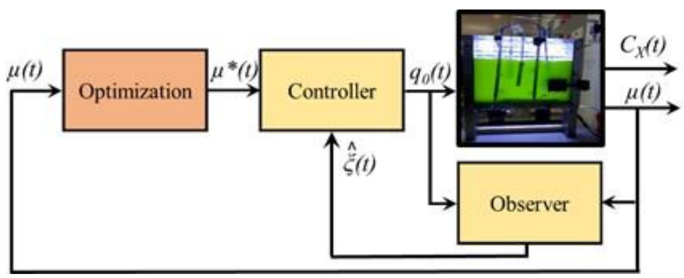

Application of strategies of advanced control under Active Disturbance Rejection Control, to produce lipids from microalgae

UNIVERSIDAD DE LA SABANA

BRIEF DESCRIPTION

This thesis proposes the application of an Active Disturbance Rejection Control (ADRC), an off-line optimization, and Model-Free Control (MFC) with on-line optimization strategies to microalgae bioprocessing. Compared to other control strategies, ADRC is less dependent of the information given by the model, and it allows working with an approximate model that only requires the knowledge of the order of the system and its gain. While using MFC and heuristic optimization, the need of any mathematical model disappears. Other advantages of ADRC and MFC are a straightforward design, an effective linear approach, and high robustness. These advantages allow working with highly uncertain plant models.

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BIOSCIENCE PhD PROGRAM

APPLICATION OF STRATEGIES OF ADVANCED CONTROL UNDER THE ACTIVE DISTURBANCE REJECTION CONTROL, TO PRODUCE LIPIDS FROM MICROALGAE

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BIOSCIENCE PhD PROGRAM
UNIVERSIDAD DE LA SABANA
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To my beloved son Daniel Camilo.

To my family for their understanding and support during these four years.

In memory of my best friend, Ruth Gisela Reina Hernández, you left us very soon.

To you

Acknowledgments

It is with great pleasure that I sincerely thank all the people who contributed to the realization of this doctoral thesis.

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I'd like to thank Universidad de La Sabana, especially the DIN for financing the development of the ING-140-2013 "Aplicación de técnicas de control proporcional integral generalizado al crecimiento de la microalga *Scenedesmus obliquus*" project. Additionally, I would like to thank COLCIENCIAS for the "Doctorado Nacional-617" and the Canadian Government ELAP (Emerging Leaders in the Americas Program) Scholarship given to me.

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Last but not least, I want to thank God and dedicate this work to my family whom I love infinitely. Hermencia and Gustavo, because I had the fortune of having them as my guide and parents, they are my strength, my joy, but more than anything else they give me the most perfect and unconditional love. To my sisters, Rocio del Pilar and Luz Adriana, to my brother, Gustavo Ernesto, because they were always there encouraging me to go ahead and supporting me unconditionally. To my brothers-in-law, Manuel and Igal, my sister-in-law María Angélica, my godparents, Alfredo and Alcira, and my aunt Isabel, for their interest in this work and in my well-being. To my son, Daniel Camilo, who is the engine, the force and the thrust of my life. To each one of you thanks for your love and great dose of patience.

In general, I want to thank all those who had me in their hearts and for their acts of support were always present to give me the necessary strength to continue this wonderful work. Truly, thank you all, this was another magnificent and enriching experience.

Abstract

In this research, advanced control strategies were designed under the Active Disturbance Rejection Control (ADRC) approach to increase the biomass production in microalgae cultures. For the above, from a control frame of reference, the development was envisaged into two stages, control and optimization. The first stage resulted in three different controllers designs: two ADRC strategies assisted by observer and a Model-Free Control (MFC). In each case, the aim was to guarantee the tracking of the reference signal. In the second stage, the design of two optimization strategies were achieves to increase the biomass production, off-line and on-line. Comparing, at a simulation level, these strategies with other existing proposals, the following was found: 1) the ADRC strategies assisted by observer had a few dependence on the model, letting us to work with an approximate model that only required knowing of the system order and the input gain; 2) the off-line optimization, despite maximizing the biomass production, required knowing the model and 3) the proposal that combines MFC with on-line optimization, may act on any microalgae culture since it does not need a model. All the proposals are robust front to disturbances and variation of parameters allowing to increase the biomass production when an optimization strategy is used.

Keywords: Active Disturbance Rejection Control, Model-Free Control, optimization, nonlinear system, microalgae culture, biomass production

Resumen

En esta investigación se diseñaron estrategias de control avanzado bajo el enfoque del rechazo activo de perturbaciones (ADRC, Active Disturbance Rejection Control) para incrementar la producción de biomasa en cultivos de microalgas. Para lo anterior, desde el punto de vista del control, esta investigación se planeó en dos etapas: control y optimización. La primera etapa resultó en tres diseños diferentes de controladores: dos estrategias ADRC asistida por observador y un control libre de modelo (MFC, Model-Free Control). En cada caso, el objetivo fue garantizar el seguimiento de la señal de referencia. En la segunda etapa, se realizaron dos diseños de estrategias de optimización con el fin de incrementar la producción de biomasa, una fuera de línea y una en línea. Al comparar, a nivel de simulación, estas estrategias con otras propuestas ya existentes, se encontró que: 1) las estrategias ADRC asistidas por observador tienen poca dependencia del modelo, permitiendo trabajar con un modelo aproximado que solo requiere conocer el orden del sistema y la ganancia de entrada; 2) la optimización fuera de línea aunque logra maximizar la producción de biomasa requiere conocer el modelo y 3) la propuesta que combina MFC con la optimización en línea, puede actuar sobre cualquier cultivo de microalgas ya que no necesita de un modelo. Todas las propuestas son robustas frente a perturbaciones permitiendo incrementar la producción de biomasa cuando se hace uso de una estrategia de optimización.

Palabras clave: Control de rechazo activo de perturbaciones, control libre de modelo, optimización, sistema no lineal, cultivo de microalgas, producción de biomasa

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CHAPTER 1. INTRODUCTION

Control Engineering plays a fundamental role in modern technological systems. This has been motivated by the fact that many real-world control problems involve nonlinear effect (Goodwin, Graebe, & Salgado, 2000), which require control systems to deal with changes in the operating conditions of the plant. Some examples of nonlinear systems are: braking systems in sport motorcycles (Formentin, De Filippi, Tanelli, & Savaresi, 2010), helicopter system (Boubakir, Labiod, Boudjema, & Plestan, 2014), in the medical area in the regulation of glycemia of Type-1 Diabetes (MohammadRidha & Moog, 2015), and in general biological processes as the based-on microalgae (Bernard, 2011).

Microalgae are unicellular organisms that have become a new natural resource with great potential and of high interest for the industry as for the research centers (Ben-Amotz, 2008; Bernard, 2011). The processes based on microalgae imply new challenges in both modeling and control, owed to their non-linear and time-varying behavior mainly due to the attenuation of light given by cell growth (Abdollahi & Dubljevic, 2012; Bernard, 2011). The determination of the process variables that help increase biomass and metabolite production is a more elaborate control problem and has not been fully explored (Vonshak & Torzillo, 2004). It is possible to establish that the composition of the biomass, the total specific growth rate and the formation of metabolites as the lipids, depend strongly of the culture conditions as: temperature, pH-CO_2, stirring, light/dark cycles and the most important: light intensity. See Table A.1.

Therefore, to increase production it is possible handle the problem with a control strategy, where the variable to be maximized is the reference signal. An appropriate optimization of the reference signal and an adequate control of the process will guarantee an increase of the biomass production. There are many proposals that involve the use of different strategies of advanced control used in microalgae cultures, see Table A.2. In most of these cases, it is necessary the knowledge of a precise model that can lead to an inadequate management of uncertainties. Additionally, those proposals imply greater complexity in the tuning and implementation of the controller. Therefore, the admission of a certain level of uncertainty in the system parameters cannot be guaranteed, and in this case, the performance of the control will not be satisfactory (Fliess & Join, 2013; Gao, 2006; Mandonski, Gao, Lakomy, 2015). In terms of optimization, all proposals have high dependence of the information given by the model, some examples are shown in the Table A.3.

This poses the following question: *Is it possible to guarantee the increase of lipid production in microalgae by the application of advanced control strategies based on the active disturbance rejection?* To answer this question, the physical variables that affect the microalgae were studied; the most relevant was chosen: the intensity of the light. Subsequently, different existing models were reviewed, one was chosen for batch cultures and another for continuous cultures. With these models, different control designs were made using strategies based on the disturbance rejection. Additionally, several optimization forms were proposed, always looking for a minimum dependence on the model.

As a results of this thesis, two Active Disturbance Rejection Control (ADRC), an off-line optimization and Model-Free Control (MFC) with on-line optimization strategies to the microalgae bioprocess, were proposed. Compared to other control strategies, ADRC is less dependent of the information given by the model, and it allows working with an approximate model that only requires the knowledge of the order of the system and its input gain. While using MFC and heuristic optimization, the need of any mathematical model disappears. Other advantages of ADRC and MFC are a straightforward design, an effective linear approach, and high robustness. These advantages allow working with highly uncertain system models.

This thesis is organized in six chapters. The first chapter offers a brief presentation of the state of the art and the research question. Chapter 2 presents the objectives of the study and its relationship with each of the written articles, also, the contribution of each author. Chapter 3 contains the results obtained during the development of this research, presented as articles. Next in the chapter 4, a discussion of results is presented. Then, conclusion and future work are outlined in Chapter 5. Finally, the annexes are in Chapter 6.

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CHAPTER 2. OBJECTIVES

2.1 General Objective

Design and apply strategies of advanced control under the approach of Active Disturbance Rejection Control to increase lipid production in microalgae cultures.

2.2 Specific Objectives

- Design and apply strategies of Active Disturbance Rejection Control based on dynamic models representative of the behavior associated with the growth of microalgae
- Propose at least one simplified model based on existing models suitable for the application of strategies of Active Disturbance Rejection Control associated to the growth of microalgae
- Propose an optimal control problem based on Active Disturbance Rejection Control with the lipid optimization process
- Establish a methodology for the application of Active Disturbance Rejection Control techniques for microalgae cultures
- Evaluate the performance of the bioprocess control system with real experimental conditions

2.3 Objectives-articles relationship

The objectives of this thesis, its relationship with each of the sections of chapter 3 and the published papers derived from this work are presented in Fig 2.1.

The first article, offers a proposal to control the intensity of light applied to a batch culture, using the strategy of Active Disturbance Rejection Control (ADRC) with the goal of increasing biomass concentration (C_X). Additionally, the total specific growth rate ($\mu(t)$) was established as reference. The optimal reference signal, μ_{opt}, was obtained from the one Haldane type model.

In the second article, an ADRC approaches the dilution rate (D) in a continuous culture of microalgae *Chlorella vulgaris*, to attain reference biomass (C_X^*) was proposed. Hence, the plant model was solved under steady state conditions to find the values of productivity ($P(t)$) for different light intensities. The reference was chosen as the practically achievable value that allowed the highest productivity.

These two articles are the result of the work done to achieve the first two specific objectives. The articles are presented in 3.1 and 3.2 sections.

The third article presents a method to find an optimal decision curve to manage the incident light intensity ($q_0(t)$) that is applied to a batch microalgae cultivation to maximize C_X. This is an off-line optimization strategy, in other words an optimal value is determined a priori.

Next, the fourth article shows the application of Model Free Control (MFC) strategy and a heuristic optimization on-line proposal to optimally control of growth of the microalga *Chlamydomonas reinhardtii* in a batch culture. The control signal was the incident light intensity. In both cases, the total specific growth rate ($\mu(t)$) was established as reference.

These two articles are the result of the work done to achieve the third and fourth specific objectives. The articles are presented in 3.3 and 3.4 sections.

Numerical simulations evaluated the performance of four proposals. The simulations showed that the proposals are robust towards parametric uncertainties, un-modeled dynamics, and disturbances.

In the fifth article, the optimal medium for the growth of *A. obliquus* was determined. In addition, four extraction methods were compared: classical, microwave assisted (MW), Soxhlet, and ultrasound assisted (US). The above to establish of the most suitable method for the extraction of lipids from the microalga *Acutodesmus obliquus* (*Scenedesmus obliquus* UTEX 393), was presented.

Finally, *A. obliquus* was cultivated under illumination with LEDs emitting λ = 620-750 nm (red), λ = 570-590 nm (yellow), λ = 495-570 nm (green), and λ = 380-450 nm (violet) light. Additionally, each these cultures were illuminated at different light intensities (I_1=40 $\mu E \cdot m^{-2} \cdot s^{-1}$, I_2=65 $\mu E \cdot m^{-2} \cdot s^{-1}$ and I_3=90 $\mu E \cdot m^{-2} \cdot s^{-1}$). The effect both wavelength and light intensity, on the growth as well the production of essential fatty acids, in cultures of *Acutodesmus obliquus* (*Scenedesmus obliquus*) were present in the sixth article.

These two articles are the result of the work done to achieve the fifth specific objective. The articles are presented in 3.5 and 3.6 sections.

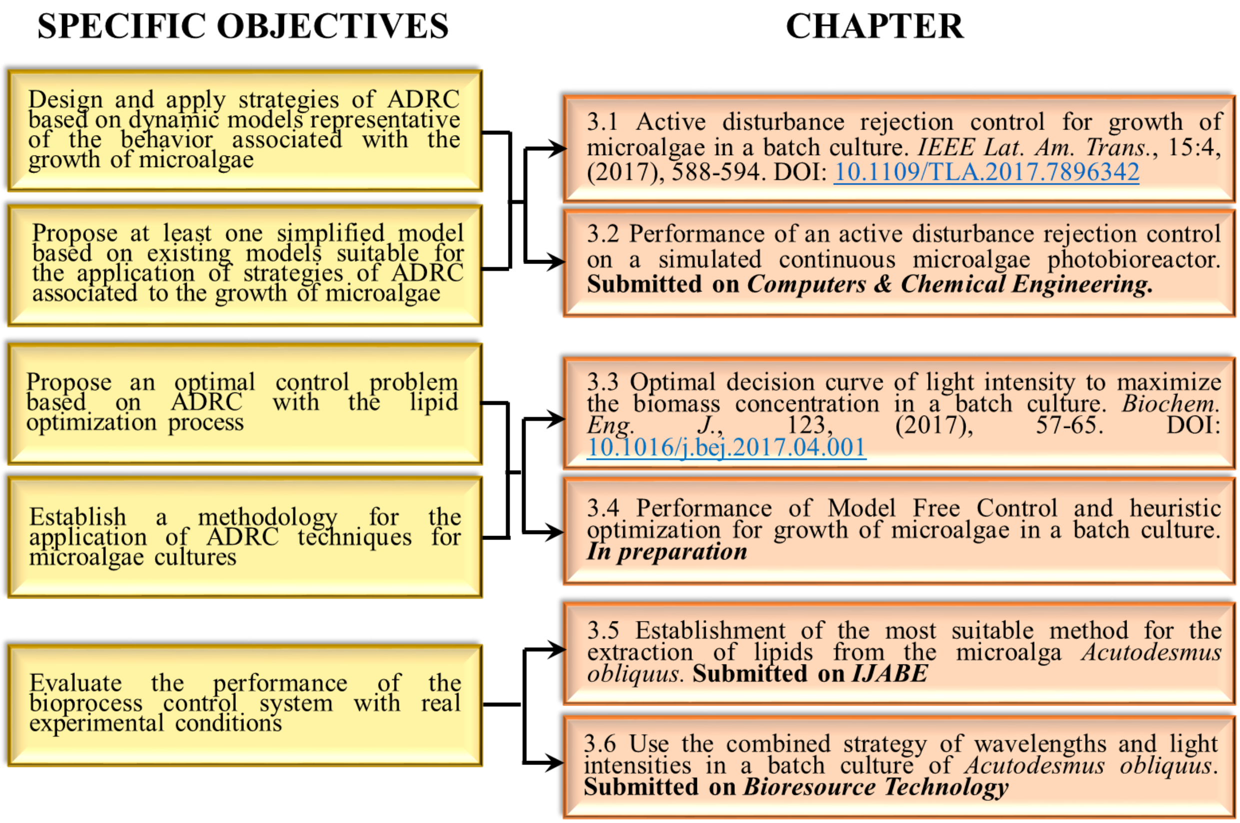


Figure 2.1. Structure of the document and published papers

2.4 Status of written articles

As result of the present work, in the Table 2.1 is the information of the state of each or the research articles that have been written. Each article corresponds to a section of the results chapter of this document.

Table 2.1. Status of written articles

SECTION	PAPER INFORMATION	PUBLICATION STATUS	PUBLICATION DETAILS
3.1	Garzón C. L., Cortés J. A., Tello E. (2017). Active Disturbance Rejection Control for growth of microalgae in a batch culture. *IEEE Latin America Transactions*, *15* (4), 588-594. https://doi.org/10.1109/TLA.2017.7896342	Published	Date: April 12, 2017 SCOPUS: Q2 ISI: Q4 Publindex: B Impact factor: 0.631 H: 15
3.2	Garzón-Castro C. L., Delgado-Aguilera E., Cortés-Romero J. A., Tello E. & Mazzanti G. Performance of an Active Disturbance Rejection Control on a simulated continuous microalgae photobioreactor.	Submitted on *Computers & Chemical Engineering*	Date: October 5, 2017 SCOPUS: Q1 ISI: Q1 Publindex: A1 Impact factor: 3.024 H: 113

3.3	Garzón-Castro C. L., Cortés-Romero J. A., Arcos-Legarda J, Tello E. (2017). Optimal decision curve of light intensity to maximize the biomass concentration in a batch culture. *Biochemical Engineering Journal*, *123*, 57-65. https://doi.org/10.1016/j.bej.2017.04.001	Published	Date: April 3, 2017 SCOPUS: Q1 ISI: Q1 Publindex: A1 Impact factor: 2.892 H: 95
3.4	Garzón-Castro C. L., Cortés-Romero J. A., Tello E. Performance of Model Free Control and heuristic optimization for growth of microalgae in a batch culture.	In preparation	
3.5	Hurtado-Varela X., Garzón-Castro C. L., Cortés J. A., Tello E. A comparison of lipid extraction methods for the microalgae *Acutodesmus obliquus.*	Submitted on *International Journal of Agricultural and Biological Engineering (IJABE)*	Date: August 22, 2017 SCOPUS: Q2 ISI: Q3 Publindex: A2 Impact factor: 0.835 H: 15
3.6	Hurtado-Varela X., Garzón-Castro C. L., Cortés J. A., Tello E. Use the combined strategy of wavelengths and light intensities in a batch culture of *Acutodesmus obliquus*	Submitted on *Bioresource Technology*	Date: April 26, 2018 SCOPUS: Q1 ISI: Q1 Publindex: A1 Impact factor: 5.651 H: 216

In addition, the following work was presented in scientific congress:

- Hurtado X., Garzón-Castro C. L., Cortés-Romero J. A., & Tello, E. (2017). Establishment of the most suitable method for the extraction of lipids from the microalga *Acutodesmus obliquus*. In: Algal Biofuels. Miami, USA, June 18-21, 2017.
- Garzón-Castro C., Cortés-Romero J. & Tello E. (2018). Optimización en línea y fuera de línea aplicada al crecimiento de microalgas. In: Jornada de Actualización en Biotecnología Algal. Bogotá, Colombia, February 1-2, 2018.

2.5 Individual's contributions

The information corresponding to the contribution made by each one of the authors in the written articles can be consulted in Table 2.2.

Table 2.2. Individual's contribution

SECTION	PAPER INFORMATION	CONTRIBUTION
3.1	Garzón C. L., Cortés J. A., Tello E. (2017). Active Disturbance Rejection Control for growth of microalgae in a batch culture. *IEEE Latin America Transactions*, *15* (4), 588-594. https://doi.org/10.1109/TLA.2017.7896342	All authors made substantial contributions to conception and design of project. These contributions were materialized in the publication of this article. Garzón participated in the design of the control proposal; carried out the simulations, made the analysis of them; and wrote the manuscript. Cortés participated in the design of the control proposal and helped revise the manuscript. Tello helped to revise the manuscript. All authors read and approved the final manuscript.

3.2	Garzón-Castro C. L., Delgado-Aguilera E., Cortés-Romero J. A., Tello E. & Mazzanti G. Performance of an Active Disturbance Rejection Control on a simulated continuous microalgae photobioreactor.	Garzón-Castro, Cortés-Romero and Tello made substantial contributions to conception and design of project. These contributions were materialized the writing of this manuscript. Garzón-Castro did the design of the control proposal; carried out the simulations, made the analysis of them; and wrote the manuscript. Delgado-Aguilera participated in the review of design of the control proposal and help to draft the manuscript. Cortés-Romero and Tello-Camacho helped to revise the manuscript. Mazzanti participated both in the review of mathematical model of the bioprocess and in the analysis of simulations. Additionally, he helped in the writing of the manuscript. All authors read and approved the final manuscript.
3.3	Garzón-Castro C. L., Cortés-Romero J. A., Arcos-Legarda J, Tello E. (2017). Optimal decision curve of light intensity to maximize the biomass concentration in a batch culture. *Biochemical Engineering Journal*, *123*, 57-65. https://doi.org/10.1016/j.bej.2017.04.001	Garzón-Castro, Cortés-Romero and Tello made substantial contributions to conception and design of project. These contributions were materialized in the publication of this article. Garzón-Castro participated both in the design of the optimization proposal as in carried out the simulations. Additionally, she made the analysis of the simulations and wrote the manuscript. Cortés-Romero participated both in the design of the optimization proposal as in carried out the simulations. He also helps to revise the manuscript. Arcos-Legarda replicated the optimization with which the proposed design was compared, and he helped to revise the manuscript. Tello-Camacho helped to revise the manuscript. All authors read and approved the final manuscript
3.4	Garzón-Castro C. L., Cortés-Romero J. A., Tello E. Performance of Model Free Control and heuristic optimization for growth of microalgae in a batch culture.	All authors made substantial contributions to conception and design of project. These contributions were materialized the writing of this manuscript. Garzón-Castro participated both in the design of the proposal as in carried out the simulations. Additionally, she made the analysis of the simulations and wrote the manuscript. Cortés-Romero participated both in the design of the optimization proposal as in carried out the simulations. He also helps to revise the manuscript. Tello-Camacho helped to revise the manuscript. All authors read and approved the final manuscript
3.5	Hurtado-Varela X., Garzón-Castro C. L., Cortés J. A., Tello E. A comparison of lipid extraction methods for the microalgae *Acutodesmus obliquus.*	Garzón-Castro, Cortés-Romero and Tello made substantial contributions to conception and design of project. These contributions were materialized the writing of this manuscript. Hurtado-Varela carried out all experiments and participated both in the analysis of the data as in the writing of the manuscript. Garzón-Castro chosen the nitrogen amount in the culture medium. Additionally, she participated both

		in the analysis of the some of the data as in writing of the manuscript. Cortés-Romero helped to revise the manuscript. Tello-Camacho chose what extraction methods should be compared. Additionally, he participated both in the analysis of the data as in the writing of the manuscript. All authors read and approved the final manuscript
3.6	Hurtado-Varela X., Garzón-Castro C. L., Cortés J. A., Tello E. Use the combined strategy of wavelengths and light intensities in a batch culture of *Acutodesmus obliquus*	Garzón-Castro, Cortés-Romero and Tello made substantial contributions to conception and design of project. These contributions were materialized the writing of this manuscript. Hurtado-Varela participated in design of the experiments. She carried out all experiments and participated in the writing of the manuscript. Garzón-Castro participated in design of the experiments. Additionally, she participated both in the analysis of the data as in writing of the manuscript. Cortés-Romero helped to revise the manuscript. Tello-Camacho participated both in the analysis of the data as in the writing of the manuscript. All authors read and approved the final manuscript

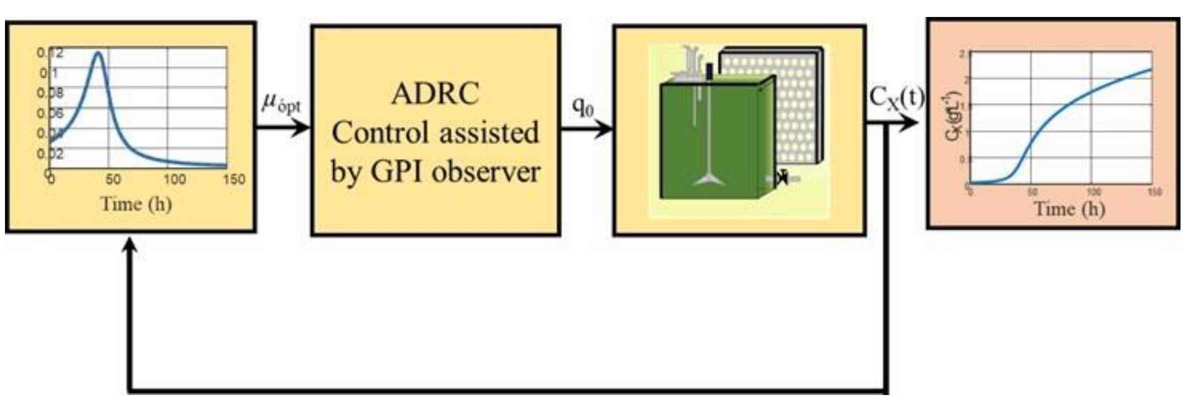
CHAPTER 3. RESULTS

3.1 Active Disturbance Rejection Control for Growth of Microalgae in a Batch Culture

Abstract. The processes based on microalgae pose new challenges on modelling as well as control, due to the complex nonlinear dynamics and variants during the course of time. The microalgae can be cultivated in closed systems called photobioreactors (PBR), through which it is possible to control the conditions of the culture, with the purpose of increasing the production of biomass or metabolites. The optimization of the conditions of growth in relation to light is essential for the increase of biomass. In this paper, a proposal is presented to control the intensity of light applied to a batch PBR, using the strategy of active disturbance rejection control (ADRC) with the goal of increasing biomass. Under this paradigm: 1) a substantial simplification of the design of the laws of control is obtained, 2) the complexity of the system is reduced to a linear disrupted system, 3) the high uncertainty of the internal dynamics are worked liked equivalent disturbances at the entrance, 4) the internal and external disturbances that affect the behavior of the system are worked in a unified manner and 5) the estimation of the unified disturbances is done in line, by a linear observer of extended state, in schemes of control based on the observer. The proposal of control is illustrated and evaluated at a simulation level. In the analysis, the performance of the strategy was studied against the variation of parameters. The simulations show the excellent performance against the different perturbations considered, highlighting the strength of the strategy.

Keywords: Active Disturbance Rejection Control, Nonlinear System, Robust Control, Bioprocess, Batch Culture, Microalgae.

Graphic abstract



Link: https://ieeexplore.ieee.org/document/7896342/

588 IEEE LATIN AMERICA TRANSACTIONS, VOL. 15, NO. 4, APRIL 2017

Active Disturbance Rejection Control for Growth of Microalgae in a Batch Culture

C. L. Garzón, *Member, IEEE,* J. A. Cortés and E. Tello

Abstract— **The processes based in microalgae imply new challenges on modelling as well as control, due to the complex nonlinear dynamics and variants during the course of time. These can be cultivated in closed systems called photobioreactors, through which it is possible to control the conditions of the crop, with the purpose of increasing the production of biomass or metabolites. The optimization of the conditions of growth in relation to light is essential for the increase of biomass. In this paper, a proposal is presented to control the intensity of light applied to a batch PBR, using the strategy of active disturbance rejection control (ADRC) with the goal of increasing biomass. Under this paradigm: 1) a substantial simplification of the design of the laws of control is obtained, 2) the complexity of the system is reduced to a linear disrupted system, 3) the high uncertainty of the internal dynamics are worked liked equivalent disturbances at the entrance, 4) the internal and external disturbances that affect the behavior of the system are worked in a unified manner and 5) the estimation of the unified disturbances are done in line by a linear observer of extended state in schemes of control based on the observer. The proposal of control is illustrated and evaluated on a simulation level. In the analysis, the performance of the strategy was contemplated against the variation of parameters. The simulations show the excellent performance against the different sensibility analysis considered, highlighting the strength of the strategy.**

Keywords— **Active Disturbance Rejection Control, Nonlinear System, Robust Control, Bioprocess, Batch Culture, Microalgae.**

I. INTRODUCCIÓN

LAS MICROALGAS son organismos fotosintéticos que al igual que las plantas terrestres, asimilan CO_2, nitrógeno y fósforo para la formación de biomasa [1]. Además, son fuente de: ácidos grasos saturados y poliinsaturados, polisacáridos, vitaminas, carotenos, pigmentos [2], hidrógeno [3] y alimento para los organismos marinos [4]. Por lo que se han convertido en un recurso natural con gran potencial, de alto interés tanto para la industria [5], [6] como para la comunidad científica.

Las diversas aplicaciones que tiene la biomasa obtenida a partir de microalgas han generado un incremento en las investigaciones que buscan, identificar las condiciones óptimas de crecimiento y aumentar así la productividad, a partir de cultivos cerrados [7], [8]. Los cultivos cerrados, se llevan a cabo en fotobiorreactores (FBR), que pueden operar en modo continuo o discontinuo. Los FBR se caracterizan por requerir: nutrientes (nitrógeno, fosfato y micronutrientes), una fuente inorgánica de carbono (CO_2), agitación, suministro de luz (fuente solar o artificial), pH y temperatura constantes. Siendo la intensidad y la distribución espacial de la luz, la variable que más afecta la velocidad de crecimiento de las microalgas [9]–[12]. Esta variable puede afectar la velocidad de crecimiento de la microalga causando una disminución en la misma, debido al uso de: 1) intensidades bajas que generen fotolimitación y/o 2) intensidades altas, que puede generar daño celular y llevar a la fotoinhibición [13].

Desde el punto de vista de la ingeniería de biosistemas, los procesos basados en microalgas implican nuevos desafíos tanto en el modelado como en el control, debido a su comportamiento no lineal y variante en el tiempo [6], [14]. Diversos autores han planteado el uso y/o combinación de diferentes estrategias de control [15]–[18]. En estos desarrollos se requiere el conocimiento de un modelo preciso, lo cual puede conllevar a un mal manejo de las incertidumbres y por otro, a una mayor complejidad en la sintonización e implementación del controlador. Adicionalmente, la mayoría de investigaciones se han enfocado en el control de FBR continuos, en los que se controla la velocidad de dilución. Son pocos los reportes que plantean el control de la intensidad de luz en cultivos por lotes. Las propuestas realizadas por [19], [20] son interesantes y muestran que es posible incrementar la producción de biomasa realizando control de la intensidad de la luz, pero al requerir un modelo exacto del bioproceso no son robustas frente al cambio de parámetros y presencia de perturbaciones. Por lo tanto, el control y la operación óptima de un FBR tipo batch para aumentar la producción de biomasa sigue siendo un desafío para abordar. En lo relacionado con la optimización fuera de línea, lo reportado por [20] es novedoso.

En este artículo se presenta una propuesta de control bajo el enfoque del Rechazo Activo de Perturbaciones (ADRC, por sus siglas en inglés), en la que a partir de un valor óptimo de la tasa de crecimiento, se controla la intensidad de la luz incidente en un cultivo tipo batch, con el fin de incrementar la biomasa. El objetivo es mejorar aún más la eficiencia del bioproceso por medio de estrategias de control avanzado. El diseño de la ley de control se basa en un modelo simplificado que se caracteriza por ser lineal perturbado, de acuerdo a lo propuesto por [21]- [22]. Esta propuesta resulta robusta frente a la variación de parámetros e incertidumbre de dinámicas internas no modeladas. La propuesta de control se ilustra y se evalúa, a nivel de simulación, con el modelo completo para un cultivo de *Chlamydomonas reinhardtii* en un FBR toroidal.

La organización del trabajo es la siguiente. En la Sección II se presenta el modelo del bioproceso. La Sección III describe la estructura del control basado en observador. En la Sección IV se encuentran los resultados de las simulaciones. Finalmente, se consignan las conclusiones en la Sección V.

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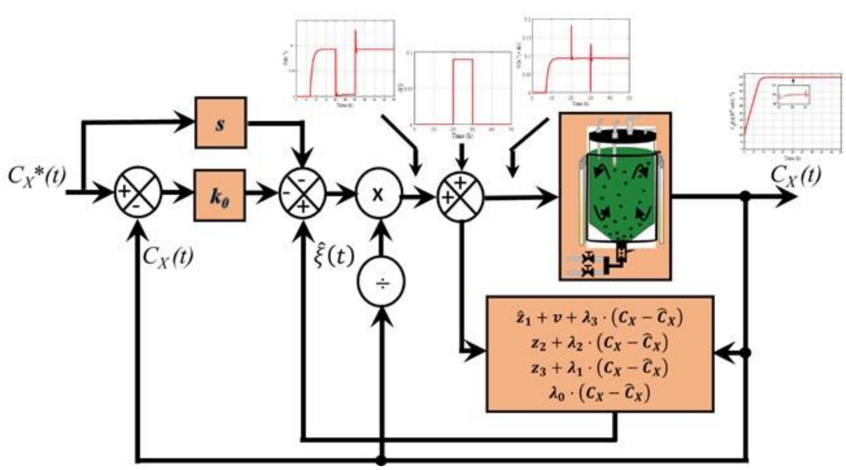
3.2 Performance of an active disturbance rejection control on a simulated continuous microalgae photobioreactor

Abstract. Microalgae are used for the industrial production of high value compounds. The aim in continuous bioreactors is to obtain the highest biomass production. It is necessary to guarantee that the bioprocesses attain and maintain the optimal reference biomass $C_X^*(t)$, despite endogenous and exogenous disturbances. This paper describes the numerical simulation of the application of Active Disturbance Rejection Control (ADRC) to control the dilution rate ($D(t)$) in a continuous culture of the microalga *Chlorella vulgaris*. To reduce the bioprocess to a "SISO" system, the authors chose the dilution rate, $D(t)$, to be the only control signal. The control proposal was illustrated and evaluated through a numerical simulation using MATLAB/Simulink™ environment. The performance of the ADRC was tested by the application of external perturbations and variation of parameters over a nominal case. At nominal conditions, $D(t)$ was always maintained within the physical limits imposed by the bioprocess. Step and smooth type signals, at 96.4%$\cdot|D_{max}(t)|$, were imposed as external perturbation on the control signal input, $D(t)$. The controller response kept the output signal $C_X(t)$ within an insignificant 0.0043%$\cdot|C_{Xmax}(t)|$. The algal culture had a strongly asymmetric response to variations of the ideal maximum growth rate, $\mu_{max}(t) \pm 30\%\cdot|\mu_{max}(t)|$, and of the nominal light intensity, $I_{in}(t) \pm 30\%\cdot|I_{in}(t)|$. Nonetheless, the controller promptly returned the output signal to its reference value, $C_X(t)^*$. The numerical test of the control proposal, in summary, showed that the ADRC strategy ensures excellent reference tracking capability and robustness towards parametric uncertainties, un-modeled dynamics, and external disturbances.

Keywords: active disturbance rejection control, nonlinear system, robust control, growth of microalgae, continuous culture.

Graphic abstract



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Abstract: Microalgae can produce high value compounds in continuous bioreactors, operated at optimal biomass production i.e. reference. This reference, CX*(t), must be kept, despite endogenous and exogenous disturbances. A numerical simulation served to evaluate the performance of Active Disturbance Rejection Control (ADRC) to keep CX*(t) of a Chlorella vulgaris culture. ADRC operated on the dilution rate, D(t). At nominal conditions, D(t) remained within the limits of the bioprocess. Step and smooth signals, at 96.4%·|D_max (t)|, were imposed as external perturbation on D(t). The controller kept CX(t) within 0.0043%·|C_Xmax (t)|. The response to variations of the ideal maximum growth rate, µmax(t) ± 30%·|µmax(t)|, and of the light intensity, Iin(t) ± 30%·|Iin(t)|, were strongly asymmetric. Nonetheless, the controller promptly returned CX(t) to CX(t)*.
This numerical test showed that the ADRC strategy ensures excellent reference tracking capability and robustness towards parametric uncertainties, un-modeled dynamics, and external disturbances.

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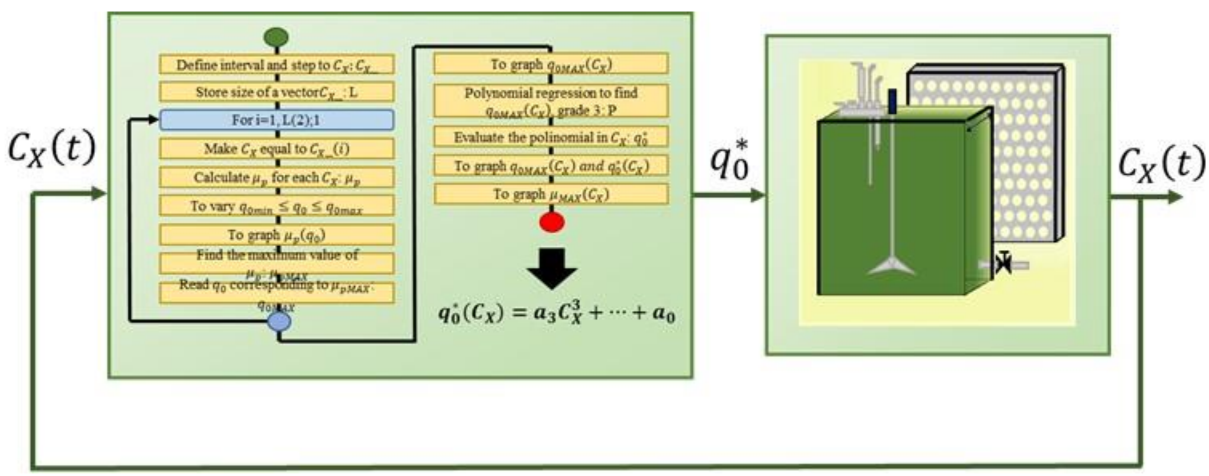
3.3 Optimal decision curve of light intensity to maximize the biomass concentration in a batch culture

Abstract. This paper proposes a method to find an optimal decision curve to manage the incident light intensity (q_0) that is applied to microalgae cultivation to maximize biomass concentration (C_X). Microalgae are characterized by the production of high value compounds of interest to industry; the challenge is to obtain the highest biomass concentration. Optimization of the performance of microalgae culture systems is important to guarantee the viability of the economical process. The advantages of this optimization proposal are to attain to C_X maximum productivities, as well as its simplicity and its robustness against perturbations. The optimization proposal is illustrated and evaluated on a numerical simulation in a batch culture of microalga *Chlamydomonas reinhardtii*. Additionally, it was compared to a conventional constant light operation and with an optimization approach based on finding the ratio between q_0 and C_X (light-to-microalga ratio). In the analysis, the performance of the optimal decision curve was contemplated in presence of the perturbations and variation of parameters. The simulations of proposal in this paper shows an optimal behavior in terms of a maximum production C_X. In addition, this would have a better behavior in front of robustness and disturbance rejection capabilities, compared to both, constant light operation and light-to-microalga ratio.

***Keywords**:* optimal decision curve, nonlinear system, robustness front perturbations, growth of microalgae, batch culture.

Graphic abstract



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Regular article

Optimal decision curve of light intensity to maximize the biomass concentration in a batch culture

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ABSTRACT

This paper proposes a method to find an optimal decision curve to manage the incident light intensity (q_0) that is applied to microalgae cultivation to maximize biomass concentration (C_X). Microalgae are characterized by the production of high value compounds of interest to industry; the challenge is to obtain the highest biomass concentration. Optimization of the performance of microalgae culture systems is important to guarantee the viability of the economical process. The advantages of this optimization proposal are to attain to C_X maximum productivities, as well as its simplicity and its robustness against perturbations. The optimization proposal is illustrated and evaluated on a numerical simulation in a batch culture of microalga *Chlamydomonas reinhardtii*. Additionally, it was compared to a conventional constant light operation and with an optimization approach based on finding the ratio between q_0 and C_X (light-to-microalga ratio). In the analysis, the performance of the optimal decision curve was contemplated in presence of the perturbations and variation of parameters. The simulations of proposal in this paper shows an optimal behavior in terms of a maximum production C_X. In addition, this would have a better behavior in front of robustness and disturbance rejection capabilities, compared to both, constant light operation and light-to-microalga ratio.

1. Introduction

Microalgae are unicellular organisms that assimilate carbon dioxide (CO_2), nitrogen (N) and phosphorus (P) to form their biomass [1]. These organisms contain significant amounts of micronutrients, such as proteins, carbohydrates and lipids, as well as pigments, polyphenols and minerals. These metabolites can be transformed into products of interest for the energy [2,3], nutraceutical [4], cosmetic [5] and food industries [6]. Additionally, they are used in diverse applications related to environmental restoration [7,8]. This has turned microalgae into a new natural resource with great potential and high interest to industry, but with the challenge of improving efficiency to achieve an increase of the biomass concentration (C_X).

Microalgae can be cultivated in closed systems called photobioreactors (PBRs). Usually the PBR requires: nutrients, an inorganic carbon source, agitation, light supply, pH and temperature, constants. Light energy is the most important factor for microalgae growth cultures photosynthesis; it must be supplied in a continuous manner because radiative energy may not be accumulated [9,11]. Usually the problem in cultivating microalgae is related to the light intensity, the growth rate of photosynthetic microalgae depends on the light energy absorbed by cells, a low intensity causes photolimitation and higher intensity causes photoinhibition [12,13]. On the other hand, when the C_X increases, the mutual shading enhances and the photolimitation takes places, which decreases the light energy available per cell, starts playing an important role. As a result, light utilization efficiency decreased and so does the specific growth rate. Moreover, it's not possible to increase the light intensity over a certain level because a high light energy per cell

Abbreviations: C_X, biomass concentration; HNN, hybrid neural network; IPOPT, interior point optimizer; LED, light-emitting diode; PBR, photobioreactor; PMP, Pontryagin's maximum principle; q_0, incident light intensity; q_0^*, optimum value for q_0; μ, total specific growth rate; μ_p, photosynthetic growth rate; μ_{pMAX}, maximum μ_p; μ_s, respiration growth rate.

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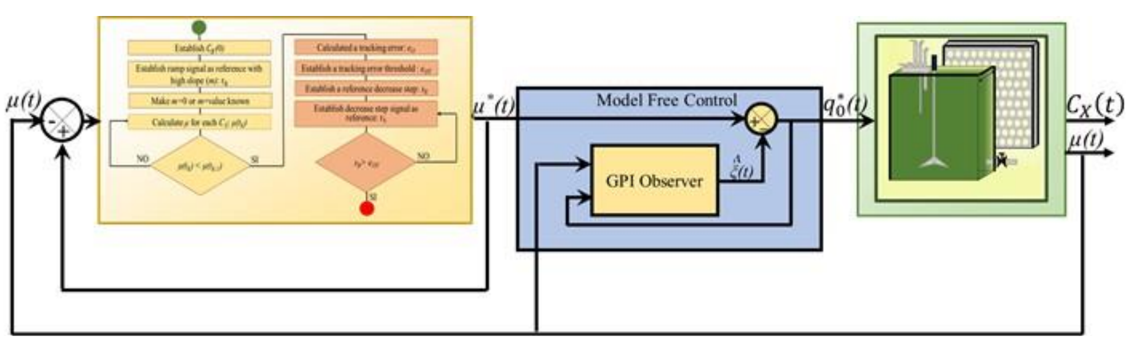
3.4 Performance of Model-Free Control and heuristic optimization for growth of microalgae in a batch culture

Abstract. This paper shows the performance of Model Free Control (MFC) strategy and a heuristic optimization on-line proposal to optimally control of growth of the microalga *Chlamydomonas reinhardtii* in a batch culture. The aim of the proposed strategy consists in controlling the incident light intensity so that the biomass concentration $C_X(t)$ is maximized. The proposed MFC consist of: 1) a linear control law used to specify the dynamics of system and 2) a high gain linear observer, known as Generalized Proportional Integral (GPI) that assist any extra unknown or partially known dynamics, effects of uncertainties and external disturbances. An advantage of this control strategy, as well as, not needing a model is its simplicity and its robustness. The strategy proposed is illustrated and evaluated on a numerical simulation level. Additionally, MFC (on-line optimization) was compared to a conventional constant light operation and with an optimal decision curve (off-line optimization). In the analysis, the performance of the strategies were contemplated against nominal conditions, disturbances and variation of parameters. The simulations showed the excellent performance of both MFC and optimal decision curve (ODC) in terms of a maximum production $C_X(t_f)$, compared to conventional constant light operation. Determining that both MFC and ODC would have a better behavior in front of robustness towards uncertainties compared to conventional constant light operation. However, in front of the unknowing of some plant parameters, the MFC strategy is better compared with the other two alternatives.

Keywords: Model-Free Control, Heuristic Optimization, Nonlinear control, Robust Control, growth of the microalgae, batch culture.

Graphic abstract

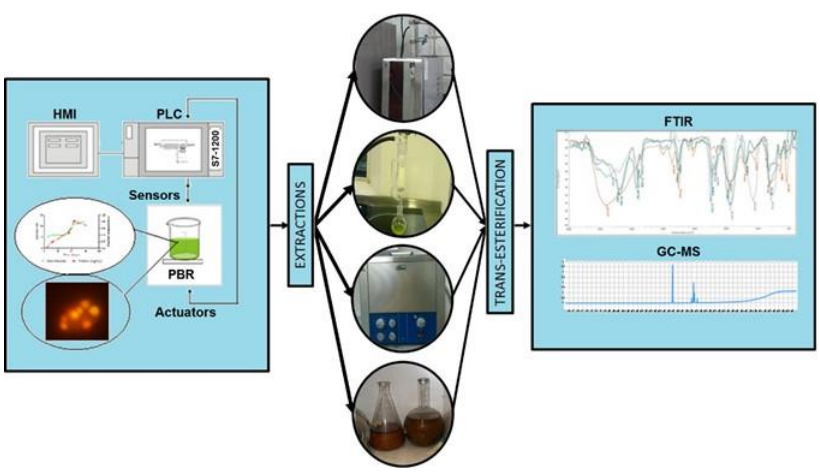


3.5 A comparison of lipid extraction methods for the microalgae A*cutodesmus obliquus*

Abstract. Because microalgae are widely used in the pharmaceutical and energy industries, the conditions for their cultivation and extraction methods play an important role in the profiling and acquisition of lipids. The efficiency of lipid extraction from microalgae has attracted great interest from industry because of the wide variety of lipids and amounts that can be obtained. *Acutodesmus obliquus* (*Scenedesmus obliquus* UTEX 393) was used in this study. It was cultivated in Bold 3N medium modified with 75% nitrogen at 25 °C, pH 6.8, 125 rpm, and a photoperiod of 18/6 h and illuminated with white light provided by a Light-Emitting Diode Surface Mount Device extensions (LED SMD) with an intensity of 1200 $\mu E \cdot m^{-2} \cdot s^{-1}$. The cells were stained with the Red Nile (RN) technique to indicate lipid production. Four extraction methods were compared, classical, microwave assisted (MW), Soxhlet, and ultrasound assisted (US), using the same solvent proportions (hexane:chloroform:methanol 1:2:3). All samples were analyzed with Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). The results showed: 1) lipid production detected by RN was consistent with microalgal growth; 2) the MW technique was the best extraction method, according to the statistical analysis through Randomized Complete Block (RCB) design and performance of 4.6%; and 3) the presence of saturated and unsaturated acids was indicated by FTIR spectra. GC-MS was able to identify palmitic and linoleic acids as the likely major constituents of the sample.

Keywords: *Acutodesmus obliquus*, lipid extraction, trans-esterification, dye with Red Nile.

Graphic abstract



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Abstract

Because microalgae are widely used in the pharmaceutical and energy industries, the conditions for their cultivation and extraction methods play an important role in the profiling and acquisition of lipids. The efficiency of lipid extraction from microalgae has attracted great interest from industry because of the wide variety of lipids and amounts that can be obtained. Acutodesmus obliquus (Scenedesmus obliquus UTEX 393) was used in this study. It was cultivated in Bold 3N medium modified with 75% nitrogen at 25 °C, pH 6.8, 125 rpm, and a photoperiod of 18/6 h and illuminated with white light provided by a Light-Emitting Diode Surface Mount Device extensions (LED SMD) with an intensity of 1200 μE.m-2.s-1. The cells were stained with the Red Nile (RN) technique to indicate lipid production. Four extraction methods were compared, classical, microwave assisted (MW), Soxhlet, and ultrasound assisted (US), using the same solvent proportions (hexane:chloroform:methanol 1:2:3). All samples were analyzed with Fourier Transform Infrared Spectroscopy (FTIR) and Gas Chromatography coupled to Mass Spectrometry (GC-MS). The results showed: 1) lipid production detected by RN was consistent with microalgal growth; 2) the MW technique was the best extraction method, according to the statistical analysis through Randomized Complete Block (RCB) design and performance of 4.6%; and 3) the presence of saturated and unsaturated acids was indicated by FTIR spectra. GC-MS was able to identify palmitic and linoleic acids as the likely major constituents of the sample.

Keywords	Acutodesmus obliquus, lipid extraction, trans-esterification, dye with Red Nile.
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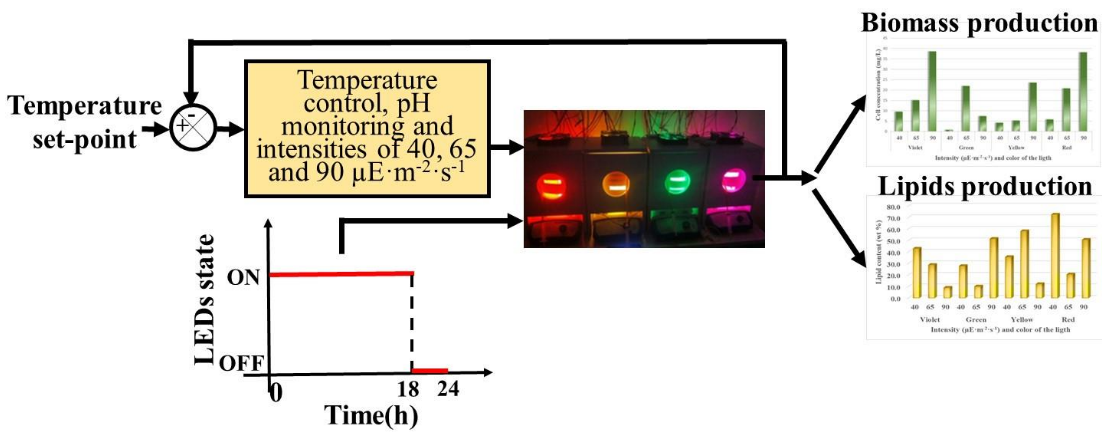
3.6 Use the combined strategy of wavelengths and light intensities in a batch culture of *Acutodesmus obliquus*

Abstract. The quality of light, such as wavelength and the intensity affect the performance of the algal growth and the lipids production. Moreover, these variables too affect the lipids profile. To study the interrelation among these variables, *Acutodesmus obliquus* (*Scenedesmus obliquus* UTEX 393) was cultivated in Bold 3N medium modified with 75% nitrogen at 25 °C, pH 6.8, 125 rpm, and a photoperiod of 18/6 h. The illumination was provided by a Light-Emitting Diode Surface Mount Device extensions (LED SMD) emitting red (λ = 620-750 nm), yellow (λ = 570-590 nm), green (λ = 495-570 nm) and violet (λ = 380-450 nm) light. Additionally, each culture was illuminated at different light intensities (I_1=40 $\mu E \cdot m^{-2} \cdot s^{-1}$, I_2=65 $\mu E \cdot m^{-2} \cdot s^{-1}$ and I_3=90 $\mu E \cdot m^{-2} \cdot s^{-1}$). The extraction method was microwave assisted (MW) using hexane:chloroform:methanol in proportions 1:2:3. All samples were analyzed with Gas Chromatography coupled to Mass Spectrometry (GC-MS). The results showed: 1) the biomass production is directly proportional to the light intensity under the parameters stablished, e.g. at 90 $\mu E \cdot m^{-2} \cdot s^{-1}$ with violet, yellow and red light showed the major biomass production; 2) with violet light produced minor amount of lipids when there was major light intensity; and 3) *A. obliquus*, under the parameters stablished, is good producer of palmitate, linolenate and linoleate methyl ester.

Keywords: *Acutodesmus obliquus*, wavelength, light intensity, biomass production, lipids production, linoleate methyl ester.

Graphic abstract



CHAPTER 4. DISCUSSION

In the present investigation a design and apply strategies of advanced control under the approach of Active Disturbance Rejection Control (ADRC) on microalgae cultures, was proposed. For the above, from a control frame of reference, two stages were considered. The development of the first stage resulted in three different designs controllers, all inspired by the active disturbance rejection philosophy. In each case, the aim was to guarantee the tracking of the reference signal. This tracking had been ensured in the presence of internal or external disturbances and variation of parameters. The second stage, in which the design of at least one optimization strategy based on ADRC was looking to increase the biomass production, was contemplated. During the development of this stage, two optimization heuristics were proposed, off-line and on-line strategies. For the design and implementation at simulation level, real experimental data reported by different authors was used.

Both control and optimization proposal are heavily dependent on the mathematical system model of the bioprocess, which limits their applicability. Therefore, both control and optimization strategies that did not have a strong dependence on the model were proposed. It is important to note that the ADRC strategy is less dependent of the given information by the mathematical model, and it allowed proposing a simplified model that only requires the knowledge of the order of the system and its input gain. Thus, ADRC is among the model-dependent control methods and model-free control methods (MFC).

4.1 Controllers and optimizations designed

The first control design was a strategy ADRC assisted by a General Proportional Integral (GPI) observer. The proposed control was characterized by the simplified model linearly disturbed. This design manages to control the intensity of incident light (q_0) in a batch culture, to increase the concentration of biomass ($C_X(t)$), and the total specific growth rate ($\mu(t)$) that was reference signal. The ADRC proposal was compared with a proposal of Predictive Controller for Nonlinear Models (NMPC). The results show that the ADRC strategy that achieved the maximum growth rate was also achieved in less time than NMPC strategy. Front to variation of parameters, ADRC was able to follow the reference without problem. These changes in the reference signal obviously involved a variation in the output of the bioprocess but the variation in biomass concentration was not significant.

Next, the second design was an ADRC assisted by a GPI observer approaches the dilution rate in a continuous culture, to attain reference biomass. The efficiency of the ADRC strategy for tracking the reference value in nominal conditions can be appreciated. The ADRC maintained the control input ($D(t)$) within the physical limits imposed by the bioprocess. Moreover, the proposed controller performance was tested with equivalent disturbances ($\xi(t)$) and the effect on $C_X(t)$ wasn't significant. The ADRC strategy kept track of $C_X(t)$, within the ability of the natural response of the microalgae growth. This showed that the ADRC strategy maintained tracking of the desired reference value under the presence of the applied disturbances. Additionally, under variation of parameters, it could be saw that the behavior of the control signal changes according to the variation given, in order to keep track of the reference signal. Although the parameter variation was similar in both positive and negative directions, the response was plain. This strong asymmetric response of biological systems is often given due to their non-linear growth regime while the ADRC was able to ensure the reference tracking according with this dynamic.

Then, an off-line optimization strategy was design and tested at simulation level. This proposal is a method to find an optimal decision curve (ODC) to manage the $q_0(t)$ that is applied to a batch microalgae cultivation according to cell growth. The optimization proposal was compared with a conventional constant light operation and an optimization approach based on finding the ratio between a q_0 and C_X (light-to-microalga ratio). In conventional constant light operation, the incident light is always in the upper bound. While in the cases of light-to-microalga ratio and ODC, the intensity of the incident light is increased gradually until it reached its upper bound. Conventional constant light operation had a lower C_X than the other methods in nominal conditions and with variation of parameters. The light-to-microalga ratio showed a lower C_X than the others did methods under disturbances. The production of C_X in ODC was always higher than light-to-microalga ratio and than conventional constant light operation. ODC is a very simply and efficient method. The disadvantage is that has heavily dependent on the mathematical model of the bioprocess, which limits their applicability.

Finally, a design of a MFC and a heuristic optimization on-line strategy that controlling q_0 for increase of C_X on a batch culture, was proposed. In contrast to existing control strategies, this method does not need the knowledge of the mathematical bioprocess's model. Moreover, the heuristic proposed optimization was also a Model-Free optimization. The MFC proposal was evaluated, at simulation level, by comparing a conventional constant light operation and ODC. The efficiency and the robustness of the three batch cultures were tested under variation of parameters. It was assumed that only the ideal maximum growth rate (μ_0) and the respiration kinetics (μ_S) were known. The results showed that the C_X production in MFC strategy was 96.11% higher than ODC and 5.55% than conventional constant light operation. This result confirmed that ODC requires knowledge of the values of the model parameters while that MFC does not have this advantage. Additionally, the proposed control is characterized for presenting a linear simplified control law. This suggest that it is a simple and easy proposal to implement.

After all the proposed designs, the contribution of an MFC proposal, controller and the optimization, is highlighted. It is also considered that with it, it is possible to maximize the biomass production of any microalgae culture since it does not need a model.

Preliminaries

Although the choice of the control signal was determined by literature, some preliminary experiments in batch cultures were done. The results in the preliminary experiments are outlined below.

First, a modified Bold 3N broth with a content of 75% nitrogen for the growth of *A. obliquus* was chosen. The microalga was grown at 25 °C, pH 6.8, 125 rpm, and a photoperiod of 18/6 h and with white light illumination provided by a Light-Emitting Diode Surface Mount Device extensions (LED SMD) of while light. These experimental conditions showed that the highest rate of lipid production occurred in the exponential phase, but similar to biomass, the lipid content was higher in the stationary phase. Additionally, four extraction methods were compared, classical, microwave assisted (MW), Soxhlet, and ultrasound assisted. It was determined that MW-assisted extraction was the best method for the extraction of lipids.

After, some microalgae cultures with constant light operation throughout the cultivation time were made. The illumination was provided by LED SMD emitting red, yellow, green, and violet light. Additionally, each culture was illuminated at different light intensities (I_1=40 $\mu E \cdot m^{-2} \cdot s^{-1}$, I_2=65 $\mu E \cdot m^{-2} \cdot s^{-1}$ and I_3=90 $\mu E \cdot m^{-2} \cdot s^{-1}$). With these experiments, it was possible to determine that the production of biomass of microalga *A. obliquus* is directly proportional to the light intensity. Moreover, it was also possible to see that the amount of lipids produced with respect to dry biomass was higher when the cultures were illuminated at 40 $\mu E \cdot m^{-2} \cdot s^{-1}$ with red and violet light.

These results confirmed that to achieve an increase of the biomass and lipids production, the intensity of the light had to be controlled. In addition, the results allowed to establish the hypothesis that to achieve a higher production of biomass and lipids, the intensity of light should be variable.

CHAPTER 5. CONCLUSION AND FUTURE WORKS

5.1 Conclusion

The results reported in this thesis showed that each one of the control proposals are robust against disturbances and variation of the parameters. Moreover, the ADRC proposal has little dependence on the model, while the MFC proposal and online optimization heuristics do not require any model. Additionally, the optimization proposals allowed ensuring an increase in biomass production. After the study, at a simulation level, the MFC strategy with online optimization heuristics is proposed to use, because the advantages exposed let´s it establish that it is possible to work with any microalgae species using this type of methodology.

5.2 Future works

Further research could explore to evaluate the effect of wavelength in a batch culture, but by making use of Model-Free Control (MFC). The effect would be evaluated on the production of biomass and lipids, additionally a profile of the methyl esters could be made. This with the aim to establish if the results are better than when the conventional constant light operation is used.

Further work is to design and probe MFC with an on-line heuristic optimization for a continuous culture. In this case, it should be checked if the variable control would be the light intensity or dilution rate. Maybe will be necessary to propose a multivariable control.

CHAPTER 6. ANNEXES

Table A.1. Physical variables that affecting the microalgae

VARIABLE	EFFECTS
Light intensity	The intensity light acts as a guide and helping factor to cell proliferation and it helps cellular respiration and photosynthesis (Daliry, Hallajsani, Roshandeh, Nouri, & Golzary, 2017). However, under high light intensity conditions, reduces the production of biomass, because the extra light can no be absorbed for photosynthesis and it may damage to microalgae and stop its growth (Khoeyi, Seyfabadi, & Ramezanpour, 2012).
Stirring	This variable helps aeration and mixing include preventing precipitation of microalgae, homogenization of cultivation environment so that all of the cells can reach light and food, avoiding temperature differences and facilitating the exchange of gases between the cultivation environment and air (Daliry et al., 2017; Kumar et al., 2010). Unfortunately, high mixing rates can cause damage to cells (Sobczuk, Camacho, Grima, & Chisti, 2006).
Light/dark cycles	In the light phase the cells utilise the light to produce their storage nutrients to increase in biomasa and photosynthetic pigments (Tsygankov, Kosourov, Seibert, & Ghirardi, 2002). Under dark conditions it utilise the stored nutrients to survive giving a loss of biomasa (Ogbonna & Tanaka, 1998).
Temperature	Temperature may affect photosynthesis, respiration, growth rate and optical properties (Schabhüttl et al., 2013). Microalgae are typically able to develop over a wide range of temperatures between 20 and 35 °C, with a highly variable response between species (Daliry et al., 2017; Serra, Bernard, Gonçalves, Bensalem, & Lopes, 2016). Low temperature limits cell growth speed and therefore reduces the biomass production (Daliry et al., 2017). At high temperature, the balance among the various biochemical reactions in phytoplankton cells can be disrupted and damage may be irreversible (Schabhüttl et al., 2013).
pH-CO_2	Most microalgal species are favored by neutral pH, whereas some species are tolerant to higher pH or lower pH. (Daliry et al., 2017). High concentration of CO_2 promotes photosynthetic efficiency of microalgae to reproduce within a shorter time and thus more quantity of microalgae biomass could be attained (Lam, Lee, & Mohamed, 2012). While, when microalgae grow at low pH it may significantly retard their survival rate when growing (Chiu et al., 2008).

Table A.2. Some control strategies used for microalgae cultures

OBJECTIVE	RESULTS
Generalized Predictive Controller	
This work addresses effective utilization of flue gases trough the proper pH control in raceway reactors using a Generalized Predictive Controller (GPC) (Pawlowski et al., 2014).	The authors found that is possible to reduce the control effort, and at the same time saving control resources. This controller can be tuned to supply only necessary amount of CO_2 to keep the pH close to its optimal value.
Nonlinear model predictive controller	
This article shows a nonlinear model predictive controller (NMPC) law to create an anticipated effect, based on the prediction of the future behavior of the systems. The aim was the tracking of a reference trajectory in nonlinear systems (Tebbani, Titica, Ifrim, Barbu, & Caraman, 2015).	The controller maintains the system at its optimal growth capacity. The increase of the biomass growth induces an increase of the applied light intensity. Nevertheless, simulations highlighted the sensitivity of this approach to the model accuracy.
This wok proposes the design of a Robust Predictive Control strategy which guarantees robustness towards parameters mismatch for a continuous PBR model (Benattia, Tebbani, & Dumur, 2015)	The developed control law is compared to classical and robust predictive controllers. Its efficiency and robustness against parameter uncertainties are illustrated through numerical results.
Feed-forward controller	
This study shows a feed-forward inversion control scheme for maintaining an optimum incident irradiance on PBR during batch cultivation (Kandilian, Tsao, & Pilon, 2014).	The feed-forward inversion control adjusted the incident irradiance with respect to the in-process measured mass concentration to maintain the optimum average fluence rate inside the PBR. The method demonstrated in this investigation can be used for any microorganism species and PBR design.
Adaptative Control	
The authors proposed nonlinear control laws which regulate the light attenuation factor in a microalgae culture (Mairet, Muñoz-Tamayo, & Bernard, 2015).	It was shown through numerical simulations that the adaptive controller present good performances in terms of regulation under constant light. Finally, numerical simulations too illustrate how the adaptative controller can be used to optimize biomass productivity under day-night cycles.
Filtered Smith Predictor	
In this work was sought to maintain the constant pH using NaOH or H_2SO_4. The model presents delay and uncertainty in the parameters (Romero-García, Guzmán, Moreno, Acién, & Fernández-Sevilla, 2012).	A Filtered Smith Predictor (FSP) is proposed as control strategy. The FSP has reduced the Integral Absolute Error and the time for the solution addition in more tan 25% and it has increased 5% the production of L-aminoacids compared to an on-off control, which is the controller most used in these processes. In addition, it has an acceptable performance despite the uncertainty of the systems, presenting a robust behaviour.

Table A.3. Some optimization methods applied in cultures of microalgae

PROPOSAL	RESULTS
The authors considered a optimal control problem that consists in finding the time evolution of manipulated variables maximizing a given criterion on a finite time horizon (Muñoz-Tamayo, Mairet, & Bernard, 2013).	This work shows that the quasi optimal (QO) controller performs as well as the optimal controllers, confirming the hypothesis that controlling the efficiency of light absorption makes it possible to attain high productivities both in lipid and biomass. The response of the QO controller suggests that an optimal strategy consists in driving the biomass concentration to a certain value and to allow it to oscillate around this point.
This study shows a data-based model-free optimization using quadratic fit to rapidly estimate the optimum average fluency rate set point value that rendered maximum microalgae growth rate and biomass productivity (Kandilian et al., 2014).	This approach can rapidly identify the optimum average fluence rate for any given species, reduce the lag time, and increase the growth rate and productivity of microalgae.
In this paper the authors solving min-max optimization problem for set-point trajectory tracking. Next they proposed a reduction the basic min-max problem into a regularized optimization problem based on the use of linearization techniques, to ensure a good trade-off between tracking accuracy and computation time (Benattia et al., 2015).	Tests in simulation show good performance of the proposed strategy with respect to worst-case model uncertainties. Moreover, it allows to significantly reduce the computational load with a good tracking trajectory accuracy.
This paper proposes a control structure for the optimization of the microalgae cultivation process in PBR, which uses a performance criterion that includes productivity and light use (Ifrim, Titica, Barbu, Ceanga, & Caraman, 2016).	In this work was focuses on the optimization through the dilution rate, *D*. In this case, the extremum seeking procedure was used. The authors found that the PBR optimization through extremum seeking method is theoretically possible but with poor performances which severely limit its applicability.
This work demonstrated a procedure to optimize the harvest time and the pond depth to make the process economically viable (Jayaraman & Rhinehart, 2015).	A Bootstrapping analysis was done to estimate the uncertainty associated with model parameters, and to evaluate the range of optimized harvest time and pond depth. This work also demonstrated leapfrogging as a viable technique for optimizing stochastic processes.