 1999; Gu et al., 1996). However, this approach is both costly and time-consuming. Furthermore, because the fluorescent and luminescent response is frequently used as an indicator for gene expression, the very nature of the experimental design dictates that a large number of experiments are needed.

The ability to measure fluorescence and luminescence in integrated, multiplexed microbioreactors would allow an experimenter to run multiple small-scale experiments in parallel, thus greatly decreasing the resources needed per experiment as well as increasing the number of experiments that could be run. In addition, the use of a microbioreactor with integrated sensors would allow the collection of additional data such as growth kinetics, dissolved oxygen over time, and pH over time. This information is generally not available when shake flasks and microtiter plates are used. The microbioreactor system that we previously described could potentially provide the needed platform (Zanzotto et al., 2004).

Our model system for measurements of luminescence is a collection of *E. coli* strains provided by DuPont Company. These strains were created using transcriptional fusions of the *luxCDABE* operon to bioluminesce in response to specific environmental stresses (Rupani et al., 1996; Van Dyk and Rosson, 1998; Van Dyk et al., 2001b). We have shown that we can induce and detect bioluminescence in microbioreactors by exposing the bacteria to a known stress (e.g., low oxygen concentration), and we have compared the results to those obtained in bench-scale bioreactors under similar conditions. In addition, we have examined the reproducibility of luminescence measurements in microbioreactors. Our model system for measurements of fluorescence is an *E. coli* strain that carries a constitutive promoter fused to *gfp*. We have demonstrated measurements of fluorescence in microbioreactors and compared these measurements to results from shake flasks.

MATERIALS AND METHODS

Microbioreactor

Fermentations were carried out in 50 μL poly(dimethylsiloxane) (PDMS) microbioreactors in which oxygenation occurred through a gas-permeable membrane (Fig. 1). Each microbioreactor was fabricated out of three PDMS layers and a glass slide. The glass slide functioned as a rigid base. The bottom PDMS layer contained embedded sensors for dissolved oxygen and pH. The middle PDMS layer was the body of the microbioreactor; the thickness of this layer was 300 μm. The top PDMS layer was the aeration membrane and had a thickness of 100 μm. The three layers were attached to

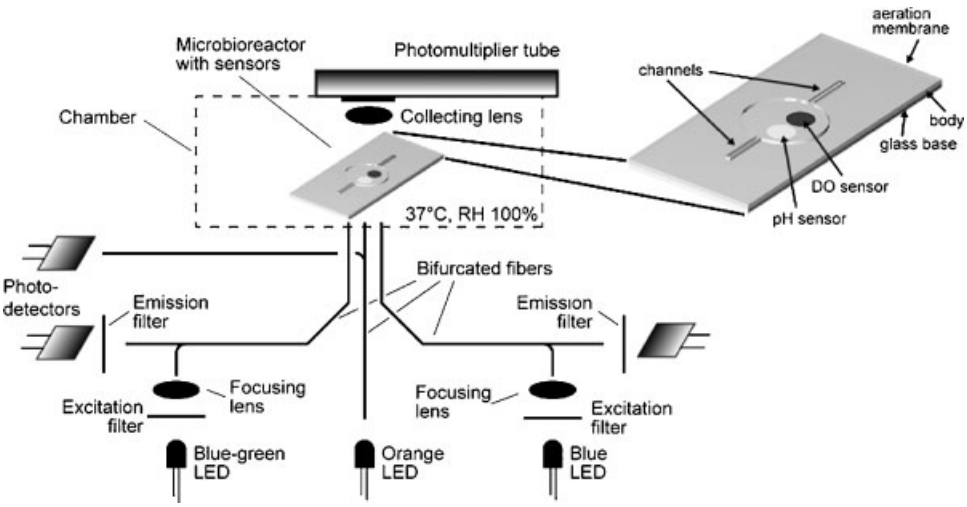

Figure 1. Schematic of the microbioreactor and experimental setup. Both the dissolved oxygen (DO) sensor and the pH sensor are used during luminescence measurements. Only the DO sensor is used during fluorescence measurements because of the overlap between the excitation and emission spectra between green fluorescent protein (GFP) and the pH sensor.

each other and the glass slide using silicone adhesive (ASI 502, American Sealants, Inc., Fort Wayne, IN). During experiments, the microbioreactor was housed in a chamber that controlled temperature and maintained high humidity. Additional details on fabrication and sensor placement may be found in Zanzotto et al. (2004).

Analytical Methods

Dissolved Oxygen

Dissolved oxygen (DO) was measured using fluorescence lifetime (Bacon and Demas, 1987; Demas et al., 1999; Klimant and Wolfbeis, 1995). The DO sensor (PSt3, PreSens, Regensburg, Germany), located at the bottom of the microbioreactor, was excited with a square-wave modulated blue-green LED (505 nm, NSPE590S, Nichia America, Southfield, MI). An exciter bandpass filter (XF1016, Omega Optical, Brattleboro, VT) and an emission longpass filter (XF3016, Omega Optical) separated the excitation and emission signals and minimized cross-excitation. Data switches (8037, Electro Standards Laboratories, Cranston, RI) multiplexed the output signal and the input signal of the function generator (33120A, Agilent Technologies, Palo Alto, CA) and the lock-in amplifier (SR830, Stanford Research Systems, Sunnyvale, CA), respectively. The lock-in amplifier measured the phase shift between the excitation and emission signals for the DO measurement, which was correlated to a dissolved oxygen concentration.

pH

During experiments in which luminescence was monitored, pH was measured using fluorescence lifetime (Kosch et al., 1998; Lin, 2000). The pH sensor (HP2A, PreSens), located at the bottom of the microbioreactor, was excited with a square-wave modulated blue LED (465 nm, NSPB500S, Nichia). An exciter bandpass filter (XF1014, Omega Optical) and an emission longpass filter (XF3018, Omega Optical) separated the excitation and emission signals and minimized cross-excitation. The signal was collected and analyzed using the same procedure as for the DO measurement. pH measurements were not made during runs in which GFP fluorescence was measured due to the overlap between the excitation and emission spectra of GFP and the pH sensor. (GFP absorbs light at two wavelengths: 395 and 470 nm). We found stronger emission intensity when the excitation wavelength of 470 nm was used. The maximum intensity of the resulting emission signal was at 510 nm).

Optical Measurements Using a Photomultiplier Tube

A photomultiplier tube (PMT) (R928, Hamamatsu, Bridgewater, NJ) located directly above the microbioreactor was used to measure luminescence, fluorescence, and optical density. Initial experiments used a hand-held multimeter (Fluke 189, Fluke, Everett, WA) to take direct current measurements. In later experiments, the current was passed through a low-noise current preamplifier (Model SR570, Stanford Research Systems) that converted the signal to a voltage (at a sensitivity setting of 20 μA/V) that was then passed to an automated multimeter (Fluke 45, Fluke). Luminescence was measured continuously and readings for optical density were taken every 10 min. Operational characteristics of the PMT were determined to ensure correct numerical analysis. The PMT was found to have an anode luminous sensitivity of 1419 A/lm and an anode dark current of 0.03 nA. The signal-to-noise of the PMT with a dark chamber was approximately 30 (after sufficient warm-up time for the PMT, approximately 1 h), and the calculated minimum detectable luminescence signal was approximately 100 photons/s (two standard deviations above the mean background signal).

Luminescence

Light was collected above the microbioreactor using a plano-convex lens (LA1131-A, Thorlabs, Newton, NJ) and passed to the PMT. Luminescence was measured as the total signal minus the background, in the absence of all other light. Measurements of luminescence light intensity are presented in arbitrary units. For figures in which luminescence appears with other measurements, it was scaled to fit on the same axis as the optical density. In comparisons of luminescence signals from multiple runs, the time courses were scaled to have the same luminescence intensity range and offset for clarity. Because the microbioreactors are not centered exactly beneath the collecting lens, the absolute magnitude of the luminescence signal varies between experiments, however, the relative signal intensity over time is the important factor.

Fluorescence

Excitation light from a blue LED (465 nm, NSPB500S, Nichia) was passed through a collimating lens (F220SMA, Thorlabs), a bandpass filter (XB78, Omega Optical), and a collecting lens (F220SMA, Thorlabs) before being split using a bifurcated cable. Half of the light was sent to a photodetector to monitor the excitation intensity, and the other half was passed into the chamber, directly beneath the microbioreactor. The emitted light (maximum intensity at 510 nm) was collected using a plano-convex lens (LA1131-A, Thorlabs) and passed through a longpass filter (XF3092, Omega Optical) before it was collected by the PMT. Measurements of fluorescence intensity were scaled for the purpose of graphing, and are presented in arbitrary units.

Optical Density

Optical density, calculated from a transmission measurement at 600 nm, was used to monitor biomass. Light from an orange LED (600 nm, Epitex L600-10V, Kyoto, Japan) was

passed through the microbioreactor, collected by a plano-convex lens (LA1131-A, Thorlabs), and sent to the PMT. During experiments with luminescent bacteria, the optical density was calculated from the total measured signal minus the magnitude of the signal due to luminescence and background. During experiments with fluorescent bacteria, the measured signal was not corrected for alternate sources of light since the energy of the incident light at 600 nm was insufficient to excite GFP. As a result, fluorescence was not present during these measurements. The optical density was calculated using Equation 1.

$$OD = 33.33 \log_{10}\left(\frac{I_{\text{reference}}}{I_{\text{signal}}}\right) \quad (1)$$

In this equation I_{signal} is the intensity of the signal and $I_{\text{reference}}$ is the intensity of the first measurement for a given experiment. The multiplication factor of 33.33 in Equation 1 is a normalization for the pathlength of 300 μm in the microbioreactor which allows direct comparison with results from conventional cuvettes with pathlengths of 1 cm. Calibration data from the microbioreactor using known concentrations of *E. coli* show that the measurements are within the linear region.

Biological Methodology

Organisms and Medium

Experiments involving bioluminescence were carried out using *Escherichia coli* strains DPD2276 and DPD2417, obtained from DuPont Company. Plasmids pDEW257 and pDEW657 are members of a collection of plasmids containing random *E. coli* genomic fragments fused to a *Photorhabdus luminescens luxCDABE* reporter that has been previously described (Van Dyk et al., 2001b). Plasmid pDEW257 contains the *gyrA* promoter region, *E. coli* nucleotides 2336048–2337993 (Blattner et al., 1997), joined to the *luxCDABE* reporter in the appropriate orientation. Plasmid pDEW657 contains the *nirB* promoter region, *E. coli* nucleotides 3490135–3491711 (Blattner et al., 1997). DPD2276 and DPD2417 are the transformants of *E. coli* strain DPD1675 (Van Dyk and Rosson, 1998) containing plasmid pDEW257 and pDEW657, respectively. Initial characterization of DPD2276 revealed that it produced very bright, essentially constitutive bioluminescence. By contrast, the bioluminescence of strain DPD2417 was dramatically increased under oxygen-limiting conditions, due to the regulation of *nirB* expression by the anaerobic regulatory protein Fnr (Lynch and Lin, 1996). Both of these *E. coli* strains contain an ampicillin resistance marker on the plasmid.

Experiments involving fluorescence were carried out using an *E. coli* strain that constitutively expresses green fluorescent protein: strain JM83 [F^-ϕ80d*lac*ZΔM15Δ(*lac pro*AB) *ara rps*L] (Yanisch-Perron et al., 1985), transformed with plasmid pCF56. This plasmid was constructed by cloning *gfp* under the control of the constitutive promoter CP25 (Jensen and Hammer, 1998), into plasmid pKAN (Hayes et al., 2002), which carries kanamycin and ampicillin resistance markers.

Stock cultures were maintained at −80°C in 20% (vol/vol) glycerol. Prior to fermentation experiments, single colonies were prepared by streaking out the frozen cell suspension onto LB plates containing 2% (wt/vol) agar and either 100 μg/mL of ampicillin (DPD2276 and DPD2417) or 100 μg/mL of ampicillin and 100 μg/mL of kanamycin (JM83). The plates were then incubated overnight at 37°C to obtain single colonies, and subsequently stored at 4°C for up to a week or used immediately to inoculate precultures.

Luria-Bertani medium was used for all fermentations and contained: 10 g/L tryptone (Difco Laboratories), 5 g/L yeast extract (Difco Laboratories), and 5 g/L NaCl. The solution was autoclaved for 40 min at 120°C and 150 kPa.

Precultures

For all experiments, 5 mL of sterile LB medium were transferred into test tubes. The appropriate antibiotics were added and each tube was inoculated with a single colony of *E. coli*. These cultures were incubated on a roller at 60 rpm and 37°C. Once the cultures reached an OD of 1, medium was transferred to a 500 mL baffled shake flask containing 35 mL of fresh medium, to a starting optical density of 0.05. The inoculated shake flasks were incubated on shakers (150–200 rpm) at 37°C and grown to OD ∼1. The medium was then used to inoculate bench-scale bioreactors, shake flasks, or microbioreactors to a starting OD of 0.05. For the inoculation of the microbioreactors, the transfer of the inoculum to fresh medium was carried out in a Falcon tube using a medium volume of 10 mL. The medium, at the correct OD of 0.05, was then transferred to a microbioreactor.

Bench-Scale Bioreactor

Batch cultures were grown in 500 mL SixFors bioreactors (Infors, Switzerland) with a starting medium volume of 450 mL. Dissolved oxygen probes (405 DPAS-SC-K8S/200, Mettler Toledo) were calibrated with nitrogen gas (0% DO) and air (100% DO) prior to each run. pH probes (InPro 6100/220/S/N, Mettler Toledo) were calibrated with buffer at pH 7.0 and 4.0 (VWR, West Chester, PA).

The aeration rate of gas was set to 0.25–1 VVM and the impeller speed was set to 500 rpm. The temperature of the vessels was maintained at 37°C for all fermentations. Dissolved oxygen and pH were not controlled, so as to simulate the batch microbioreactor. The time course of temperature, dissolved oxygen, and pH was recorded every 10 min throughout all fermentations. Biomass was monitored by removing samples from the bioreactor at defined time intervals and measuring the optical density at 600 nm on a spectrophotometer (Spectronic 20 Genesys, Spectronic Instruments, Rochester, NY). Luminescence was measured with an off-line luminometer (Optocomp I, MGM

Instruments, Cambridge, MA). Luminescence is presented in arbitrary units, and was scaled to fit on an existing axis.

Shake Flasks

Shake flasks with a volume of 500 mL, containing 35 mL of fresh medium, were inoculated to a starting optical density of 0.05. Between readings they were housed in an incubator at 37°C and 150 rpm. Samples were removed periodically to measure OD and fluorescence. Fluorescence intensity measurements were taken using a fluorimeter (Fluorescence Spectrophotometer, F-4500, Hitachi Instruments, San Jose, CA). An excitation wavelength of 470 nm was used with a slit width of 5 nm. Emission was measured at a wavelength of 510 nm with a slit width of 5 nm. The detector voltage was 700 V.

Microbioreactor

Inoculation of the medium for the microbioreactor was carried out outside of the bioreactor. Ten milliliters of fresh medium were transferred to a Falcon conical tube, and to this was added the preculture medium from a shake flask for a starting optical density of 0.05. This inoculated medium was then introduced into the microbioreactor by injecting the liquid via channels (Fig. 1). The headspace of the chamber was filled with air for all experiments except for the fermentation of DPD2417 in which oxygen depletion was avoided by using pure oxygen.

RESULTS AND DISCUSSION

The bioluminescence of *E. coli* DPD2276 is shown in Figure 2. This strain contains a plasmid-borne *gyrA-luxCDABE* gene fusion resulting in intense, essentially constitutive bioluminescence. The total measured luminescence would therefore be expected to increase with biomass, as the figure demonstrates. Luminescence appears to be associated with cell growth, and drops off as the culture reaches stationary phase. This is in agreement with previous studies (Rupani et al., 1996).

Fermentation of *E. coli* DPD2417 under conditions in which oxygen becomes limiting is shown in Figure 3. *E. coli* DPD2417 carries an anaerobically-regulated *nirB-luxCDABE* gene fusion that is expressed as the oxygen level in the medium drops. In a microbioreactor the strain experiences a sharp peak in specific luminescence (luminescence in arbitrary units/OD) when the dissolved oxygen in the medium depletes (Fig. 3a). A similar response can be seen in a 500 mL bench-scale bioreactor (Fig. 3b). By contrast, no luminescence response is seen when pure oxygen is supplied to the microbioreactor (Fig. 4).

Replicates of the oxygen-level induction in the microbioreactor were performed to examine the reproducibility of the bioluminescence response and measurement (Fig. 5). When the raw luminescence (a.u.) is plotted as a function of time (Fig. 5a), a strong initial bioluminescence peak is seen as the oxygen level depletes. A secondary peak occurs as the oxygen level begins to recover. This is consistent with the growth association of the luminescence response. As stated previously, expression of the *lux* operon, as well as the different rates of synthesis and degradation of the five *lux* proteins, is closely correlated with cellular growth. In

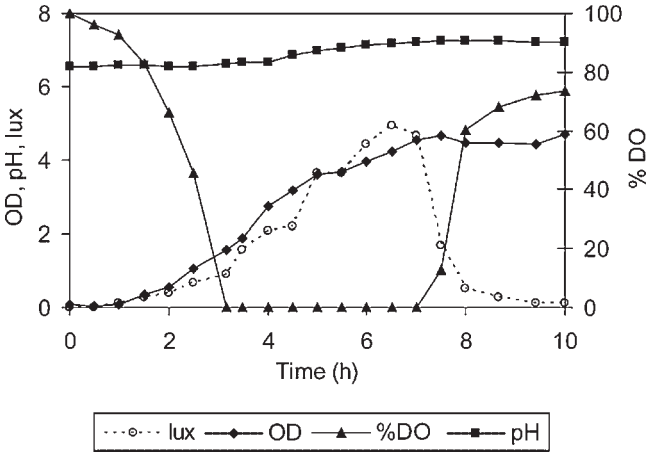

Figure 2. In situ detection of total luminescence (lux), optical density (OD), dissolved oxygen (%DO), and pH in a 50 µL microbioreactor for an *E. coli* strain constitutive for the expression of the *lux* operon. Values of lux have been scaled.

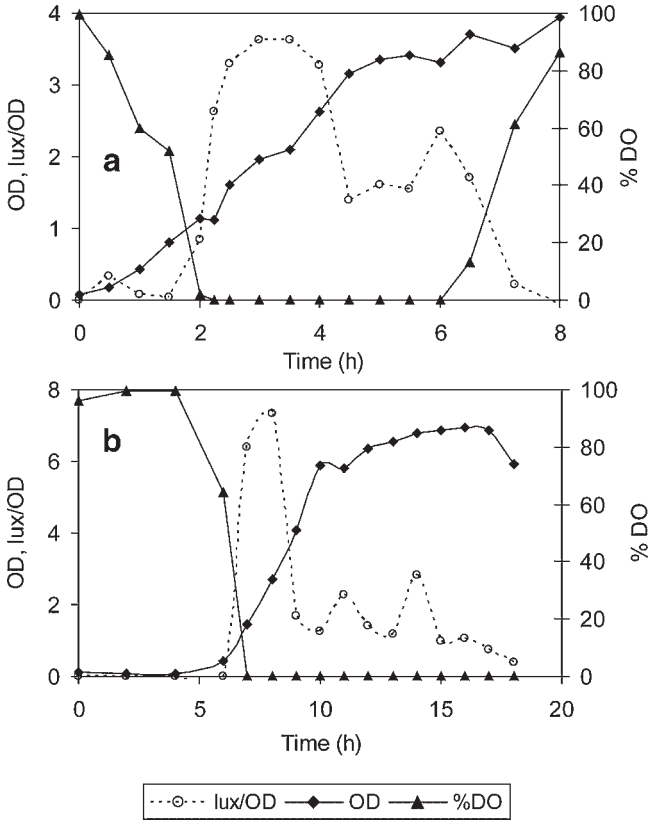

Figure 3. Specific bioluminescence (lux/OD), optical density (OD), and dissolved oxygen (%DO) for an *E. coli* strain that carries an anaerobically-regulated *lux* fusion, grown in (**a**) a microbioreactor and (**b**) a bench-scale bioreactor. Values of lux have been scaled.

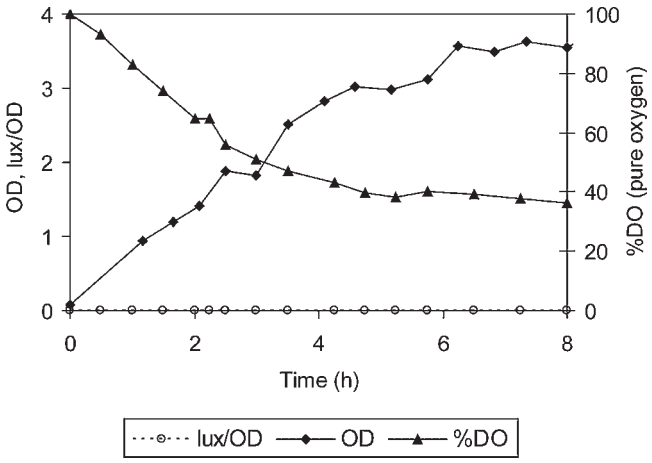

Figure 4. Specific bioluminescence (lux/OD), optical density (OD), and dissolved oxygen (%DO) for an *E. coli* strain that carries an anaerobically-regulated *lux* fusion, grown in a microbioreactor where pure oxygen is used as the contacting gas.

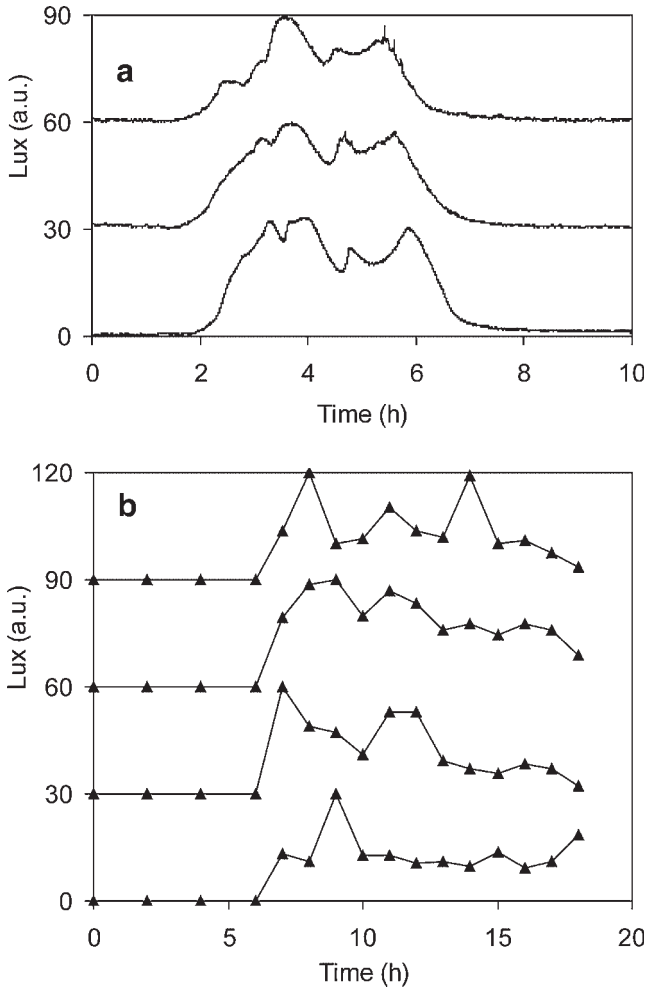

Figure 5. Measurements of emitted luminescence for an anaerobiosis-sensitive strain of *E. coli* during independent experiments in (**a**) microbioreactors and (**b**) bench-scale bioreactors operated at aeration rates of 0.25–1 VVM. All curves were scaled to have the same luminescence intensity range. Curves on each plot are offset for clarity.

addition, oxygen is necessary for the luciferase-catalyzed reaction to occur. Therefore, it is expected that the bioluminescent signal would increase temporarily as oxygen levels in the microbioreactor begin to recover. By contrast, results from bench-scale bioreactors show the limitations of off-line sampling (Fig. 5b).

These results illustrate one potential advantage of on-line luminescence monitoring in microbioreactors. Typically, accurate on-line measurements of luminescence and fluorescence in bench-scale bioreactors are complicated by the inner filter effect (IFE). The IFE occurs because while cells emit light (emission) they also absorb and scatter this emitted light (extinction), thereby diminishing the measured quantity. The IFE becomes more significant at higher cell densities and must be accounted for if the true level of bioluminescence is to be known. For example, it has been found that in a luminescing bacterial culture at an optical density of 40, the light is attenuated three to fourfold (Konstantinov et al., 1993). The IFE also affects fluorescence readings made in turbid media. Researchers have presented data that suggest that extremely large errors appear at OD values close to 1.0 (Lutz and Luisi, 1983). Other researchers have shown that significant deviations have been found with OD values as low as 0.2 (Parker, 1968). When the IFE of bacterial bioluminescence has been studied, it was found that OD values above 0.3–0.6 caused a decrease in the measured luminescence (Konstantinov et al., 1993).

In measurements of bioluminescence, the IFE can be compensated for in one of two ways. In direct, on-line measurements, an algorithm can be used to account for the light attenuation (Konstantinov et al., 1993; Lutz and Luisi, 1983). The drawback is that a model must first be fit to existing data and validated, thus necessitating previous knowledge of bacterial behavior. Alternatively, samples can be removed, diluted to a sufficiently low cell concentration, and measured off-line in a luminometer (Fig. 5b). The difficulty with this approach, apart from the obvious increase in labor that this entails, is that the bioluminescence reaction is extremely sensitive. In particular, dilution of the culture fails to accurately represent the bioluminescence of the original solution. It is hypothesized that this may be caused by a decrease in the concentration of the bioluminescence reaction substrates, most probably the long-chain aldehyde (Konstantinov et al., 1993). Therefore, there is no simple, accurate method with which to account for the IFE in a large bioreactor at high cell density.

On the other hand, the wide aspect ratio of the microbioreactor results in a much shorter pathlength that is ''seen'' by the measuring instrument. The result is that absolute optical densities in the microbioreactor (before a pathlength correction factor is applied) are typically below 0.5. The IFE is therefore diminished and accurate measurements of bioluminescence can be made, allowing real-time gene expression measurements. In this way, one of the most promising features of gene expression reporters can be realized.

Detection of *E. coli* JM83 fluorescence is shown in Figure 6. It can be seen that in both microbioreactors and

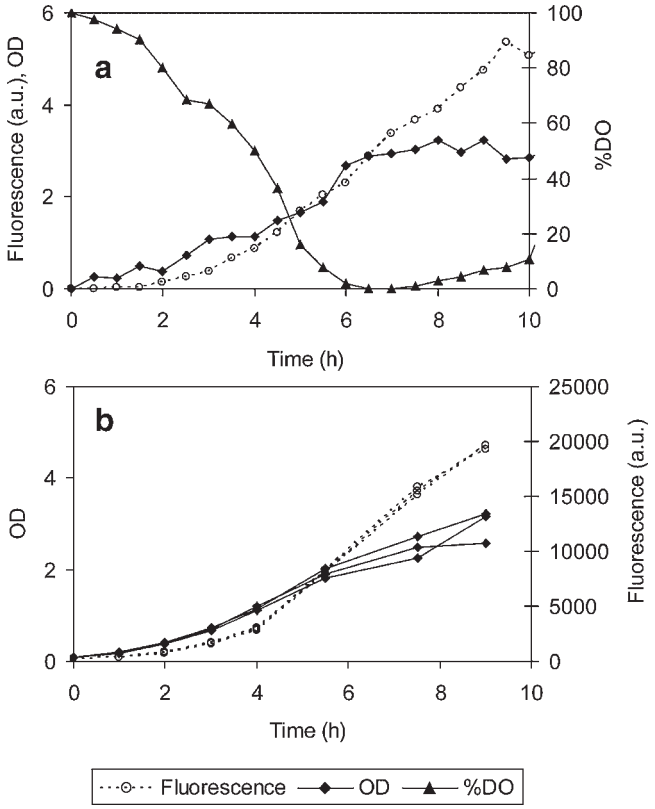

Figure 6. Optical density (OD), dissolved oxygen (%DO), and fluorescence for a strain of *E. coli* that expresses GFP constitutively in (**a**) a microbioreactor and (**b**) shake flask.

shake flasks, specific fluorescence (fluorescence/optical density) increases throughout the bacterial growth cycle. As with the measurement of bioluminescence, it is possible that in the microbioreactors the effects of high cell density on light attenuation are minimized as a result of the low absolute optical density as calculated from transmittance measurements.

CONCLUSIONS

Real-time monitoring of bioluminescence and fluorescence of bioprocesses is an important tool as gene expression markers become widely used in toxicity assays, chemical detection, and gene expression profiling. In particular, the ability to link bioluminescence and fluorescence measurements to multiple, parallel studies of bacterial growth (Szita et al., 2005) could provide great flexibility in applying these methods, particularly in the area of gene expression analysis where a large number of experiments must be run.

We have demonstrated the measurement of both bioluminescence and fluorescence in a microbioreactor. We have also used in situ luminescence detection with an *E. coli* strain sensitive to anaerobiosis to indicate oxygen depletion, and compared the response to growth in the absence of oxygen limitation to demonstrate the feasibility of using reporter strains as environmental markers. In addition, we have examined the reproducibility of the luminescence response and its measurement in microbioreactors. Our results suggest that the design and configuration of the microbioreactor may allow direct, on-line readings in which the inner filter effect is minimized, thus providing a unique advantage over larger bioreactors that require off-line analysis. One area in which this ability could have a great impact is that of genome-wide expression profiling. Currently, samples are removed at certain time points and each dataset is analyzed separately using a DNA microarray chip. The ability to monitor the expression of a gene in real-time (and, with parallel scale-out, potentially all of the genes for a given cell), would obviate the need for discrete analysis at different times and, together with knowledge of the time response of various promoters, provide a true dynamic picture of cellular gene expression.

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