On-line State Estimation of Microalgal Photobioreactors

A THESIS SUBMITTED TO THE GRADUATE DIVISION OF THE UNIVERSITY OF HAWAII IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE DEGREE OF

MASTER OF SCIENCE

IN

BIOSYSTEMS ENGINEERING

December 2002

By

Jian Li

Thesis Committee:

Wei-Wen Su, Chairperson
Pingyi Yang
Roger W. Babcock Jr.
ACKNOWLEDGEMENTS

I am grateful to Marine Bioproduct Engineering Center for providing fund for this project and its efforts to educate engineering students.

I am grateful to my advisor, Dr. Wei-wen Su, for his support, suggestion and encouragement during this project. I appreciate the opportunity of working under his supervision.

I am grateful to Professor Ningshou Xu for his invaluable guidance and indispensable contributions to the fulfillment of the project and the publications. Without his effort, these achievements would be impossible.

I am grateful to Dr. Loren Gautz, Charles Nelson and Daniel Paquin for their professional advices and engineering support that, I believe, were more than essential.

I am grateful to my lab mates and office mates for the joyful moments that we spent together either in laboratory, office or in the tennis courts.

I would like to thank my committee members Dr. Pingyi Yang and Dr. Roger Bobcock for their insightful advices and suggestions that greatly facilitated my thesis work.

Finally, I would like to thank Ms. Joanne Kurosawa and other departmental staff for their great help during my study in the department.
ABSTRACT

Photobioreactors are indispensable technical systems to cultivate photoautotrophic microorganisms. Sensing and control of photobioreactors are an essential and integral part of photobioreactor technology. The requirements of low instrumentation cost and multiple parameter on-line sensing call for innovative approach to address the sensing problems of photobioreactors. In this research, we apply on-line estimation methodology to photobioreactor systems. Two types of on-line estimators were developed and tested, using the extended Kalman filter as the optimizing algorithm. One was based the measurement of the dissolved oxygen level, and the other was based on the measurement of local irradiance level. Both estimators were proved to be able to track major cultural states, and detailed development of system models, implementation of the estimators, and the tuning of the parameters involving in the extended Kalman filters were reported in this thesis.
# TABLE OF CONTENTS

Acknowledgements ........................................................................................................... iii
Abstract ............................................................................................................................ iv

**Chapter 1.** Introduction ................................................................................................. 1
  1.1 Photobioreactor and Its applications ............................................................................ 1
  1.2 Bioprocess monitoring and sensing ............................................................................. 1
  1.3 On-line estimation of bioreactors .................................................................................. 2
  1.4 The scope of this research ......................................................................................... 2

**Chapter 2:** On-line estimation of stirred-tank microalgal photobioreactor cultures based on dissolved oxygen measurement

Abstract ............................................................................................................................... 5
  1. Introduction ................................................................................................................... 5
  2. Materials and methods ................................................................................................. 7
    2.1 Microorganism and medium ....................................................................................... 7
    2.2 Bioreactor Culture Conditions and Measurements .................................................. 7
    2.3 Extended Kalman filter ............................................................................................ 9
  3. Results and discussions ............................................................................................... 10
    3.1 Light model development ....................................................................................... 10
    3.2 Dynamic process model development and parameter estimation .......................... 12
    3.3 State estimation ...................................................................................................... 15
      3.3.1 Basic EKF ......................................................................................................... 15
      3.3.2 Adaptive EKF .................................................................................................. 19
      3.3.3 Auxiliary internal model EKF ............................................................................ 20
  4. Conclusion ..................................................................................................................... 22

Nomenclature ...................................................................................................................... 23
Reference ........................................................................................................................... 24
Appendix .............................................................................................................................. 28
Figures ................................................................................................................................ 30
Table ................................................................................................................................... 35
Chapter 3: Application of a submersible quantum sensor in the state estimation of microalgal photobioreactor cultures

Abstract ........................................................................................................... 37
1. Introduction........................................................................................................ 38
2. Materials and methods .................................................................................... 39
3. Results and Discussion..................................................................................... 40
   3.1 Light measurement using the $4\pi$ submersible quantum sensor ............. 40
   3.2 Development of the measurement model ..................................................... 42
   3.3 Process model development ........................................................................ 43
   3.4 Formulation of the estimation model ............................................................ 46
   3.5 Effect of Kalman filtering on state estimation ............................................. 48
   3.6 State estimation of photobioreactor culture with constant incident illumination ........................................................................................................ 48
   3.7 Filter tuning .................................................................................................. 49
   3.8 State estimation of photobioreactor culture with changing incident illumination ........................................................................................................ 52
4. Conclusion ........................................................................................................ 53

Nomenclature ....................................................................................................... 54
Reference ............................................................................................................... 55
Figures ................................................................................................................ 61
Table .................................................................................................................... 69

Chapter 4: Conclusions and Recommendations .................................................. 70
4.1 Comparison between the two sensing approaches ....................................... 70
4.2 Roles of the extended Kalman filter ............................................................... 71
4.3 Quantum sensor .............................................................................................. 71
4.4 Applying the sensing approach in production systems ................................ 72
4.5 Recommended research topics ....................................................................... 73

Appendix 1. A representative Matlab program realizing the simulation of software sensing ................................................................. 74
Chapter 1. Introduction

1.1 Photobioreactor technology and its applications

Photobioreactor (PBR) is a term referring to a variety of technical systems used for cultivation of photoautotrophic microorganisms. Photobioreactors can be divided into two categories, open systems and closed systems, based on the exposure of the culture to the environment. Open systems include natural waters and artificial ponds or containers of different shapes, while most large-scale closed systems adopt either tubular or flat plate types of design. Closed systems provide a closed and controllable cultural environment at the expense of high construction and maintenance cost.

Phototrophic microorganisms are of great importance in biotechnology industry. Effective and efficient photobioreactor systems would not only facilitate the commercial production of microalgae with current applications as feed for aquaculture, food supplement and sources of nutraceuticals but also serve as the potential technical platform to produce bioactive molecules such as innovative antibiotics or antitumor agents, to remedial the environment by sequestrating CO2 either from the waster gas or atmosphere and removing heavy metal from waster water, and even to provide next generation sustainable and environmentally friendly energy sources.

1.2 Bioprocess monitoring and sensing

Bioprocess monitoring or sensing is an essential and integral part of implementing bioprocesses. In early process development stages, as many as process parameters are needed to understand and optimize the process. Once the process is established, it still requires to monitor and control of system variables to achieve consistent, desirable results. Commonly monitored bioprocess parameters are temperature, pH, dissolved oxygen, cell mass, and dissolved CO2 et al.
Sensing of photobioreactor processes presents more challenges than that of other bioreactors. Some new physical parameters such as light regime and physiological states such as photosynthetic efficiency and photoinhibition are not encountered in conventional bioprocess. In addition, most current photobioreactor processes are of low profit margin. Installing of a manifold of hardware sensors would make the photobioreactors prohibitively expensive both in construction and maintenance. Thus, sensing of photobioreactors calls for both inexpensive sensing strategy and comprehensive inclusion of arrays of process parameters.

1.3 On-line estimation of bioreactors

On-line estimation approach is promising technology to meet the requirements of photobioreactor sensing. On-line estimation, also called software sensing, is combination of in situ hardware sensor measurements with estimation algorithms to provide on-line estimates of noise-filtered measurements and unmeasured variables. The estimation algorithms usually consist of an optimizing algorithm and a system model which represent our knowledge on the system behavior. This approach requires only minimal hardware instrumentation, but provides parallel estimates of an array of essential process parameters or variables.

On-line estimation approach has been intensively applied to bioprocesses for the last two decades. The estimators or software sensors have been developed for various of biological processes to estimate otherwise unmeasurable variables. According to the system models involved in the estimation algorithm, the developed estimators can be classified into three categories. 1. estimators based on mechanistic models; 2. estimators based on black box model; 3. estimators based on the combination of mechanistic and black box models.

1.4 The Scope of this research
We apply on-line estimation approach to photobioreactor systems based on dissolved oxygen measurement and local irradiance level measurement. We developed a system dynamic model for the stirred tank photobioreactor in our laboratory. Then we constructed the estimator using the extended Kaman filter as the optimizing algorithm based the model in conjunction with the dissolved oxygen measurement. The development of this estimator is detailed in Chapter 2. Because the model was specific to the system, the estimator would only be able to be implemented to systems with similar geometry. To widen the application of our approach, we developed a black box model for cell growth in photobioreactors, using an internal model approach. An observation equation was established empirically by correlation the local irradiance level inside of the photobioreactor and cell concentration. Based on the model and on-line local irradiance level measurement, the estimator was optimized by the extended Kalman filter. Chapter 3 reports this estimator in details. Conclusions and recommendations are written in Chapter 4. A representative MatLab program is documented in Appendix A.
Chapter 2. Online Estimation of Stirred-Tank Microalgal Photobioreactor Cultures based on Dissolved Oxygen Measurement

Abstract

Photobioreactor sensing presents unique challenges not met in conventional fermentors. First and foremost, photobioreactor processes are governed by photosynthesis, and hence many parameters important to photobioreactors are not considered in conventional fermentors. Furthermore, photobioreactor processes are typically associated with stringent cost constraints, and thus the use of complex sensing hardware is precluded. This calls for innovative approaches to address sensing problems in photobioreactors. Here we report the development of an effective model-based estimator that is capable of tracking key culture states in stirred-tank microalgal photobioreactor systems. A marine micro-alga Dunaliella salina was used as a model organism in this study. Extended Kalman filter (EKF) was applied here to provide optimal estimates of photobioreactor states, based on a dynamic process model in conjunction with online dissolved oxygen measurement. The process model consists of a growth model and a light transport model. The former associates growth to average light intensity in the reactor, while taking into account both photoinhibition and oxygen inhibition. The latter is based on a modified radial model to estimate the average light intensity. The estimator is capable of estimating biomass density, specific growth rate, dissolved oxygen concentration, photosynthetic efficiency, and average light intensity in the photobioreactor illuminated either with constant incident light at different intensity levels or with time-varying incident lights. For the latter, an auxiliary internal model EKF was used to accurately track the variation rate of the incident lights. This paper also presents a detailed analysis on the tuning of EKF for optimal estimation. This state estimation system offers a cost-effective means for monitoring the process dynamics of microalgal photobioreactor cultures online, through which the productivity of such a process could be optimized.

The material presented in this chapter has been accepted for publication in Biochemical Engineering Journal.
1. Introduction

Photobioreactors are used for culturing photosynthetic microorganisms such as microalgae, cyanobacteria, plant cells, and photosynthetic bacteria, for various biotechnological applications. Large-scale production of cyanobacteria biomass as food supplement has been in commercial operation for many years. There also exist several microalgal-based processes for producing nutraceutical-type products, such as polyunsaturated fatty acids (e.g., eicosapentaenoic acid and docosahexaenoic acid) and carotenoids (e.g., astaxanthin), as well as specialty chemicals (such as radio-labeled compounds). In aquaculture, microalgal cultures are used as feed. Large-scale microalgal cultures also find applications in energy (e.g., biohydrogen) production and environmental remediation (e.g., wastewater treatment and removal of CO₂ from power plant stack gas). In addition, bioreactor-based photosynthetic microalgal cultures are being considered as a part of the Closed Ecological Life Support System [1].

Process sensing is imperative for optimizing photobioreactor productivity. With the ability to accurately monitor the process dynamics, timely control actions can be taken to assure an optimal environment in the photobioreactor. The aim of this work is to develop an effective sensing system for estimating key culture parameters in microalgal photobioreactors. Process sensing in photobioreactors presents unique challenges unparalleled in conventional fermentors. First and foremost, photobioreactor processes are governed by photosynthesis, and hence many parameters important to photobioreactors are not considered in conventional fermentors. Furthermore, photobioreactor processes are typically associated with stringent cost constraints, and thus the use of complex sensing hardware is precluded. This calls for innovative approaches to address sensing problems in photobioreactors. In microalgal photobioreactors, pH, dissolved oxygen, temperature, and light intensity are commonly monitored. Measurement of these parameters alone, while useful, does not provide much in-depth information on culture physiological states. Meanwhile, electronic sensors for measuring a variety of other culture and process parameters (such as culture turbidity, sugar concentration, and metabolic activities) are available, however, they are quite costly. In order to monitor multiple parameters, a manifold of sensors will need to be installed, which makes it impractical.
In this study, we took a state estimation approach for online detection of key state variables in microalgal photobioreactors. State estimation entails the use of process models together with limited process measurements to provide noise-filtered measurements, estimates of system states that are not readily measurable, and identification of uncertain system dynamics [2]. Specifically, extended Kalman filter (EKF) was used here to provide optimal estimates of relevant culture states in the photobioreactor, based on a dynamic process model in conjunction with readily available online process measurements. This sensing approach requires only minimal instrumentation and cost. In addition, it is versatile enough that several culture states can be estimated in parallel without needing complex sensor hardware. In this study, an EKF estimation scheme was developed that is capable of monitoring biomass density, specific growth rate, dissolved oxygen level, photosynthetic efficiency (in terms of photosynthetic oxygen evolution), and average light intensity in the reactor, based on incident light information and online dissolved oxygen measurement. A marine micro-alga *Dunaliella salina* was used as the model organism.

2. Materials and Methods

2.1. Microorganism and Medium

The green alga *Dunaliella salina* (Teod.) UTEX 1644 was cultured in the chemically defined hypersaline liquid medium containing 1.5 M NaCl, 15 mM KNO3, 1 mM K2HPO4, 1 mM CaCl2, 5 mM MgSO4, 25 mM NaHCO3 and 40 mM Tris-HCl at pH 7.4, supplemented with a mixture of micronutrients containing, in final concentration, 50 μM H3BO3, 50 μM EDTA, 10 μM MnCl2, 5 μM FeCl3, 2 μM Na2MnO4, 1.5 μM NaVO3, 0.8 μM ZnSO4, 0.4 μM CuSO4, 0.2 μM CoCl2, and CO2 as carbon source. This medium was modified from that used by Pick *et al.* [3], and the modification was made based on biomass composition of major nutrient elements and verified via growth studies (Joanne Radway, personal communication). The medium was autoclaved at 121°C for 20 minutes before use. The culture was maintained in Erlenmeyer flasks, illuminated using dim cool-white fluorescent lights (< 100 μE m⁻² s⁻¹) under a 12/12 h light/dark cycle at room temperature without shaking, and sub-cultured once every two weeks.
2.2. Bioreactor Culture Conditions and Measurements

Bioreactor cultures of *D. salina* were conducted in an instrumented bench-top photobioreactor. This reactor was modified from a 3L stirred-tank fermenter (BiofloIII, New Brunswick Scientific, Edison, NJ). The original BioFloIII culture vessel contains a dished stainless steel bottom chamber which also serves as a heat exchanger. This chamber takes up about 1/5 of the reactor working volume, preventing uniform illumination throughout the reactor, and hence it was replaced with a flat stainless steel base plate (Fig. 1). In addition, a jacketed and lengthened glass cylinder (with an inner diameter and height of 13.5 cm and 25.0 cm, respectively) was used in place of the original BioFlo glass jar. The photobioreactor was illuminated by an external light source. The light source was constructed using six compact fluorescent light bulbs (Biax™ D/E, F26DBX/840/4P, GE Lighting, Cleveland, OH) connected to two dimmable ballasts (Mark X® REZ-3S32, Advance Transformer Co., Rosemont, IL), and the light intensity could be adjusted manually via a dimmer (NF-10WH, Lutron Electronics, Coopersburg, PA). Culture pH was precisely maintained at pH 7.4±0.05 by controlled supplementation of the air feed stream with CO₂ gas based on a PID control system. The pH control system consists of a pH sensor (405-DP-AS-KSC-200, Metter Toledo, Urdorf, Switzerland), a pH meter (model 430, Corning, Corning, New York), a CO₂ mass flow controller (FMA-A2404, Omega Engineering, Stamford, CT), and a supervisory computer which receives pH signals from the pH meter and activates the mass flow controller through a data acquisition board (AT-MIO-16DE-10, National Instruments, Austin, TX). The PID algorithm was coded in LabVIEW (National Instruments, Austin, TX). The flow rate of the aeration stream was controlled using a similar mass flow controller via LabVIEW. The photobioreactor was also equipped with a dissolved oxygen sensor (InPro6000, Ingold-Metter-Toledo, Wilmington, MA) connected to an oxygen meter (model 01971-00, Cole Parmer, Vernon Hills, IL), and the signals were logged into the supervisory computer through the data acquisition board. The volumetric oxygen transfer coefficient, \( k_{\text{la}} \), in the photobioreactor was measured using the gassing-in method [4].
All the experiments were performed at a temperature of 29°C which was controlled by the BioFloIII thermostat unit. The cell concentration of *D. salina* culture was determined by measuring the culture turbidity at 750 nm using a spectrophotometer (UV60, Shimadzu, Columbia, MD). The turbidity data were converted to cell dry weight based on a calibration curve. Cell dry weight was determined directly in some of the culture samples (those with higher cell concentrations) to validate the dry weight data derived from turbidity measurement. The cell dry weight concentration of *D. salina* culture was determined by filtering the culture suspensions through pre-dried and pre-weighed 0.45 μm membrane filters. The cells on the filter were then rinsed with a 0.3 M NaCl solution to remove excess salts from the culture medium, and dried in an oven at 90°C till constant weight. We have confirmed experimentally that the growth of *D. salina* was not limited by the macro nutrients (phosphate, iron, and nitrate) under the culture conditions employed in this study, indicated by the residual nutrients detected in the medium after each culture experiments (data not shown) and the increased biomass production corresponding to the increases in illumination intensities. Furthermore, by considering the equilibrium concentrations of dissolved carbon dioxide species, molecular carbon dioxide should also be in excess, under the condition that the culture pH was controlled at 7.4 by bubbling CO₂ gas [4]. The incident light intensity on the reactor surface was measured using a flat-surface quantum sensor (LI-190SA, LI-COR, Lincoln, NE) connected to a micrologger (21X, Campbell Scientific, Logan, UT). Because the average light intensity in the reactor cannot be measured directly, we measured the local light intensity at 30 distinct locations inside the photobioreactor using a micro quantum sensor (US-SQS/LI, Heinz Walz GmbH, Effeltrich, Germany) linked to a light meter (LI-250, LI-COR, Lincoln, NE). This fiber-optic micro light sensor has a spherical sensor tip (with a diameter of 3 mm) that can sense light from all directions, as opposed to the flat-surface quantum sensors which detect unidirectional light. The light measurements were taken at five horizontal planes uniformly located along the vertical axis of the reactor, and with each plane containing six evenly distributed measurement points along the radial axis. The average light intensity was calculated by taking the weighted average of all measurements.
2.3. Extended Kalman Filter

The dynamics of a non-linear biochemical process can be expressed in the following general form:

\[
\dot{\xi}(t) = \phi[\xi(t)] + w(t) ; \quad \xi(t)\big|_{t=0} = \xi_0
\]

\[
Y(t) = h[\xi(t)] + v(t)
\]  

where \( \xi(t) \) is the state vector with an initial value of \( \xi_0 \), \( Y(t) \) is the measurement vector, \( w(t) \) is system noise, representing modeling error and unknown disturbances, and \( v(t) \) is measurement noise. Both system and measurement noises are assumed to be independent random white noises with zero mean, with corresponding covariance matrices \( Q \) and \( R \). When the measurements are taken continuously in time, the extended Kalman filter (EKF) algorithm for calculating the optimal state estimate \( \hat{\xi}(t) \) based on the available measurement up to current time \( t \) is given by [5]:

\[
\dot{\hat{\xi}}(t) = \phi[\hat{\xi}(t)] + K(t) \left( Y(t) - h[\hat{\xi}(t)] \right) ; \quad \hat{\xi}(t)\big|_{t=0} = \hat{\xi}_0
\]

with

\[
K(t) = P(t) C(t) R^{-1}
\]

where \( K(t) \) is the filtering gain matrix, and \( P(t) \) is the covariance matrix of filtering error satisfying the following matrix Riccati equation:

\[
\dot{P}(t) = A(t)P(t) + P(t)A^T(t) - K(t) \cdot R \cdot K^T(t) + Q ; \quad P(t)\big|_{t=0} = P_0
\]

where

\[
A(t) = \frac{\partial \phi[\xi(t)]}{\partial \xi(t)} \bigg|_{\xi(t)} ; \quad C(t) = \frac{\partial h[\xi(t)]}{\partial \xi(t)} \bigg|_{\xi(t)}
\]

3. Results and Discussions

3.1. Light Model Development

Light availability plays a key role in photobioreactor operation. In modeling photobioreactors it is a common practice to link cell growth with the average light intensity in the reactor [6,7,8]. Quantification of light distribution in photobioreactors is a
prerequisite for estimating the average light intensity. However, modeling of light propagation and attenuation inside a bioreactor is complicated by several factors such as spectral and physical properties of the light source, the geometrical properties of the reactor, optical properties of the culture, and physiology of the cells [9]. Mathematical modeling of the radiant field in photoreactors has been the subject of numerous studies [10,11,12,13,14]. Basically, the light models developed thus far differ in how light propagates and attenuates inside the reactor. For cylindrical reactors with uniform external illumination, light is assumed to propagate inside the photobioreactor with various degrees of light diffusion [15]. In the absence of any light diffusion (i.e., light ray travels in one direction), it results in the so-called “radial” model [16]. In cases where light rays are diffused (i.e., light travels in all directions), a “diffuse” model has been proposed [15]. The local light intensity is calculated by considering light rays from all directions. When light diffusion is considered to occur only in certain region of the reactor, such as the center, this leads to the partially diffuse model. Notice that when the diffuse region is restricted only to the center of the reactor, the diffuse model becomes the radial model [14]. In terms of light attenuation through cell culture, most published formulas were based upon the Lambert-Beer’s law. Detailed mechanistic modeling approaches have been taken to reflect the actual light absorption and scattering phenomena occurred in the heterogeneous photobioreactor containing cell particles [12,14,17]. To this end, mechanistic models considering either isotropic [18] or nonisotropic scattering [11] have been reported. These mechanistic models however are difficult to use in an online state estimation system due to the introduced computational complexity.

Average light intensity models for a cylindrical reactor with uniform external illumination can be derived by first calculating the local light intensity \( I(r) \) (at a radial position \( r \)) based on a particular mode of light propagation and the formula of light attenuation. Using the assumption that light distribution inside the bioreactor vessel is vertically uniform, the average light intensity, \( I_{av} \), can then be calculated as an integral of \( I(r) \) across the horizontal plane of the cylindrical reactor, divided by the reactor cross-sectional area:
In the case of radial light propagation, the following formula of $I(r)$ was deduced by adopting Lambert-Beer's law and energy shell balance [15]:

$$I(r) = \frac{I_0 R}{r} \left[ e^{-K_a X (R - r)} + e^{-K_a X (R + r)} \right]$$  \hspace{1cm} (8)

where $I_0$ is the incident light intensity on the reactor external surface, $X$ is the biomass concentration, and $K_a$ is the light extinction coefficient. Based on the Lambert-Beer's law, $K_a$ was estimated from the light absorption data of the same culture used in the experiment for measuring average light intensity.

Substituting Eq. (8) into Eq. (7) and integrating the equation, an analytical solution is reached as follows.

$$I_{av}(I_0, X, K_a) = \frac{2I_0}{K_a X R} \left( 1 - e^{-2K_a X R} \right)$$  \hspace{1cm} (9)

Another formula of $I(r)$ was derived for the case of diffused light propagation with the assumption of Lambert-Beer's law [9].

$$I(r) = \frac{I_0}{\pi} \int_0^\pi e^{-K_a X \left[ r \cos \theta \sqrt{r^2 - r^2 \sin^2 \theta} \right]} d\theta$$  \hspace{1cm} (10)

Combination of Eq. (10) with Eq. (7) gives another average light intensity model:

$$I_{av}(I_0, X, K_a) = \frac{2I_0}{\pi R^2} \int_0^\pi \int_0^{2\pi} e^{-K_a X \left[ r \cos \theta \sqrt{r^2 - r^2 \sin^2 \theta} \right]} d\theta dr$$  \hspace{1cm} (11)

The above two $I_{av}$ models (Eqs. 9 and 11) were tested against the measured data of average light intensity. The radial and diffuse models were simulated, with the resulting $I_{av}$ vs. $X$ curves presented in Fig. 2, which indicated that the former overestimated the average light intensity while the latter did the opposite. A number of factors may contribute to this discrepancy: 1) the behavior of the actual light source is likely to be between purely radial and purely diffused; 2) due to varying degrees of light scattering, light attenuation in the culture may not be fully described by the Lambert-Beer's law especially in dense cultures and long light path conditions; and 3) light intensity distribution in the bioreactor is not entirely equal in the vertical direction as...
assumed in the calculation. In addition, $K_a$ might not be a constant during the cultivation process due to changes in cellular pigment content [6]. Considering the complexity of the system, a semi-empirical approach was taken here to derive a model based upon the structure of the radial model (mainly because of its mathematical simplicity) but included three new parameters ($E_0$, $E_1$ and $n$):

$$I_{av} = \frac{I_0 E_0}{X^n} \left(1 - e^{-E_1 X^n}\right)$$  \hspace{1cm} (12)

As the biomass concentration $X$ approaches zero, $I_{av}$ approaches $I_0 E_0 E_1$. This semi-empirical three-parameter model can fit the experimental data well, within a large range of cell concentrations (Fig. 2). Compared with entirely empirical second-order polynomial models, which also contain three adjustable parameters, Eq. (12) provides a better fit to the experimental results (data not shown). This new model implicitly compensates for some of the deficiencies encountered in the radial model, such as light diffusion and deviation from the Lambert-Beer’s law. Eq. (12) is simple to use, which makes it well suited for incorporation into the state estimator since it reduces the computational load of EKF. Moreover, when used as a part of the state estimator, the light model can be tuned dynamically to cope with uncertainty of the model structure and/or parameter values, via model parameter evolution (i.e., by including the model parameters in the state vector with other state variables). This will be discussed further in section 3.3.2.

3.2. Dynamic Process Model Development and Parameter Estimation

A central element of a state estimator is a state model that adequately depicts the process kinetic behavior. In developing such state models, it is customary to keep the model structure as simple as possible while retaining the model's capability to reflect the overall dynamics of the process [2]. By doing so it should reduce the computational load of the state estimator, which is particularly significant when the dimension of the state vector is large. With this in mind, we developed an unstructured model to simulate the dynamics of cell density, dissolved oxygen concentration, and average light intensity in the photobioreactor for culturing *D. salina* under light-limited growth conditions. Light and oxygen are two of the most important factors that affect the productivity of
microalgal photobioreactor processes. The majority of photobioreactor processes are, for the most part, operated under light-limited conditions. However, photobioreactor cultures may sometimes be exposed to excessive light intensity. Under such condition, protein D1 in photosystem II can be damaged leading to photoinhibition and reduced growth rate due to reduction in the number of active photon traps [19]. In addition, accumulation of high levels of dissolved oxygen resulting from photosynthesis, especially in poorly mixed high-density cultures, may lead to growth inhibition [20]. In the growth experiments reported here, substrate limitation was precluded, indicated by the residual nutrients detected in the medium after each culture experiments (data not shown) and the increased biomass production corresponding to the increases in $I_0$ (refer to the data in Fig. 3). Other culture variables, such as temperature, media pH, and oxygen mass transfer, were precisely controlled in our photobioreactor system at constant levels.

The process model reported here was formulated based on mass balances and saturation-type kinetics, with assumption of unsegregated, balanced growth [4]. Among the three state variables included in the model (i.e., $X$, $O$ and $I_{av}$), the dissolved oxygen level ($O$) could be continuously measured online. Biomass and average light intensity are closely linked to photosynthesis (and thus photosynthetic oxygen evolution), and hence both of these state variables should be observable via dissolved oxygen measurement. For a well-mixed batch photobioreactor, the following mass-balances were used to describe the process dynamics in terms of the kinetic behaviors of biomass and dissolved oxygen concentrations:

$$\dot{X} = \mu X$$

(13)

$$\dot{O} = R_o - k_ia(O - O^*)$$

(14)

where $\mu$ is specific growth rate, $O$ is dissolved oxygen level, $O^*$ is the dissolved oxygen level in the media that is in equilibrium with the bulk gas phase in the sparged air stream, $k_ia$ is volumetric oxygen transfer coefficient, and $R_o$ is the net oxygen production rate which reflects the combined effect of photosynthetic oxygen evolution and respiration.

In modeling the specific growth rate, we have taken into account light limitation, light inhibition, and oxygen inhibition, and the model was based on the average light intensity. Hyperbolic equation was widely used to model light-limited algal growth [10,
On the basis of the hyperbolic model, a light inhibitory term and an oxygen inhibitory term were included in our specific growth rate model:

\[
\mu = \mu_{\text{max}} \left( \frac{I_{\text{av}}}{K_f + I_{\text{av}}} \right) \left( 1 - \frac{I_{\text{av}}}{I_m} \right) \left( 1 - \frac{O}{O_m} \right)
\]  

(15)

where \( \mu_{\text{max}} \) is the maximum specific growth rate, \( K_f \) is the light saturation constant, \( I_m \) represents the light intensity level at which the cell growth is totally inhibited, and \( O_m \) is the oxygen level at which the cells cease to grow. Similar models with various modifications have been used successfully in modeling other microalgal cultures [7, 22]. Some of the reported models accounted for the maintenance requirement but left out the oxygen inhibitory effect. Considering that all cultures in this study were conducted under continuous illumination and underwent active cell growth, the maintenance energy was assumed negligible in our model. The net oxygen production rate, \( R_o \), was modeled as follows:

\[
R_o = (R_{\text{ave}} - R_{\text{ore}})X = \left( R_{\text{omax}} \left( \frac{I_{\text{av}}}{K_f + I_{\text{av}}} \right) \left( 1 - \frac{I_{\text{av}}}{I_m} \right) \left( 1 - \frac{O}{O_m} \right) - R_{\text{omin}} \right)X
\]

(16)

Here the specific photosynthetic oxygen evolution rate, \( R_{\text{ave}} \), was assumed to be linearly correlated with the specific growth rate, with a ratio of \( R_{\text{omax}}/\mu_{\text{max}} \) between the two rates. Such linear correlation has been reported in the literature, for example, by Rebolloso Fuentes et al. [23] in their study of *Porphyridium cruentum*, and it is justified by the fact that cell growth is directly coupled with photosynthesis. The specific respiration rate of many algal species was observed to be influenced by the average light intensity [24] as well as by the history of the illumination that the cells experienced [25]. However, the respiration rate was typically less than 10% of the oxygen evolution rate. Therefore \( R_{\text{ore}} \) was considered as a constant here, represented by \( R_{\text{omin}} \).

The state model (i.e. the growth model plus the light model) was fitted to data from culture experiments conducted under various light conditions, utilizing only one set of model parameters. Among the model parameters, the oxygen mass transfer coefficient, \( k_o \), was determined experimentally. For the rest of the parameters, the estimation was done based on a combination of literature data and non-linear least square fitting of the state model to the data derived from the short-term experiments conducted at four
different incident light intensity conditions. To initiate the non-linear model regression, initial values of the parameters were estimated from the literature data, and in one case from unpublished results. The initial values for $\mu_{\text{max}}$ was estimated from the batch growth data published by Cao et al. [26], $K_r$ and $I_m$ were estimated from the data of Baroli and Melis [27] and Neidhardt et al. [28], $R_{\text{omax}}$ and $R_{\text{omin}}$ were estimated from the data of Cao et al. [26] and Loeblich [29], and $O_m$ from unpublished data (Joanne Radway, personal communication). All these data were obtained using *D. salina* culture. The multivariable regression was carried out as follows. First, second-order polynomials were used to fit the original experimental data (to obtain smoothed profiles of $X$ and $O$ together with their derivatives, $\dot{X}$ and $\dot{O}$). The original differential model equations were then transformed into an algebraic form (by rearranging the terms in the equations). Subsequently, the equations were subject to the regression analysis by using the Marquardt non-linear least squares method [30]. Values of the model parameters obtained from the regression analysis are presented in Table 1. These values are generally in good agreement with the literature values. For instance, the value of $R_{\text{omax}}$ from regression was 324.2 mg g$^{-1}$ h$^{-1}$, while 370 and 468 mg g$^{-1}$ h$^{-1}$ were reported by Loeblich [29] and Cao et al. [26], respectively. Using parameters in Table 1, the model simulation results are presented in Figs. 3 and 4 for short-term ($< 15$ h) and long-term ($> 100$ h) experiments, respectively. As indicated in Figs. 3 & 4, the model could capture the overall trends of the key culture states, namely $X$ and $O$, in both short- and long-term cultures, although some discrepancy between the model and the experiment was apparent, especially as the cultivation progressed. As to be seen later, the model discrepancy can be reduced by using EKF.

A parameter sensitivity analysis was conducted to quantitatively determine the influence of each parameter on the model. The sums of squared differences of the simulation results (on $X$ and $O$) upon a 5% increase or a 5% decrease from the base value (as indicated in Table 1) of each parameter were examined. The results were normalized and the relative sensitivity of all parameters presented in Fig. 5. Base on this analysis, the model was found to be most sensitive to $\mu_{\text{max}}$ for biomass estimation, and $R_{\text{omax}}$ for dissolved oxygen estimation. The estimated values for these parameters were in accordance with the literature data. Moreover, we have tested an adaptive EKF that included $\mu_{\text{max}}$ and $R_{\text{omax}}$ in the state vector, to compensate any error that may exist in the
values assigned for these model parameters. This will be discussed further in section 3.3.2.

3.3. State Estimation

3.3.1. Basic EKF

The extended Kalman filter algorithm was used to estimate cell density ($X$), dissolved oxygen concentration ($O$), and average light intensity ($I_{av}$), based on the dynamic growth and light transfer models described above, and to eliminate noise in the measured state (in this case, $O$). Dissolved oxygen was chosen as the measured state because it is readily measurable in a continuous mode. In addition, dissolved oxygen dynamics is closely related to photosynthesis and cell growth. As such, bioreactor states could be updated continuously. The photobioreactor states are also greatly influenced by the average light intensity. However, $I_{av}$ could not be measured directly and the determination of its value could be affected by several factors such as modeling error associated with the $I_{av}$ vs. $X$ correlation (Eq. 12), random disturbance on $I_o$ due to ambient lights, and potential variation in the biomass light absorption efficiency resulted from different extents of pigmentation during the cultivation process [6]. Therefore, $I_{av}$ was included as one of the estimated states. Combining Eqs. (12) ~ (16) gives the following state model:

$$
\begin{bmatrix}
\dot{X} \\
\dot{O} \\
\dot{I}_{av}
\end{bmatrix} =
\begin{bmatrix}
\mu X \\
\left( \frac{R_{o_{\text{max}}}}{\mu_{\text{max}}} - R_{o_{\text{min}}} \right) X + k_I a(O^* - O) \\
I_o \mu \cdot I(X) + \frac{\hat{i}_{av}}{I_o}
\end{bmatrix} +
\begin{bmatrix}
W_X \\
W_O \\
W_{I_{av}}
\end{bmatrix}
$$  

(17)

where

$$
I(X) = n \left[ E_0 E_i e^{-E_i x^*} \frac{E_0}{X} (1 - e^{-E_i x^*}) \right] = n \left[ E_0 E_i e^{-E_i x^*} - \frac{I_{av}}{I_0} \right]
$$  

(18)

Since dissolved oxygen (i.e. $O$) was used as the only measured state, the measurement equation becomes:

$$
Y(t) = O(t) + V_o(t) = C \xi(t) + V_o(t)
$$  

(19)
where \( C = [0 \ 1 \ 0] \). In this case \( Q = diag[VarW_x, VarW_o, VarW_i] \) and \( R = VarV_o \), and the detailed expression of \( A \) used in Eq. (5) for calculating \( P(t) \) is given by Eq. (A1) in the Appendix.

The covariance matrix \( R \) can be determined on the basis of the standard deviations corresponding to the noises of the instruments that were used to measure the states. In our system, as dissolved oxygen was the only measured state and the measurement was assumed to be characterized by a Gaussian noise with a standard deviation of ca. 3%, we set \( R = [VarV_o] = [0.001] \). The system noise covariance matrix \( Q \) can be set based on the variance of the model uncertainty of each state. For the photobioreactor system, \( Q \) was chosen as: \( diag[10^{-5}, 10^{-3}, 4] \). Here the covariance matrix \( Q \) was chosen in a diagonal form according to the usual assumption that the individual components in \( w \) (i.e., system noise vector) are uncorrelated. Based on the same reasoning, \( R \) was also chosen as a diagonal matrix. For the covariance matrix of initial estimation error, \( P_0 \), a diagonal form was assumed at first. The initial value of the states was estimated based upon off-line measurements. The values of the diagonal elements in the \( P_0 \) matrix should be set close to the variance of the initial state estimation errors. Based on this rule, \( P_0 \) was chosen as: \( diag[P_{X0}, P_{O0}, P_{IavO}] = diag[0.0001, 0.05, 6] \). The choice of covariance matrices is important for state estimation. If fast tracking is required, more weight should be placed on the error term in Eq. (3), which means small \( R \) and large \( P \) should be chosen. However, weighing too much on the error term will cause the system unstable. As the system is non-linear and time variant, the stability of system at different time will not be the same.

When \( I_0 \) is kept at a constant level, \( \dot{I}_0 = 0 \). The state estimation results under this condition are given in Figs. 3 and 4 for short-term (<15 h) and long-term (>100 h) experiments, respectively. Figs. 3 and 4 show that while the model alone can generally fit the experimental results for \( X \) under different \( I_0 \) values and different culture durations, propagation of modeling error was augmented as the cultivation period was extended. Note that after applying EKF, the modeling errors could be rectified. To further illustrate this point, Fig. 6 shows that when a significant initial estimation error exists in \( X \), the
model output retains a large error throughout the entire culture period, while the EKF can correct the initial estimation error immediately, provided that the value for \( P_{xo} \) is set correctly (as stated before, \( P_{xo} \) should be set at a value close to the variance of the initial state estimation error). This distinctive advantage of EKF is mainly due to the fact that the EKF is able to make full use of the feedback information picked up from the online measurement of \( O \), even if the measurement is somewhat noisy.

Upon a closer examination of the estimation results for cases where the initial estimation of \( X \) is inaccurate, several interesting observations were noted. If estimation of \( X \) is the primary concern, then increasing \( P_{xo} \) alone (up to 0.1), while keeping \( P_{av0} \) unchanged, may well cope with very large initial estimation errors in \( X \) (up to 0.3 g L\(^{-1} \); note that 0.1 \( \approx (0.3)^2 \)), although in this case the estimation of \( I_{av} \) becomes distorted (Fig. 6A). In the photobioreactor state model presented here, two of the state variables, \( X \) and \( I_{av} \), are actually negatively correlated (cf. Eq. 12 and Fig. 2), therefore the assumption of a diagonal \( P_0 \) matrix may not be entirely correct. With such an assumption, the initial estimation errors are considered uncorrelated. As seen in Figs. 3 and 4, however, this assumption did not lead to erroneous estimation since the initial estimation of \( X \) was reasonably accurate in those simulations. If a significant error exists in the initial estimate of \( X \), as shown in Fig. 6A, the assumption of a diagonal \( P_0 \) matrix may lead to erroneous estimation of \( I_{av} \) in the starting stage (during the first 25 h). To overcome this problem, a non-zero covariance term \( \text{Cov}(X_0, I_{av0}) = -\sqrt{P_{xo} \cdot P_{av0}} \) was incorporated into the matrix \( P_0 \). This way, the estimation of \( I_{av} \) was improved compared with the case where diagonal \( P_0 \) was used, as seen from Fig. 6B. In Fig. 6B, \( P_{xo} = 10^{-4} \), and \( P_{av0} = 6 \). It should be pointed out, however, when the initial estimation errors in \( X \) exceeded ca. 0.15 g L\(^{-1} \), the estimation of \( I_{av} \) remained distorted even if the additional covariance term was incorporated into the matrix \( P_0 \).

Next, the effect of \( Q \) on the state estimation was examined. Recall that \( Q = \text{diag} [\text{Var} W_X, \text{Var} W'_X, \text{Var} W'_W] \), the estimation results under various \( \text{Var} W_X \) values are presented in Fig. 7. When \( \text{Var} W_X \) was set too high (>0.01) it resulted in a significant
overestimation of \( X \). Conversely, when the value of \( VarW_X \) was set too low (<10^{-7}), the estimation of \( X \) almost reduced to the model output. Also noted in Fig. 7, when \( VarW_X \) was set too low, the estimation of \( I_{av} \) was distorted. The estimation result was not so sensitive to \( VarW_O \) and \( VarW_i \), and hence the results are not presented here. Fig. 8 illustrates the effect of \( R \) on the state estimation. When the value for \( R \) was set either too high (>0.1) or too low (<0.00005), notable underestimation of \( X \) was observed, with substantial distortion in \( I_{av} \) and even \( \hat{O} \). The simulations shown in Figs. 6 ~ 8 depicted the sensitivity of the estimator to the filter settings and could be used as guidelines for tuning the extended Kalman filter.

We observed in numerical simulations that, if the initial state estimation error variance \( P_0 \), the dynamical noise variance \( Q \), and the measurement noise variance \( R \) were all adjusted proportionally, the state estimation results remained unchanged. We show here this phenomenon can be explained by analyzing Eqs. (3) ~ (5). Eq. (3) implies that for a given process the state estimation result depends mainly on the gain matrix \( K(t) \), which is proportional to \( P(t) \) and inversely proportional to \( R \) as shown by Eq. (4). Inserting Eq. (4) into Eq. (5) gives:

\[
\hat{P}(t) = A(t)P(t) + P(t)A^T(t) - P(t)C(t) \cdot R^{-1} \cdot C^T(t)P(t) + Q ; \quad P(t)\big|_{t=0} = P_0 \tag{20}
\]

Then, with \( P_0 = \alpha P'_0 \), \( Q(t) = \alpha Q'(t) \), and \( R(t) = \alpha R'(t) \) (i.e., \( P_0 \), \( Q \) and \( R \) are adjusted proportionally), Eq. (20) becomes:

\[
\frac{1}{\alpha} \hat{P}(t) = A(t)\frac{1}{\alpha} P(t) + \frac{1}{\alpha} P(t)A^T(t) - \frac{1}{\alpha} P(t)C(t) \cdot (\alpha R')^{-1} \cdot C^T(t)P(t) + \alpha Q' ; \quad P(t)\big|_{t=0} = \alpha P'_0 \tag{21.a}
\]

or

\[
\frac{1}{\alpha} \hat{P}(t) = \alpha A(t)\frac{1}{\alpha} P(t) + \frac{1}{\alpha} P(t)A^T(t) - \frac{1}{\alpha} P(t)C(t) \cdot (R')^{-1} \cdot C^T(t)P(t) + \frac{1}{\alpha} P(t) + Q' ; \quad P(t)\big|_{t=0} = \frac{P'(t)}{\alpha} \tag{21.b}
\]

Therefore, the new filtering error variance obtained under the same \( A(t) \) and \( C(t) \) should be \( P'(t) = \frac{1}{\alpha} P(t) \). This leads to the following conclusion:
\[ K'(t) = P'(t) C(t) (R')^{-1} = \frac{1}{\alpha} P(t) C(t) \left( \frac{1}{\alpha} R \right)^{-1} = K(t) \] (22)

and hence the state estimates \( \hat{\xi}(t) \) remain unchanged. According to Eq. (6), this in turn guarantees that \( A(t) \) and \( C(t) \) will not change. The above analysis reveals that, when choosing \( P_0, Q \) and \( R \), it is important to pay attention to their relative values.

3.3.2. Adaptive EKF

In practice, uncertainty may exist in model structure and measurement precision, and certain model parameters may possess slowly time-varying property. To cope with these factors, two versions of adaptive EKF were considered here to allow parameter evolution, by including in the state vector, \( \mu_{\text{max}} \) and \( R_{\text{max}} \) in one case, and \( E_0 \) and \( E_1 \) in the other, in addition to the common state variables \( X, O \) and \( I_{av} \) (i.e.,
\[ \xi = [X, O, I_{av}, \mu_{\text{max}}, R_{\text{max}}]^T \) or \( \xi = [X, O, I_{av}, E_0, E_1]^T \)). The parameters, \( \mu_{\text{max}} \) and \( R_{\text{max}} \) were selected based on results from the sensitivity analysis, while \( E_0 \) and \( E_1 \) were chosen due to their potential time-varying property. Here we consider \( \mu_{\text{max}} \) and \( R_{\text{max}} \) or \( E_0 \) and \( E_1 \) to be relatively stationary throughout the cultivation, and thus the dynamics of the parameter change can be modeled as \( \dot{\theta}_i = 0 + W_i \) where \( \theta_i \) and \( W_i \) represent the adaptive model parameters and the noises, respectively. Here the corresponding measurement equation remains the same as Eq. (19) in form, except that
\[ C = [0, 1, 0, 0, 0]. \] The detailed expressions of \( A \) used in Eq. (5) for calculating \( P(t) \) in the two versions of adaptive EKF are given by Eqs. (A2) ~ (A4) in the Appendix.

The parameter evolution process for \( \mu_{\text{max}} \) and \( R_{\text{max}} \) is illustrated in Fig. 9 while setting \( P_{\mu_0} = 0.1, \ Var W_\mu = 0.000031 \) for \( \mu_{\text{max}} \), and \( P_{R_0} = 3, \ Var W_R = 3125 \) for \( R_{\text{max}} \). If we intentionally set the initial values of \( \mu_{\text{max}} \) and \( R_{\text{max}} \) incorrectly (in this case, we set \( \mu_{\text{max}} = 0.099 \) h\(^{-1}\) and \( R_{\text{max}} = 300 \text{mg g}^{-1} \text{h}^{-1}\)), Fig. 9 shows that the adaptive EKF is capable of adjusting these parameters towards their correct values (\( i.e., \mu_{\text{max}} = 0.119 \) h\(^{-1}\) and \( R_{\text{max}} = 324.2 \text{mg g}^{-1} \text{h}^{-1}\)) at a satisfactory rate. Similarly, the adaptive EKF was also
able to rectify deviations in the initial settings of $E_o$ and $E_1$ with respect to their correct values ($E_o = 0.26 \text{ g}^{0.87} \cdot \text{L}^{0.87}$ and $E_1 = 10 \text{ L}^{0.87} \cdot \text{g}^{0.87}$) as shown in Fig. 9, while setting $P_{e_0} = 0.1$, $VarW_{E_0} = 0.0000002$ for $E_o$, and $P_{e_1} = 1$, $VarW_{E_1} = 0.31$ for $E_1$. The estimation curve for $O$ in these cases is similar to that in Fig. 4 and thus not presented here again.

### 3.3.3. Auxiliary Internal Model EKF

In outdoor applications, the light intensity on the photobioreactor surface, $I_o$, is time-varying. Under such a condition, one needs accurate information for both $I_o$ and its derivative $I_r$, in order to achieve precise state estimation (recall Eq. (17) which represents the state model for the photobioreactor process). This is complicated by the fact that, under outdoor conditions, $I_o$ measurement is typically associated with substantial noise, and hence calculation of its derivative can be prone to errors. To cope with the filtering problem associated with $I_o$ and $I_r$, we took the following internal model approach. Successful implementation of such an approach in state estimation has been reported in discrete-time systems [31]. With the internal model approach, we assume that $I_o$ satisfies a sinusoidal function with an unknown but almost constant angular frequency $\omega$ within a small time interval around current time instant $t$, i.e.

$$I_o = I_M \sin(\omega t + \psi)$$  \hfill (23)

where $I_M$ is the amplitude, and $\psi$ is the phase angle. Then,

$$I_r = \dot{I}_o = \omega I_M \cos(\omega t + \psi)$$  \hfill (24)

and

$$\ddot{I}_r = \ddot{I}_o = -\omega^2 I_M \sin(\omega t + \psi) = -\omega^2 I_o$$  \hfill (25)

Thus by setting a state vector as $\xi_t = [I_o, I_r, \omega]^T$ to include the unknown but approximately constant frequency $\omega$, an internal model state equation for $I_o$ can be derived as follows
and the corresponding measurement equation is

\[
Y_t = I_o + V_I = C_I \xi_I + V_I
\]  

where \( C_I = [1, 0, 0] \).

Notice that here the amplitude \( I_M \) and the phase angle \( \psi \) do not appear in the internal model state Eq. (26). We need only to consider the angular frequency \( \omega \). This is an advantage of the internal model approach. Since this state equation is non-linear, an auxiliary EKF is also needed for estimating \( \xi_I \). The filter parameters for such an auxiliary EKF are: \( Q_I = \text{diag} [\text{Var} W_{o}, \text{Var} W_{u}, \text{Var} W_{\omega}] \), \( R_I = \text{Var} V_I \), and

\[
A_I = \begin{bmatrix}
0 & 1 & 0 \\
-\omega^2 & 0 & -2\omega I_0 \\
0 & 0 & 0
\end{bmatrix}
\]  

(28)

Once \( I_o \) and its derivative \( I_r \) are calculated from the internal model, the values are plugged into Eq. (17) for bioreactor state estimation. Fig. 10 shows the state estimation results under stepwise varying \( I_0 \), which approximately simulates the daily sunlight variation. The parameters for the auxiliary EKF were selected as follows. Considering that the time-varying \( I_0 \) function contains a first angular frequency component \( \omega_1 \approx \frac{2\pi}{18} \approx 0.35 \text{ rad h}^{-1} \) with a period of about 18 h (corresponding to the total culture period), and a second angular frequency component \( \omega_2 \approx \frac{2\pi}{1} \approx 6.28 \text{ rad h}^{-1} \) with a period of 1 h (corresponding to the hourly stepwise change in \( I_0 \)), an intermediate value \( 1.4 \text{ rad h}^{-1} \) was chosen as the initial estimation of the angular frequency \( \omega \). Since \( \dot{I}_0 \) by definition is exactly equal to \( I_r \), and hence \( \text{Var} W_{I_0} = 0 \). \( R_I = \text{Var} V_I = 40 \) was chosen based on the precision of the light meter. The remaining parameters do not affect the estimation result significantly and were chosen empirically as \( \text{Var} W_{I_r} = 10 \).
\[ Var W_o = 0.007, \ P_{I_0} = 100, \ P_{I_r} = 100, \ P_{\omega_0} = 0.007. \]

It can be seen from Fig. 10 that, by incorporating such an auxiliary internal model EKF whose outputs can well track the \( I_0 \) and its variation rate \( I_r \), the EKF offers satisfactory state estimations under time-varying \( I_0 \) condition, while the model alone fails to give proper outputs.

4. Conclusion

A simple unstructured process model coupled with a semi-empirical light model was shown here to give a reasonable representation of the *D. salina* photobioreactor process dynamics, although with the model alone it was not possible to accurately track all culture states, especially under long-term cultivations and time-varying illuminating conditions. By incorporating EKF with the model, and based only on online measured dissolved oxygen concentration, a state estimator was successfully developed to track the major states of *D. salina* culture grown in a stirred-tank photobioreactor. The estimator was scrutinized under a wide range of operating conditions including short- (less than 15 h) and long-term (over 100 h) cultures under different levels of constant incident light intensities, as well as under the condition of time-varying incident light intensities. This state estimation system offers a cost-effective means for monitoring the process dynamics of microalgal photobioreactor cultures online, and could substantially improve the productivity of such a process.

Acknowledgements

The *D. Salina* strain was obtained from Dr. Anastasios Melis at the University of California, Berkeley. This work was supported by the NSF ERC Program. Contract grant number: EEC-9731725.

Nomenclature

\begin{itemize}
  \item \textbf{A}: \ \text{partial derivative matrix, defined in Eq. (6)}
  \item \textbf{C}: \ \text{partial derivative matrix for calculating the filtering gain, defined in Eq. (6)}
\end{itemize}
$E_0$: parameter in the light model (Eq. 12)
$E_I$: parameter in the light model (Eq. 12)
$h[\bullet]$: measurement function
$I_{av}$: average light intensity in the culture
$I_{m}$: average light intensity at which cell growth is ceased
$I_M$: amplitude in Eq. (23)
$I_0$: incident light intensity on the reactor external surface
$I_v$: changing rate of incident light intensity
$K$: filtering gain matrix
$K_a$: biomass light absorption coefficient
$K_i$: saturation constant
$k_{fa}$: volumetric oxygen mass transfer coefficient
$n$: parameter in the light model (Eq. 12)
$O$: dissolved oxygen concentration
$O^*$: saturated oxygen conc. in the media
$O_m$: dissolved oxygen conc. at which cell growth is ceased
$P$: covariance matrix of state estimation error
$Q$: covariance matrix of system noise
$R$: covariance matrix of measurement noise
$R$: reactor radius (a constant)
$R_o$: net oxygen production rate
$R_{oe}$: specific photosynthetic oxygen evolution rate
$R_{or}$: specific respiration rate
$R_{omax}$: max. oxygen generation rate
$R_{omax}$: equivalent to the specific respiration rate
$v$: measurement noise vector
$V_o$: measurement noise of dissolved oxygen concentration
$V_I$: measurement noise of $I_0$
\[ w : \] dynamic noise vector
\[ W_f : \] noise
\[ X : \] cell density (cell dry weight)
\[ Y : \] measurement vector
\[ Y : \] measurement of dissolved oxygen concentration
\[ Y_f : \] measurement of \( I_0 \)
\[ Y_o : \] yield coefficient
\[ \phi[\cdot] : \] dynamic state function
\[ \mu : \] specific growth rate (\( h^{-1} \))
\[ \mu_{\text{max}} : \] maximum specific growth rate
\[ \omega : \] instantaneous angular frequency of \( I_0 \) variation
\[ \psi : \] phase angle in Eq. (23)
\[ \xi : \] state vector
\[ \hat{\xi} : \] state estimate
\[ \xi_f : \] internal model state vector

References


Appendix: Detailed expressions of $A$ for calculating $P(t)$

In the basic EKF (section 3.3.1), $A$ is expressed as:

$$
A = \begin{bmatrix}
\frac{R_{o_{\text{max}}}}{\mu_{\text{max}}} \hat{\mu} - R_{o_{\text{min}}}/\mu_{\text{max}} & \frac{R_{o_{\text{max}}}}{\mu_{\text{max}}} \frac{\partial \hat{\mu}}{\partial \hat{X}} \hat{X} - k_i a & \frac{R_{o_{\text{max}}}}{\mu_{\text{max}}} \frac{\partial \hat{\mu}}{\partial \hat{I}_{\text{av}}} \hat{X} \\
I_0 \frac{\partial \left(I(\hat{X})\right)}{\partial \hat{X}} & I_0 I(\hat{X}) \frac{\partial \hat{\mu}}{\partial \hat{O}} & I_0 I(\hat{X}) \frac{\partial \hat{\mu}}{\partial \hat{I}_{\text{av}}} + I_0
\end{bmatrix} = \bar{A} 
$$

(A1)

In the two versions of adaptive EKF (section 3.3.2):

$$
A = \begin{bmatrix}
\bar{A} & B \\
0_{2 \times 3} & 0_{2 \times 2}
\end{bmatrix}
$$

(A2)

for $\xi = [X, O, I_{ao}, \mu_{\text{max}}, R_{o_{\text{max}}}]^T$, $B$ is expressed as:

$$
B = \begin{bmatrix}
\frac{\hat{\mu}}{\mu_{\text{max}}} \hat{X} & 0 \\
0 & \frac{\hat{\mu}}{\mu_{\text{max}}} \hat{X} \\
I_0 I(\hat{X}) \frac{\hat{\mu}}{\mu_{\text{max}}} \hat{X} & 0
\end{bmatrix}
$$

(A3)

and for $\xi = [X, O, I_{ao}, E_0, E_1]^T$, $B$ is expressed as:

$$
B = \begin{bmatrix}
\frac{\partial \hat{\mu}}{\partial \hat{E}_0} \hat{X} & \frac{\partial \hat{\mu}}{\partial \hat{E}_1} \hat{X} \\
\frac{R_{o_{\text{max}}}}{\mu_{\text{max}}} \frac{\partial \hat{\mu}}{\partial \hat{I}_{\text{av}}} \hat{X} & \frac{R_{o_{\text{max}}}}{\mu_{\text{max}}} \frac{\partial \hat{\mu}}{\partial \hat{I}_{\text{av}}} \hat{X} \\
\Lambda_0 & \Lambda_1
\end{bmatrix}
$$

(A4)

where

$$
\frac{\partial I(\hat{X})}{\partial \hat{X}} = nE_0 \left(1 - \frac{1}{\hat{X}_{n+1}} - nE_1^2 \hat{X}_{n+1} - \frac{1}{\hat{X}_{n+1}} \right) e^{-E_0 \hat{X}_{n+1}}
$$

(A5)
\[
\frac{\partial \hat{\mu}}{\partial \hat{O}} = -\frac{\mu_{\text{max}} \hat{I}_{av}}{O_m (K_f + \hat{I}_{av})} \left(1 - \frac{\hat{I}_{av}}{I_m}\right) \tag{A6}
\]

\[
\frac{\partial \hat{\mu}}{\partial \hat{I}_{av}} = \left(1 - \frac{\hat{O}}{O_m}\right) \frac{\mu_{\text{max}}}{K_f + \hat{I}_{av}} \left[\frac{K_f}{K_f + \hat{I}_{av}} \left(1 - \frac{\hat{I}_{av}}{I_m}\right) - \frac{\hat{I}_{av}}{I_m}\right] \tag{A7}
\]

\[
\frac{\hat{\mu}}{\mu \text{max}} = \frac{\hat{I}_{av}}{K_f + \hat{I}_{av}} \left(1 - \frac{\hat{I}_{av}}{I_m}\right) \left(1 - \frac{\hat{O}}{O_m}\right) \tag{A8}
\]

\[
\frac{\partial \hat{\mu}}{\partial \hat{E}_i} = \frac{\partial \hat{\mu}}{\partial \hat{I}_{av}} \frac{\partial \hat{I}_{av}}{\partial \hat{E}_i}, \quad i = 0, 1 \tag{A9}
\]

\[
\Lambda_i = I_o \left[I(\hat{X}) \frac{\partial \hat{\mu}}{\partial \hat{E}_i} + \hat{\mu} \frac{\partial I(\hat{X})}{\partial \hat{E}_i}\right] + I_o \frac{\partial \hat{I}_{av}}{I_o \partial \hat{E}_i}, \quad i = 0, 1 \tag{A10}
\]

\[
\frac{\partial \hat{I}_{av}}{\partial \hat{E}_0} = \frac{I_o}{X^n} \left(1 - e^{-E_i \hat{X}^n}\right) = \frac{\hat{I}_{av}}{E_0} \tag{A11}
\]

\[
\frac{\partial \hat{I}_{av}}{\partial \hat{E}_1} = \frac{I_o \hat{E}_0 e^{-E_i \hat{X}^n}}{E_0} \tag{A12}
\]

\[
\frac{\partial I(\hat{X})}{\partial \hat{E}_0} = -n \left(n E_i e^{-E_i \hat{X}^n} - \frac{1}{I_o} \frac{\partial I_{av}}{\partial \hat{E}_0}\right) \tag{A13}
\]

\[
\frac{\partial I(\hat{X})}{\partial \hat{E}_1} = n \left[E_o (1 - E_i \hat{X}^n) e^{-E_i \hat{X}^n} - \frac{1}{I_o} \frac{\partial I_{av}}{\partial \hat{E}_1}\right] \tag{A14}
\]
Fig 1 Schematic representation of the photobioreactor system.

Fig 2. Comparison of three light models.
Fig 3. Modeling and filtering results from four short-term experiments. From the top to the bottom, the incident light intensities are 500, 400, 300 and 200 \( \mu \text{E m}^{-2} \text{s}^{-1} \) respectively. Symbol: \( \circ \) experimental data \( X \) \( \longrightarrow \) experimental data \( O \) \( \longrightarrow \) model output \( \longrightarrow \) EFK filter results

Fig 4. Modeling and filtering results from two long-term experiments. From the top to the bottom, the incident light intensities are 60 and 100 \( \mu \text{Em}^{-2} \text{s}^{-1} \) respectively. Symbol: \( \circ \) experimental data \( X \) \( \longrightarrow \) experimental data \( O \) \( \longrightarrow \) model output \( \longrightarrow \) EFK filter results
Fig 5. Model parameter sensitivity analysis. Symbol: ■ on X  ■ on O

Fig 6. Effect of Po on state estimation. Symbol: o experimental data X
--- experimental data O --- model output for A --- $P_{X_0} = 0.0001$
--- $P_{X_0} = 0.001$ --- $P_{X_0} > 0.01$ for B --- Cov($X_0, I_{avo}$) = 0 ---
Cov($X_0, I_{avo}$) < 0
Fig 7. Effect of Q on state estimation. Symbol: ○ experimental data X
      experimental data O  — model output  . . . Var$W_X$ = $10^{-7}$
      Var$W_X$ = 0.0001  —- Var$W_X$ = 0.01  — Var$W_X$ = 0.1

Fig 8. Effect of R on state estimation. Symbol: ○ experimental data X
      experimental data O  — model output  filter results
Fig 9. Adaptive EKF state estimation. Symbol: adaptive $\mu_{\text{max}}$ and $R_{o\text{max}}$
--- adaptive $E_0$ and $E_1$

Fig 10. State estimation under varying incident light intensity. Symbol: o experimental data X experimental data O model output
--- EFK filtering results
Table 1. Model Parameter Values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\mu_{\text{max}}$</td>
<td>$0.119\ h^{-1}$</td>
</tr>
<tr>
<td>$K_I$</td>
<td>$202.1\ \mu\text{E}\ \text{m}^{-2}\ \text{s}^{-1}$</td>
</tr>
<tr>
<td>$I_m$</td>
<td>$2005\ \mu\text{E}\ \text{m}^{-2}\ \text{s}^{-1}$</td>
</tr>
<tr>
<td>$O_m$</td>
<td>$47.9\ \text{mg}\ \text{L}^{-1}$</td>
</tr>
<tr>
<td>$R_{o\text{max}}$</td>
<td>$324.2\ \text{mg}\ \text{g}^{-1}\ \text{h}^{-1}$</td>
</tr>
<tr>
<td>$R_{o\text{min}}$</td>
<td>$22.2\ \text{mg}\ \text{g}^{-1}\ \text{h}^{-1}$</td>
</tr>
<tr>
<td>$k_{\alpha}$ for short-term experiments</td>
<td>$13.5\ \text{h}^{-1}$</td>
</tr>
<tr>
<td>$k_{\alpha}$ for long-term experiments</td>
<td>$6.9\ \text{h}^{-1}$</td>
</tr>
<tr>
<td>$E_0$</td>
<td>$0.26^{0.87} \cdot 0.87\ \text{g}\ \text{L}^{-1}$</td>
</tr>
<tr>
<td>$E_1$</td>
<td>$10.0^{0.87} \cdot 0.87\ \text{L}\ \text{g}^{-1}$</td>
</tr>
<tr>
<td>$n$</td>
<td>$0.87$</td>
</tr>
</tbody>
</table>
Chapter 3. Application of a Submersible Quantum Sensor in the State Estimation of Microalgal Photobioreactor Cultures

Abstract

Local photosynthetic photon flux fluence rate (PPFFR) determined by a submersible $4\pi$ quantum micro-sensor was used in developing a versatile on-line state estimator for stirred-tank microalgal photobioreactor cultures. A marine micro-alga Dunaliella salina was used as a model organism in this study. A dynamic model for the photobioreactor was derived based on mass-balance equations of relevant states, whereas an internal model approach was used to provide a generic description of the specific growth rate without the need of state-based kinetic expression. The observation equation was established based on an empirical correlation between the microalgal biomass concentration and the local PPFFR measured at a fixed point inside the photobioreactor. On-line state estimation was realized using the extended Kalman filter (EKF), based on the state model and the on-line local PPFFR measurement. The estimator was proved to be capable of estimating biomass concentration, specific growth rate, as well as phosphate and dissolved oxygen concentrations in the photobioreactor illuminated either with fixed incident radiation at a wide range of intensity levels or with time-varying incident radiations. The quantum sensor was shown to be robust and able to quickly respond to dynamic changes in local PPFFR. In addition, the quantum sensor outputs were not affected by bubble aeration or agitation within the typical operating range. The strong filtering capacity of EKF provides the state estimator with a superior performance over direct calculation from the empirical biomass/local PPFFR correlation on the estimation of specific growth rate and dissolved oxygen concentration. This state estimation system offers a cost-effective means for monitoring the process dynamics of microalgal photobioreactor cultures on-line, through which the productivity of such a process could be optimized.

Keywords: microalgae, photobioreactor, quantum sensor, state estimation
1. Introduction

Photobioreactors are key to bioprocesses involving phototrophic microorganisms, such as microalgae and cyanobacteria, which have been used or proposed for a variety of biotechnological applications. Cultivation of cyanobacteria and microalgae in open pond photobioreactors as food supplement and feed for aquaculture has been in commercial operation for decades. Industrial production of nutraceuticals such as polyunsaturated fatty acids and carotenoids by photobioreactors is reported to be commercially viable (Olaizola, 2000). The exploitation of microalgae and cyanobacteria as sources of novel antibiotics and anticancer agents is expected to yield promising discoveries that could lead to applications of commercial values (Borowitzka, 1995; Skulberg, 2000). Large scale cultivation of microalgae is also being considered as a renewable and environmentally-friendly means for energy production (Asada and Miyake, 1999; Scragg et al., 2002). In addition, microalgae are useful in bioremediation applications such as removal of heavy metal pollutants, reduction of carbon dioxide from industrial waste gas, and even sequestration of carbon dioxide from atmosphere to alleviate the green house effect (Benemann, 1997; Usui and Ikenouchi, 1997; Matsunaga et al., 1999). Because most of the above applications necessitate large-scale cultivation of photosynthetic microalgae, photobioreactors, as the indispensable production system for phototrophic organisms, have been undergoing intensive studies in the past decades. Until recently, the open pond systems still dominate the commercial operation, but the closed systems are being developed for new applications especially for high value products such as pharmaceuticals and nutraceuticals (Borowitzka, 1999; Pulz, 2001).

Process sensing is an essential and integral component of the photobioreactor technology. Industrial photobioreactor systems stipulate accurate tracking of multiple key culture parameters to correctly reflect the process dynamics, and from which timely control actions can be implemented to enhance the process efficiency. Whereas sensors for measuring a variety of culture and process parameters (such as culture turbidity, sugar concentration, and even affinity sensors for monitoring specific compounds) are available, most of these sensors suffer either from high cost or poor long-term stability. Furthermore, the requirement of installing a manifold of electronic hardware sensors would make the photobioreactor system prohibitively expensive not only in terms of
design and construction but also in maintenance. In practice, only a few process parameters such as temperature, dissolved oxygen, and pH can be measured directly in bioprocesses with sufficient reliability and reasonable cost (Harms et al., 2002). These considerations call for innovative approaches to address sensing problems in photobioreactors.

The present study is an extension from our earlier work (Li et al. 2002) in which process sensing in microalgal photobioreactors was achieved via state estimation in connection with dissolved oxygen measurement. State estimation, or software sensing, entails the use of process models together with limited and oftentimes simple process measurements to provide noise-filtered measurements, estimates of immeasurable system states, and identification of uncertain system dynamics (Stephanopoulos and Park, 1991). Here we report the application of a submersible quantum sensor coupled with process modeling and extended Kalman filter (EKF) in establishing a versatile state estimator for on-line estimation of multiple state variables in microalgal photobioreactors. Quantum sensors are more affordable and robust than most electrochemical sensors, and they are especially suited for photobioreactors. The submersible quantum micro-sensor was utilized in this study to determine the local photosynthetic photon flux fluence rate (PPFFR), which served as the measurement state in our EKF-based state estimator. Photon flux fluence rate represents the integral of the photon flux radiance at a point over all directions about the point. When the wavelength range of the photons measured is limited to the 400-700 nm range (photosynthetically active radiations; PAR), the term "photosynthetic photon flux fluence rate" is used (Biggs, 1984). Another important element of the state estimator is the photobioreactor state model, which was derived based on mass-balance equations of the relevant culture states, while an internal model approach was used to provide a generic description of the specific growth rate without the need of any specific kinetic expression. Adequate estimation of biomass concentration, specific growth rate, as well as phosphate and dissolved oxygen concentrations in the photobioreactor illuminated either with fixed incident radiation at a wide range of intensity levels or with time-varying incident radiations was demonstrated in a series of growth experiments.

2. Materials and Methods
The green alga *Dunaliella salina* (Teod.) UTEX 1644 was used throughout this study. The organism was cultured in a chemically defined hypersaline liquid medium as previously described (Li et al. 2002). Bioreactor cultures of *D. salina* were conducted in an instrumented bench-top photobioreactor. This reactor was modified from a 3-L stirred-tank fermenter (BiofloIII, New Brunswick Scientific, Edison, NJ). The photobioreactor setup, the basic extended Kalman filter algorithm, as well as the determinations of biomass density and dissolved oxygen concentration, were also described in literature (Li et al. 2002). The incident light intensity on the reactor surface was measured using a cosine (2π) quantum sensor (LI-190SA, LI-COR, Lincoln, NE) connected to a micrologger (21X, Campbell Scientific, Logan, UT), and expressed as photosynthetic photon flux density (PPFD). The local irradiance level at a fixed point within the photobioreactor was measured using a submersible spherical (4π collector) quantum micro-sensor (US-SQS/LI, Heinz Walz GmbH, Effeltrich, Germany) connected to a micrologger (21X, Campbell Scientific, Logan, UT), and expressed as photosynthetic photon flux fluence rate. This fiber-optic micro sensor has a spherical sensor tip (with a diameter of 3 mm) that can sense light from all directions underwater, and the sensor reading was logged into a supervisory computer through a data acquisition board (AT-MIO-16DE-10, National Instruments, Austin, TX). To protect the sensor from the highly corrosive hypersaline medium, the sensor was inserted into a glass optical well that was fixed to the reactor head plate and submerged in the culture broth. The sensor tip was placed 2 cm inward from the reactor periphery and 6 cm below the culture surface. A schematic drawing of the sensor is shown in Fig. 1. Phosphate concentration in the culture medium was determined by the molybdate method (Tara, 1991).

3. Results and Discussion

3.1. Light measurement using the 4π submersible quantum sensor

Light availability plays a key role in photobioreactor performance. As such, it is necessary to accurately assess this process parameter to assure adequate photobioreactor operation. Quantum sensors have been widely used to measure the level of irradiance either on the external photobioreactor surface (Acién Fernández et al., 1997; Janssen et al., 1999), or inside the photobioreactors (Katsuda et al., 2000; Li et al., 2002; Sánchez
Miron et al., 1999). Two major types of quantum sensors are commonly used in photobioreactor applications, and they differ both in the geometrical appearance and in the physical characteristics of irradiance sensed. The $2\pi$ sensors measure the photosynthetic photon flux density (PPFD), which is defined as the number of PAR photons incident per unit time on a unit flat surface. Whereas the $4\pi$ sensors with a spherical shape measure the photosynthetic photo flux fluence rate (PPFFR), which is the integral of photon flux radiance at a point over all directions (Biggs, 1984). The former sensors are suitable to quantify the incident radiation on the reactor surface, indicating the level of radiation supplied to the photobioreactor, and the latter are suited for measuring the level of PAR inside the reactor to indicate the photon flux available to the cells from all directions at certain point inside the photobioreactor.

In our previous study (Li et al. 2002), we developed a state estimator for stirred-tank photobioreactor cultures using dissolved oxygen concentration as the measured state. While the estimator worked well, signal drift from the polarographic dissolved oxygen probe during prolonged cultivation was observed. Quantum sensors offer many advantages over electrochemical type dissolved oxygen sensors in on-line sensing of photobioreactor states. First and foremost, the signal from the electrochemical type dissolved oxygen sensors is known to drift substantially during long-term cultivation. Drift is commonly caused by accumulation of hydroxyl or metal ions, chloride depletion, or external fouling of the probe membrane surface (Baily & Ollis, 1986). This means that the sensor needs to be re-calibrated frequently. However, frequent calibration could be difficult to implement during the cultivation. Moreover, the membrane body of the electrochemical type dissolved oxygen sensor is very fragile, and it is susceptible to physical damages and biofouling. Therefore, periodic replacement of the membrane bodies is necessary, which makes the maintenance of the sensors quite costly. On the contrary, the quantum sensors require no frequent calibrations and are very robust in their construction.

The stability of the quantum sensor was confirmed experimentally in this study. Quantum sensor data under fixed external illumination were followed for a period of three days. The variation of the data was less than 2% (data not shown). This is true for
both $2\pi$ and $4\pi$ sensors. If one considers the irradiance fluctuation caused by the power supply to the fluorescent lights, the sensor drift could be considered negligible. Because the $4\pi$ sensor was inserted into the bioreactor culture to measure the local PPFFR, it is possible that aeration and agitation could affect the measurements. To examine these effects, sensor data were recorded at different aeration and agitation rates with either culture broth or cell-free media inside the bioreactor, under fixed external illumination. The data showed that within the operating conditions comparable to those used in this study (i.e., aeration from 0.1 to 0.8 vvm and agitation from 75 to 200 rpm), aeration and agitation have little, if any, effect on the quantum sensor readings (data not shown).

3.2. Development of the measurement model

A measurement model links the on-line acquired measurement signals with the culture states to be estimated. The dependence of local irradiant (PPFFR) level in the photobioreactor on biomass concentration forms the basis for state estimation using the submersible quantum sensor. Although mechanistic modeling of radiant light distribution in photobioreactors has been reported (Evers, 1991; Kurata et al., 1993; Comet et al., 1994; Brucato and Rizzuti, 1997; Katsuda et al., 2000), use of such complex models in a state estimator would considerably complicate the computation. The inherent assumptions associated with such models also inevitably limit their general applicability in spite of their mathematical complexity. The correlation between local PPFFR level and biomass concentration was therefore established empirically in this research. The local PPFFR level at different locations inside the bioreactor responses differently to changes in biomass concentration. In order to determine a location at which the quantum sensor gives the best resolution in response to changes in biomass concentration, we estimated the radiant field in the photobioreactor using a simple radial light model (Huff and Walker, 1962), and from which the average light intensity and light intensity at any given point in the reactor could be calculated. This calculation indicated that at a radial position 2 cm inward from the reactor surface (reactor diameter is 13.5 cm), the ratio of the local irradiance level over the average irradiance intensity remained essentially constant within the biomass range of interest in this study, namely from $0.05 \text{ g L}^{-1}$ to $1.5 \text{ g L}^{-1}$ (dry cell weight). The location revealed by the calculation was then verified experimentally by
measuring and comparing local and average light intensities in the photobioreactor, using techniques described in our previous report (Li et al. 2002). Based on the model structure we used previously to depict the correlation between the average light intensity and the biomass concentration (Li et al. 2002), the local irradiance \(I_L\) in terms of PPFFR) data were correlated with the biomass data \(X\) using the following equation:

\[
I_L = \frac{I_L e^{-\frac{E}{X^a}}}{X^a} (1 - e^{-\frac{E}{X^a}})
\]  

This correlation was checked against the data \((I_L\) and \(X)\) collected during two repeated batch culture experiments. These experiments were conducted at an external illuminating level of 450 \(\mu\)E-m\(^{-2}\)-s\(^{-1}\). Equation (1) was used to fit the data via the Marquardt’s non-linear least squares method (Marquardt, 1963). Values of the measurement model parameters are listed in Table 1. Fig. 2 indicates that the measurement model closely agrees with the data throughout the entire biomass range tested.

3.3. Process model development

Several photobioreactor models have been reported in the literature (Evers, 1991; Cornet et al., 1992; Cornet and Albiol, 2000; Taya et al, 1995; Acien Fernández et al., 1998; Camacho Rubio et al., 1999; Csögör et al., 1999; Baquerisse, et al., 1999; Rorrer and Mullikin, 1999). In many of these models, light-limited growth was implicated, and cell growth rate was assumed to be governed by the average light intensity in the photobioreactor. As such, accurate modeling of the radiant field inside the photobioreactor is necessary in order to provide a truthful estimation of the average light intensity. This could be a very challenging task considering that detailed mechanistic modeling of radiant distribution in photobioreactors would entail considerations such as selective absorption of photosynthetically active radiation, non-isotropic light scattering, as well as size and shape of the cells. The radiant absorption and the energy conversion of the absorbed radiances are dynamic processes, whose rates vary substantially under different illumination conditions. This is due in part to the fact that the antenna size of the cellular photosystems changes with the irradiance levels (Baroli and Melis, 1996). In addition to the irradiance considerations, one may need to consider other nutrient limitation and inhibitory factors as well. Putting together, it is desirable to establish alternative approaches to model cell growth in photobioreactors other than using
constitutive models (Blanch and Clark 1996) to relate the growth response of a cell to its environment (e.g. irradiance, nutrient, and inhibitor levels).

Having a proper measurement model that links the local irradiance level and biomass concentration in the photobioreactor system, a precise mechanistic growth model coupling cell growth with environmental conditions is no longer necessary because the biomass concentration is observable through the measurement model. This situation allows us to use an adaptive approach for depicting the specific growth rate using EKF without the need to relate the growth rate to the environmental conditions. Such approach for modeling the specific growth rate greatly broadens the applicability of the state estimator. Similar approaches had been successfully applied to the on-line estimation of yeast and bacterial fermentations (Stephanopoulos and San, 1984a; Takiguchi et al. 1997). In these earlier studies, it was commonly assumed that the dynamics of $\mu$ could be represented by a second-order system with certain random disturbances. In this study, we developed an alternative adaptive model for the specific growth rate ($\mu$). Consider that $\mu$ changes smoothly during the cultivation process, and assume that it satisfies a sinusoidal function with an unknown but almost constant angular frequency $\omega$ within a small time interval around the current time instant $t$, i.e.

$$\mu = M \sin(\omega t + \varphi) \quad (2)$$

where $M$ is the amplitude, and $\varphi$ is the phase angle. Then,

$$\dot{\mu} = \omega M \cos(\omega t + \varphi) = \gamma \quad (3)$$

and

$$\ddot{\mu} = -\omega^2 M \sin(\omega t + \varphi) = -\omega^2 \mu = \dot{\gamma} \quad (4)$$

By considering $\gamma$ and $\omega$ as additional states and assuming frequency $\omega$ to be approximately constant, the following adaptive state equation for $\mu$ can be obtained:

$$
\begin{bmatrix}
\dot{\mu} \\
\dot{\gamma} \\
\dot{\omega}
\end{bmatrix} =
\begin{bmatrix}
\gamma \\
-\omega^2 \mu \\
0
\end{bmatrix} +
\begin{bmatrix}
W_\mu \\
W_\gamma \\
W_\omega
\end{bmatrix}
$$

(5)
where $W_p, W_f,$ and $W_u$ represent system noises. Notice that by introducing the new state variable $\gamma$, the parameters $M$ and $\phi$ are eliminated in the adaptive state equation for $\mu$.

By incorporating biomass concentration, $X$, into equation (5), the main state equation is established:

$$
\begin{bmatrix}
\dot{X} \\
\dot{\mu} \\
\dot{\gamma} \\
\dot{\omega}
\end{bmatrix}
= 
\begin{bmatrix}
\mu X \\
\gamma \\
-\omega^2 \mu \\
0
\end{bmatrix}
+ 
\begin{bmatrix}
W_x \\
W_\mu \\
W_\gamma \\
W_\omega
\end{bmatrix}
\begin{bmatrix}
X \\
\mu \\
\gamma \\
\omega
\end{bmatrix}
+ 
\begin{bmatrix}
X_0 \\
\mu_0 \\
\gamma_0 \\
\omega_0
\end{bmatrix}
$$

(6)

According to the measurement model (eq. 1), the observation equation is set as:

$$
Y = I_L + V_t = \frac{I_o E_o}{X^*} \left(1 - e^{-E_o X^*}\right) + V_t
$$

(7)

In addition to the above state variables, the following dynamic models are employed as auxiliary equations to estimate the dissolved oxygen level ($O$) and nutrient level ($S$), specifically phosphate concentration. The model for dissolved oxygen was based on a dynamic mass balance as discussed elsewhere (Li et al., 2002), and the phosphate model was derived based on basic saturation-type uptake kinetics commonly seen in microalgal species (Nyholm 1977).

$$
\begin{bmatrix}
\dot{O} \\
\dot{S}
\end{bmatrix}
= 
\begin{bmatrix}
(R_{um} \mu - R_{omin}) X + k_i a (O^* - O) \\
- m_s \frac{S}{k_m + S} X
\end{bmatrix}
\begin{bmatrix}
O \\
S
\end{bmatrix} = 
\begin{bmatrix}
O_0 \\
S_0
\end{bmatrix}
$$

(8)

where $R_{um} = \frac{R_{vmax}}{\mu_{vmax}}, R_{omin}, k_i a, m_s, k_m$ and $O^*$ are constant parameters, and $\mu$ and $X$ are estimated by the EKF. The values of these parameters were either calculated ($O^*$), measured ($k_i a$), or fitted against two sets of experimental culture data under 450 $\mu$E·m$^{-2}$·s$^{-1}$ incident radiance, using Marquardt’s non-linear least squares method (Marquardt, 1963). To enable model fitting, we used the biomass data ($X$) from the two experiments, and calculated $\mu$ based on a second-order polynomial fit of the growth curves (i.e. $X$ vs. $t$), and the fitting results for $O$ and $S$ using equation (8) are shown in Fig. 45.
3. The values of the auxiliary model parameters are listed in Table 1. As to be discussed below, the poor model fitting of the dissolved oxygen data seen in Fig. 3 could be substantially improved by the EKF.

3.4. Formulation of the estimation model

Based on the main state model (i.e. eq. 6), an adaptive EKF estimation model can be derived as follows

\[
\begin{bmatrix}
\dot{\hat{X}} \\
\dot{\hat{\mu}} \\
\dot{\gamma} \\
\dot{\omega}
\end{bmatrix} =
\begin{bmatrix}
\hat{\mu}
\gamma
-\hat{\omega}^2
0
\end{bmatrix} + K [ Y - \hat{I}_L ]
\]

\[
E \begin{bmatrix}
\hat{X}_0 \\
E\mu_0 \\
E\gamma_0 \\
E\omega_0
\end{bmatrix} = [ EX_0 ]
\]

where

\[
\hat{I}_L = I_0 \frac{E_0}{\hat{X}_0} \left(1 - e^{-\hat{X}_0}\right)
\]

is the estimated local PPFFR, while

\[
K = PC^T R^{-1}
\]

is the Kalman filter gain matrix, and \(P\) is the covariance matrix of filtering error satisfying the following matrix Riccati equation:

\[
\dot{P} = AP + PA^T + Q - KCP, \quad P_{|t=0} = P_0
\]

where

\[
P = \begin{bmatrix}
P_X & P_{X\mu} & P_{X\gamma} & P_{X\omega} \\
P_{\mu X} & P_{\mu} & P_{\mu\gamma} & P_{\mu\omega} \\
P_{\gamma X} & P_{\gamma\mu} & P_{\gamma} & P_{\gamma\omega} \\
P_{\omega X} & P_{\omega\mu} & P_{\omega\gamma} & P_{\omega}
\end{bmatrix}, \quad P_{|t=0} = P_0
\]

\[
= \text{diag}[P_{X0}, P_{\mu0}, P_{\gamma0}, P_{\omega0}]
\]

\[
= \text{diag}[\text{Var}X_0, \text{Var}\mu_0, \text{Var}\gamma_0, \text{Var}\omega_0]
\]

\[
Q = \text{diag}[q_X, q_\mu, q_\gamma, q_\omega] = \text{diag}[\text{Var}W_X, \text{Var}W_\mu, \text{Var}W_\gamma, \text{Var}W_\omega]
\]

\[
R = \text{Var}V
\]
Furthermore, using the auxiliary equation (8) leads to the following estimation model for $O$ and $S$:

$$
\begin{bmatrix}
\hat{O} \\
\hat{S}
\end{bmatrix} = \begin{bmatrix}
(R_{\text{un}} \hat{\mu} - R_{\text{min}}) \hat{X} + k_i a (O^* - \hat{O}) \\
-m_x \frac{\hat{S}}{k_m + \hat{S}} \hat{X}
\end{bmatrix}, \quad \begin{bmatrix}
\hat{O} \\
\hat{S}
\end{bmatrix}_{r=0} = \begin{bmatrix}
O_0 \\
S_0
\end{bmatrix}
$$

(18)

It should be noted that $O$ and $S$ are not observable from $I_L$ measurement. Hence $O$ and $S$ were estimated using the auxiliary estimation model (eq. 8) with the EKF estimates of $X$ and $\mu$. In this study, $I_L$ (i.e. local PPFFR) was used as the online measured signal input for the state estimator. It is reasonable to include $I_L$ as one of the states in the system model provided an adequate model for $I_L$ is available. Here a dynamic state equation for $I_L$ can be conveniently derived based on the empirical measurement model (eq. 1). The measurement state $I_L$ could then be estimated by the EKF estimator. With this approach, it is conceivable to alleviate interferences such as modeling error associated with the measurement equation, random disturbance on $I_o$ due to ambient lights, and potential variation in the biomass light absorption efficiency resulted from different extents of pigmentation during the cultivation process. Alternatively, as in this study, $I_L$ was not included as one of the states in the system model. Rather, $I_L$ was estimated directly from the measurement model (eq. 10) using the optimal estimates of $X$. Since $X$ is observable through the measurement of $I_L$, as indicated in the measurement model, the difference between the measured and estimated $I_L$ could be used with the Kalman gain to generate optimal state estimates according to eq. (9). Here the measurement model is considered quite accurate, and the slight model error could be
partly compensated by the noise $V_I$ in eq. (15). By not including $I_L$ in the state vector the dimension of the state vector is reduced, resulting in simplified estimator computations.

3.5. Effect of Kalman filtering on state estimation

According to the measurement model, $I_L$ is directly related to $X$, and therefore $X$ and $\mu$ can be directly calculated from the measurement model without using EKF, given that $I_L$ is continuously monitored online. To determine whether state estimation can be improved by using the EKF-based estimator over the direct calculation from equation 1, the two approaches were compared and the results are presented in Fig. 4, using the experimental culture data obtained under 450 $\mu$E·m$^{-2}$·s$^{-1}$ incident radiance. The results revealed considerable improvement by using EKF over direct model calculation on the estimation of certain state variables, particularly $\mu$ and $O$. The estimation results from direct measurement model calculation are too noisy to convey any clear trend for $\mu$ and $O$. The superior performance of EKF primarily resulted from its filtering capacity. Given that with direct model calculation, estimation of all other states was based on the estimation of $X$, which in turn was based on the $I_L$ signals and the measurement model, the $I_L$ measurement noise was transmitted to the estimation of $X$ and subsequently propagated and amplified in the estimation of $\mu$ and $O$. Although simpler filtering algorithms such as the moving-average filter could also potentially reduce the estimation noise, they generally are less effective than EKF (Stephanopoulos and San 1984a, b). Moreover, estimation based on the moving-average filter is associated with time lag, which is not ideal for timely control.

3.6. State estimation of photobioreactor culture with constant incident illumination

Using model parameters in Table 1 and filter parameters determined based on the estimation of the experimental culture data under 450 $\mu$E·m$^{-2}$·s$^{-1}$ incident radiance, the estimation results for three additional illumination conditions (70, 100 and 300 $\mu$E·m$^{-2}$·s$^{-1}$) are presented in Fig. 5. In these experiments, the external illumination level was fixed throughout the cultivation (each lasted for about 2 days). The estimation results indicated effective tracking of all four culture states examined (i.e., $X$, $\mu$, $O$, and $S$). Among these
states, tracking of $X$ is most accurate. Since $X$ is included in the main state estimation vector, it is anticipated that probable modeling errors in depicting cell growth could be compensated by the EKF algorithm. The accurate tracking of $X$ also indicated that it is appropriate to use the adaptive internal model for $\mu$. EKF also helped to filter out the strong noise associated with the estimation of $\mu$, as indicated in Fig. 4 and discussed in section 3.5. The state estimator reported here does have certain limitation. The observability of the system is built on the relationship between $I_L$ and $X$, as indicated in the measurement model. Since $I_L$ decreases as $X$ increases during the culture, $I_L$ becomes less sensitive to $X$ at high biomass concentrations (Fig. 2). As a result, the system becomes less observable through measurement of $I_L$. The practical limit of biomass concentration within which the state estimator reported here would work depends on the cellular light absorption properties as well as $I_o$. As shown in Figs. 2 and 4, the state estimator worked well at biomass concentration as high as 2 g·L$^{-1}$ (dry cell weight) at a $I_o$ of 450 µE·m$^{-2}$·s$^{-1}$ for the *D. salina* culture.

As discussed earlier $O$ and $S$ are not observable from $I_L$ measurement, and hence these states were estimated using an auxiliary estimation model (eq. 18) with the EKF estimates of $X$ and $\mu$. As seen in Fig. 5, the estimation results of these two states generally agreed with the experimental data and only began to deviate from the data during the later stage of culture. This suggested that the model for $O$ and $S$ are reasonably accurate, since the model errors were not corrected by EKF as in the case of $X$. However, as the culture progressed, the errors existed in the model structure/parameter and in the initial state measurement/estimation accumulated, leading to the observed estimation discrepancy.

3.7. Filter tuning

An appropriate setting of the filter parameters, namely $P_0$, $Q$ and $R$, is essential to ensure the convergence of EKF simulation and the optimal estimation results. Equations (9)–(11) indicate that these three parameters influence the estimation result through the gain matrix $K$ which is proportional to the filtering error covariance matrix $P$ and inversely proportional to $R$. Thus, simultaneously increasing $P_0$ and $Q$ (and hence $P$) is
therefore equivalent to decreasing R. According to its definition, R can be roughly estimated by calculating the variance between a set of actual measurement data and a least square fitted polynomial curve (Wilson et al., 1998). For all of our estimation, we set R at 130 that is obtained from the batch cultivation data under 450 μE·m⁻²·s⁻¹ illumination. Simulation results showed no significant difference in the date range from R=30 to R=900, given that other parameters were fixed at appropriate values (data not shown). P₀ and Q were empirically determined by balancing between their effect on the effectiveness of state-tracking and their effect on the stability of estimation results. Since both P₀ and Q are positive related to the filter gain, if P₀ and Q are set to 0 or R is set to extremely large, the filtering error covariance matrix P and hence the filtering gain K keeps 0. Then, the IMEKF simply reduces to a pure internal model, both ̂μ and ̂γ vary sinusoidally with a constant frequency ω = ̂ω₀ and ̂X may seriously distorted (as shown in Fig. 7). While, if P₀ and Q are set extremely large, the EKF will become too sensitive to measurement noise and even unable to give stable estimate (as shown in Fig. 6). In general, both P₀ and Q may be diagonal matrices whose diagonal components represent the variances of the initial state values and variances of the system noises, respectively. Specifically, here pₓ₀ was estimated by considering the measurement error variance of the initial state value X₀, and qₓ was estimated by calculating the mean-square value of the deviation of the measurement data and their least square fitted 2nd or 3rd order polynomial for the whole cultivation process as its rough lower limit.

However, there were no directly measurable data available for μ, γ and ω, and thus Pₓ₀, Pᵧ₀, Pₓ₁₀, qₓ, qᵧ and qω were estimated by try-error process based on our first batch of experimental data under 450 μE·m⁻²·s⁻¹. All the final values of filter parameter are tabulated in Table 1 and the effects of those parameter values were illustrated in Fig. 6 and Fig. 7. Generally speaking, the bigger the initial filtering error variances P₀ and the system noise q, the quicker the estimates converge to their true values especially in case unbiased initial state estimates are set, while the more the estimates are being disturbed. It can be seen from the left column of Fig. 6 that in case of an under-estimated ̂μ₀ = 0.089, with the increment of Pₓ₀ from 0.0001 to 0.01, the estimate ̂μ and hence ̂O
converges to their corresponding curves obtained from a proper initial setting (i.e., \( \mu_0 = \mu_{\text{max}} = 0.119 \) and \( p_{\mu_0} = 0.0001 \)), although significant variation appears when \( p_{\mu_0} \) is larger. To isolate the effect of \( p_{\mu_0} \) from that of \( p_{\gamma_0} \) and \( p_{w_0} \), we set \( p_{\gamma_0} = p_{w_0} = 0 \).

Similarly, from the right column of Fig. 6 we see that in case of an under-estimated \( \hat{\gamma}_0 = -0.0025 \), with the increment of \( p_{\gamma_0} \) from \( 10^{-6} \) to \( 10^{-4} \), the estimate \( \hat{\gamma} \) and hence \( \hat{\mu} \) and \( \hat{O} \) converges to their corresponding curves obtained from a proper initial setting (i.e., \( \hat{\gamma}_0 = -\gamma_{\text{max}} = -0.002 \) and \( p_{\gamma_0} = 10^{-6} \)), although significant oscillatory variation also appears when \( p_{\gamma_0} \) is larger. Also, to isolate the effect of \( p_{\gamma_0} \) from that of \( p_{\mu_0} \) and \( p_{w_0} \), we set \( p_{\mu_0} = p_{w_0} = 0 \).

After the EKF parameters were determined based on the first set \( (I_0=450 \, \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}) \) of batch experimental data, they were applied to the on-line estimation of three batch culture processes under different illumination conditions (i.e., \( I_0=70, 200 \) and \( 300 \, \mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1} \)) as shown in Fig. 5. But because the initial conditions for each process were different, the initial values of the cultural states needed to be given individually for each estimator. The initial values of biomass \( X \), oxygen level \( O \) and phosphate concentration \( S \) could be obtained by relatively accurate measurements, while \( \mu, \gamma \) and \( \omega \) could not be directly measured.

To make a proper initial setting for \( \mu_0 \) and \( \gamma_0 \), a general guidance is given in Table 2 based on our experiences (Li et al., 2002). For a particular kind of algae cell, if its cultivation begins with very low biomass concentration (say, less than \( 0.01 \, \text{g}\cdot\text{L}^{-1} \) dry weight in our case) under constant incident light intensity \( I_0 \), then the cell grows at light saturation. In this case, it may be assumed that there exist certain thresholds for \( I_0 \) at which \( \mu_0 \) and \( \gamma_0 \) take their max. or zero values. For example, as the incident light intensity is strong enough (say, \( I_0 \geq I_{01} \)), the growth rate can start with its max. value and keep it for a certain period of time, so \( \mu_0 = \mu_{\text{max}} \) and \( \gamma_0 = 0 \). As the light intensity decreases down to a critical level \( I_0 = I_{02} (< I_{01}) \), the growth rate starts to reduce with the increasing of biomass at the max. slope, so \( \mu_0 = \mu_{\text{max}} \) and \( \gamma_0 = -\gamma_{\text{max}} \). Clearly, as the light
intensity furthermore decreases (i.e., $I_0 \to 0$), we finally have $\mu_0 \to 0$ and hence $\gamma_0 \to 0$. For the intermediate light intensity between 0 and $I_{02}$ or between $I_{02}$ and $I_{01}$, we may approximately infer that $\mu_0$ and $\gamma_0$ make corresponding linear transforms. For instance, as the light intensity becomes lower and lower than $I_{01}$, the growth rate reduces quicker and quicker from its max. value, thus $\mu_0 = \mu_{\text{max}}$ and $\gamma_0 = -\gamma_{\text{max}} (I_{01} - I_0) / (I_{01} - I_{02})$ may be chosen. Similarly, as the light intensity is within a range of $(0, I_{02})$, $\mu_0$ and $-\gamma_0$ vary respectively within the ranges $(0, \mu_{\text{max}})$ and $(0, \gamma_{\text{max}})$ at the same rate $I_0 / I_{02}$. In our case we took $I_{01} \approx 260$, $I_{02} \approx 200$, $\mu_{\text{max}} \approx 0.119$ (Li et al., 2002) and $\gamma_{\text{max}} \approx 0.002$, which is approximately estimated from the average decreasing rate of $\mu$ ($\mu_{\text{max}}/48$ hours $\approx 0.002$ h$^{-2}$).

As for the initial setting of $\omega_0$, by studying the properties of the dynamic curve of $\mu$ in Fig. 4a and fitting the curve with a sinusoidal function, a rough estimation of $\omega_0 \approx 0.001$ (rad·h$^{-1}$) can be obtained. We found that the estimation results are not so sensitive to the initial setting $\hat{\omega}_0$, since acceptable results were achieved for all estimates of $X$, $\mu$, $O$ and $S$, if $\hat{\omega}_0 \in (0.01, 0.0001)$ was chosen together with $p_{\omega_0} = 0.0001$ and $q_\omega = 0.000001$.

3.8. State estimation of photobioreactor culture with changing incident radiation

Outdoor microalgal cultures are commercially important and they are typically operated under diurnal cycles. It is hence of value to test whether the state estimator developed here can function well under conditions of time-variant incident radiation. Because the local irradiance level changes synchronously with the level of incident radiation as indicated in the measurement model, the same state estimator structure described above can be used in conditions of time-variant incident radiation. However, the filter parameters need to be retuned, and the initial values of certain states (i.e. $\mu$, $\gamma$, and $\omega$) need to be reset to suit the time-varying radiant conditions. In this case, the variances $p_{\xi_0} = 10^{-6}$, $p_{\mu_0} = 10^{-7}$ and $q_\xi = 10^{-5}$, $q_\mu = 10^{-7}$ were set lower, while $p_{\omega_0} = 0.01$, $q_\gamma = 10^{-7}$, $q_\omega = 0.001$ and $R = 700$ were set higher, compared with the
respective values used in the case of fixed incident radiation. Unlike the situations in which $I_0$ was fixed, here $I_0$ increased from a very low level at the onset of the cultivation. Therefore, a smaller initial value was chosen for $\mu (\hat{\mu}_0 = 0.08)$, and a positive initial value was chosen for its changing rate $\gamma (\hat{\gamma}_0 = 0.005)$, while a large initial value was chosen for the frequency $\omega (\hat{\omega}_0 = 0.05)$. A typical set of state estimation results under time-varying $I_0$ is presented in Fig. 8. The estimator was able to truthfully track the biomass and phosphate concentration and correctly predict the specific growth rate. The estimation of dissolved oxygen concentration deviated substantially from the measurement although they did share a similar trend. The less than satisfactory estimation performance on $O$ might be explained by the following two plausible factors. First, the simple conservation balance model for $O$ may not be able to accurately express the actual physiological response of the cells upon rapid changes of incident light radiation. Second, $O$ was not directly observable from $I_L$ measurement and its estimation was based on the direct integration of the corresponding dynamic mass balance with the filtered estimates of $X$ and $\mu$. As such, model errors could not be compensated by the filter, leading to accumulation of modeling errors as discussed in the preceding sections. Estimation of $O$ might be improved by incorporating additional measurement variable(s) with which $O$ becomes directly observable.

4. Conclusion

A state estimator was developed in this work capable of effectively estimating a number of culture states in microalgal photobioreactors, including $X$, $\mu$, $O$, and $S$, from the online measurement of local PPFFR (i.e. $I_L$) in the reactor, under constant or time-varying incident radiation conditions. Submersible quantum sensor was shown to give reliable continuous measurement of $I_L$. The biomass concentration was observable through the measurement of $I_L$ and an adaptive internal model was shown to provide good estimates of the specific growth rate. For the adaptive model to work properly, having certain knowledge a priori about the overall dynamics of the growth rate would be useful in setting the appropriate initial values of the state variables (i.e. $\mu$, $\gamma$, and $\omega$) implicated in the adaptive model. With the general applicability of submersible quantum
sensors in microalgal bioreactor cultures, and the generic nature of the state models, the
state estimation system developed here is expected to be useful for monitoring a wide
range of phototrophic microalgal processes.

Acknowledgement

The *D. Salina* strain was obtained from Dr. Anastasios Melis at the University of
California, Berkeley. This work was supported by the NSF ERC Program. Contract grant
number: EEC-9731725.

Nomenclature

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>matrix defined in Eq. (16)</td>
</tr>
<tr>
<td>C</td>
<td>matrix for calculating the filtering gain, defined in Eq. (17)</td>
</tr>
<tr>
<td>$E_o$</td>
<td>parameter in the light model (Eq. 1) $(g^n \cdot L^{-n})$</td>
</tr>
<tr>
<td>$E_i$</td>
<td>parameter in the light model (Eq. 1) $(L^n \cdot g^{-n})$</td>
</tr>
<tr>
<td>$I_L$</td>
<td>local irradiance light intensity in the culture $(\mu E \cdot m^{-2} \cdot s^{-1})$</td>
</tr>
<tr>
<td>$I_0$</td>
<td>incident light intensity on the reactor external surface $(\mu E \cdot m^{-2} \cdot s^{-1})$</td>
</tr>
<tr>
<td>$K$</td>
<td>Kalman filtering gain matrix defined in Eq. (11)</td>
</tr>
<tr>
<td>$k_{pa}$</td>
<td>volumetric oxygen mass transfer coefficient $(h^{-1})$</td>
</tr>
<tr>
<td>$k_m$</td>
<td>constant in Eq. (8) $(mg \cdot L^{-1})$</td>
</tr>
<tr>
<td>$M$</td>
<td>amplitude in Eq. (2)</td>
</tr>
<tr>
<td>$m_s$</td>
<td>max. specific phosphate uptake rate</td>
</tr>
<tr>
<td>$n$</td>
<td>parameter in the light model (Eq. 1)</td>
</tr>
<tr>
<td>$O$</td>
<td>dissolved oxygen concentration $(mg \cdot L^{-1})$</td>
</tr>
<tr>
<td>$O^*$</td>
<td>saturated oxygen concentration in the media $(mg \cdot L^{-1})$</td>
</tr>
<tr>
<td>$P$</td>
<td>covariance matrix of state estimation error</td>
</tr>
<tr>
<td>$p_{\xi}$</td>
<td>variance of state variable $\xi$</td>
</tr>
<tr>
<td>$p_{\xi\xi}$</td>
<td>covariance between state variables $\xi$ and $\zeta$</td>
</tr>
<tr>
<td>$Q$</td>
<td>covariance matrix of system noises</td>
</tr>
<tr>
<td>$q_{\xi}$</td>
<td>variance of system noise $W_\xi$</td>
</tr>
</tbody>
</table>
\( R \): covariance matrix of measurement noises \((\mu E^2 \cdot m^{-4} \cdot s^{-2})\)

\( R_{o_{\text{max}}} \): max. oxygen generation rate \((\text{mg} \cdot \text{g}^{-1} \cdot \text{h}^{-1})\)

\( R_{o_{\text{min}}} \): equivalent to the specific respiration rate \((\text{mg} \cdot \text{g}^{-1} \cdot \text{h}^{-1})\)

\( R_{\text{am}} \): ratio between max. \( O_2 \) generation rate and \( \mu_{\text{max}} \) \((\text{mg} \cdot \text{g}^{-1})\)

\( S \): phosphate concentration \((\text{mg} \cdot \text{L}^{-1})\)

\( t \): time \((\text{h})\)

\( Var \): variance

\( V_I \): measurement noise of \( I \) \((\mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1})\)

\( W_\xi \): system noise of state variable \( \xi \)

\( X \): cell density (cell dry weight) \((\text{g} \cdot \text{L}^{-1})\)

\( Y_I \): measurement of \( I \) \((\mu \text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1})\)

\( \gamma \): changing rate of specific growth rate \((\text{h}^{-2})\)

\( \gamma_{\text{max}} \): max. changing rate of specific growth rate \((\text{h}^{-2})\)

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

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

\( \omega \): instantaneous angular frequency of \( \mu \) variation \((\text{rad} \cdot \text{h}^{-1})\)

\( \varphi \): phase angle in Eq. (2) \((\text{rad})\)

\( \hat{\xi} \): estimate of state variable \( \xi \)

\( \xi_0 \): initial value of state variable \( \xi \)

References


Sphärischer Mikro Quantumsensor

WALZ
Mess- und Regeltechnik

SQS

4 - 393 - 094
Fig 2. Empirical relationship between local irradiance level and biomass concentration. Legend: experimental data set A (○), experimental data set B (●), fitted curve (—).
Fig 3. Model fitting of two batch experiments under 450 μE m\(^{-2}\) s\(^{-1}\).
Legend: experimental biomass data or phosphate data (O), experimental dissolved oxygen data (---), fitted model curve (--).
Fig 4. Effects of the EKF. The left column is EKF estimates, and the right is direct calculation results.
Fig 5. Sensor performance under different light conditions
Fig 6. Effects of initial values of states and Po.
Fig 7. Effects of $P_0$ and $Q$ on estimation

1. $P_0$, $Q$ increased or $R$ decreased by 100 times
2. $P_0$, $Q$ and $R$ normal as shown
3. $P_0$, $Q = 0$ or $R$ infinite
Fig 8. Sensor performance under changing light conditions
Table 1. Model parameters

<table>
<thead>
<tr>
<th>Parameter symbol</th>
<th>Parameter value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$R_{um}$</td>
<td>1147 mg g$^{-1}$</td>
</tr>
<tr>
<td>$R_{omin}$</td>
<td>7.308 mg g$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$k_{a}$</td>
<td>13.50 h$^{-1}$</td>
</tr>
<tr>
<td>$m_{s}$</td>
<td>0.844 mg g$^{-1}$ h$^{-1}$</td>
</tr>
<tr>
<td>$k_{m}$</td>
<td>5.100 mg L$^{-1}$</td>
</tr>
<tr>
<td>$O^{*}$</td>
<td>5.376 mg L$^{-1}$</td>
</tr>
<tr>
<td>$E_0$</td>
<td>0.749 g L$^{0.925 - 0.925}$</td>
</tr>
<tr>
<td>$E_1$</td>
<td>3.873 L$^{0.925 - 0.925}$</td>
</tr>
<tr>
<td>$n$</td>
<td>0.925</td>
</tr>
</tbody>
</table>
Chapter 4. Conclusions and Recommendations

4.1 Comparison between the two sensing approaches

Both sensors, either the one based on dissolved oxygen measurement or the one based on local irradiance level measurement, were proved to be able to track the major photobioreactor states under constant or varying incident radiant conditions. The former sensor can on-line sense biomass concentration, dissolved oxygen level, and average light intensity of microalgal cultures grown in a stirred-tank photobioreactor. The latter sensor outputs biomass concentration, specific growth rate, dissolved oxygen level, and phosphate concentration. Both kinds of sensors can track biomass concentration accurately, while the first group outperformed the second one in terms of estimating dissolved oxygen level.

The two kinds of software sensors are based on different dynamic models. An unstructured process model coupled with a semi-empirical light model specifically for the stirred-tank photobioreactor was developed and utilized for the oxygen measurement sensor. The model might not be able to be applied to other photobioreactor systems. Thus to apply this approach, new specific photobioreactor models would in most cases be required. This requirement would limit the applicability of the approach due to the strenuous and time-consuming model developing processes. A black box model composed by sinusoidal functions was developed and termed the internal model. The model is only based on the slowly time varying characteristics of specific growth rate of microalgae, but not on the specific photobioreactor systems. Therefore it should be applied to other systems cultivating microalgae. An observation equation was required to be established to realize on-line estimation based on local irradiance measurement. Usually the observation equation can be easily obtained by empirical fitting of off-line biomass concentration and local irradiance level data.

The two kinds on-line estimators utilized different hardware sensors for actual measurement inputs. The hardware for the first approach is oxygen sensor, and the
irradiance sensor for the second approach. Both sensors are readily available and affordable, while the maintenance cost of oxygen sensor is much more than irradiance sensors. In addition, drifting is a serious problem for oxygen sensors. In terms of observability, irradiance sensors might be only good enough for a narrow range of biomass concentrations, while oxygen sensors are indifferent to biomass concentrations.

4.2 Roles of the extended Kalman filter

The extended Kalman filter worked as optimizing algorithms for both sensors. The necessity of the filter was undoubtedly proved in both cases. The oxygen sensor alone could give any information about the state parameters other than dissolved oxygen level. On the other hand, although the dynamic model could predict major cultural states, the predicted results based on model alone deviated substantially from real measurements especially at long cultivation conditions. The extended Kalman filter reconstructed the state by computing the weighted average of the measurements and model output. For the irradiance measurement based estimators, the state could be identified solely based on the measurements. In this case the extended Kalman filter was still necessary and the best choice among various of filter algorithms in order to reduce the noise and timely sense the state variables. In the second cases, the extended Kalman filter was almost indispensable for the software sensing system to predict the specific growth rate and oxygen level. Without the filter, the prediction of those parameters solely based on model was too noisy to provide, if any, system information. Again, other filters might be also able to reduce the noise substantially, but the timely response of the sensor might also be delayed, for example, in the case of moving average filter.

4.3 Quantum sensors

Two kinds of quantum sensors used in the study have different geometrical shapes and physical properties. The flat sensor measures the photosynthetic photon flux density (PPFD) which is defined as the number of PAR photons incident per unit time on a unit surface, While the sensors with a spherical shape measure the so called Photosynthetic
Photo Flux Fluence Rate (PPFFR), which is the integral of photon flux radiance at a point over all directions. The former sensors are suitable to quantify the incident radiation on the cultural surface, namely external irradiance, to measure the overall energy supply of the photobioreactor and the latter are able to measure the level of scalar irradiance inside of culture to quantify the actual photo flux available to cell at certain point inside of the photobioreactor.

4.4 Application of the sensing approach in production systems

It is still not common to monitor either oxygen level or irradiance level in large-scale outdoor photobioreactors for production purposes. In order to realize the on-line estimation approach, at least one of the two sensors is required to be installed. For the first approach, a specific system model for outdoor reactors is needed. While, this is not necessary for the second approach although an empirical relationship between biomass and irradiance level is necessary to be established. The latter can be easily done by empirically fitting off-line historical biomass and irradiance level measurement data. With regard to the maintenance of the estimators, the irradiance based one also outdoes the oxygen based one because of the easier maintenance of irradiance hardware sensors.

For outdoor systems and Due to the diurnal change and daily variation of solar light, at least two sensor need to be installed to effectively sensing culture states. One sensor senses the incoming solar energy at the surface of the photobioreactor system, and the other one can be put under water. The exact position of the sensors would be determined individually based on the systems. Devices might be needed to overcome the difficulties caused by the fact that solar light changed directions. The installation position would also affect the range of the cultural parameters that the sensor can estimate.

Based on the sensing results, appropriate manual or automatic control measures can be taken to either enhance the productivity. The irradiance level, the pH of the culture, the substrate level of the culture and the mass transfer rate of the system are commonly used control parameters. It is known that high irradiance level and oxygen level harm the
microalgal growth. When the sensor senses very high irradiance level, a sunscreen can be used to cover the photobioreactor. When the oxygen level is high, more air can be blew into the bioreactor to strip oxygen. Of course, nutrients can be added according the sensing results to ensure the optimal nutrient level. In addition, at industrial level, the control of pH by bubbling Carbon dioxide can roughly realized by an on-off valve and a pH meter. The effects of pH on the microalgal physiological states can be readily measured by software sensors.

4.5 Recommended research topics

The sensor based on both oxygen level and irradiance level measurements is expected to be able to outperform the both sensors developed in this research. Software sensing system with double hardware will definitely increase the cost, but it might be necessary when high sensing precision and reliability are desirable.

The tuning of the extend Kalman filter represents a difficulty in realizing the filtering. A systematic examination of theories and experiences of the tuning of the filter applied to bioreactor systems will facilitate the application of the on-line estimation approach.

The radiant model has been of central importance in the effort of modeling photobioreactors for decades. The irradiance distribution inside bioreactors can still not be satisfactorily mechanistically explained and mathematically described. Further mechanism modeling is still an intriguing topic.

The relationship between irradiance level and the pigment content can be further quantified. It is well know that the pigment level changes with the illumination level in microalgae, but no quantitative relationship between the two factors has been reported.
Appendix 1. A representative Matlab program realizing the simulation of software sensing

The main program

```matlab
% File name: mainIo_B_1e_2b_r.m (Io=200) (043002)
% The main program for Microalgal estimation system for model version 1e using Ie % instead of Ia
% The estimation is implemented via EKF & internal model approach (Io is % generated under 6 line-type lamp around) using subroutines: "dk_B_1a_2r.m" & "dk_B_1e_2r.m"
% The code is written by Ningshou Xu

clear;

% TOM=[Ie O t] % original experimental data set
% stored in files "TOM_Ie_O_t_2b.m" & "C:/matlab l l/work/Alg_TOM_Ie_O_t_2b.mat"
load Alg_TOM_Ie_O_t_2b;
load t_X_uu_2b;
Ioo=200;

% JOHN is the manually measured data set
% JOHN=[t X S Ioo]
JOHN=[
  0 0.0808 33.9495 Ioo
  5 0.1014 33.8389 Ioo
  8.5 0.1312 33.0644 Ioo
 20.0 0.3283 30.6304 Ioo
 24.0 0.4566 29.0815 Ioo
 27.0 0.5417 28.4176 Ioo
 31.5 0.6806 26.6474 Ioo
 43.5 1.0493 22.8857 Ioo
 48.0 1.1060 21.6687 Ioo
];

% Xle=[X Ie]
Xle=[
  0.103514 466
  0.190721 354
  0.34032 262
  0.57429 205
  0.82244 162
  1.032304 136
  1.24784 113
];

% calculate the length of experiment data
len=length(t_X_uu)-1;

EO=0.7487; E1=3.873; na=0.925; %--- #450r (1a,1b)
Rum=1147.36; Romin=7.3075; kla=13.5; Os=5.376; % from 1a,1b (-9)
Yxs=10000; ms=0.8444; km=5.1; % from 1a,1b

pr=[EO E1 na];
pr2=[Rum Romin kla Os Yxs ms km];
% q1=[Qx Qu Qrg Qw]; r1=VarVi;
q1=[10^(-3) 10^(-5) 10^(-8) 10^(-6)];
```
r1=130; %---450r (-9r)

X0=JOHN(1,2);

u0=0.119; %umax=0.119
rg0=-2*10^(-3);
w0=10^(-2);

O0=TOM(1,2); S0=JOHN(1,3); %exact initial conditions
le(1)=loo*E0*(1-exp(-E1*X0'na))/X0'na;

%initialize
Zo=[X0 u0 rg0 w0];

ZO=Zo;

VarX0=0.01;

Varu0=10^(-4);

Varrg0=10^(-6);

Varw0=10^(-4);

%normal setting of initial state variances

Z1=[P10

% X0 u0 rg0 w0]

Z1=[VarX0 0 0 0 ...

Varu0 0 0 ...

Varrg0 0 ...

Varw0 ...]

Z0);

tt(1)=0;
ty(1,:)=Z1;

Z2=[O0 S0];

tt2(1)=0;
ty2(1,:)=Z2;

h=1/60;

for i=1:len-1

%call 'dk_B_1a_2r' function implementing hybrid Extended Kalman Filter
[tl, yl]=ode45('dk_B_1a_2r', [TOM(i,3) TOM(i+1,3)], Z1, [], ...

[TOM(i,3) TOM(i+1,3) TOM(i,1) TOM(i+1,1) i i00 pr q1 r1]);

k1=length(tl);

if yl(k1,14)<0

yl(k1,14)=0;
end

%save data

tti(i+1)=ti(k1);
ty(i+1,:)=yl(k1,:);

Z1=yl(k1,:);

X=ty(i+1,11);

dX=(ty(i+1,11)-ty(i,11))/h; ux(i+1)=dX/X;

le(i+1)=loo*E0*(1-exp(-E1*X'na))/X'na;

%call 'dk_B_1e_2r' function implementing estimation of O and S

[t2, y2]=ode45('dk_B_1e_2r', [TOM(i,3) TOM(i+1,3)], Z2, [], ...

[TOM(i,3) TOM(i+1,3) y(i,11) y(i+1,11) ...]
k2 = length(t2);
t2(i+1) = t2(k2);
ty2(i+1,:) = y2(k2,:);
Z2 = y2(k2,:);

if mod(i+1,20) == 0
  i+1
end
end

L = 300; h = 0.01;
X1(1) = 0; r = (h/100)*na;
le1(1) = 100*(E0/r)*(1-exp(-E1*r));
for i = 2:L
  X1(i) = (i-1)*h;
r = X1(i)*na;
le1(i) = 100*(E0/r)*(1-exp(-E1*r));
end

disp('Show the result of B_1 e_2b')

% plot the results.
subplot(4,2,1);
plot(JOHN(:,1),JOHN(:,2), 'o', tt, ty(:,11));
title ('VarXo=0.01/Qx=10^-3(Io=200)');
ylabel('X');

subplot(4,2,2);
plot(tt, ty(:,12));
title ('Var\mu=10^-4/Q\mu=10^-5');
ylabel(\mu');

subplot(4,2,3);
plot(tt, ty(:,13));
title ('Var\gammao=10^-6/Q\gamma=10^-8');
ylabel(\gamma'');

subplot(4,2,4);
plot(tt, ty(:,14));
title ('Var\omegao=10^-4/Q\omega=10^-6');
ylabel(\omega');

subplot(4,2,5);
plot(TOM(:,3), TOM(:,2), 'g', tt2, ty2(:,1));
ylabel('O');

subplot(4,2,6);
plot(tt2, ty2(:,2), JOHN(:,1), JOHN(:,3), 'o');
xlabel('time (h)');
ylabel('S');

subplot(4,2,7);
plot(JOHN(:,1), JOHN(:,4), 'r', tt, TOM(:,1), tt, le, 'g');
title ('Rvi=130'; 'Rvi=450r (-9r')
xlabel('time (h)');
ylabel(le, Io');

76
Subroutine 1

function dy1=dk_B_1a_2r(t,y1,init,fTro)

%initial values
tr = (t-fTro(1))/(fTro(2)-fTro(1));
Iy = fTro(3)+(fTro(4)-fTro(3))*tr;
i = fTro(5);
Io=fTro(6); E0=fTro(7); E1=fTro(8); na=fTro(9); Qx=fTro(10);
Qu=fTro(11); Qr=fTro(12); Qw=fTro(13); Rv1=fTro(14);

X = y1(11); %4 states
u = y1(12);
r = y1(13);
w = y1(14);

%Q1=diag[Qx Qu Qr Qw];
Q1=diag([Qx Qu Qr Qw]);

% calculate A and C
xe=10^(-15);
if X>=xe
   Xn=X*na;
else
   Xn=(xe)^na;
end
erx=exp(-E1*Xn);
rkx=E0*(1-erx)/Xn;
%--- when na=1 may not be true
%erx=exp(-E1*X);
%rkx=(E0/X)*(1-erx);
%--- when na=1 is true

F11=u; F12=X; F13=0; F14=0;
F21=0; F22=0; F23=1; F24=0;
F31=0; F32=-w^2; F33=0; F34=-2*w*u;
F41=0; F42=0; F43=0; F44=0;

A1=[F11 F12 F13 F14
  F21 F22 F23 F24
  F31 F32 F33 F34
  F41 F42 F43 F44];

% when choosing O as the 1st measured data
C11=Io*na*(E0*E1*erx-rkx)/X;
C12=0; C13=0; C14=0;
C1=[C11 C12 C13 C14];

PR1=[y1(1) y1(2) y1(3) y1(4)
y1(2) y1(5) y1(6) y1(7)
y1(3) y1(6) y1(8) y1(9)
y1(4) y1(7) y1(9) y1(10)];

KG1=PR1*C1*inv(Rv1);
DPR1=PR1*A1'+A1*PR1-KG1*C1*PR1+Q1;
FX1=[u*X
    rg
    -w^2*u
    0];

le=Io*rkx;

% pause;

%DXK1=[DXK1(1); DXK1(2); DXK1(3); DXK1(4)]
% when choosing le as the 1st measured data
DXK1=FX1+KG1*[Iy-le];

dy1=[DPR1(1); DPR1(2); DPR1(3); DPR1(4); ...
    DPR1(6); DPR1(7); DPR1(8); ...
    DPR1(11); DPR1(12); ...
    DPR1(16); ...]
    DXK1(1); DXK1(2); DXK1(3); DXK1(4)];

Subroutine 2

function dy2=dk_B_2e_2r(t,y2,init,ftro)

% initial values
tr = (t-ftro(1))/(ftro(2)-ftro(1));
X = ftro(3)+(ftro(4)-ftro(3))*tr;
u = ftro(5)+(ftro(6)-ftro(5))*tr;
i = ftro(7);
Rum=ftro(8);
Romin=ftro(9); kla=ftro(10); Os=ftro(11);
Yxs=ftro(12); ms=ftro(13); km=ftro(14);

O = y2(1);
S = y2(2);

RR=Rum*u-Romin; RRX=RR*X; kso=kla*(Os-O);
% [u RR X RRX O kso]
% pause;
uYm=-(uiYxs+ms*(S/(km+S)));

dy2=[RRX+kso
    uYm*X];